Ecophysiology and ecological impacts of an Antarctic invader: The chironomid, *Eretmoptera murphyi*.

By

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Thesis Abstract

Antarctica has entered a period of rapid, and potentially drastic, change. The combined pressures of anthropogenic climate change, which disproportionately affects the polar regions, and an increase in human activity and connectivity in and around the Antarctic, is opening the least invaded continent on the planet to new species. As ice retreats, terrestrial habitats ripe for colonisation by both humans and non-native species are increasing, and so must our knowledge of the biology, ecology and impact of invading species. This thesis explores these issues through the model invasive species, the chironomid, *Eretmoptera murphyi* Schaeffer (Diptera: Chironomidae), which has successfully colonised Signy Island in the maritime Antarctic, following introduction by humans in the 1960s. Through whole organism experiments and field observations, we confirm parthenogenesis and adult emergence throughout summer on Signy. Physiological studies are employed to assess the midge's potential to establish further south, and/or cope with climate change. Differing responses to temperature are identified in different life stages, which at various points in the life cycle must endure microclimate temperatures from +30 ºC to -20 ºC, on Signy Island. The impact of microhabitat temperature and moisture conditions on development and overwintering survival is examined, with oviposition sites found to be an important factor in determining reproductive success, especially considering a warming climate. The extent of *E. murphyi'*s distribution on Signy is updated, doubling previous estimates of its range, and finding that it is on the brink of moving into new valley systems. Where it occurs, the midge is capable of increasing soil nitrates by as much as five times the background levels, bringing nitrogen levels up to that seen in association with seal colonies. As the only true insect on the island, and a significant detritivore, *E. murphyi* has the potential to affect change to local vegetation and is arguably a new keystone species in this nutrient-poor ecosystem. Existing biosecurity measures in place seem unlikely to limit its spread which appears to be tracking footpaths used by researchers on the island. Larval stages are also able to survive several weeks in sea water, suggesting there is little impediment to its eventual colonisation of other islands and the Antarctic Peninsula, where it would likely flourish. This body of work encompasses a range of disciplines from whole organism biology through to ecosystem function, and highlights the impact that a single, and seemingly innocuous invasive species can have on an Antarctic terrestrial ecosystem.

“Difficulties are just things to overcome, after all.”

- Ernest Shackleton

"A human being should be able to change a diaper, plan an invasion, butcher a hog, conn a ship, design a building, write a sonnet, balance accounts, build a wall, set a bone, comfort the dying, take orders, give orders, cooperate, act alone, solve equations, analyse a new problem, pitch manure, program a computer, cook a tasty meal, fight efficiently, die gallantly. Specialization is for insects."

- Robert Heinlein, Time Enough for Love.

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Author Contributions

This thesis is presented in the 'alternative format', being made of six experimental chapters and one appendix that are written, or already published/submitted, as scientific papers. I am first author of all chapter papers, and second author on the appended paper, of which the latter details author contributions separately. Supervison from SH and PC applies to all chapters. Authors and their contributions to each experimental chapter are as follows:

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Chapter 1: Thesis Introduction

1.1 Overview

Biological invasions are one of the largest threats to global biodiversity, with claims that in some areas, the impacts of non-native species outweigh those of habitat loss and climate change (Clavero and Garcia-Berthou, 2005; Kearney et al., 2018). As human activities and influences become more globalised there is a greater risk of biological homogenisation of species across continents, exacerbated by the effects of climate change (Banks et al., 2014). In order to predict these changes, and potentially limit them, they must first be understood across a breadth of disciplines: studying the life history, physiology, habitat requirements, and phenology, with potential human linkages will all aid in forecasting range limitations and, thus, the boundaries of potential spread (e.g. Pertierra et al., 2017). Such knowledge can inform whether similar species may cause concern, and even identify 'weak-spots' in an invasive species' biology that can be exploited through control measures (Klassen and Curtis, 2005). Furthermore, understanding the invaded ecosystem is important as the influence that just a single species can have on entire food chains and ecosystems is increasingly recognised (e.g. David et al., 2017; Feit et al., 2018; Hill et al., 2003; Quist et al., 2014). By assessing the ecological impacts that a non-native species has on an ecosystem, both the role of that taxon, and the risks that the species may present can be identified.

Establishing ecological impact not only aids understanding of ecological function, but also helps policy makers and practitioners prioritise which non-native species pose the highest risk. In vulnerable and simple ecosystems, such as those of terrestrial Antarctica, control can be particularly challenging. This is not only because of the severe environment and its practical and logistical challenges, but also the continent's governance system, which requires that any course of action cannot result in any greater detriment to the environment (Grant et al., 2012; Hughes et al., 2019; Secretariat to the Antarctic Treaty, 2011). Therefore, obtaining a full understanding of an introduced

species and its biology is the primary route to enabling future management, and the prevention of similar invasions (Hughes et al., 2019). Antarctica is today the least biologically invaded continent on the planet, and the Antarctic Treaty places a strong responsibility on its signatory nations to ensure the future protection of the Antarctic environment and its ecosystems, including minimising the threat of human-assisted biological invasions.

Using the introduced midge *Eretmoptera murphyi* (Schaeffer, 1914) as an 'invasive model species', and the colonised Signy Island as a 'model ecosystem', this thesis investigates the risk it poses to Antarctic ecosystems through assessment of its life history, phenology, stress physiology, distribution and dispersal potential, ecological impacts and, finally, assesses the efficacy of existing biosecurity measures in place in Antarctica.

1.2 Antarctica and Signy Island

The Antarctic region can be broadly divided into three major biogeographic zones based on overall environmental and biological characteristics (Convey, 2013; Smith, 1984): the continental Antarctic which includes the main body of the Antarctic continent, the maritime Antarctic which includes the western Antarctic Peninsula and the archipelagos of the South Shetland Islands, South Sandwich Islands, South Orkney Islands, and Bouvetøya and Peter Øya, and the sub-Antarctic islands which include the major island groups of the Southern Ocean (Fig. 1.1). However, recent studies have divided ice-free habitats in Antarctica into 16 distinct biogeographic regions, known as "Antarctic Conservation Biogeographic Regions (ACBR's)" (Terauds et al., 2012; Terauds and Lee, 2016). For the purposes of this thesis, the three widely used zones of continental, maritime and sub-Antarctic will primarily be referred to due to the geographical scale of the work.

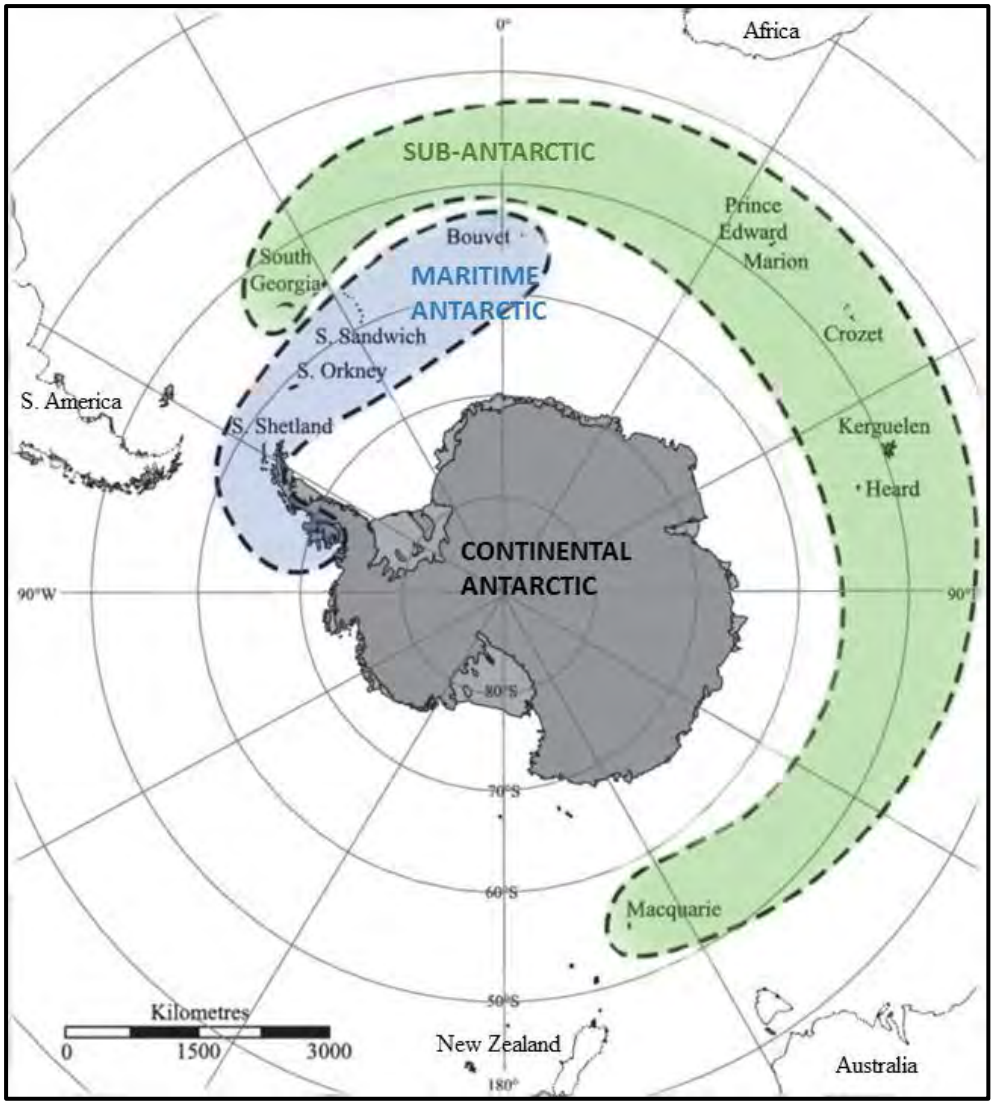


Figure 1.1. *The three main Antarctic biogeographical zones depicting continental, maritime and the sub-Antarctic regions. Redrawn from Thomas et al., 2008.*

1.2.1 Terrestrial Antarctica

The Antarctic continent is unique, both in its geography and climate: encircled by the Southern Ocean, it is geographically isolated by over 4,000 km from Australasia and Africa, and 1,000 km from its nearest neighbour South America (Fig 1.1). This isolation is further accentuated by the strong oceanic and atmospheric circulations surrounding the continent and dividing it from lower latitudes (Clarke et al., 2005). Whilst the oceans surrounding Antarctica are rich in life, the landmass is very different as the extreme environment limits the life that can survive there: Over 99.7% of Antarctica is permanently covered by snow or ice (Burton-Johnston et al., 2016), on average 2 km thick, and

terrestrial organisms face long winters lasting six to nine months (Convey and Block, 1996). Air temperatures during winter fall below −40 °C in the continental Antarctic and often below −10 °C in the maritime Antarctic (Block et al., 2009; Convey, 2017). The little ice-free land that is available has been scoured during repeated glacial cycles, and water is locked away as ice for most of the year, leaving habitats low in nutrients and liquid water (Block et al., 2009).

The extreme isolation of Antarctica, combined with the extremely patchy distribution of terrestrial habitats separated by barren glaciated areas, has led to very low rates of biological colonisation. Most of Antarctica's terrestrial biodiversity is limited to the less extreme ice-free habitats found around the coasts, at the edges of the ice sheets, and on the maritime and sub-Antarctic islands, comprising just 0.21% of all terrestrial area within the Antarctic region (Burton- Johnson et al., 2016; Convey et al., 2008, 2009). Yet despite the hostility of the region, terrestrial life has persisted in the Antarctic regions for tens of millions of years, with extant microbes, flora, and fauna surviving through periods of intense glaciation (Bennett et al., 2016; Biersma et al., 2018; Chong et al., 2015; Convey et al., 2009a; Iakovenko et al., 2015). Antarctica has few native terrestrial species, but higher life forms do exist: ice-free areas are dominated by a cryptogramic vegetation of moss, lichen and algae, which typically supports a micro-arthropod and micro-invertebrate community of springtails and mites, tardigrades, rotifers and nematodes (Convey, 2017). Ecosystems are simple and truncated, with the most extreme examples supporting food webs including as little as five animal species (Convey and McInnes, 2005). The largest terrestrial organism native to the Antarctic continent is the chironomid midge, *Belgica antarctica* (Jacobs, 1900), which is present along the western Antarctic Peninsula and in the South Shetland Islands. Antarctic terrestrial organisms experience, and are well adapted to, multiple environmental stresses including extreme low and variable temperature, desiccation and lack of water availability, low nutrients, ultraviolet radiation (Convey, 1996a; Convey et al., 2014a; Michaud et al., 2008; Strathdee and Bale, 1998), as well as osmotic and salinity stress (Elnitsky et al., 2009).

1.2.2 Signy Island

1.2.2.1 Geography and climate

Sitting exposed in the Southern Ocean at 60° 30'S, 45° 30'W, the South Orkney Islands consist of four major islands, of which Signy Island is the smallest (Fig 1.2). Most of the South Orkney Islands are heavily glaciated with only steep buttresses and some coastal areas free of ice. The archipelago is subject to the strong circumpolar westerly airflow that surrounds the Antarctic continent, and its annual climate is cooler than might be expected from its latitude due its location well to the south of the Antarctic Circumpolar Current, and to the winter expansion of sea ice northwards from the Weddell Sea. Its climate is comparable with much of the western Antarctic Peninsula, up to eight degrees of latitude and 600 km further south along the Scotia Ridge (Hughes et al., 2013; Walton, 1987). Winter temperatures on Signy average -10 °C (Cannone et al., 2017; Davey et al., 1992; Royles et al., 2013a; Walton, 1987). High annual precipitation in the South Orkney Islands (Cannone et al., 2017; Royles et al., 2013a), mean that soil layers are buffered from the low air temperatures in winter, with snow cover providing insulation from the harshest of conditions (Convey et al., 2018; Davey et al., 1992). Annual precipitation on Signy is around 400 mm y^{-1}, occurring on average of 280 days of the year, and increasingly falling as rain in summer (Holdgate, 1967; Royles et al., 2013a; Walton, 1982). Substrate moisture content is thus generally high in the spring and summer months, due to the combination of precipitation and the melt of snow and ice (Bokhorst et al., 2007a; Gardiner et al., 1998). Signy has positive summer monthly mean air temperatures between 0-3 °C and benefits from the warming effects of Foehn winds from the adjacent Coronation Island that account for some of the island's maximum recorded temperatures (King et al., 2017). Microhabitats within the island's soil and vegetation can easily reach temperatures in excess of 20 °C for short periods, with peaks as high as 38.5 °C recorded as a result of insolation effects (Convey et al., 2018; Walton, 1982; Davey et al., 1992).

Signy is approximately 6 km by 5 km and is dominated by an icecap that divides it along a mountain ridge of a maximum altitude 288 m a.s.l. This currently covers almost half of the island in permanent ice, although this is reducing annually with rising temperatures (Cannone et al., 2017; Smith, 1990).

Two glaciers spill from the ice-cap to reach the sea, the McLeod and Orwell, with the Orwell glacier terminating in a large tidal bay (Fig. 1.3). The island's geology is predominantly metamorphic quartz-mica schist, with occasional outcrops of marble and amphibolite, and is characterised by glacial cirques which create steep areas of unstable rubble and scree that provide poor habitats for terrestrial life (Cannone et al., 2017; Matthews and Marling, 1967). Periglacial activity on the island is a key driver in the formation of mineral soils, with permafrost and aspect major influencers on the development of primary flora (Cannone et al., 2017; Gugelimen et al., 2008).

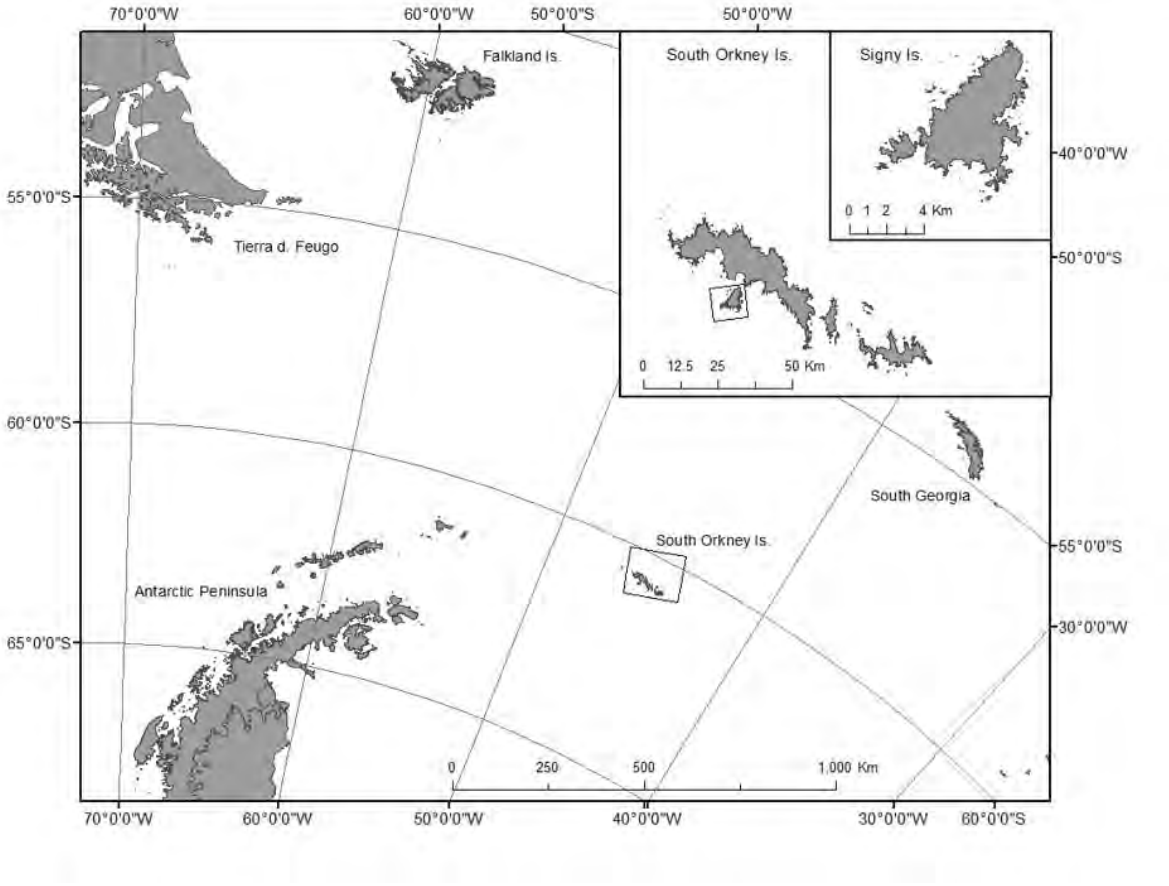


Figure 1.2. *Location of the South Orkney Islands, and Signy Island, in the Southern Ocean. Created using ArcMap®10.4.1 software by Esri. Copyright © Esri.*

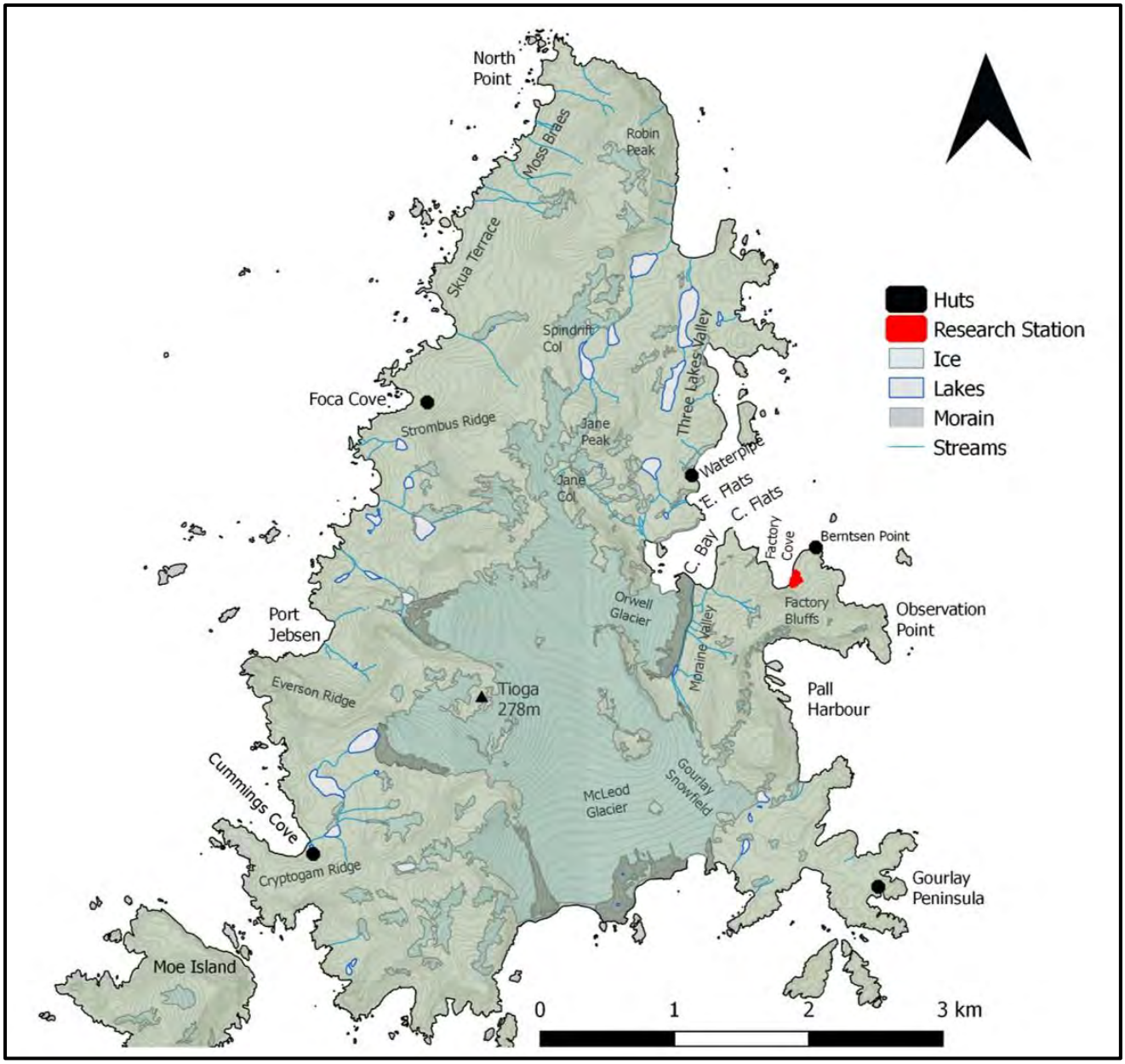


Figure 1.3. *Signy Island shown with key geographical features, locations, and infrastructure. Created using QGIS v2.18 'Las Palmas' (QGIS Development Team 2016. QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org).*

1.2.2.2 Ecology and biota

The ice-free areas of Signy are typically comprised of barren fellfield habitats which are dominated by scree and moraine (Block et al., 2009). Some areas of more stable ground have well-developed moss banks that reach depths of up to 3 m (Allison and Smith, 1973; Smith, 1981). Whilst hosting Antarctica's two native vascular plants, Antarctic pearlwort (*Colobanthus quitensis)* and Antarctic hair-

grass (*Deschampsia antarctica*) (Smith 1972, 1990; Cannone et al., 2017), Signy's vegetation is dominated by cryptogams – typically acrocarpous mosses and lichens. The island is considered to host exceptional examples of Antarctic flora, in particular the extensive moss banks found along the north-western coasts (Ochyra et al., 2008; Smith, 1972, 1990).

Native terrestrial fauna in the maritime Antarctic, and thus Signy also, is limited to the micro-arthropods and micro-invertebrates. The former includes Acari and Collembola, which are found in association with vegetation and soils, but also in areas of high guano deposition. Certain Acari and Collembola are associated with areas of penguin activity (Bokhorst et al., 2016), typically on or under rocks, or within the folds of the commonly occurring foliose alga, *Prasiola crispa*. Micro-invertebrates including nematodes, tardigrades and rotifers are also found in soil and vegetation (Velasco-Castrillión et al., 2014). Two species of Diptera are native to the maritime Antarctic, *Parochlus steineni* and *B. antarctica*, but neither are present on Signy due to natural dispersal limitations between their native ranges and the South Orkney Islands (Chown and Convey, 2016; Convey and Block, 1996).

The lack of significant herbivory and predation in the Signy ecosystem, as for all terrestrial Antarctic systems, increases the strength of any trophic cascades caused by the loss or gain of a new species (Borer et al., 2005). The limited number of interactions compared to a more temperate or even sub-Antarctic ecosystem allows lower trophic levels, such as microbes and detritivores, a great level of efficiency and dominance in the food web (Polis, 1999; Strong, 1992). Similarly, high resource availability through the lack of competition or resource abundance will also promote cascading effects by increasing consumption rates of primary producers (Leibold, 1989; Polis, 1999). However, it is abiotic factors, like temperature and water stress, in these environments that are considered dominant influencing factors, meaning that ecological interactions such as competition and predation are deemed negligible (e.g., Block, 1994; Hayward et al., 2003; Worland, 2005; Teets and Denlinger, 2014).

The low biodiversity, dominance of mosses, cold annual temperatures and high levels of precipitation mean the breakdown of organic matter is slow. This results in low humus content in soils of all Antarctic terrestrial ecosystems. Soil on Signy, and in Antarctica in general, is thin with little humus content and is typically derived from mineral or guano sources (Beyer and Bölter, 2002; Thomas et al., 2008). Consequently, soil layers on Signy are friable and nutrient-poor in those areas away from wildlife colonies. Most nutrient deposition on the island is orthinogenic, as Signy hosts several substantial and globally important sea-bird colonies (Harris et al., 2011). In particular, large colonies of chinstrap (*Pygoscelis antarcticus*), Adélie (*P. adeliae*) and to a lesser extent, gentoo (*P. papua*) penguins, which are all found largely along the south and west coast of the island, along with southern giant petrels (*Macronectes giganteus*) and blue-eyed shags (*Phalacrocorax atriceps*) (Dunn et al., 2015, 2016) (Fig. 1.3). In several areas around the island, including Factory Bluffs immediately adjacent to the research station, bird cliffs and boulder/scree slopes with smaller prions, cape, storm and snow petrels, and southern brown skua (*Stercorarius antarcticus*) are also contributors. In 2017 the island hosted 3,554 fur seals (*Arctocephalus gazella*), and 1,417 elephant seals (*Mirounga leonina*) – although as many as 21,000 fur seals have previously been recorded in any one season (BAS, 2018; Waluda et al., 2010). Both species are major contributors to the terrestrial nutrient budget, *via* direct and wind-blown deposition of faeces and shedded skin (Waluda et al., 2010). Signy's vertebrate wildlife are also contributors to its soil microbiota (Yew et al., 2018), and the addition of nutrients from penguin colonies also attracts native soil micro-arthropods (Bokhorst and Convey, 2016). The composition of soil microbiota communities on the island is largely driven by soil pH but is also influenced by variations in soil/substrate disturbance (Chong et al., 2010), as well as the wildlife colonies and the presence of vegetation (Chong et al., 2009). Thus, Signy represents an exceptional example of a maritime Antarctic terrestrial ecosystem and can be considered a paradigmatic system from which to study polar ecology.

1.3 Polar invertebrates: An overview

1.3.1 Life history and phenology

As ectotherms, invertebrate developmental rates and general physiology are directly influenced by the prevailing environmental conditions and variation therein. Life history strategies have evolved in response to this, which in polar regions may include coping with both short-term exposure to extreme events, and chronic exposure to a range of factors such as low temperature, freezing and/or desiccation. Seasonality at latitudes above the polar circle is extreme: with the sun below the horizon for many months during winter, contrasting with 24 h daylight in mid- summer (Convey, 2010). Because of the environmental conditions, polar invertebrates have 'adversity selected' life-history strategies (Greenslade, 1983), which focus on investment in stress-tolerance strategies, low reproductive investment, limited competitive or dispersal abilities and typically flexible and often extended life cycles (Block, 1990; Cannon and Block 1988; Convey, 1996b, 1997).

Life cycle length is perhaps one of the most distinguishing features of polar invertebrate biology, with only short summer conditions suitable for development combined with long harsh winters driving some level of dormancy, dictating an extension of life cycles compared to temperate equivalents (Convey, 1996b). Thus, development required to complete the life cycle is rarely completed within a single summer season and species will need to overwinter in one or more life stages. For example, the Arctic woolly moth (*Gynaephora groenlandica*) may take more than 10 years to complete its life cycle, compared to just 2-3 in warmer latitudes, whilst the Antarctic mite, *Alaskozetes antarcticus,* can extend its life cycle to 7 years (Danks, 2007).

True diapause is a common feature of high latitude species of the Arctic (Bale et al., 1997; Danks, 2004), serving as a way of synchronising life cycles – an important feature in sexually breeding chironomids for example (Armitage et al., 1995). However, in the southern polar regions this is not the case, with fewer higher-invertebrate/insect species and less clear environmental cues to induce diapause outside of the sub-Antarctic regions (Arnold and Convey, 1998; Convey, 2010). Previous studies have found that the limited seasonal variation in some polar regions also results in a lack of

higher invertebrate species emergence in early summer for reproductive purposes (Chown and Klok, 2003; Chown et al., 2006), whilst greater seasonality permits synchronised, and also, annual life cycles. These annual, or univoltine, life cycles do occur in the regions where species are also found with extended life cycles, however they are rarely seen in the more extreme polar regions. On the Antarctic Peninsula, the native midge *B. antarctica*, takes two years to become reproductive. A study by Harada et al. (2014) found that this extended life-cycle is a result of low-temperature, and that the species is physically capable of emerging sooner, if warmer conditions prevail.

Typically, as is characteristic of adversity selected life-history strategies, polar invertebrates have low reproductive output, compared to temperate species. Moreover, many of the major groups present in the polar regions exhibit parthenogenesis (Chown and Convey, 2016). Asexual reproduction removes the need to synchronise adult emergence with a distinct environmental cue in order to find a mate and retains effective adaptation to the local environment and associated stressors. In environments where physical stresses are less important than biotic interactions (competition for instance), increased genetic variation *via* sexual reproduction is more likely to be selected over asexuality, but in extreme environments parthenogenic species are disproportionately common (Duckhouse, 1985). Furthermore, parthenogenesis can potentially increase species numbers rapidly, and is a trait found in many successful colonising invertebrate species (Block et al., 1984; Frenot et al., 2005)

1.3.2 Role of invertebrates in polar ecosystems

Invertebrates have an essential role in ecosystems globally, accounting for more than 75% of global biodiversity (Larsen et al., 2017). Terrestrial arthropods, dominated by the Class Insecta, are the largest contributors to invertebrate species richness and are essential to terrestrial ecosystem function: for example, they are central to litter and nutrient turnover, aiding the degradation of organic matter (De Groot et al., 2002; Fincher et al., 1981), the pollination of flowering plants (James and Pitts-Singer, 2008) including vitally, food crops. They act as seed dispersers (Farwig and Berens, 2012), pest controllers (Landis et al., 2000), and prey to the higher food chain (Koltz et al., 2017).

In the Arctic, invertebrates are key ecosystem engineers with Arthropoda dominating here also (Roslin et al., 2013; Várkonyi and Roslin, 2013; Wirta et al., 2015a, b, 2016): above ground arthropod species alone make up 83% of all Arctic terrestrial species (Wirta et al., 2016). This is largely due to the abundant Diptera, that take part in many trophic interactions (Schmidt et al., 2016). Indeed, Arctic chironomid diversity outnumbers that of mammals by such a great ratio that it exceeds that of the Diptera-mammal ratio found in the tropics (Wirta et al., 2016 *vs*. Basset et al., 2012). This huge insect community also underpins the success of some Arctic birds who predate on them (Wirta et al., 2015a), as well as larger invertebrates such as predatory spiders that rely on Chironomidae in particular (Brten et al., 2012). Adult stages not only underpin bird populations, but are also key pollinators (Kevan, 1972), and can move nutrients from lakes to soils, aiding soil fertility (Dreyer et al., 2015).

In the Antarctic, terrestrial communities within all the three biogeographic 'zones' are also dominated by arthropods. Whilst insects do occur even in the maritime Antarctic, there are few species represented, with once again the Diptera dominating. However, it is typically the micro-arthropods, such as Collembola and Acari that are the most abundant in both the continental and maritime zones (Spain, 1971; Treonis et al., 1999; Wharton and Brown 1989). As with any ecosystem, microbial autotrophs form the basis of the Antarctic ecological process (Wynn-Williams, 1996), but with little to no vegetation cover, the role that they play in primary colonisation and continued establishment of other biota is crucial. Studies that focus on the role that invertebrates play in Antarctica are largely focussed on the sub-Antarctic islands. On sub-Antarctic Marion Island, the most influential invertebrates are chironomid detritivores that are capable of significantly affecting soil nutrients and litter turnover (Hänel and Chown, 1998). Whilst still very low in biodiversity on a global scale, the sub-Antarctic region is more species rich than the maritime or continental Antarctic regions, with 19 indigenous insect species occurring on Marion Island alone (Chown and Convey, 2016; Smith, 1977), compared to just two in the entire maritime Antarctic region. Therefore, any function played by an individual species in the sub-Antarctic will be much harder to disentangle from other invertebrates in these more complex ecosystems. However, the introduction of non-native species to sub-Antarctic

islands has seen entirely new guilds established, with pollinators (Convey et al., 2010), and predators (Chevrier, 1996; Convey et al., 2011; Ernsting et al., 1993, 1995) introduced as well as more herbivorous (Chown and Avenant, 1992) and detritivorous species (Hänel and Chown, 2010)

In the maritime Antarctic, micro-arthropods such as Collembola and Acari typically dominate terrestrial ecosystems, certainly in terms of population size (Sinclair and Sjursen, 2001; Spain, 1971; Treonis et al., 1999; Wharton and Brown, 1989). However, once more, Chironomidae in the form of *B. antarctica* will likely be larger contributors to litter turnover, as the largest native terrestrial organism in Antarctica (Peckham, 1971).

1.4 Polar invertebrates: Physiological response to stress

Invertebrates are poikilothermic ectotherms, thus their body temperature is directly influenced by the surrounding environmental conditions (Speight et al., 2008). As they can occupy a range of microhabitats, it is these microclimate conditions that are important to characterise, over large-scale climate data. Microhabitats are particularly important in polar regions, where they can ameliorate the harshest of conditions. Vegetation type, colour, snow depth and water content can all vary across a habitat and polar invertebrates will seek out those that best suit their physiological needs. (Davey et al., 1992; Convey et al., 2018; Hayward et al., 2003, 2004; Holmstrup and Zachariassen, 1996). For instance, the Antarctic mite *Alaskozetes antarcticus*, will choose a lower temperature microhabitat in order to maintain a cold-hardened state, whilst the collembolan *Cryptopygus antarcticus* will seek microhabitats based on hygric preferences (Hayward et al., 2003).

1.4.1 Cold tolerance

For any polar terrestrial invertebrate, a primary stressor will inevitably be low temperature, with species-specific physiological responses dependent on the characteristics of exposure, e.g. rate of temperature change, minimum temperature experienced, etc. A primary risk is injury from intracellular ice formation, and physiological responses have evolved to either remove or mask ice-nucleators which promote freezing, or to control the growth of the ice within extracellular spaces

through the directed synthesis of 'ice nucleating agents' (Lee, 2010). Species have two main strategies for surviving the cold, either 'Freeze-intolerant or avoiding' (freeze-avoiding hereafter), or 'Freeze-tolerant'(Bale, 2002; Convey, 1996a; Lee, 2010) (Fig. 1.4).

Freeze-avoiding invertebrates have been categorised further based on how proximate their lower lethal temperature (LLT) is to their freezing temperature/ supercooling point (SCP) (Bale, 1996), with most Antarctic animals either truly freeze-avoidant, or chill tolerant, chill susceptible, or opportunistic(Block, 1990; Hayward et al., 2003). Chill-tolerant, chill susceptible and opportunistic species will die after prolonged chilling at best, whilst the truly freeze-avoidant can survive extensive periods in the supercooled state (Bale, 1996). All freeze-avoiding species are not able to survive internal ice formation but can survive sudden cold shocks and low temperatures up to the point at which the body freezes (Bale, 1996; Cannon and Block, 1988; Storey and Storey, 1988; Zachariassen, 1985). (SCP) (Fig 1.4). Instead of finding means to tolerate ice formation, freeze-avoiding species depress their SCP so that they may remain unfrozen. This can happen on seasonal timescales (Bale, 1996) or across diurnal timescales, for example Worland and Convey (2001) noted a day-to-night transition of SCPs in the Antarctic springtail, *C. antarcticus* on Signy Island. Several processes can enhance supercooling, but this primarily involves removing as many ice-nucleating particles as possible, for example by ceasing feeding (e.g., Bokhorst et al., 2007b; Cannon and Block, 1988; Sømme and Block, 1982), or through moulting or ecdysis where the outer cuticle, gut lining (including microbiota) and the gut contents are removed (Hawes et al., 2007).

In contrast, freeze-tolerant invertebrates can survive the formation of ice in body fluids, although crystallisation events must be restricted to extracellular areas. They achieve this only over a specific temperature range , yet with a generally higher SCP than a freeze-avoiding species, as freezing is better controlled at higher temperatures (Duman and Horwath, 1983). Once frozen, these species can survive a broad range of sub-zero temperatures. Freeze-tolerant species accumulate ice nucleating agents to control the formation of ice, to the exclusion of solutes. As a result, the cells dehydrate and are therefore less vulnerable to damage from intracellular freezing but run the risk of desiccation stress as water moves from interacellular spaces to extracellular spaces to form ice out of harms way.

Water replacement molecules are accumulated to buffer this dehydration stress (Worland and Block, 1999). In order to reduce the risk of excessively large ice crystals, freeze-tolerant species may also utilise antifreeze proteins to reduce the size of the crystals in a process called "ice recrystallisation inhibition" (Duman et al., 2004).

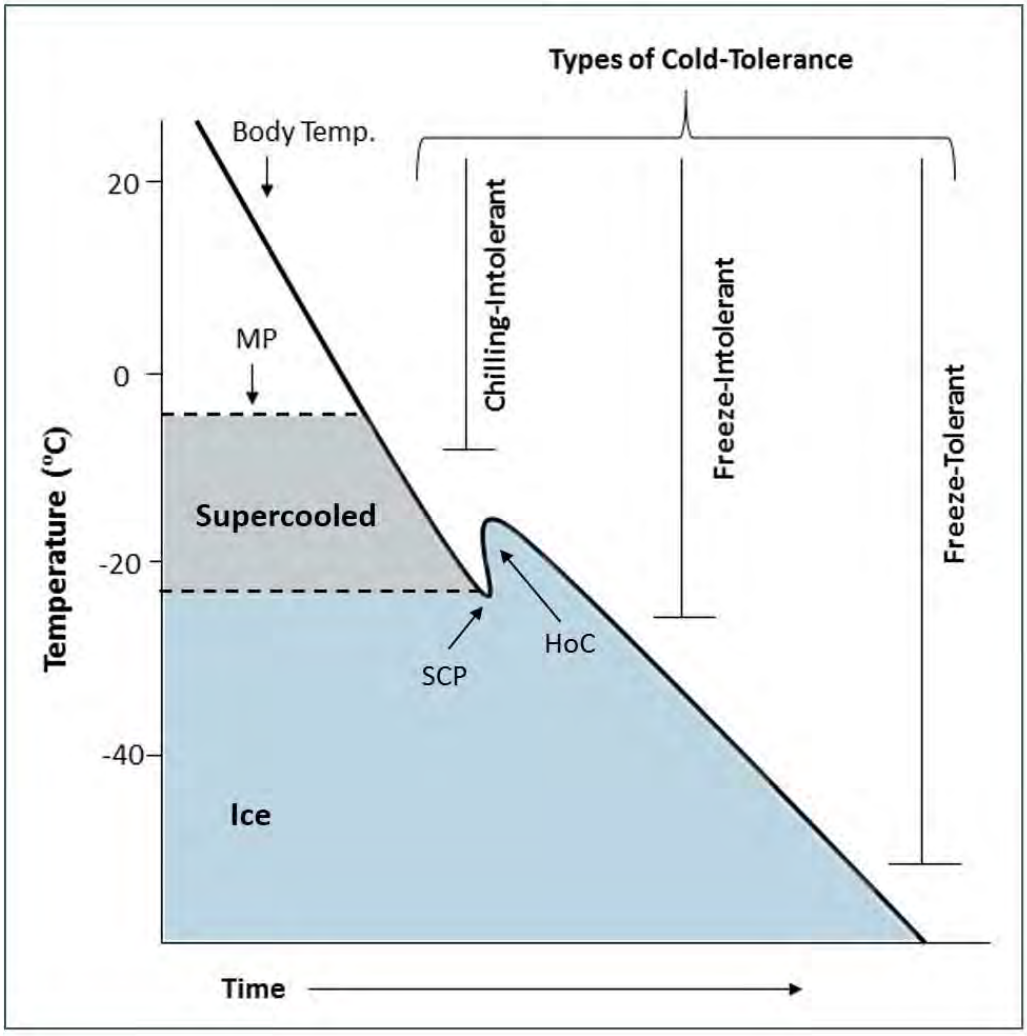


Figure 1.4. *Insect responses to low temperature and categorisation of subsequent levels of cold-tolerance. Image redrawn from Lee, 2010 and originally adapted from Lee, 1989. Body temperature in relation to the hemolymph melting point (MP), supercooling point (SCP), and release of the heat of crystallization (HoC), as body fluids freeze.*

Many polar insects survive the cold through expressing freezing tolerance strategies, including the Arctic woolly moth *Gynaephora groenlandica* (Strathdee and Bale, 1998), the sub-Antarctic coleopterans, *Hydromedion sparsutum* and *Perimylops antarcticus* (Worland and Block, 1999), and the polar dipterans, *B. antarctica* (Benoit et al., 2009a) and *Eretmoptera murphyi* (Worland, 2010). Freeze-avoiding species are geographically wide ranging and include alpine and continental species as

well as polar. Examples include the Antarctic collembolan *C. antarcticus* (Block and Worland, 2001) and mite *A. antarcticus* (Convey, 1994; Hayward et al., 2003). Where temperatures are low enough, or the temperature transition sudden enough, even freeze-avoiding or tolerant species are susceptible to injuries as a result of chilling. Hayward et al. (2014) define chilling as "cooling sufficient to induce damaging effects or even death in the absence of freezing". Such injuries can occur in more cold-tolerant polar species through brief direct chilling injuries or cold-shock, that can damage cell membranes and disrupt the movement of ions . For instance changes in hemolymph volume as a result of cold exposure, result in a change to osmotic equilibriums across the gut wall, which in turn resulted in the migration of ions crucial for muscle use. This loss of muscle excitability is associated with chill injuries and the onset of chill-coma (e.g. MacMillian and Sinclair, 2011; see also Kostál et al., 2006) Foraging, mating and overall movement are also affected by low temperatures. The point at which an invertebrate loses neuromuscular coordination is defined as the Critical Thermal Minimum (CT_{min}) (Mellanby, 1939; Semper, 1883). If temperatures continue to decline beyond this, then movement will cease altogether and the invertebrate will enter a 'Chill Coma' (Hazell and Bale, 2011). Polar invertebrates can typically remain active at temperatures a little below 0 ºC (Coulson et al., 1995), and take advantage of the cool shoulder seasons of spring and summer, such as is the case with Antarctic mites and collembolans (Block, 1990; Sinclair et al., 2006). Some Arctic dipterans (Trichocera) have even demonstrated the capacity to fly at -4 ºC and remain active at temperatures as low as -12 ºC (Hågvar, 2010). A low thermal activity threshold such as this allows species such as the midge *B. antarctica* to emerge at the first sign of snow melt in late spring/early summer, shaping the species' life history and phenology (Suggs et al., 1983).

1.4.2 Response to anoxia

Another risk of living in polar terrestrial habitats is anoxic stress, which can result from habitat flooding during spring melt or ice entrapment during freeze events/winter (Hodkinson and Bird, 2004; Sømme and Block, 1982; Lopez-Martinez et al., 2008). Polar terrestrial habitats are often waterlogged in the summer months, and/or are naturally water retentive owing to the high moss and peat composition of most ice-free habitats (Sømme and Block, 1982). As many polar invertebrates

utilise such habitats, they are vulnerable to irregular freeze-thaw cycles in early summer and the onset of winter conditions, exposing any resident fauna to periods of both flood and freeze, and in effect a semi-aquatic lifestyle (Convey, 1996a). The Arctic collembolan *Isotoma violocea*, which cannot survive more than six days of anoxic conditions, behaviourally avoids ice-entrapment by moving into the interstitial spaces in snow (Sømme and Conradi-Larsen, 1977). Other species have evolved physiological strategies to survive anoxic conditions, with some dipterans able to respire underwater (Elnitsky et al., 2009; Everatt et al., 2014a), and Collembola, mites and the midge *E. murphyi* surviving days to weeks entrapped in ice (Block and Sømme, 1982, 1983; Everatt et al., 2014a; Sømme and Block, 1984). However, winter conditions and the resulting ice-entrapment could extend to months not weeks, and tolerance to long term entrapment and resulting anoxia is untested in many polar invertebrates.

1.4.3 Heat and hygric stress physiology

Whilst a predominantly cold environment, polar regions are, like everywhere else, prone to extremes that include both unusual temperature highs as well as lows. In a warming climate, it is likely that short high temperature events will occur more frequently, along with an increased risk of warm weather drought in the summer months (Bokhorst et al., 2011; Convey et al., 2009a). Whilst hygric stress is already common during winter when liquid water is locked up as ice. The immediate habitat of polar invertebrates will also have an impact on environmental conditions experienced, for example, darker vegetation patches greatly exceeding air temperatures due to insolation and albedo effects, and different mosses and lichens providing varying levels of insulation to winter cold (Danks, 1991; Gugelimen et al., 2008). At the scale of invertebrates, even the folds of lichen and algae will create useful microhabitats in harsh and barren landscapes, retaining both water and heat (Convey, 1996a; Convey and Smith, 1997; Leather, 1993; Lindsay, 1978).

Invertebrates tend to have a large body surface area to mass ratio, leaving them vulnerable to desiccation. There are two basic physiological strategies to cope with desiccation stress that are classified in a similar way to cold-hardiness: desiccation resistance, where an organism prevents water

loss, e.g. *via* a lowered cuticular permeability (e.g., Benoit et al., 2007); or desiccation tolerance, whereby the organism can survive water loss (Danks, 1999). The most extreme example of desiccation tolerance is anhydrobiosis, and this has been noted in tropical midge species, e.g. *Polypedilum vanderplankii* (Takashi et al., 2009). Both the Antarctic chironomids, *B. antarctica* and *E. murphyi* are also highly desiccation tolerant, surviving the loss of more than 80% of their normal hydrated water content (Hayward et al., 2007; Elnitsky et al., 2009; Everatt et al., 2014c).

Studies have shown that invertebrates at high latitudes may benefit from a general increase in temperatures (Bale and Hayward, 2010; Convey, 2011), and that the thermal sensitivity of invertebrates to increases in temperature declines with increasing latitude (Addo-Bediako et al., 2000; Deutsh et al., 2008). Even Antarctic arthropods can survive above 30 ºC for short periods of time (Everatt et al., 2013a; Sinclair et al., 2006; Slabber et al., 2007), consistent with the large and rapid natural variability in ground surface temperature at microhabitat level (Convey et al., 2018; Peck et al., 2006). Responses at community level may differ from those of individual species, with climate change simulation studies finding that overall arthropod abundance decreased with warming conditions, interpreted as a consequence of increased desiccation stress (Convey et al., 2002; Day et al., 2009). Several studies have explored the effects of heat stress on polar invertebrates, finding that some arthropods have a high tolerance to warming temperatures and that survival is more likely influenced by water availability than thermal stress (Bale and Hayward, 2010; Everatt et al., 2014b; Sinclair et al., 2006). Furthermore, the benefits of cross-tolerance between cold exposure and desiccation are not seen with heat and desiccation. Particularly in species that do not undergo anhydrobiosis or vitrify, including the Antarctic dipteran *E. murphyi* , as well as nematode and Collembola species (Everatt et al., 2014c; Holmstrup and Zachariassen, 1996). This is likely because injuries associated with heat stress differ from those of desiccation, and may even be conflicting (Everatt et al., 2014b).

1.4.4 Response to salinity

With most ice-free habitats in the maritime and continental Antarctic occurring in coastal areas, exposure to wind-blown sea spray and in some cases tidal inundation are inevitable (Baust and Lee, 1987). Salinity stress is therefore variable and can include exposure to exceptionally high salinity in habitats where sea water can evaporate (Elnitsky et al., 2009). High salinity typically inflicts mortality in invertebrates, particularly terrestrial and freshwater species (Hassell et al., 2006), largely because of the disruption to ion regulation and osmotic dehydration (Benoit et al., 2009; Somero and Yancey, 1997; Sømme and Block, 1984). The Antarctic midge *B. antarctica* is known to have a high tolerance to osmotic dehydration when exposed to complete sea-water submergence in both normal and 2 x concentrated sea water (Elnitsky et al., 2009), despite dipterans in general being unable to tolerate prolonged seawater submergence (Bayly, 1972). For invertebrate populations concentrated on the coasts, there is also the potential for individuals to be washed out to sea, and this could be an important passive dispersal mechanism to new sites. There is certainly evidence of invertebrates rafting on vegetation, debris, and even the water surface, particularly Collembola species (Hawes, 2008, 2011; Hopkin, 1997; Coulson et al., 2002; McGaughran et al., 2011).

1.5 Anthropogenic influence in Antarctica

Climate change is by far the most pressing issue of our day, and the Intergovernmental Panel on Climate Change (IPCC) predicts that primarily as a result of increasing levels of anthropogenic greenhouse gases, global temperatures will increase by at least 1.5 ºC by the mid-21st Century (IPCC 2018). These temperature rises will be felt worldwide, along with forecast risks of extreme weather events, sea level rise and associated habitat loss (IPCC, 2014). Meanwhile, biological invasions as a result of increased human movement and lack of control measures, have resulted in ecological catastrophes around the globe, for example, the introduction of the cane toad in Australia (Australian TSSC, 2005), feral cats, particularly on islands (Bonnaud et al., 2012; Hilton and Cuthbert, 2010; Medina et al., 2014), and the Asian longhorn beetle (Townsend Peterson et al., 2004), to name a few. The combination of climate change and human movement will exacerbate the issues either one of

them raises, with warming climates likely to open new habitats in polar regions, and thus increase the opportunities for invasive species to establish (Hellmann et al., 2008)

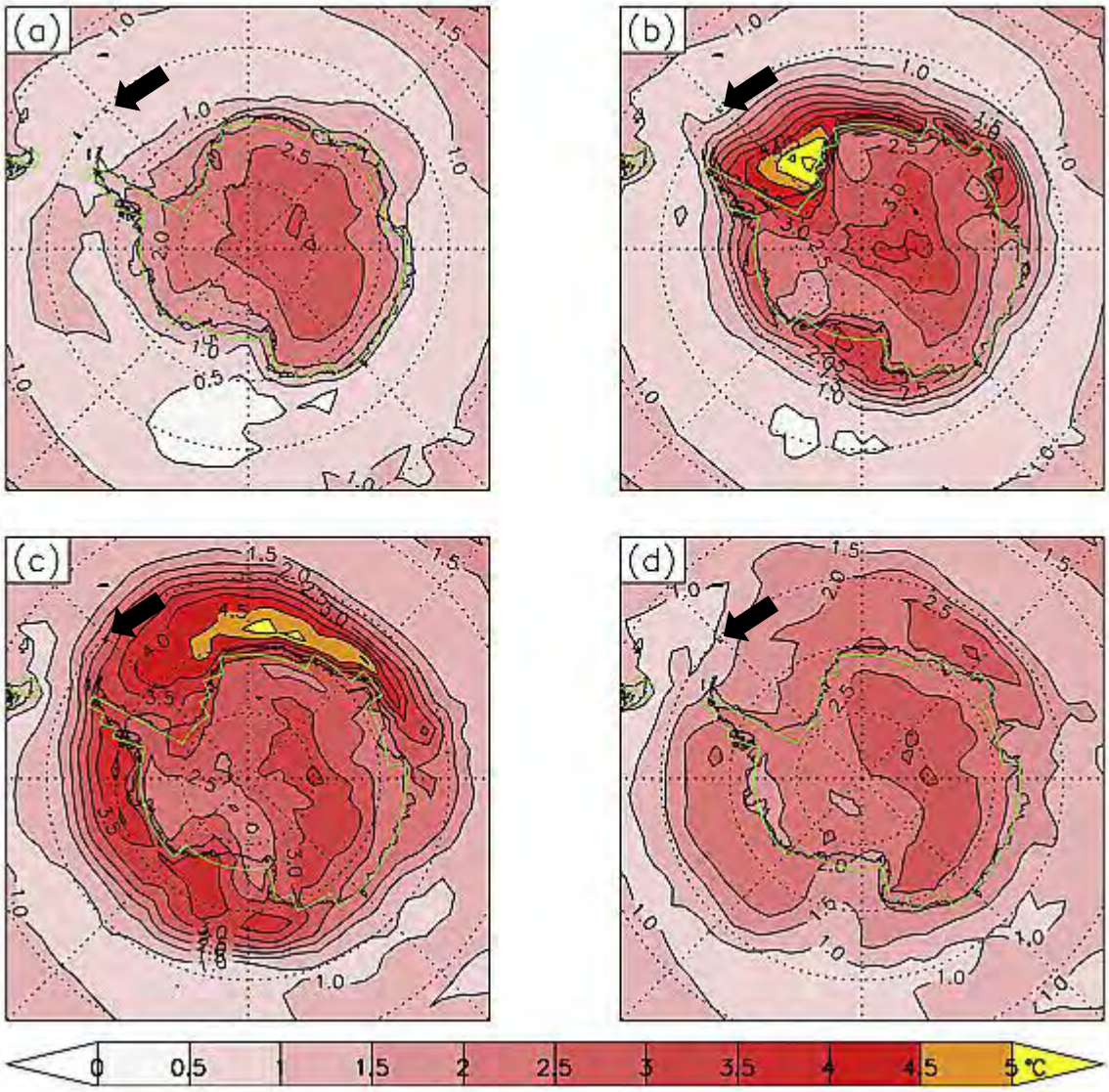
The Antarctic continent is the least biologically invaded on the planet but is at increasing risk from non-native species introductions given the inexorable increase in human activity and ongoing climatic changes that are eroding the natural barriers to invaders of the continent. This, in ecosystems that are already considerably more sensitive to changes than temperate environments (Larsen et al., 2014).

1.5.1 Climate change: Temperature

The polar regions are particularly vulnerable to climate changes due to 'polar amplification' whereby radiative feedbacks as a result of changing albedo at the poles mean that the Arctic and parts of the Antarctic are the fastest warming on the Planet (IPCC, 2013). For example, in the latter part of the 20th Century the western Antarctic Peninsula was one of the most rapidly warming regions on earth, with a surface air warming rate of 3.7 ± 1.6°C per century, and polar regions in general having increased already by 2 °C over the last 50 years (Convey et al., 2009a, b; Vaughan et al., 2003). In the maritime Antarctic Islands, Signy in the South Orkney archipelago holds the record for the highest standard meteorological air temperature in Antarctica: a startling 19.8 ºC was recorded on the 30th January 1982 (King et al., 2017), a number that will surely be exceeded in years to come. The maritime Antarctic, despite its overall low temperature, has distinct seasonal climate oscillations (Vaughan, 2006) and the predicted warming is likely to affect summer microclimate conditions, which typically stay close to 0 ºC, as well as diurnal fluctuations (Bokhorst et al., 2008, 2011; Convey et al., 2018; Davey et al., 1992; Janetschek, 1967), and a reduction in extremely cold winters (Turner et al., 2014). The western Antarctic Peninsula has been warming the most during the winter months, with an increase of +1.01 ºC per decade (Turner et al., 2012). This is likely to impact the resident flora and fauna and result in longer growing and active seasons. Consequently, Antarctic terrestrial biota

have become a prime representation for the response of organisms and ecosystems to climate change (Bers et al., 2012; Smith et al., 2008).

Figure 1.5. Antarctica climate change in the 21st century – skin temperature changes. Adapted from



Bracegirdle et al., 2008. a) DJF b) MAM c) JJA d) SON. Difference between the 2004-2023 mean and forecast 2080-2099 mean. The forecast rapid retreat of sea ice from East Antarctica leads to larger surface warming off the coast, particulary in the winter (c). South Orkney islands highlighted with black arrow.

1.5.2 Climate change: Water

As well as temperature changes, climate warming is likely to alter water availability as warmer air temperatures and associated increases in atmospheric moisture are forecast, increasing global precipitation, and levels of meltwater (IPCC-CMIP5, 2013; Turner et al., 2009). In the polar regions,

water availability is predicted to increase by up to 1% per decade (Walther et al., 2002), with more precipitation expected overall (Bintanja et al., 2017; Genthon et al., 2009; Nakiaenovia et al., 2000). An increase in precipitation as rainfall is expected in both the Arctic (Bintanja et al., 2017) and the Antarctic Peninsula as it warms (Convey et al., 2009a). Greater water availability will be of biological significance in places such as the maritime Antarctic, where liquid water is limited (Convey et al., 2006). Already changes in temperature and precipitation have increased biological production in some freshwater lakes (Queseda et al., 2006) and longer melt seasons and the potential of additional precipitation, even as rainfall, will change the dynamics and stresses experienced by both flora and fauna (Convey et al., 2009a; Turner et al., 2014). For arthropods, both in the Arctic and Antarctic, this is likely to be of benefit unless flooding is excessive, or additional snowfall results in significant melt events that could lead to prolonged submergence.

1.5.3 Human movement in Antarctica

Globally, humans have affected change on ecological systems through the introduction of new species. Antarctica's geographical isolation and challenging environment have largely acted as an effective barrier to non-native species dispersal and establishment *via* passive processes, particularly in Continental areas (Frenot et al., 2005; Hemmings, 2007; Hughes et al., 2015). However, increasing levels of tourism, scientific activity and associated support services are increasing the probability of non-native species introductions (Duffy et al., 2017; Frenot et al., 2005; Hughes et al., 2015; Tin et al., 2009). In 2018, 42,576 tourists visited the continent, an increase of 16% on the year previous (IAATO, 2019). This outnumbers visiting scientists and their support by an order of magnitude, with all Antarctic stations at peak population amounting to 4,607 from 30 National Antarctic Operators (COMNAP, 2019). However, a larger population of visitors does not equate to a larger footprint, and most introductions in recent decades have resulted from scientific activity (Frenot et al., 2005; Hughes et al., 2015). National programmes enter and move around Antarctica *via* land, sea and air, and as a result logistical hubs become high risk areas for the introduction of both indigenous and non-native species. This is of particular concern around hubs with inter-continental flights, and of areas with

multiple hubs or research stations (Houghton et al., 2014; Hughes et al., 2019; Lee and Chown, 2009; Tsujimoto and Imura, 2012).

Within Antarctica there is also potential for inter-regional dispersal: Hughes et al. (2019) find that there is much movement between ice-free areas of the ACBRs, including routes that reach between the sub-Antarctic islands, the maritime Antarctic and the continent. Chinese, Russian and Korean operators have resupply routes that reach from the East Antarctic coast to the western Antarctic Peninsula, with multiple stops in-between in some instances (Hughes et al., 2019) (Fig 1.5). However, there are currently no reports of the establishment of indigenous Antarctic species as a result of anthropogenic dispersal within the 16 ACBRs, although transfers between the larger three biogeographic regions of the sub-Antarctic and Antarctic have occurred (Hughes et al., 2010; Lee and Chown, 2011) (Fig. 1.6, Table 1.1).

Transfer of non-native organisms *via* human movement has been reported by National Antarctic Operators. for instance, during an inspection, the British Antarctic Survey found that vehicles transported by contractors into Rothera Station on the Antarctic Peninsula were contaminated with 132 kg of sub-Antarctic soil. The soil contained viable non-native species, including bryophytes, angiosperms, invertebrates, nematodes, fungi, bacteria and c. 40,000 seeds (Hughes et al., 2010). Other human associated pathways for the potential transfer of non-native species include human clothing and equipment (Chown et al., 2012a; Huiskes et al., 2014), fresh food (Hughes et al., 2011), ships (Hughes and Ashton, 2017; Lee and Chown, 2007) and also cargo, which may be the highest risk pathway into Antarctica (Bergstrom et al., 2014; Houghton et al., 2014; Newman et al., 2018; Lee and Chown, 2009; Tsujimoto and Imura, 2012). As an example, the Australian Antarctic Division reported over 1300 individual invertebrates in cargo, food and personal items bound for the Antarctic over the course of 13 years (Bergstrom et al., 2014).

Ongoing development and human activity in the Antarctic means that anthropogenic dispersal of both indigenous species within the region, and non-native species from outside the Antarctic, is increasing. With the joining of new countries to the Antarctic Treaty System and the Scientific Committee on

Antarctic Research (SCAR), and the expansion of existing research stations, it is likely that the footprint of national programmes in the Antarctic, particularly on the continent, will only grow.

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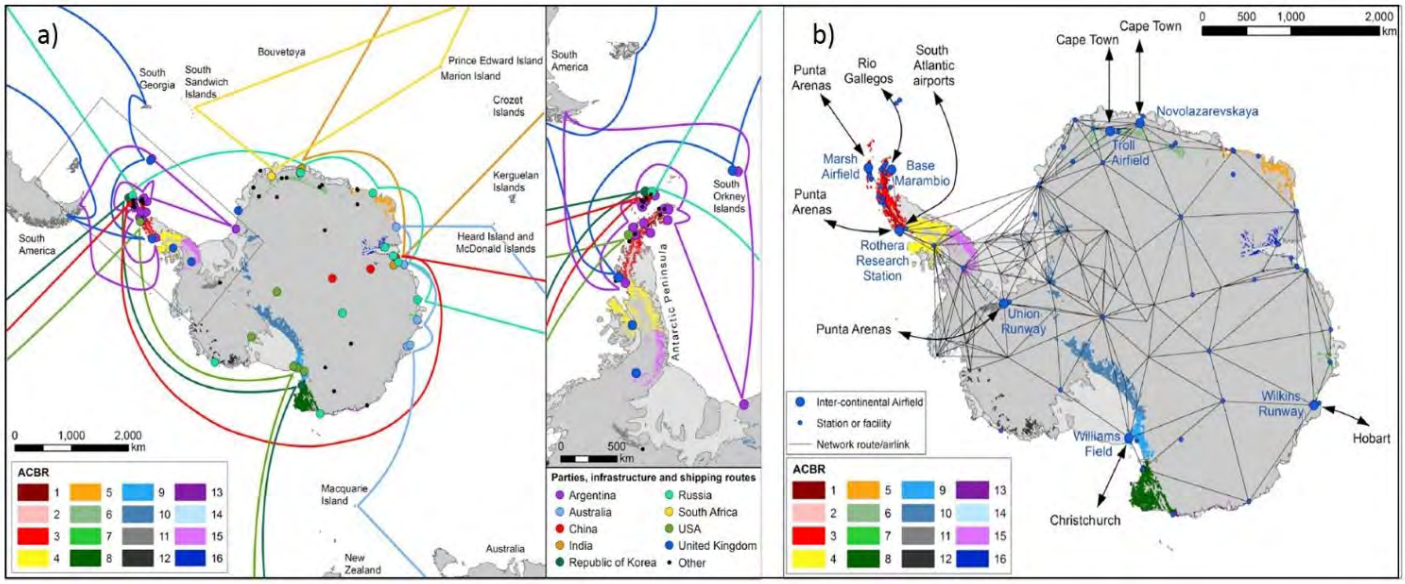

Figure 1.6. *a) Potential shipping resupply routes with more than one station on ice-free ground in Antarctica. Activities and infrastructure locations of Antarctic Treaty Parties are shown as coloured lines and circles. b) Location of intercontinental and domestic runways and other landing sites. Antarctic Conservation Biogeographic Regions are shown as coloured outcrops in both images. Taken from Hughes et al. (2019).*

1.5.4 Alien introductions

To date, human activity has already resulted in over 200 species of non-native animals and plants successfully establishing within the three Antarctic biogeographic zones (Chown et al., 2012a). Most introductions have been in the sub-Antarctic region, but 11 introductions have been recorded in the maritime Antarctic, nine of which have been invertebrates, five persistent (Frenot et al., 2005; Hughes et al., 2015) (Table 1.1, 1.2). The spatial distribution of species introductions is strongly related to that of human activity, with most maritime Antarctic introductions on the western Antarctic Peninsula where half of all Antarctic research stations and camps are located (Chown et al., 2012a; Greenslade et al., 2012; Hughes and Worland, 2010; Lee and Chown, 2009; Molina-Montenegro et al., 2012; Tin et al., 2009). Furthermore, the transfer of microbiota is a significant risk and has likely already occurred (Convey et al., 2008; Hughes et al., 2019). Of particular concern, are pathogens that may

risk disease to local wildlife populations that are 'immunologically naïve' due to evolution in microbial isolation (Curry et al., 2002; Grimaldi et al., 2014; Kerry and Riddle, 2009). The benchmark study on the status of Antarctic non-native species (Frenot et al., 2005), set out four definitions of non-native species to aid classification, based on previous work (Greene, 1964; Richardson et al., 2000; Walton and Smith, 1973). They are as follows (verbatim) and these definitions will be used hereafter:

1. *Alien*: introduced to an ecosystem as a result of human activity (including species that arrive by natural means to a specific ecosystem but are alien to that biogeographical zone)
2. *Transient alien*: survived in small populations for a short time period but either died out naturally or was removed by human intervention
3. *Persistent alien*: survived, established and reproduced for many years in a restricted locality, but has not expanded range from that location
4. *Invasive alien*: spread into native communities and displaced native species

Table 1.1 *The occurrence of persistent non-native species across Antarctic biogeographical zones (updated from Convey, 2008 and Frenot, 2005, with data from Hughes et al., 2015. Excludes species not free in the environment).*

Biological Group	**Maritime Antarctic**	**Continental Antarctic**	**Entire sub-Antarctic**	**Sub-Antarctic islands**							
				South Georgia	Marion	Prince Edward	Crozet	Kerguelen	Heard	MacDonald	Macquarie
Dicotyledons	0	0	62	17	6	2	40	34	0	0	2
Monocotyledons	2	1	45	15	7	1	18	34	1	0	1
Pteridophytes	0	0	1	1	0	0	1	1	0	0	0
Total plants	**2**	**1**	**108**	**33**	**13**	**3**	**59**	**69**	**1**	**0**	**3**
Invertebrates	**5**	**0**	**72**	**12**	**18**	**1**	**14**	**30**	**3**	**0**	**28**
Vertebrates	**0**	**0**	**16**	**3**	**1**	**0**	**6**	**12**	**0**	**0**	**6**

1.6 Non-native species in terrestrial Antarctica

1.6.1 Plants

There are currently 108 non-native vascular plant species in the sub-Antarctic, most of which are in cosmopolitan families that are successful invaders on a global scale (Pyšek, 2012). These include, but are not limited to, the grasses of Poaceae (39 spp.); flowering plants, Asteraceae (20 spp.) and Brassicaceae (8 spp.), and the flowering rush family, Juncaceae (7 spp.) (see Frenot et al., 2005 for a more extensive list). Most alien species occur on the islands of Crozet, Kerguelen and South Georgia, and many of them are not considered invasive, but are deemed persistent (Frenot et al., 2001; 2005).

On the continent, it is Poaceae that are the most successful. No records are available of bryophyte transfer, but propagules of the rush *Juncus bufonius* have been found (Cuba-Diaz et al., 2013). The cosmopolitan grass *Poa annua* is the most widespread alien vascular plant in Antarctica and is invading islands on the western Antarctic Peninsula. It is likely to succeed in further dispersal as it is capable of setting seed in this environment (Chwedorzewska et al., 2015; Rudak et al., 2018). Overcoming the environmental limits to life history events, either through rapid adaption or existing pre-adaptations, is key to a species' success. For example, the persistent introduced species *P. pratensis* was not as successful as *P. annua* as it was unable to set seed at its location on Cierva Point, Antarctic Peninsula. This enabled its successful eradication (Frenot et al., 2005; Pertierra et al., 2013, 2017; Shaw et al., 2013). However, both *P. annua* and *P. pratensis* have successfully invaded islands in the sub-Antarctic where they are widely distributed (Pertierra et al., 2017). As climate change begins to break down the barriers of environmental limitation through warmer and wetter conditions, especially on the Antarctic Peninsula, the more likely it will be that plant species like *P. pratensis* will succeed on the continent as well as in the sub-Antarctic regions (Duffy et al., 2017).

1.6.2 Invertebrates

Like plants, most alien invertebrates are found in the sub-Antarctic (72 recorded by Frenot et al., 2005), particularly on the islands of Kerguelen, Macquarie and South Georgia (Table 1.1). However,

the introduction of dipterans, enchytraeids, Mecoptera, Acari and Collembola species have occurred in the maritime Antarctic also (Table 1.2). Of the invertebrate species introduced to the maritime Antarctic, most introduction events are micro-arthropods: whilst there is only one record of a persistent non-native Collembola (Table 1.2), there are 16 recorded introductions of seven Collembolan species – eight introductions of *Hypogastrura viatica* alone. There have also been 18 recorded introductions of Acari, with *Speleorchestes* spp. accounting for seven of these (Russell et al., 2013, 2014).

However, it is the introduction of *E. murphyi* and *Christensenidrilus blocki* (Dozsa-Farkas and Convey, 1997 - Enchytraeidae) to Signy in the South Orkneys which became the first introductions to result in successful establishment of a non-native invertebrate in the maritime Antarctic. The introduction of *E. murphyi* also made it the first insect to be introduced to the maritime Antarctic. This has since been followed by the introduction of the winter crane fly, *Trichocera maculipennis* (Meigen, 1818 - Trichoceradae), to King George Island, just off the western Antarctic Peninsula. The latter species was accidentally introduced in the island in 2007 (Volonterio et al., 2013) and has since spread to many research stations, some up to 20 km away (Potocka and Krzeminska, 2018) (Table 1.2). Like *P. annua*, *T. maculipennis* is also a cosmopolitan species with a range that extends into the High Arctic in the north (Dahl and Krzeminska, 2015) meaning it is already pre-adapted to maritime Antarctic conditions.

Table 1.2. *Invertebrates that have colonised permenant station buildings and sewage treatment plants on Antarctic research stations, and non-native invertebrates that are persistent in the Antarctic environment (adapted from Hughes et al., 2015).*

Within research stations					
Order	**Species**	**Station**	**Date introduced**	**Notes**	**References**
Diptera	*Lycoriella ingénue*	Casey Station, Budd Coast, Wilkes Land	1998	Unsuccessful extensive eradication attempt in 2005	Hughes et al., 2005; Smith, 2005
Diptera	*Lycoriella* spp.	Rothera Research Station, Marguerite Bay, Antarctic Peninsula	2005	Successful eradication in 2005	Hughes et al., 2005
Diptera	Unidentified mosquito	Frei Station, Fildes Peninsula, King George Island, South Shetland Islands	Pre-2009/2010 season	Larvae persist in the sewage treatment plant. No counter measures are undertaken	Peter et al., 2013
In the environment					
Diptera	*Trichocera maculipennis*	Artigas Station, Fildes Peninsula, King George Island, South Shetland Islands	~2006	Early eradication attempt from station unsuccessful. Is now in the surrounding environment	Volonterio et al., 2013
Diptera	*Eretmoptera murphyi*	Signy Research Station, South Orkney Islands	1967, 1968	Persistent alien with expanding distribution, ~35,000 m^2	Block et al., 1984; Burn, 1982; Hughes and Worland, 2010; Hughes et al., 2013
Enchytraeid	*Christensenidrilus blocki*	Signy Research Station, South Orkney Islands	1967, 1968	Persistent	Block and Christensen, 1985; Burn, 1982; Hughes and Worland, 2010
Collembola	*Hypogastrura viatica*	Whalers Bay, Deception Island, South Shetland Islands	?	Persistent (Invasive?). Abundant in several sites. One of the most invasive Collembola in the sub-Antarctic islands with 8 separate introduction cases.	Greenslade et al., 2012; Greenslade and Convey, 2012; Hack, 1949; Russell et al.,2013, 2014
Acari	*Alicorhagia* spp.	Whalers Bay, Deception Island, South Shetland Islands	Reported 2010	Transient? Persistent?	Russell et al., 2013, 2014
Acari	*Coccotydaeolus* cf. *krantzii*	Whalers Bay, Deception Island, South Shetland Islands	Reported 2010 and 2011	Persistent? Widespread distribution in Whalers Bay	Russell et al., 2013, 2014

1.6.3 Dispersal methods

As discussed in Section 1.5, most dispersal of non-native species to Antarctica is the result of human movement. Clothing, footwear (Chown et al., 2012a; Hughes and Worland, 2010), vehicles (Hughes et al., 2010), cargo and ships (Bergstrom et al., 2014; Houghton et al., 2014; Lee and Chown 2007, 2009; Newman et al., 2018; Tsujimoto and Imura, 2012), all represent methods of introduction as well as potential routes of further dispersal across Antarctic regions (Hughes et al., 2019). Once established, natural methods of dispersal also become important. In the McMurdo Dry Valleys, the harshest of all Antarctic ice-free environments, dispersal is wind driven (aeolian) (Šabacká et al., 2012; Sakaeva et al., 2016). This is also true for larger flightless invertebrates, for instance Collembola and Oribatid mites, which can be wind dispersed (Hawes et al., 2007; Lehmitz et al., 2012; Gressitt and Yoshimoto, 1974; Joimel et al., 2018; Dunger et al., 2002). Considering that aeolian transport is the preferential theory for the colonisation of islands and remote areas (Hogg and Stevens, 2002; Peck, 1994), Hughes and Worland (2010) found that the flightless adult chironomid *E. murphyi* could potentially be wind dispersed on Signy, or *via* the feet of birds. Passive aeolian dispersal of Chironomidae has previously been suggested as a form of transoceanic movement (Krosch et al., 2011), and occurs in other arthropod groups (Washburn and Washburn, 1984). Likewise, the movement of invertebrates *via* external transport on birds, (epizoochory) has been documented in terrestrial Gastropoda species (van Leeuwen and van der Velde, 2012), and aquatic nematodes, rotifers, ostracods, copepods, tipulids, chironomids and hemipterans (Dagmar et al., 2007). Furthermore, epizoochory has been proposed as a dispersal mechanism that could help explain the distribution of some Antarctic Acari (Pugh, 1997). Finally, endozoochory (the internal dispersal of invertebrates, *via* vertebrate guts), is also well documented (see review Viana et al., 2016), but due to the lack of evidenced entomophagy amongst Antarctic birds, will not be examined further. All Antarctic biogeographic regions host a multitude of seabirds, with the South Orkney Islands, and South Georgia hosting some of the world largest and most significant colonies of migratory sea birds (Harris et al., 2011), thus epizoochory may be a method of dispersal for terrestrial, freshwater and aquatic invertebrates throughout their migratory areas.

With most ice-free habitats in the maritime Antarctic occurring around the coasts, there is also the possibility of oceanic dispersal. Antarctica's largest invertebrate, the chironomid *B. antarctica* has already been found to tolerate up to a 10-day exposure to sea water, with little to no lasting effects (Elnitsky et al., 2009), which could explain how it has colonised many islands of the Antarctic Peninsula. Hawes et al. (2008) reported that the Antarctic collembolan *C. antarcticus* was able to both raft and reproduce on seawater, using only its own exuvia as a raft. Other studies have also reported successful rafting of polar insects within oceanic debris such as feathers or driftwood (Hogg and Stevens, 2002; Coulson et al., 2002). In either instance, a species may not need to spend much time at sea in order to drift to a neighbouring, and otherwise uncolonized, island. The highly invasive carabid beetle, *Merizodus soledadinus*, is thought to have spread to neighbouring islands in the sub-Antarctic Kerguelen Island group through a combination of anthropogenic, epizoochoric and passive dispersal by oceanic flotation (Ouisse et al., 2017; Renault, 2011a). Local dispersal *via* freshwater streams is also a potential mechanism and has been considered previously as a route for *E. murphyi* to enter the oceans (Hughes et al., 2013). Studies of Antarctic Collembola have found that meltwater pools and streams are a potential dispersal route combined with their ability to raft. Hawes et al. (2011) consider the interaction with water frequent and significant enough to be considered a "widespread component of springtail ecology in Antarctica".

1.7 Ecological impacts

As previously mentioned (Section 1.2), the post-glacial terrain and extreme climate mean that only simple ecosystems currently occur in Antarctica. With low soil nutrient levels, and biodiversity that is both low in abundance and richness compared to even the Arctic, any introduction of a new species, flora or fauna, may have a notable impact on the local ecology.

1.7.1 Trophic interactions

Even in complex ecosystems a single biological introduction can be catastrophic. For example, one of the most studied and destructive terrestrial invasions in the world is that of the cane toad *(Rhinella marina),* which has been introduced in over 20 countries. Where introductions have occured, biodiversity has declined in both terrestrial and freshwater ecosystems (Greenlees et al., 2011), with trophic impacts rippling throughout the food chain, causing local extinctions (Australian TSSC, 2005; Giffiths, 2007; Feit et al., 2018). Within simpler ecosystems, such as the sub-Antarctic islands, the impacts of other single species introductions can also be devastating with invasive rodent species implicated in the decline and extinction of hundreds of endemic vertebrates (especially sea birds), the indirect suppression of terrestrial invertebrates, and effects on plant and ecosystem functioning (Chown and Froneman, 2008; Rowe-Rowe et al., 1989; St Clair, 2011) including the destabilisation of surface substrates (Eriksson and Eldridge, 2014).

Ecological impacts are not limited to invasive vertebrate species, especially in polar ecosystems. For example, the introduction of an invasive predatory carabid beetle (*Merizodus soledadinus*) to the Kerguelen Islands has had a significant impact on native invertebrate fauna, particularly Dipteran spp. (Chevrier et al., 1997; Ernsting, 1993). A similar introduction of two carabid species on South Georgia has also resulted in native invertebrate species decline, but an increase in the body size of a herbivorous invertebrate as a result of predation of smaller larvae, with implications for local vegetation food sources (Brandjes et al., 1999; Ernsting et al., 1995, 1999). The introduction of the herbivorous moth *Plutella xylostella* is significantly impacting its endemic host, the Kerguelen cabbage on Marion Island (Chown and Avenant, 1992). Also on Marion Island, predation of the native moth species *Pringleophaga marioni* by invasive mice has led to a niche gap, partially filled in turn by another invasive species, the chironomid *Limnophyes minimus* (Hänel and Chown, 1998; Smith, 2007).

Understanding the mechanisms that lead to the success of such invasive species is an important factor in establishing their role within ecosystems and impact on biodiversity. With invasions on islands

particularily detrimental to ecosystems as invaders "exploit broader ecological roles with strong indirect effects that amplify their impacts" (Narayan et al., 2015). Changes to the functional diversity of an ecosystem can in the long term, lead to changes in the functional traits of other species and change community structures (Goswami et al., 2017). Functional diversity describes the distribution and productivity of organisms within a niche and is measured through two indices: functional richness and functional evenness. The amount of an ecological niche occupied by a species within a community is the functional richness, with low levels of functional richness reducing the productivity of an ecosystem. Low species richness tends leads to low functional richness, as fewer species use fewer available resources within a niche, leading to lower niche productivity. Furthermore, the functional traits of species, such as those that govern a species' environmental tolerance, is a crucial determinant of functional richness, with extreme environments typically having lower richness (and thus functional diversity) as environmental conditions limit the ability of species' from taking full advantage of available resources. Functional evenness measures the species trait within an occupied 'trait space'. If functional traits are not evenly distributed within an ecosystem, then those traits risk being out competed, leading to niche gaps. The introduction of alien species to an ecosystem can have varied effects on functional diversity, depending on the intital productivity of said ecosystem to begin with: For example, in an extreme and inaccessible environment such as Antarctica, the ecosystem is likely to have unoccupied niches and thus low functional eveness. The introduction of a species able to utilise any niche gaps may not necessarily have a negative impact on the ecosystems overall functional diversity, rather it will increase both species richness and introduce new traits. However, a particularily aggressive or competitive invasive species may reduce functional traits within the community as seen in association with invasive ants (Wong et al., 2019). A species may even affect both richness and eveness, as seen with the highly invasive cane toad *Rhinell mariana* (Loiola et al., 2018; Jolly et al., 2015), or with the introduction of an invasive plant species that is able to drastically alter the functional diversity of the below ground microbial ecosystem, with consequence for nutrient cycles (e.g., Chen et al., 2011).

1.7.2 Influence of invertebrates on soil biochemistry

Functional diversity in the polar regions is typically low, with few species able to cope with the extreme environments, and traits amongst those species present focussed on adversity-selected life histories (Greenslade, 1983). Thus food-webs tend to be simple, and trophic cascades easily imbalanced by invaders. In particular, the low soil-nutrient levels are easily influenced by the introduction of detritivores. Detritivorous invertebrates are key influencers on nutrient cycling in any habitat as they are a large part of the decomposition process (Fig. 1.7). By physically fragmenting organic matter into smaller parts, detritivores change the chemical composition of detritus and turn organic compounds into simpler products that can be consumed by either microbes or plants. Alterations to nutrient cycling can have large consequences for ecosystem functioning, especially in polar regions where substrates are typically low in nitrogen, and litter turnover slow (Lee et al., 2009). With the dearth of herbivores and predators in polar regions, energy and nutrient cycling is driven through the detritus and associated community (Smith, 2007), through the 'brown' food web (Koltz et al., 2017) (Fig. 1.7). In the Arctic, 99.6% of carbon processed by the invertebrate food web is from microbial detrital resources ('brown' energy), resulting in invertebrate detritivores being major drivers in carbon and nitrogen cycling in tundra ecosystems (Koltz et al., 2017). Similar process are found on the Antarctic continent, only with a more truncated food web, where microbial flora, fauna and fungi are the top of the food chain and are the main drivers of nitrogen and carbon cycling (Cowan et al., 2010). Interactions between the 'brown', and the 'green' food-web of live vegetation, directly affect uptake, storage and mineralization of both carbon and nitrogen (Bardgett and Wardle, 2010), and the simpler an ecosystem the easier it is for the nutrient cycle to be disturbed. For example, on the edges of Lake Mývatn in the Arctic, it was found that an increase in the size of deceased chironomid swarms alone was capable of instigating long-term change in plant species assemblages and growth, purely by raising nitrogen levels from carcass deposition (Dreyer et al., 2015).

On sub-Antarctic Marion Island, the invasive chironomid *L. minimus*, has been found to fill the niche left behind by litter decomposer *P. marioni*. Not only does it occupy the niche gap, but it can turn over 6-10 times as much litter as its predecessor with implications for overall nutrient cycling (Hänel

and Chown, 1998). It has therefore been described as the island's new terrestrial keystone species (Klok and Chown, 1997). Furthermore, slugs introduced to the same ecosystem were found to release less nutrients than native detritivores, meaning that the quality and accumulation of peat on Marion Island are potentially altered by invasive detritivores (Smith, 2007).

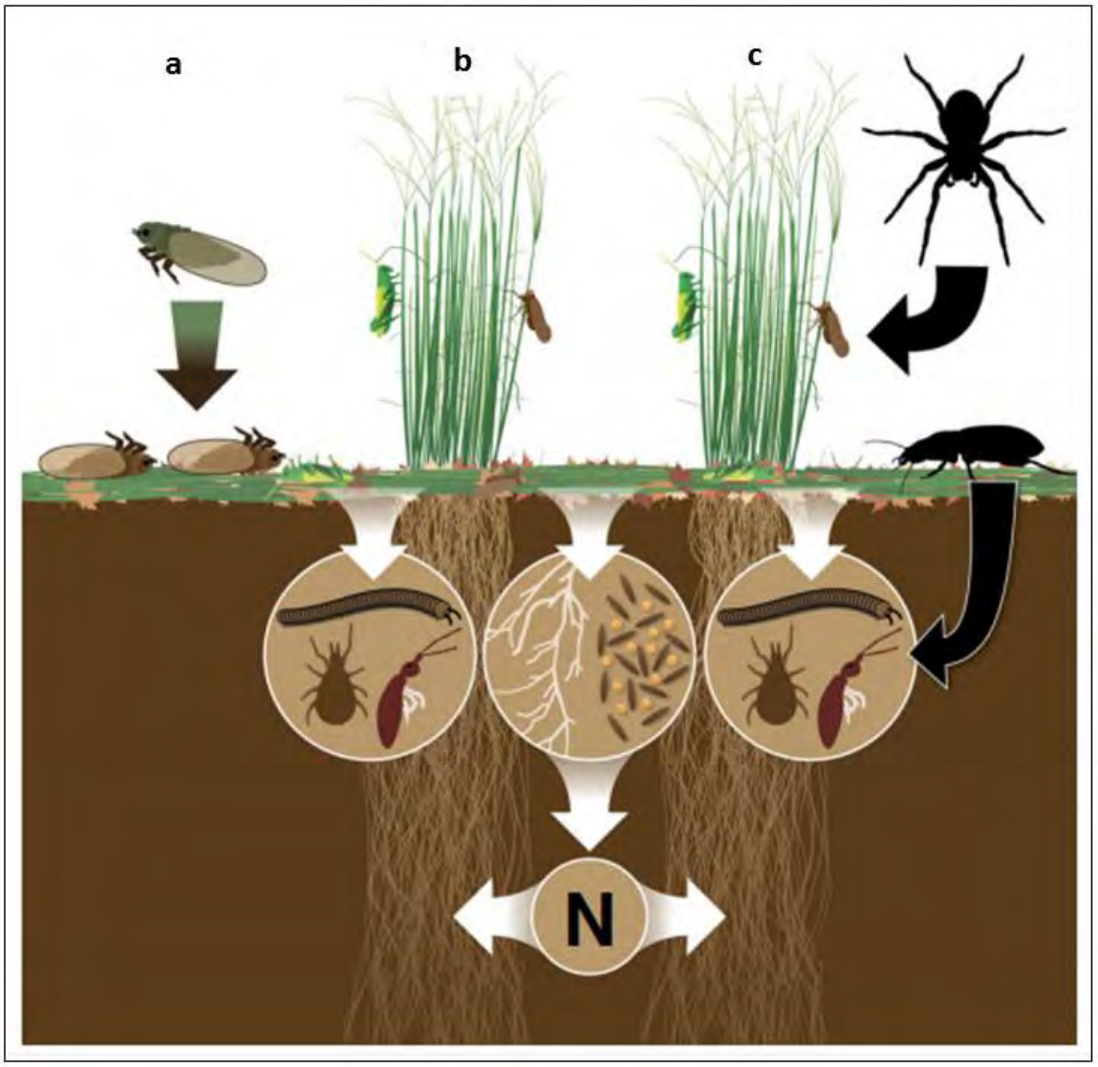

Figure 1.7. *Schematic of invertebrate effects on ecosystem functioning through modification of detrital pools. Image and the following description are taken from Yang and Gratton (2014), with only section identification labels modified. "Direct inputs (a) of insects to the detrital (dead biomass) pool can introduce copious amounts of high quality (low C:N) biomass into belowground systems. Insects and arthropods can transform live and dead (b) biomass with both positive and negative effects on ecosystem rates such as C and N cycling. Arthropod predation of decomposers (c) can create trophic cascades that alter the size of the detrital pool and decomposition rates...Ultimately, the size and quality of the detrital resource pool, both in terms of the stoichiometry of key elements (C:N:P) and secondary chemistry, are key determinants of microbial communities and activity in the soil. The ability of aboveground and soil dwelling insects and arthropod activities to alter the composition of the detrital pool therefore has the capacity to modulate ecosystem processes through the effects on microbes".*

In the maritime Antarctic, the introduction of *E. murphyi* to Signy is also altering litter function, with the chironomid capable of turning over litter by an order of magnitude more than the native soil fauna (Hughes et al., 2013). The introduction of any detritivore, particularly to an ecosystem as simple as those found in terrestrial Antarctica, can be assumed to have an impact and result in increases in C and N cycling, and therefore on the broader ecosystem function.

1.7.3 The risk of lasting consequences

As highlighted by Smith (2007), macro-invertebrate detritivores are "cardinal facilitators of mineralisation of nutrients that are locked up in peat and plant litter", and any species introductions that affect this can alter terrestrial ecosystem function. Primarily the increase in litter turnover and nutrient mineralisation will impact the vegetation community the greatest, and the longest. The release of nitrogen in particular may negatively impact endemic moss species, the 'forests of Antarctica'. Moss species in general prefer low nutrient substrates, and polar species are particularly adapted to a nutrient-poor environment. A study on long term nitrogen addition in the Arctic found a decline in all lichen and mosses evaluated (Chapin et al., 1995; Nilsson et al., 2002). However vascular plants, of which Antarctica has two, will typically take up any available nitrogen. Studies have found that the grass *D. antarctica,* and the herbaceous plant *C. quitensis* if given nitrogen fertiliser, will increase shoot biomass, leaf number and leaf area (Rabert et al., 2017). Previous work in tundra ecosystems has also found that increases in nitrogen tend to benefit grass species specifically, to the detriment of other vascular plant and bryophyte diversity, and that the extra fertilisation leads to increased microbial biomass in the soils (e.g. Chapin et al., 1995; Jonasson, 1992; Nilsson et al., 2002; Press et al., 1998; Theodose and Bowman, 1997). Any prolonged increase in the levels of soil fertility may also affect the establishment of alien propagules. At present, accidentally introduced seeds and spores have to contend not only with the Antarctic climate, but also the nutrient-poor substrates. Any alien detritivore may alleviate this problem in the long term.

1.7.4 Modelling distribution changes

By whichever means a species is distributed into, and throughout Antarctica, monitoring its local geographical expansion alone cannot predict what areas are at risk of further colonisation. Identifying areas that are climatically and environmentally suitable for a species environmental niche and life history are crucial in understanding what broad areas of habitat could support an introduced population (Ellstrand and Schierenbeck, 2000). Modelling potential geographic expansion through species distribution models (SDMs), help to identify those suitable areas and habitats that fit within a species niche, characterising the limits of its range and pattern of invasion (Guisan and Thuiller, 2005; Turbeline et al., 2017). More recent developments of open source software, using geographical information systems (GIS), allow ecologists to use known species presence data in combination with environmental niche predictors to model likely changes in distribution (Raghavan et al., 2016; Rushton et al., 2004). These types of GIS models are built on the input of 'raster' layers, which in their simplest form are a matrix of cells, with each cell containing a value that represents information, such as habitat type or temperature. Rasters can also be satellite images, or digital photographs. By weighting preferential values that are associated with a species niche, Maximum Entropy (MaxEnt - Elith et al., 2011; Phillips et al., 2006) software can predict future spatial distribution. MaxEnt uses estimations of 'probability of presence' given a series of biotic and/or abiotic variables such as terrain, habitat classification, temperature or dispersal vectors. For example: if a species is only ever found on the banks of streams within a specific temperature envelope, only these values will be carried forward into the SDM. Such models can aid understanding of species responses to climate change (e.g. Wang et al., 2018), identify habitat loss scenarios (e.g. Angileri et al., 2016), and prioritise biological conservation efforts (e.g. Pertierra et al., 2017; Pyke et al., 2005). In Antarctica, SDM's and MaxEnt have been used primarily in oceanic studies (e.g. CCAMLR MPA scientific support, Teschke et al., 2016), but recently have been used to determine the potential range distribution of the invasive grasses *P. annua* and *P. pratensis* (Pertierra et al., 2017), in the first such terrestrial study of an Antarctic species, invasive or otherwise. SDMs can be used to predict geographical expansion of an

alien species and are thus a useful tool to inform both invasive risk assessments and biosecurity measures (Jiménez-Valverde et al., 2011).

1.8 Invertebrate Control

There are three primary forms of management for introduced invertebrate species: chemical control, cultural control (interference) and biological control *via* the introduction of a predator, parasite or competitor agent (Gentz, 2009). Each method has its own merits and controversies, with biocontrol agents creating particular problems in vulnerable and island ecosystems (Howarth, 1991). Cultural or interference control is expensive, resource intensive and can tend to only be a prophylactic treatment (Myers et al., 1998). Typically, cultural control works best alongside other practices. Chemical control has previously been marked as a source of health complications in humans, with early insecticides that are no longer in use also implicated in significant environmental damage (e.g. organophosphates, organochlorines and carbamates) (Puthur, 2008). Recent developments in the manufacture of synthetic compounds with a more precise method of action have decreased the toxicity of many chemical control treatments (Nauen and Bretschneider, 2002), and there is a resurgence in their use as cost-effective control measures (Gentz, 2009). As the introduction of further species to act as a biocontrol agent would be irresponsible to consider in an Antarctic context, the remainder of this section will focus on chemical and cultural control. However, there are limitations on the chemicals that are permitted in the Antarctic Treaty Area, with practical response measures limited by the requirement to keep collateral damage to native habitats and species to a minimum (Hughes et al., 2015).

1.8.1 Chemical Control

Newer forms of chemical control can be highly specific and degrade quickly into non-toxic components. For example, a very novel insecticide has been developed from isolated spider venom compounds, and the chemical acts exclusively on insects with little toxicity to other animals (Tedford

et al., 2004). The most widely used insecticides of recent decades are the pyrethroids, neonicotinoids and insect growth regulators (IGRs).. However recent studies have led to the banning of neonicotinoids in several countries and the European Union, owing to the effects that they have on beneficial insects and their persistence in the environment (McGrath, 2014). IGRs are specific targeters of insect development at juvenile stages and are highly targeted for use against insects and arthropods in particular (e.g. Su and Scheffrahn, 1998).

Against invasive arthropods, many efforts in chemical control have been effective in several taxa, including Hympenoptera (Hoffman, 2015), Diptera (Klassen and Curtis, 2005) and Molluscsa (Wittenberg and Cock, 2001). For example, the Galapagos Islands in the South Pacific have had a 60% increase in herbivorous insect introductions over a period of 12 years, that threatens rare and endemic flora (Causton et al., 2006). An eradication programme was established focussing on one invasive species, the fire ant, *Wasmannia auropuncata* and, after repeated applications of hydramethylnon, colony size was significantly reduced (Causton et al., 2005). The 'Sterile Insect Technique' (SIT), which chemically sterilises males in combination with conventional insecticides, has also been very successful in controlling fast breeding dipterans and has led to the eradication of tse-tse flies (*Glossina* spp.) from areas over 1,000 km^2 in Africa (Klassen and Curtis, 2005). Eradication of soil invertebrates is harder, however, owing to their integration into substrates and typically only occurs as the by-product of plant-focused pesticide management, with unintentional negative impacts on valued below-ground fauna (Cortet et al., 1999; Pelosi et al., 2014).

1.8.2 Cultural control

The use of physically trapping animals (cultural control), whilst resource intensive, is often successful and leaves little impact on the environment. One of the most successful cases of cultural control on a large scale is within the Antarctic Regions: the eradication of rats and reindeer from South Georgia in the sub-Antarctic (Martin and Richardson, 2019). Cultural control of invertebrates is most effective in flying insects that can easily be drawn out. For example, trapping of the mosquito disease vectors *Culex* and *Anopheles* spp. is effective as they are drawn to heat, odour and carbon dioxide

(Chaiphingpachara et al., 2018; Kweka et al., 2013; Okumu et al., 2010). Similarly, surface-dwelling invertebrates can be eradicated through non-species specific trapping (e.g. Roda et al., 2018), but more novel ways of controlling soil invertebrates have to be developed in order to not wash a soil system with chemicals. In the case of regular agricultural pest taxa, the Nematoda, it has been found that the use of nematode-trapping fungal controls could supress the pest population (Bell et al., 2016).

1.8.3 Control of Chironomidae

Most of the large dipteran family Chironomidae are aquatic species, and all efforts to control their abundant swarms focus on water treatment over terrestrial control and viewing them as a nuisance rather than an invasive. Chironomid larvae are typically found in sediment at the base of freshwater lakes, ponds, and wastewater pools (Cranston, 1995). To pupate, the midge will rise to the surface, followed by emerging adults which swarm and mate at the water margins, eventually laying eggs back in the water (Tokeshi, 1995). Although Chironomidae do not bite, due to the huge numbers of adult midge, the swarms are a common nuisance to residents in the vicinity (Ali, 1996). They have also been known to contaminate drinking water supplies (Bay, 1993; Zhou et al., 2003) and act as a pest in 'midge friendly' agri-habitats such as rice beds (Stevens et al., 2006, 2013). Chironomids are sensitive to changes in water chemistry, and as a result have been used as a bio-indicator of freshwater pollution (Carignan and Villard, 2002; Wene, 1940). However, this sensitivity also makes them ideal candidates for chemical control, with chlorine-dioxide, organphosphates, Methoprene, Pyriproxyfen and Diflubenzuron all used to reduce midge abundance (Craggs et al., 2005; Xing-Bin Sun et al., 2006). Biocontrol measures are also used on chironomids, with the soil bacterium *Bacillus thuringiensis* var. *israelensis* found to be lethal to larvae (Craggs et al., 2005; Stevens et al., 2013). The bacteria contain a proteinaceous toxin which disintegrates the larval intestine causing death, and subsequently controlling swarm numbers (Liber et al., 1998).

1.8.4 Biosecurity in Antarctica

In 1991, the Antarctic Treaty developed the Protocol on Environmental Protection to the Antarctic Treaty, whereby it adopted (in 1998) non-native species legislation to ensure that Antarctica remained

free of widespread invasive species. All Parties to the Antarctic Treaty are responsible for developing and enacting measures to prevent or minimise the introduction of non-native species, and to control and, if feasible, eradicate any that have established (Hughes and Convey, 2014; Hughes and Pertierra, 2016). At present, available biosecurity measures are constrained by the requirement to limit damage to the environment, cost and logistic practicality. Consequently, the physical removal of soils and disinfectant boot washes are the dominant biosecurity strategy (COMNAP, 2010). Article 7 of Annex III Waste Disposal and Management explicitly bans the use of pesticides within Antarctica, except under specific circumstances (such as permethrin smoke bombs in storage units) (Hughes et al., 2015). Anti-microbial and viricidal disinfectants are also permitted and are routinely used in boot washes (Curry et al., 2002). Whilst these measures are employed by most tourist and some national programme operators, there is no evidence of widespread implementation (Hughes, 2015; IAATO, 2015).

The most widely used disinfectant by national programme operators in Antarctica is currently Virkon®. Marketed as a viricide and microbicide for use in farms, it claims effectiveness against bacteria, viruses and certain strains of fungi at temperatures as low as 4 °C (Gasparini et al., 1995; Hernandez et al., 2000). In the powder form, Virkon is easy to transport and dilutes harmlessly into non-toxic salts, should it end up in an aqueous environment (Curry et al., 2005). Virkon is the only control chemical recommended for normal use under COMNAP guidelines and is used also by International Association of Antarctica Tour Operators (IAATO), as an approved biocide. In Antarctic boot-wash scenarios it has proven effective in preventing the spread of pathogens under ambient conditions when used to wash equipment or footwear (Curry et al., 2005). Whilst not designed as an insecticide, it has been investigated against invasive invertebrate species in the past, with mixed results (e.g. Li et al., 2016; Mitchell and Cole, 2008; Paetzold and Davidson, 2011; Stockton-Fiti and Moffit, 2017; Watson et al., 2008), but never against a polar invertebrate species, so its efficacy against anything other than polar microbes is unknown.

A lack of awareness or consensus has meant that early introductions of non-native species have resulted in delayed management, if any (Hughes et al., 2019). However, there has been movement in

recent years to implement a 'rapid response' to plant introductions, such as advocated in the Committee for Environmental Protection (CEP) Non-native Species Manual (CEP, 2011). Due to the restrictions on chemical and biological control in Antarctica, cultural control through physical removal is the focus of most eradication attempts, but this is limited to the removal of plants that are in discrete areas, and is impractical as a method of removing invertebrates (Hughes and Convey, 2010, 2012; Molina-Montenegro et al., 2012; Pertierra et al., 2013; Tsujimoto, 2010). This leaves early response as the main course of action against invasive species, as highlighted extensively by Hughes et al. (2019), who list the following recommendations:

1. *Prevention*: Research dispersal vectors within Antarctica and implement biosecurity measures. Research into anthropogenic transfer between different Antarctic biogeographic regions, implement intra-regional biosecurity measures. Research alien non-native species introduction risks. Research continued policy development on the prevention of microbial introductions
2. *Monitoring*: Coordinate frequent and widespread monitoring in marine and terrestrial environments
3. *Response*: Development of contingency plans at vulnerable sites; Research eradication methods.
4. *Policy development and implementation*: Regular evaluation of progress in non-native species policy development. Regular review of biosecurity practices undertaken by all visitors and stakeholders in the Antarctic Treaty areas.

1.9 The study species, *Eretmoptera murphyi*

The brachypterous midge *E. murphyi* (Chironomidae, Orthocladiinae) (Fig 1.8) is thought to have been inadvertently introduced to Signy (Fig 1.1 and 1.2, South Orkney Islands, maritime Antarctic)

from its endemic range on South Georgia (sub-Antarctic) in the 1960s, in association with plant transplant experiments (Block et al., 1984; Convey and Block, 1996). It has successfully established and is thought to be at least a 'persistent invader' if not an 'invasive alien' (Hughes et al., 2013). As a consequence of its introduction the midge is the only macro-invertebrate on the island, with no predators or competitors.

Figure 1.8. *Adult* E. murphyi © *Roger Key*

1.9.1 Life history and phenology

Adult *E. murphyi* are short-lived, flightless (Hughes et al., 2013; Worland, 2010) and invest heavily in a single egg mass, that is twice the dry mass of the spent adult (Convey, 1992). Larvae have four instars like *B. antarctica* its closest relative (Bartlett et al., 2018a / Chapter 2; Allegrucci et al., 2012). Only one taxonomic description has been completed based on limited samples (Cranston, 1995), one

study of the egg masses and life cycle nearly 30 years ago (Convey, 1992), and one study that attempted to classify the instars (Hughes et al., 2013). Despite no males being found, parthenogenesis is assumed but is not yet confirmed (Cranston, 1995).

1.9.2 Distribution and ecology

Since its discovery on Signy in the 1980s (Burn, 1982; Block et al., 1984), the midge has successfully spread to cover an area of 35,000 m^2 (Hughes and Worland, 2010), doubling the previous distribution estimate made 12 years earlier (Dozsa-Farkas and Convey, 1997). The first comprehensive study of its distribution was not conducted until 2007-2009 (Hughes and Worland, 2010), which found sites with larval densities as high as 150,000 m^2. Later it was found that the midge may exceed the entire biomass of native micro-arthropod fauna where they co-occur (Hughes et al., 2013). Furthermore, there was evidence of the midge being moved along trails used by researchers on the island, presumably in boot treads. *Eretmoptera murphyi* is predominately associated with dead organic matter, and a study in 2013 suggested it affected litter turnover in the moss banks, increasing turnover by almost an order of magnitude compared to the local biota of Acari and Collembola (Hughes et al., 2013). Its distribution has been noted as highly patchy, possibly as a result of the high content of gravel and rock in some areas with which it is negatively correlated with (Hughes et al., 2013).

1.9.3 Physiological adaptations to stress

Extensive investigations have been conducted into the stress physiology of *E. murphyi*, which have identified several physiological traits that allow it to succeed on Signy. Its larvae have appropriate cold tolerance for the conditions on Signy and can rapidly cold-harden (Block et al., 1984; Everatt et al., 2012, 2015; Worland, 2010), as well as an ability to respire in water and withstand ice entrapment (Everatt et al., 2014a). The larvae are also desiccation tolerant (Everatt et al., 2014c), and can survive nearly 50% body water loss over 12 d. *Eretmoptera murphyi* can tolerate temperatures exceeding 30 °C for several hours, with an Upper Lethal Temperature (ULT) of 39 °C, after entering a heat coma at 31 °C (Everatt et al., 2014b). The larvae are freeze-tolerant with a mean SCP of -7.5 °C and LLT of -13 °C (Everatt et al., 2012).

1.10 Thesis Outline

1.10.1 Core objectives

The primary objective of this thesis is to build upon existing knowledge of *E. murphyi* and to understand its potential as an invasive species through examination of its life history, physiology and dispersal. The thesis seeks to establish *E. murphyi* as a model system to assess the impact of species introductions within simple Antarctic ecosystems, the importance of monitoring and modelling the spread of an invasive species, as well as the implementation of biosecurity measures. To that end, the following key objectives are addressed within this thesis:

1) Characterise the life cycle and phenology of *E. murphyi.*

2) Establish the limits of stress tolerance on the different life stages of *E. murphyi.*

3) Understand the species' ecological impact through trophic assessments and distribution change.

4) Evaluate current biosecurity measures and dispersal potential.

Chapter 2 : Life cycle and phenology of an Antarctic invader – the flightless chironomid midge, *Eretmoptera murphyi*

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2.1 Abstract

Knowledge of the life cycles of non-native species in Antarctica is key to understanding their ability to establish and spread to new regions. Through laboratory studies and field observations on Signy Island (South Orkney Islands, maritime Antarctic), we detail the life stages and phenology of *Eretmoptera murphyi,* a brachypterous chironomid midge introduced to Signy in the 1960s from sub-Antarctic South Georgia where it is endemic. We confirm that the species is parthenogenetic and suggest that this enables *E. murphyi* to have an adult emergence period that extends across the entire maritime Antarctic summer season, unlike its sexually reproducing sister species *Belgica antarctica* which is itself endemic to the Antarctic Peninsula and South Shetland Islands. We report details of previously undescribed life stages, including verification of four larval instars, pupal development, egg gestation and development, reproductive viability, and discuss potential environmental cues for transitioning between these developmental stages. Whilst reproductive success is limited to an extent by high mortality at eclosion, failure to oviposit and low egg hatching rate, the population is still able to potentially double in size with every life cycle.

2.2 Introduction

The sub-Antarctic islands, with a longer history and greater level of human influence than any other part of the Antarctic (Convey, 2013), have a greater number of non-native species than the more extreme maritime and continental Antarctic regions further south (Convey and Lebouvier, 2009; Frenot et al., 2005). However, in recent years and decades, there have been increasing records of species establishing in the maritime Antarctic with anthropogenic assistance, particularly in the South Shetland Islands and northern Antarctic Peninsula (e.g. Greenslade et al., 2012; Hughes et al., 2015; Molina-Montenegro et al., 2012; Volonterio et al., 2013). With synergy between high and increasing levels of human activity in this region of the Antarctic, and recent rapid rates of regional climate change, further establishment of non-native species is predicted, presenting fundamental challenges to the protection and conservation of Antarctic terrestrial biodiversity, and to management and governance processes in the Antarctic (Chown et al., 2012b, 2016; Hughes et al., 2010; Tin et al., 2009). The brachypterous midge *Eretmoptera murphyi* (Chironomidae, Orthocladiinae) is a non-native species on Signy Island (South Orkney Islands, maritime Antarctic), to which it is thought to have been inadvertently introduced in the 1960s in association with plant transplant experiments (Block et al., 1984; Convey and Block, 1996). Its larvae have the capacity to rapidly cold harden, cryoprotectively dehydrate (Everatt et al., 2012, 2015; Worland, 2010), respire in water and withstand ice entrapment (Everatt et al., 2014a). These traits have allowed it to succeed in the maritime Antarctic, which is more extreme in comparison with the species' native sub-Antarctic South Georgia. The sub-Antarctic has a relatively stable and chronically cool oceanic-influenced climate year-round. This presents fundamentally different pressures for terrestrial invertebrates to that of the much more extreme seasonality of the maritime Antarctic, where overwintering microhabitat temperatures can regularly fall below −10 °C, contrasting with minima only marginally below zero on South Georgia (Convey, 1996a; Convey and Block, 1996).

To date, studies of *E. murphyi* have primarily focussed on the ecophysiology of late instar larvae (Everatt et al., 2012, 2015; Hughes et al., 2013; Worland, 2010). However, a much more detailed characterisation of all life stages is required to determine how current and predicted future climate changes may affect this species' development and phenology.

2.2.1 Life history strategies of polar arthropods

Driven by the short growing seasons and environmental extremes, polar invertebrates often exhibit 'adversity selected' life history strategies in comparison with their temperate counterparts (Convey, 1996b). They have slow growth rates (Convey, 1996b), extended and free-running life cycles with reduction of obligate overwintering stages (Thomas et al., 2008), considerable investment in stress tolerance mechanisms (Convey, 1996b; Everatt et al., 2015; Hayward et al., 2003), and the ability to opportunistically take advantage of even short periods of conditions suitable for growth and activity. For instance, the Antarctic oribatid mite, *Alaskozetes antarcticus*, has a life cycle duration of up to seven years, whilst comparable temperate species are typically annual or biennial (Block and Convey, 1995; Convey, 1994). Consequently, multi-year life cycles are common in polar arthropods and many lack a true diapause, instead entering a state of temporary quiescence during winter or other shorter periods of unsuitable conditions. Thus, the most common shared life history feature across polar arthropods is the flexibility which enables the challenges of adverse conditions to be overcome, although some 'programmed' elements may remain so that key life stages can take advantage of regular environmental triggers each season (Convey, 1996a, b; Danks, 1999; Worland and Convey, 2008).

Chironomid midges are a group of higher insects that are particularly well represented at high latitudes in both Hemispheres, relative to other insect groups (Chown and Convey, 2016; Convey and Block, 1996; Coulson et al., 2014). Polar representatives typically conform to the normative polar life-history strategy as defined by Danks (1999), having a fixed and synchronous spring emergence after overwintering in a late larval stage, and a brief adult reproductive stage during summer, but an otherwise flexible life history. Asexual reproduction is prevalent in all major polar arthropod and

micro-invertebrate groups (Convey, 1996a; Chown and Convey, 2016) and especially so in sub-Antarctic Psychodidae, a family of biting midges (Duckhouse, 1985). However, asexual reproduction has not yet been definitively proved in any maritime Antarctic insect species (Convey, 1996a) despite being strongly suspected in *E. murphyi* (Convey, 1992; Cranston, 1985).

2.2.2 Life histories of Antarctic chironomids

The life histories and biology of the native Antarctic chironomids *Parochlus steinenii* (Gercke, 1889) (Podonominae) and *Belgica antarctica* (Jacobs, 1900) (Orthocladiinae) have been well-studied (e.g. Allegrucci et al., 2006, 2012; Convey and Block, 1996; Hahn and Reinhardt, 2006; Harada et al., 2014; Sugg, 1983; Usher and Edwards, 1984). These are typically characterised by larval development taking place over two years, overwintering as either early or late instars, followed by synchronised mass emergence of adults in summer (Convey and Block, 1996; Harada, 2014; Richard et al., 1985; Sugg, 1983). *Belgica antarctica* occurs along the Antarctic Peninsula and is the only higher insect endemic to the Antarctic continent (Convey and Block, 1996; Kelley et al., 2014). It experiences environmental conditions similar to those of *E. murphyi* on Signy, and the assumption is that both species have similar ecological niches. Recent molecular evidence also suggests that *E. murphyi* should be assigned to the genus *Belgica* (Allegrucci et al., 2012), further supporting likely common life history strategies. However, questions remain as to whether the long evolutionary history of *E. murphyi* on sub-Antarctic South Georgia has provided the opportunity for the evolution of a temperate-style life history pattern that would show less flexibility than that of a more typical polar insect.

In the field on Signy *E. murphyi* is thought to emerge *en masse*, possibly in response to abiotic factors such as increased spring daylength, the seasonal melt of basal snow (Block et al., 1984; Gardiner et al., 1998), or as a heritage trait from related chironomids (Armitage et al., 1995). Convey (1992) showed that rates of egg development decrease with an increase in temperature (2-12 °C) and that the females invest greatly in reproduction with ca. 85 eggs being laid in a single hydro-sensitive egg sac – representing a dry mass twice that of the female post-oviposition. Once larvae hatch, they are thought

to overwinter twice (Hughes et al., 2013; Worland, 2010), once in an early larval stage and later in the fourth instar, although this has not been explicitly demonstrated. It is assumed that *E. murphyi* has four larval instars like *B. antarctica*, although previous size class distribution analyses and taxonomic studies have identified only two distinct classes *via* assessments of larval mass or field observations (Cranston, 1985; Hughes et al., 2013). One reason underlying the current lack of explicit knowledge of *E. murphyi's* life history has been the challenge of establishing a long-term laboratory culture, with all data obtained to date derived from short periods of field observations combined with laboratory experiments relying on field collected material (Convey, 1992; Everatt et al., 2014 a, b, c; Hughes et al., 2013).

2.2.3 Patterns of climate change in the western Antarctic Peninsula and Scotia Arc

In recent decades, rapid regional warming and other physical environmental changes have been documented in parts of Antarctica, in particular in the region of the western Antarctic Peninsula and Scotia Arc (Convey et al., 2009a; Turner et al., 2014), including Signy (Cannone et al., 2016; Royles et al., 2012; Smith, 1990). Signy was recognised early on as a paradigmatic location at which to study terrestrial biological processes in the maritime Antarctic, and how these might change under the influence of changing environmental drivers (Smith, 1990). Within terrestrial ecosystems, the primary consequences of these environmental changes are longer active seasons (earlier spring thaw combined with later autumn freeze), greater integrated thermal energy availability (increased temperatures), and greater availability of liquid water to terrestrial organisms. Thus, and unlike the general consequences in many regions of the world, regional warming in parts of the Antarctic relaxes the current extreme environmental constraints on biological processes, and recent syntheses recognise that many of the native biota in these regions, including polar terrestrial invertebrates, are likely to benefit from the changes being observed (Bale and Hayward, 2010; Convey, 2011; Convey et al., 2014a). It is also increasingly recognised that this relaxation of environmental constraints, with or without the direct influence of human assistance in transporting propagules, will lower the barriers to new species arriving and establishing in Antarctica (Frenot et al., 2005; Hughes et al., 2006).

2.3.4 Aims of this study

Against this background, the primary aims of this study are to provide the first detailed characterisation of different developmental stages within the life cycle of *E. murphyi*, and to investigate the potential role of abiotic triggers in the timing of major life history transitions on Signy, such as pupation, adult eclosion or oviposition. These are then considered in the context of the implications of climate change for this species' life history and distribution on the island and, potentially, more widely in the maritime Antarctic.

2.3 Materials and methods

2.3.1 Sample collection and processing

All samples were either obtained from, or observed *in situ*, on the Backslope and in the immediate vicinity of the British Antarctic Survey (BAS) research station on Signy Island (Section 1.2.2.1, Fig. 1.2 and Fig. 1.3). Samples collected during the 2014/15 austral summer by BAS staff were returned to the United Kingdom by ship in +4 °C cold storage (10 weeks), and then maintained at +4 °C at the University of Birmingham until use. Studies were conducted in the field on Signy between December 2016 and March 2017. All laboratory cultures and experiments, both at the University of Birmingham and on Signy, were maintained on local Signy peat soil substrate, which is both the species' habitat and food source on the island. The substrate was kept moist with a soil solution comprising 3:1 deionised water to Signy soil (hereafter termed 'field water') to ensure that conditions deviated as little as possible from the natural environment.

Eretmoptera murphyi's current distribution on Signy is centred around the research station and adjacent Backslope, and therefore all monitoring and sampling occurred within a few hundred meters of the station (Fig. 1.3). All images and morphological measurements were obtained using a Leica EZ4 digital microscope and associated software. Individual larvae or adults were extracted from the soil/moss substrate by washing through stacked sieves (2-mm, 0.5-mm mesh sizes) and hand-picked

from the remaining soil solution. Moss and peat substrate were broken apart with fine tweezers prior to washing to ensure individuals were not trapped amongst the fibres. Weather conditions were noted in association with all field experiments and collection days, with particular attention to recording strong sunshine and significant precipitation events. Temperature was recorded every 30 minutes from the 28th December 2016 to the 10th March 2017 using a TinyTag Plus II temperature logger. The logger was placed on the ground surface on the backslope, amongst areas of know *E. murphyi* distribution (Appendix II).

2.3.2 Measurement of larvae

Larvae were assigned to instars based on size. They were initially separated into approximate size classes by eye, followed by detailed width and length measurements using images taken with a digital microscope with in-built camera (Leica EZ4). The microscope software was calibrated for each image using a micrometre stage graticule. Width measurements were taken by measuring the length of the intersegmental groove between segment IV (SIV) and segment V (SV) - the intersection of the cephalothorax and abdomen where the larvae was consistently the widest (Cranston, 1985) . Length measurements were taken from head to anus but did not include mandibles or posterior parapods (the latter only in the case of L1). This information on distinct size classes then informed the selection of L4 larvae for studies of pupal development and larval instar occurrence in phenological surveys, described below.

2.3.3 Environmental triggers for pupation

In laboratory samples maintained at 4 °C under constant darkness, progression to pupae from the L4 instar is infrequent and unpredictable (P. Convey, J. Bartlett, pers. obs.). We therefore hypothesized that other environmental signals might be required to trigger pupation. In a simple test of this, batches of L4 larvae (n = 20) were placed under the following conditions representing different temperature, light and water availability scenarios on Signy during the transitions from winter to spring and summer (Table 2.1). Control conditions were constant darkness at 5 °C. To determine if light was a trigger for pupation, samples were transferred to 19:5/L:D (5 °C), which approximates summer

photoperiods on Signy. A fluctuating temperature regime of 5 °C during illumination and 0 °C during darkness was also used, to approximate typical Signy summer diurnal conditions. To determine if spring melt/access to water was a trigger for pupation, larvae were maintained either under "wet" conditions (1:1 soil mass to 'field water' volume ratio), or "dry" conditions (no additional water was added to the substrate) in Petri dishes.

Larvae were maintained under these experimental conditions for 60 days from 18 December 2015 to 18 February 2016, and cumulative pupation recorded. Any pupae obtained were maintained under the same temperature and light conditions, but with saturated soil, until either death or eclosion to imago.

Table 2.1. *Environmental treatments used to assess influence of temperature, light and soil saturation on pupation*

Dominant light condition	Temperature (°C)	Light regime (L:D)	Soil moisture
Light	5	19:5	Wet
			Dry
Light	2	19:5	Wet
			Dry
Dark	5	0:24	Wet
			Dry
Dark	5-0	19:5	Wet
			Dry

2.3.4 Pupal development

Initial observations suggested morphologically distinct phases of pupal development, individual pupae (n = 31)were observed and imaged as they occurred in laboratory stocks throughout the study (i.e. from both the 2014/15 BAS collection and 2016/17 collections). To clearly document the discreet phases, digital images were taken of all pupae under the different treatments applied, with width and length data recorded as well as other key morphological and physiological changes including development of gonads, development from stemmata to compound eyes, and changes to cuticle

pigmentation (Table 2.2). This definition of pupal phases informed the experimental design for field monitoring of pupal and imago development during the 2016/17 season.

2.3.5 Pupal and imago development in the field

Field monitoring of pupal development took place during January 2017 adjacent to Signy Research Station. Individual pupae (n = 20) were placed in open Petri dishes containing local substrate within a larger arena placed on the ground, and temperature data were recorded for the duration of the observations. The arena was constructed using 2-L plastic tubs with modified lids of nylon mesh, in order to keep the arena open to the environment whilst preventing damage by local wildlife, predominantly the brown skua (*Stercorarius antarcticus*). Pupae were assessed daily from 20 December 2016 to 6 January 2017. Temperature readings inside the arena were taken each day at the time of surveying using a soil temperature probe and digital thermometer (RS Pro- 206-3738 with K type thermocouple probe) and ambient readings collected with an adjacent temperature logger external to the arena (Tinytag Transit TG-0050). Pupae were followed through their development *via* assessment with a hand lens and allocated to the developmental stages as noted above in Section 2.3.4 and in Table 2.2. Pigmentation was noted as a darkening of the cuticle from cream-yellow to a gray-black. Pupal development wasfollowed by recording their eclosion date and any subsequent oviposition asadults.

Pupal Phase	Size (mm)	Physically mobile	Eye type	Pigmentation	Legs	Gonapophysis	Reproductively viable	Development time in phase (days)
1	1-1.5	Very	S	None	Sheathed	None	No	3.00 ±0.82
2	1.5	Somewhat	S&C	Cephalothorax & legs	Sheathed	Yes	No	4.67 ±2.75
3	2	Sessile	C	Full, opaque	Sheathed	Yes	No	2.14 ±2.59

4	>2	Somewhat	C	Full, opaque	Free	Yes	Yes	1.00 ±1.31

Table 2.2. *Description of pupal phases and classification guide for development tracking. Average development time within each phase ± SEM. n=31 pupae assessed in laboratory conditions (5 °C, saturated soil, dark). Eye type: S = stemmata, C = compound eye. Size is total body length.*

2.3.5.1 Adult emergence

Three 0.5 m^2 quadrats were set out on a moss bank adjacent to the research station and all were monitored twice daily for 5 min at 1000 and 1600 local time, noting the presence of adults active on the surface, between 20 December 2016 and 6 January 2017. Ground surface temperature readings were taken each day during the observation periods, as described above.

2.3.6 Egg maintenance and larval development

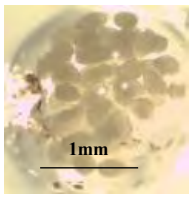
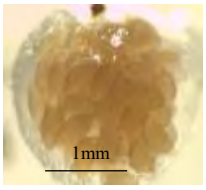
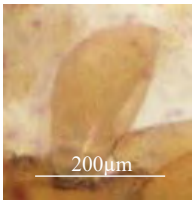
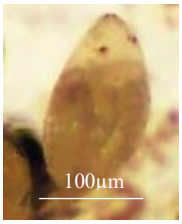
Laboratory cultures established from the 2014/15 stocks enabled the rearing of larvae to pupation and subsequent emergence with successful oviposition. From these laboratory eggs an initial four-phase classification system of embryonic development was established to aid development stage identification (Table 2.3). All laboratory adults that emerged and then oviposited under one of the experimental environmental conditions described above, were subsequently maintained with their egg sacs at 5 °C on saturated substrate to maintain sac structure. Hydrated egg sac diameters were measured, and numbers of eggs were recorded. Eggs were assessed every 48 h, and developmental stage recorded.

2.3.7 Monitoring of egg development in the field

Recently laid egg sacs (n = 11) collected from field samples were placed in open 2 cm petri dishes with saturated substrate in an external arena and monitored every 48 h until development ceased, or the eggs hatched (a maximum of 39 d), and then again on day 45 to confirm that no further delayed development had occurred. Temperature readings inside the arena were noted daily as above. The

development stage of each egg (Table 2.3) within each egg sac was recorded using a dissecting microscope. On day 45, all egg sacs were dissected, and any remaining unhatched eggs individually inspected. A total of n = 740 eggs were assessed.

Table 2.3. *Description of egg development stages and classification guide for development tracking. Typical duration of each stage given and mean success rate/progression to next phase ± SEM. Success rate = % that successfully complete each development stage.*

Egg Stage	Image	Description	Development (days)	Success rate (%)
1 – Opal		Opaque white eggs granulated and slightly iridescent in appearance. No pigmentation	10-14	44 ±2.72
2 – Yellow		Outer-casing turning yellow/brown. Still granulated and no sign yet of embryonic form.	5-7	100
3 - Early Embryo		Shape of embryo becomes clearer and red stemmata eyes become evident.	7-10	92.2 ±0.97
4 - Late Embryo		Pharate larva visible with some evidence of internal organs, eyes and mandibles clearly visible.	7-10	82 ± 2.29

2.3.8 Phenology of summer-occurring life-stages

Weekly soil cores (n = 5) were taken from a site adjacent to the research station where *E. murphyi* was abundant, using a steel 5-cm x 10-cm corer. This took place between 23 January and 6 March 2017 (= late summer season). Soil cores were returned to the Signy laboratory in a sterile sealed bag and processed within 24 h. Cores were divided into vegetation and soil/peat substratum and weighed using a Sartorius precision balance (E - 6202) before being carefully washed separately through stacked sieves as described above. All life stages were extracted, sorted into groups (adults, pupae, L4 larvae, L3 larvae, L2 larvae, eggs unhatched, eggs hatched) and counted. L1 larvae were not included as their small size would have resulted in sample processing being too time consuming. All sieved substrate was dried for 24 h at 60 °C and re-weighed to obtain constant dry mass, against which all counts were normalised. An additional core was collected each week, divided into vegetation and soil components, and used to make pH and salinity measurements with a Hanna combi water reader (HI-98129). Throughout the field period in 2016/17, the presence of pupae or adults on the surface were recorded, from which the final dates of sighting of both pupae and adults were established.

2.4 Results

2.4.1 Environmental description

The Signy field site was generally very stable throughout the 2016/17 season. The mean pH in the vegetation layer was 5.3 ± 0.13 SEM, n = 7, and underlying soil pH was 5.5 ± 0.11 SEM, n = 7. Salinity was also largely stable, with only one spike during a week of high storm activity detected in the vegetation layer, when it rose to 425 µS from an average of 174 ± 45 µS SEM, n = 7. Salinity in the soil layer remained close to 70 ± 10 µS SEM, n = 7. Temperatures fluctuated from -2.8 ºC to +27.1 ºC, with the largest diurnal fluctuation between -2.8 and +22.6 ºC on the 5th January 2017 (Appendix II).

2.4.2 Larval classification

Size class analysis proved to be suitable for separating the four larval instars, with each size class being significantly different in both width at SIV and length, and no overlap between instar size classes (Fig. 2.1).

2.4.3 Larval survival and environmental triggers for pupation

Data was first deemed to be parametric with a Shapiro-Wilkes test before further analysis. Larval survival (Fig. 2.2) was greatest in the control/dark 5 °C conditions (70% survival after 60 d), and was significantly different from the light treatments (two-way ANOVA with Tukey's multiple comparisons column factor, $F_{(3,20)} = 9.71$: Light 2 °C - 32.5% survival after 60 d, $p = 0.008$; Light 5 °C - 30% survival after 60 d, $p < 0.001$). Overall survival dropped signifcantly over time across all treatments (two-way ANOVA with Tukey's multiple comparisons, $F_{(4, 20)} = 11$, $p < 0.0001$) and soil moisture only had a small effect on survival in the warmest lit conditions of Light 5 °C (Unpaired t-test, $df = 8$, $p = 0.02$). Of all environmental conditions tested, the fluctuating freeze/thaw cycle of +5 °C/0 °C with corresponding 19:5/L:D, which is the condition most reflective of Signy summer conditions, led to the greatest level of pupation , although this was not significantly different from other treatments (ANOVA $p = 0.6$)

2.4.4 Pupal classification and development

Pupae exhibited four distinct phases of development before completing the moult to imago (Table 2.2). Broadly, the 1st and 2nd phases were differentiated by an increased level of pigmentation (darkening to a gray-black) and development of the gonads. The 3rd phase was quite sessile, deeply pigmented and with the legs still encased in leg sheaths. There was a thickening of the cuticle in this stage. The final 4th phase was a partial eclosion, where the legs were free of the sheath but the imago not fully eclosed from the exuvia. Mean development time in the field from initial pupation to eclosion was 14 days (± 5 d, $n = 12$), with the longest period spent in the 2nd phase of pupation (Table 2.2). There was no difference in development rates of pupae incubated at constant or fluctuating

temperature in laboratories in the UK, compared with those in the field conditions with a fluctuating temperature on Signy (Kruskal-Wallis, H = 1.3, $p = 0.54$)

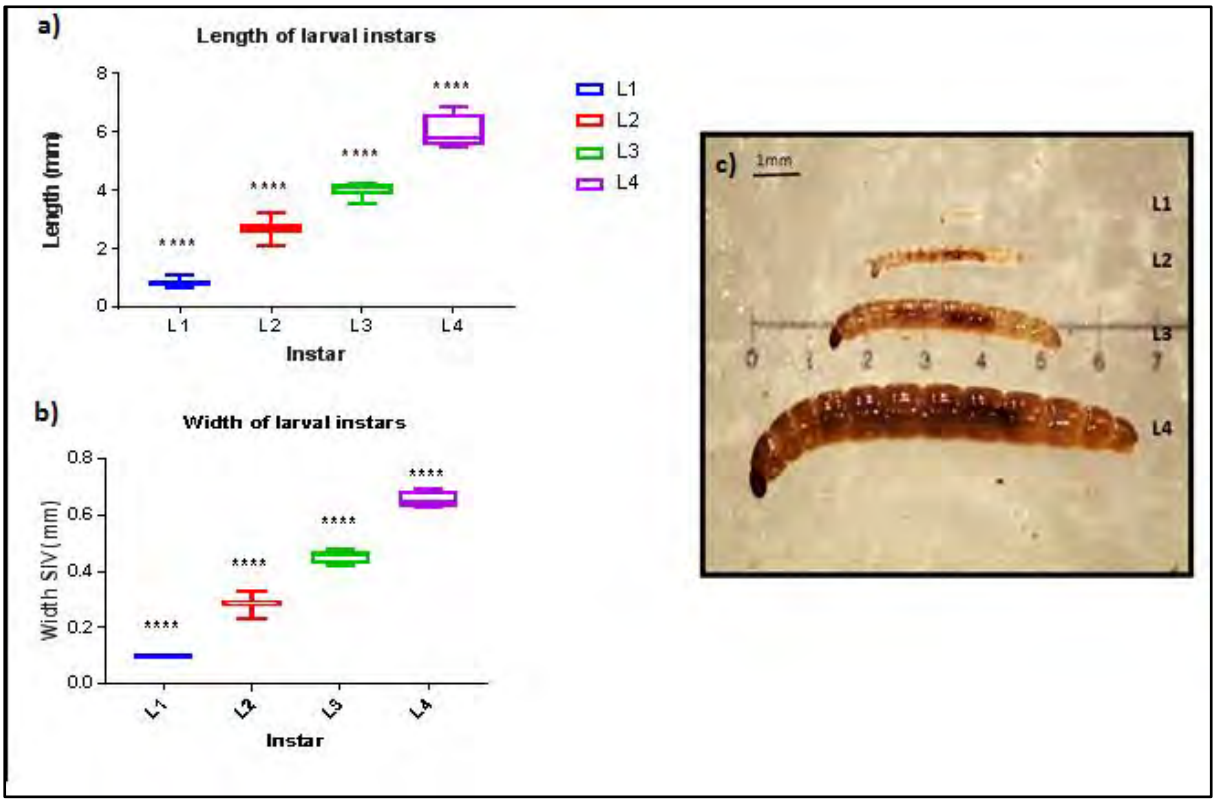


Figure 2.1. *Classification of the four larval instars by* ***(a)*** *total body length and* ***(b)*** *width at segment four/five intersection; n = 10 individuals for each of L1, 3 and 4, n = 12 individuals for L2. All instars significantly different from each other for length: ANOVA* $F_{(3, 31)} = 372.2$, $p < 0.0001$; *width: ANOVA* $F_{(3, 34)} = 780.7$, $p < 0.0001$. ***(c)*** *Larval instars side by side: Top to bottom, L1 to L4.*

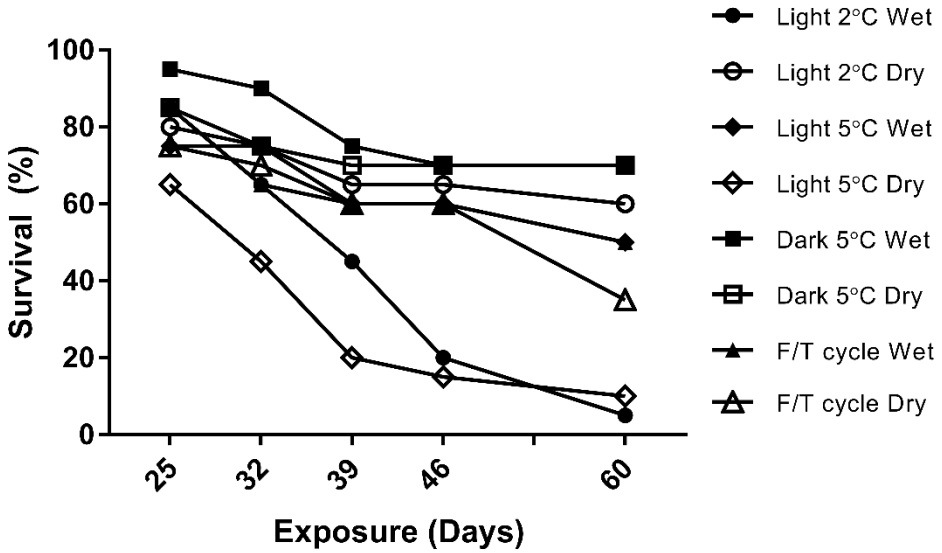


Figure 2.2. *Larval survival over 60 days after exposure to varied light, temperature and substrate saturation levels (n = 20 for each condition) Survival over time across all treatments (2-way ANOVA Tukey's post hoc comparisons, $F_{(4, 20)} = 11$, $p < 0.0001$). Soil moisture effect the only significant variable in Light 5°C (Unpaired t-test p = 0.02).*

2.4.5 Eclosion, imago development and phenology.

Only 45% of pupae ($n = 20$) placed within the external field arenas successfully eclosed, and 55% of these adults oviposited. There was no correlation between numbers of individuals eclosing and either ambient temperature on the ground surface over the preceding 24 h or within the pupation arena at the time of sampling (surface temperature: $r_s = 0.21$, $p = 0.4$; arena temperature: $r_s = 0.31$, $p = 0.2$). There was, however, a strong correlation between the temperature outside the arena and the spot temperature taken within it at the time of surveying, verifying that the arena did not increase temperature artificially ($r_s = 0.88$, $p < 0.0001$). Monitoring of quadrats for the presence of adults showed no correlation with daily ambient mean temperatures ($r = 0.07$), although anecdotally adult presence was associated with calm, clear days (Appendix I).

2.4.6 Egg classification and monitoring

Egg sacs ($n = 30$) had a mean dry mass of 0.14 mg (± 0.06 mg) and water content of 96% (± 1.29%) fresh mass. Egg sacs contained a mean of 48 (± 12.48 $n = 30$) individual eggs and had a diameter of 1.78 (± 0.4 $n = 30$) mm, with size not being significantly correlated to either number of eggs or water content ($r = 0.08$ and 0.009, respectively). Changes in the proportion of different egg stages within egg sacs (Table 2.3) until hatching are presented in Fig. 2.3a. Stage 1 (opal) spanned c. 14 days across all samples, although very consistently across all egg sacs approximately 40% of eggs did not develop beyond this stage (Fig. 2.3b). The eggs then turned yellow, before the early embryo with stemmata evident become visible around day 19. Late embryos, with visible mandibles and pharate larvae, appeared around day 25 and eggs hatched by day 31. Development within an individual egg sac was not tightly synchronised. After 45 days of field observations of $n = 740$ eggs, 40% (± 2.72%) did not progress past the first 'opal' stage (Fig. 2.3b). Nearly all remaining eggs progressed through the yellowing phase, but 7.6% (± 0.97%) did not progress beyond the early embryo and 16% (± 2.29%) beyond the late embryo, with only 35% of all eggs (± 2.44%) going on to hatch. There was no

difference in development rates of eggs incubated at constant temperature compared with those under field conditions with fluctuating temperature (Mann-Whitney U = 17, $p = 0.62$) (Fig. 2.4)

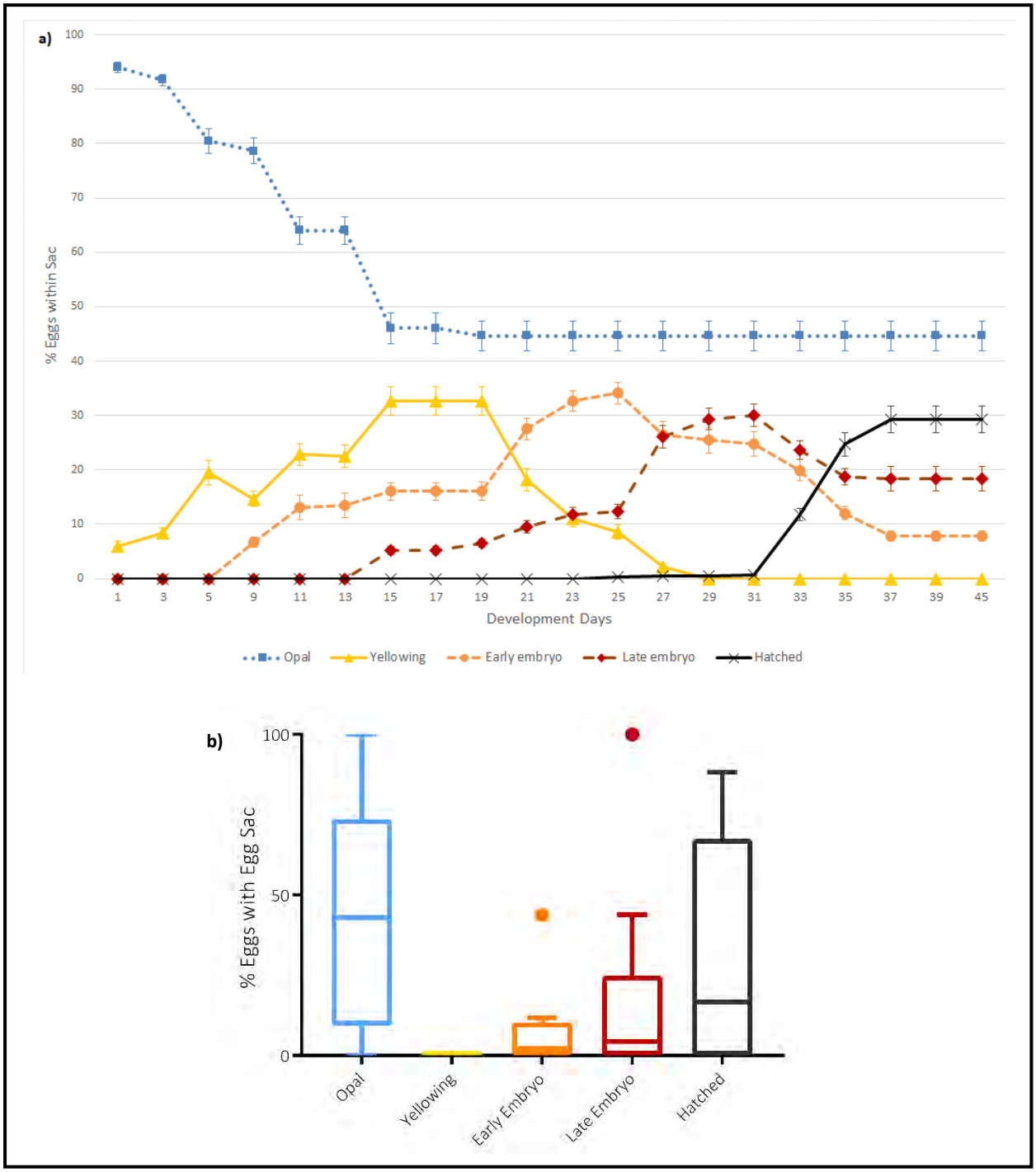


Figure 2.3. ***a)*** *Egg development in field conditions on Signy Island. Stages of individual egg (n = 740) development within each egg sac (n = 11) recorded over time, shown with CI of 95%.* ***b)*** *Maximum stage reached after 45 days of monitoring (Tukey's plot) with outliers.*

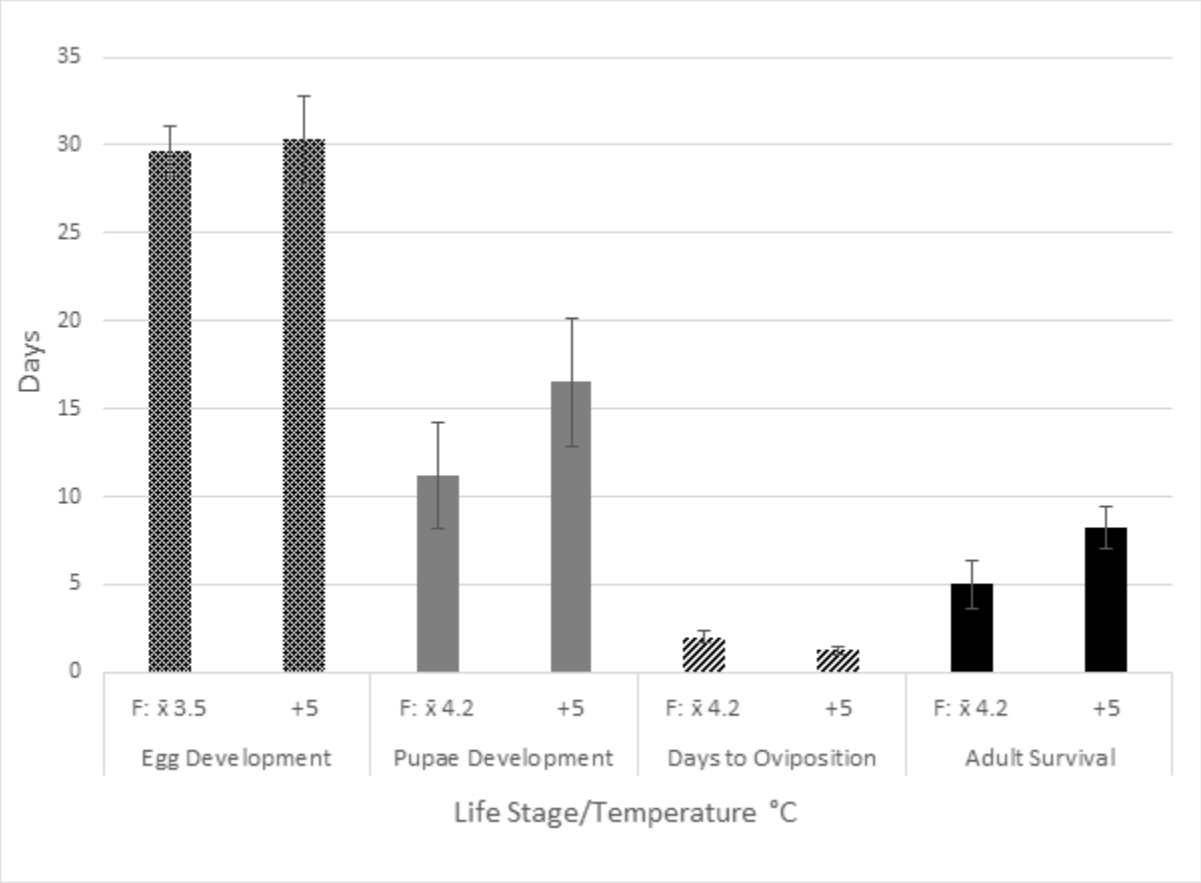


Figure 2.4. *Mean (+/- SEM) time (days) taken to complete egg or pupal stage under different temperature conditions. Also shown, the mean period from eclosion to oviposition and adult longevity post eclosion. Field conditions are shown as the mean temperature experienced (F: x̄) over the relevant period. Pupal and adult development periods overlapped and thus were subject to the same average field temperatures. Laboratory temperatures were either static 5°C or 12h 5/12 h 0°C. Sample sizes - Egg development: "F: x̄ 3.5", n = 6 egg sacs; "+5" n = 7. Pupal development: "F: x̄ 4.2" n = 5; "+5" n = 4. Oviposition "F: x̄ 4.2" n = 5; "+5" n = 4. Adults: "F: x̄ 4.2" n = 5; +5 n = 4.*

2.4.7 Phenology and Environmental Factors

Seasonal life stage monitoring in the field over the late summer showed that the *E. murphyi* population had progressed through pupal and adult stages before the end of January (Fig. 2.5). The last pupae were found on January 3rd, 2017 and the last adult was seen on January 19th, 2017 (Fig. 2.5). By February 13th, 2017 no further viable unhatched eggs were found in the samples (determined by the presence of hatched eggs alongside those that had stalled development at a much earlier stage), with only hatched eggs collected thereafter. Unhatched eggs that were considered undeveloped/ unviable appeared to decompose, becoming opaque, soft and bloated, often combined with visible

fungal development. The phenological soil core surveys did not include any counts of adults or pupae (Fig. 2.6), so these were discounted from analysis. There was no significant difference in the appearance of the larval instars or trend over time (Kruskal Wallis, H = 1.3, $p = 0.53$; $r = 0.1$). The appearance of unhatched and then hatched eggs did show a visible difference (Fig. 2.6), with a distinct increase in the number of hatched eggs over time compared to a decline in unhatched (Mann Whitney U = 202, $p = 0.008$).

2.4.8 Population success

Analysis of reproductive output from this study suggests that the *E. murphyi* population can potentially double with each life cycle. Considering the percentage of larvae that survive over 60 d, pupae that successfully eclose, adults that successfully oviposit and the viability of eggs laid, this will amount to an average 50,000 additional L1 added to the population every two years, based on the most recent distribution data reported by Hughes and Worland (2010) (Table 2.4).

Table 2.4. *Life stage success table using population densities of larvae reported by Hughes et al. (2010). Average larval densities are taken from the whole sample site. ~70% of L4 larvae survive, 45% of larvae successfully eclose, 55% then oviposit, with a mean of 48 eggs per oviposition, and 35% go on to hatch.*

Population density	Larvae density (m^{-2})	Approximate larvae survival (m^{-2})	Successfully eclose (m^{-2})	Successfully oviposit (m^{-2})	Total Eggs laid (m^{-2})	Total Hatched (m^{-2})
High	150,000	105,000	47,250	25,987	1,247,400	361,746
Average	21,000	14,700	6,615	3,638	174,636	50,644
Low	500	350	157	87	4,158	1,205

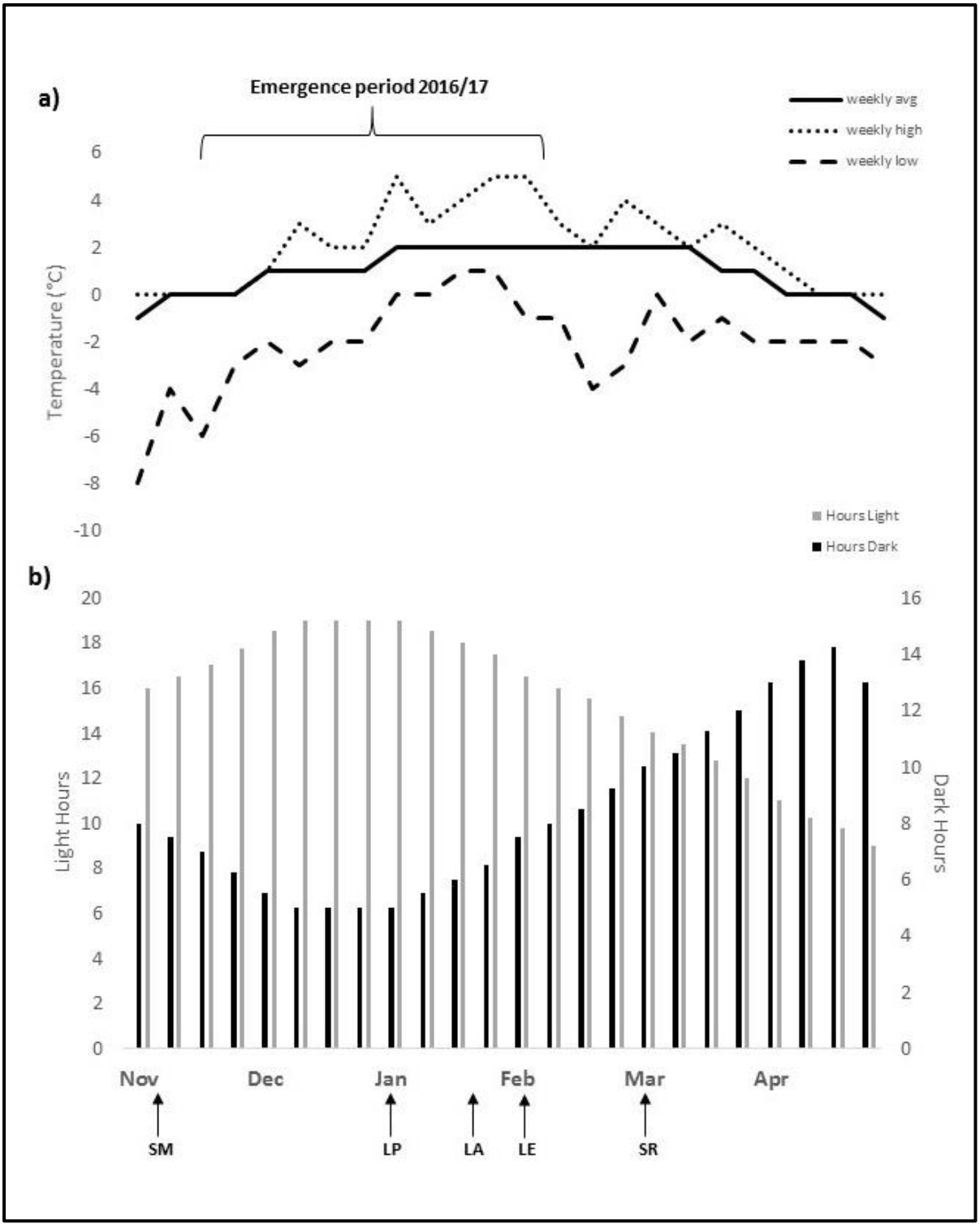


Figure 2.5. *Mean (weekly) hours of daylight and darkness as well as high and low air temperatures on Signy Island, annotated with key points in the development of* E. murphyi *life stages: SM = Basal snow melt, mid Nov 2016; LP = Last pupae seen 3 Jan 2017; LA = Last adult seen 19 Jan 2017; LE = Last unhatched eggs seen 30 Jan 2017; SR = Snow returned 27 Feb 2017.*

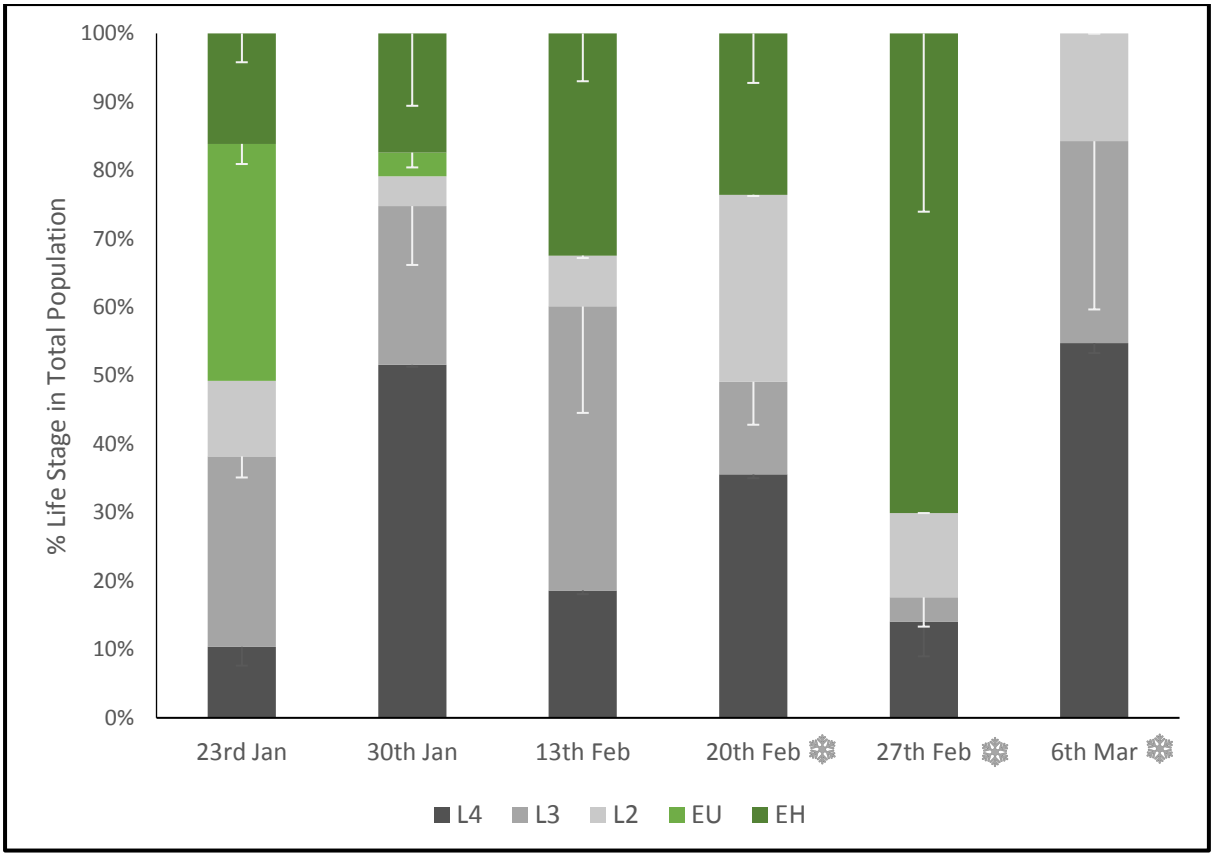


Figure 2.6. *Abundance of different* E. murphyi *life stages found in soil cores from a single site collected from late January to the beginning of March 2017, shown as mean percentage of total population (+/- S. E.). L2, L3 and L4 = larval instars; EU = egg sacs where majority (>50%) of eggs were unhatched; EH = egg sacs where the majority (>50%) of eggs had hatched. No adults or pupae found. Snowflake indicates snow cover on survey days.*

2.5 Discussion

The developmental stages described here for larval instars are consistent with the only taxonomic study of *E. murphyi* (Cranston, 1985) and provide a first description of the L1 and L2 instars. Like the sister species *B. antarctica* (Suggs et al., 1983), and typical for chironomids, there are four larval instars, which in our data do not overlap in size classes for either width or length and provide a clearer assessment of all instars than the use of body mass classes (cf. Hughes et al., 2013). Our observations of pupae indicate that metamorphosis takes around 14 d, and can be divided into four morphologically distinct phases, providing a new level of detail for *E. murphyi* and for chironomids in general, whose pupal stage is understudied (Armitage et al., 1995). Apparent obligate parthenogenesis in *E. murphyi*

enables oviposition to occur prior to the completion of eclosion. Whilst this alone is not unique among asexual chironomids (Armitage et al., 1995; Langton et al., 1988), it does offer *E. murphyi* a distinct advantage in a polar habitat such as on Signy, enabling it to reproduce even if conditions or physiology do not permit eclosion. Our data do not provide evidence for a temperature or moisture/spring thaw trigger for pupation. Fluctuating temperatures of the spring season (+5/0 °C), did result in more pupae, however, this was not statistically different from other treatments. Block et al. (1984) reported that pupae of *E. murphyi* appeared shortly after spring thaws, and a similar observation has been reported in an aquatic Antarctic midge, *Parochlus steinenii* (Hahn and Reinhardt, 2006; Rauschert, 1985), but further early season sampling will be required to clarify the cues required to initiate pupation.

The emergence of *E. murphyi* adults does not take place in a synchronous mass event as reported in *B. antarctica* (Sugg et al., 1983) but continues over a 2-3-month period until later January. In the 2016/17 summer season, adults were already noted to be active when Signy station was opened in mid-November (Station Leader M. Jobson, pers. comm.), coinciding with an early spring thaw. Hatched eggs were already present in the soil in late December 2016 which, based on the egg development times recorded in this study, means they would have been laid in late November at the earliest. This could give *E. murphyi* a distinct advantage over sexual reproducers, such as *B. antarctica*, the only chironomid that successfully completes its life cycle on the Antarctic Peninsula. Whilst *B. antarctica* is limited by the need to have males and females emerge synchronously to reproduce sexually, *E. murphyi* is not even limited by the need to complete eclosion. Staggering the emergence period means that any adverse weather encountered in the summer months would not necessarily impede the species' continued survival on the island, as posited by Hughes et al. (2013). The lack of mass emergence also suggests that *E. murphyi* has a more flexible life history and emergence may be triggered by significant environmental cues for favourable conditions rather than any obligate physiology.

Parthenogenetic reproduction is a common feature within the Chironomidae, and in the Orthocladiinae usually takes the complete form of parthenogenesis known as thelytoky (Moller Pillot,

2014; Scholl, 1956; Thienemann, 1954). In thelytoky, genetic fertilization is absent and so females only produce female progeny, as is seen in *E. murphyi* (Convey, 1992; Cranston, 1985). It is likely that *E. murphyi* exhibits apomictic thelytoky, the most widespread from of thelytoky in Orthocladiinae (Scholl, 1956, 1960) and that the lack of progression of a significant proportion of eggs beyond the initial development stage seen here is the result of a mechanical failing at an early maturation stage of mitosis (Porter and Martin, 2011), possibly as the result of an environmental stressor. It is thought that the adoption of thelytoky by arthropods is an advantageous strategy. It may particularly benefit polar species, through the elimination of males, which have been shown to be more susceptible to the cold and extremes in temperature (Colinet, 2009; David, 2005; Oliver and Danks, 1972; Reinhart et al., 2000). Thus, the need for synchronous mass emergence is redundant. (Downes, 1962; Porter and Martin, 2011).

A total of 740 individual eggs were studied to document embryogenesis and hatching. Unusually for the sub-family Orthocladiinae, eggs are laid in an almost uniform spherical mass rather than in a rope-like mass or bale, a trait that is otherwise used to define the group (Nolte, 1993) and that is also exhibited by *B. antarctica*. Individual egg morphology is consistent with previous descriptions of Orthocladiinae (Armitage et al., 1995; Nolte, 1993; Thienemann and Strenzke, 1940), and particularly that of *B. antarctica* (Harada et al., 2014). *Eretmoptera murphyi* individuals produced an average clutch size of 48 eggs in this study, of which only 35% hatched successfully. This is a small clutch size for a chironomid midge, which typically produce hundreds if not thousands of eggs, but not unusual for terrestrial Orthocladiinae which have been recorded to lay eggs in numerous small batches or even individually (Nolte, 1993). Another Antarctic chironomid, *P. steinenii* lays an average of 191 eggs per batch, sometimes over multiple batches (Hahn and Reinhardt 2006), whilst Harada et al. (2014) describe a mean batch size of 41 eggs per string for *B. antarctica* with a gestation of just 16 d. Neither of these studies documented the percentage of eggs that go on to hatch.

With ground surface temperature variation of as much as 24.8 °C (see Appendix II) in a 12 h period on Signy, temperature stress may account for the long egg development time and high mortality observed. Despite the low clutch size and hatching success, our data indicate that at least 13 eggs will

hatch for every *E. murphyi* adult. With population densities as high as 150,000 individual larvae per m^2 (L/m^2) (Hughes et al., 2010), this could result in as many as 1.2 million eggs being laid per m^2 in parts of the species' current distribution and, consequently, further local dispersal if no checks are held on the population at other points in the life cycle. However, as nearly half of all pupae failed to eclose to adult under field conditions, and 55% of adults failed to successfully oviposit, these two life stages appear to represent a major limitation. Whether this indicates lower stress tolerance in these life stages requires further study, given that only larval stages have been studied in detail to date. Even taking this into account, with an average density of *E. murphyi* within its distribution on Signy of 21,000 individual L/m^2 (Hughes et al., 2010), we estimate that the species could have the potential to double its population over every life cycle of two years. If so, a simple back-calculation would suggest an introduction date of a single individual in the early 1970s, which is generally consistent with the assumed introduction event with plant transplants in the latter part of the 1960s (Block et al., 1984; Burn, 1982). However, it is worth noting that, Convey (1992), found higher egg counts of up to 85 eggs per batch, which could lead to an even higher population growth rate than calculated here.

Although all larval instars were present throughout the study period, their relative densities were highly variable over time. These data appear to reflect more the patchiness of larval distribution than the sequential occurrence of particular instars over time. Whilst eggs are immobile and thus easier to represent through repeated sampling in a fixed location, larvae are mobile. Patchiness in larval distribution, including aggregations of larvae, was also reported by Hughes et al. (2010), and is a characteristic of Antarctic terrestrial invertebrate communities (Usher and Booth, 1984). Any increase in hatched eggs naturally infers an increase in the number of L1 in the soil but, due to the very small size of this instar, it was not practicable to include them in this survey. Unhatched eggs do not overwinter. *Belgica antarctica* is also thought to overwinter only in the larval stage and in all four instars (Sugg, 1983). However, during soil core analyses, L1 and L3 *E. murphyi* larvae were noted to be moulting towards the end of the season, indicating that L2 and L4 are likely to be the primary overwintering larval instars, as suggested by Convey (1996a) and Hughes et al. (2013).

A mid-range climate forecast for the Antarctic Peninsula and South Orkney Islands suggests that mean annual air temperatures are expected to increase by 1.5-2 °C by 2100 (Larsen et al., 2007). Temperature warming is thought to benefit polar terrestrial invertebrates by reducing the stress of low-temperature extremes and giving greater liquid water availability (Bale and Hayward, 2010; Convey, 2006, 2011; Convey et al., 2014a) and, in the case of non-native species, making available locations that were previously uninhabitable. By increasing knowledge of *E. murphyi*'s life cycle, we can better understand any threat it may pose to Signy's terrestrial ecosystems, and its potential as an invasive invertebrate at other at-risk areas, such as along the Antarctic Peninsula.

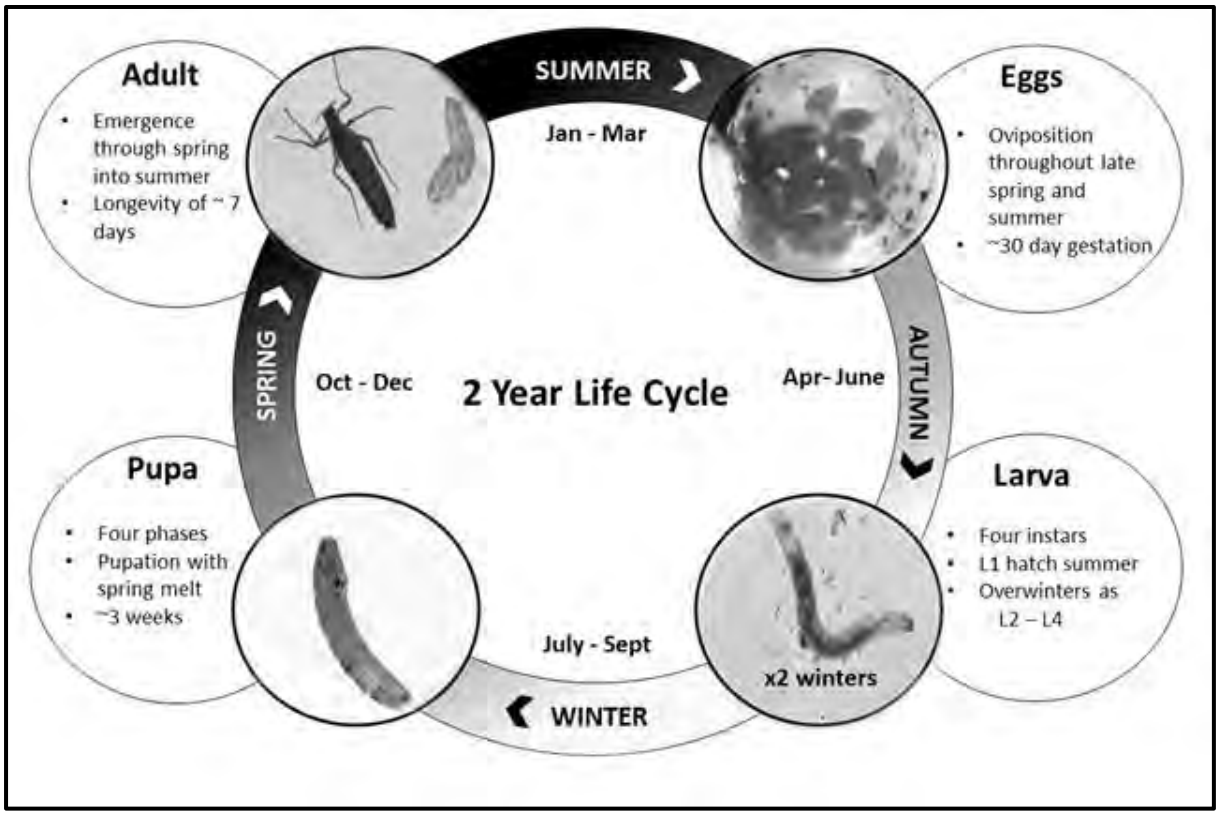


Figure 2.7. *Summary of* E. murphyi's *life cycle*

2.6 Conclusions

This study provides the first comprehensive documentation of the life cycle of *E. murphyi*, a flightless chironomid midge that is currently expanding its distribution following anthropogenic introduction to Signy Island. The species' reliance on parthenogenesis is confirmed and new information provided on the characteristics of all life stages, their development rates, the phenology of previously undescribed eggs and pupae, and the emergence of adults. Adults do not show synchronised emergence, rather appearing throughout the first half of the summer season, which suggests a flexible life history strategy where emergence is not dependent on any discrete environmental cue. Ground temperature variability and spikes in field temperature may explain the long development time of eggs compared to previous laboratory studies, and their low percentage of successful maturation. Despite the limitations on survival at each of the life stages, the population is potentially able to double in size every life cycle/two years, highlighting the ability of this species to further expand its population and distribution on Signy. This study provides a springboard for further description and physiological studies of all life stages of this species, which will increase our understanding of the risks it poses as a non-native species on Signy Island, and the potential to colonise new areas, if given opportunity

Chapter Transition

The following two chapters takes the knowledge of *E. murphyi* life history and phenology and assesses how environmental conditions at relevant life stages are survived. The next chapter evaluates how oviposition site choice impacts egg development, particular the influence of ground surface temperatures, and how differing habitats may influence the overall reproductive success due to variability in experienced environmental conditions.

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Chapter 3: Not So Free Range? Oviposition microhabitat and egg clustering affects *Eretmoptera murphyi* (Diptera: Chironomidae) reproductive success.

The work presented in this chapter has been published in Polar Biology as: Bartlett JC, Convey P, Hayward SAL (2018) Not So Free Range? Oviposition microhabitat and egg clustering affects *Eretmoptera murphyi* (Diptera: Chironomidae) reproductive success. Polar Biology. DOI: 10.1007/s00300-018-2420-4

3.1 Abstract

Understanding the physiology of non-native species in Antarctica is key to elucidating their ability to colonise an area, and how they may respond to changes in climate. *Eretmoptera murphyi* is a chironomid midge introduced to Signy Island (Maritime Antarctic) from South Georgia (Sub-Antarctic) where it is endemic. Here we explore the tolerance of this species' egg masses to heat and desiccation stress encountered within two different oviposition microhabitats (ground surface vegetation and underlying soil layer). Our data show that, whilst oviposition takes place in both substrates, egg sacs laid individually in soil are at the greatest risk of failing to hatch, whilst those aggregated in the surface vegetation have the lowest risk. The two microhabitats are characterised by significantly different environmental conditions, with greater temperature fluctuations in the surface vegetation, but lower humidity (% RH) and available water content in the soil. Egg sacs were not desiccation resistant and lost water rapidly, with prolonged exposure to 75% RH affecting survival for eggs in singly oviposited egg sacs. In contrast, aggregated egg sacs (n = 10), experienced much lower desiccation rates and survival of eggs remained above 50% in all treatments. Eggs had high heat tolerance in the context of the current microhabitat conditions on Signy. We suggest that the atypical (for this family) use of egg sac aggregation in *E. murphyi* has developed as a response to environmental stress. Current temperature patterns and extremes on Signy Island are unlikely to affect egg survival, but changes in the frequency and duration of extreme events could be a greater challenge.

3.2 Introduction

Eretmoptera murphyi is a flightless midge endemic to the sub-Antarctic island of South Georgia (54°S, 36°W), from where it was introduced to Signy Island (Section 1.2.2.1) in the 1960s (Block et al. 1984; Convey and Block, 1996). It is one of Antarctica's few holometabolous insects, and the only macro terrestrial invertebrate and true insect found on Signy (Chown and Convey, 2016; Convey and Block, 1996). Insects, being small ectotherms, are especially vulnerable to environmental stressors such as temperature extremes, and a high surface area to volume ratio means that they are also at risk of desiccation (Gaston and Chown, 1999; Gibbs et al., 1997; Hayward et al. 2004). The ability to tolerate both of these stresses is particularly relevant to species survival and distribution patterns in polar terrestrial environments (Convey, 1996b; Convey et al., 2014a; Hayward et al., 2004).

3.2.1 Environment and terrestrial habitats of Signy Island

At a latitude of 60 °S, Signy is subject to the strong, circumpolar westerly airflow that surrounds the Antarctic continent. Combined with the winter expansion of Antarctic sea ice from the Weddell Sea that typically extends north of the South Orkney Islands, Signy experiences an annual climate that is more extreme than might be expected for its latitude, and comparable with that of Marguerite Bay more than eight degrees of latitude further south (Hughes et al., 2013; Walton, 1987). However, within the South Orkney Islands, Signy does benefit from the warming effects of the Foehn winds that are drawn down from the mountains of the adjacent Coronation Island (King et al., 2017). As a result, Signy has positive summer monthly mean air temperatures between 0-3 °C and experiences microhabitat temperatures that can be easily exceed 20 °C on the moss or soil surface, with spikes as high as 38.5 °C recorded (Convey et al., 2018; Davey et al., 1992; Walton, 1982).

The ice-free areas of Signy Island are largely comprised of scree and moraine, with some areas of well-developed and diverse moss and lichen communities, as well as large moss banks and populations of the two Antarctic flowering plants, Antarctic pearlwort (*Colobanthus quitensis)* and Antarctic hair-grass (*Deschampsia antarctica*) (Cannone et al., 2017; Smith, 1972, 1990). Vegetation is dominated by cryptogams, predominately turf and cushion forming mosses that are generally > 5cm

in depth (Cannone et al., 2017; Smith, 1972), whilst soils on Signy, and in Antarctica in general, are typically thin with little humus content (Campbell and Claridge, 1987). The depth and density of vegetation cover not only influences the formation of soils, but also microhabitat temperatures, acting as insulating blankets over the friable soil layer, keeping temperature more stable and preventing loss of water through evapotranspiration (Tenhunen et al., 1992). Typical annual precipitation on Signy is around 400 mm y^{-1} and occurs on average on 280 days per year, nowadays normally as rain in summer (Holdgate, 1967; Royles et al., 2013a; Walton, 1982). Substrate moisture content on Signy is generally higher in summer, due to the combination of summer precipitation events in summer and melt of snow and ice (Bokhorst et al., 2007c; Gardiner et al., 1998).

3.2.2 Terrestrial invertebrate microhabitat selection and stress physiology

Antarctic terrestrial ecosystems present a challenging habitat for invertebrates, with low water availability from freeze and evaporative drought events, and low temperature seen as the two principle stressors (Block et al., 2009; Cannon and Block, 1988; Convey, 1996b; Convey et al., 2014a). Thus, microhabitat selection is a trade-off between maximizing heat budgets for development and limiting the daily risk of desiccation or freezing (Hayward et al., 2003). Air temperatures on Signy can range by as much as 60 ºC annually, from around -40 °C to +20 ºC (Walton, 1982), whilst the substrates in which *E. murphyi* is found also experience considerable temperature variation, with 21.8 °C diurnal fluctuation in the summer, ground temperatures below freezing in winter (Walton, 1982), and an RH range of 37-100% (Worland and Block, 1986). Bokhorst et al. (2008) reported that Signy soils have a greater number of summer freeze-thaw events than similar substrates on Anchorage Island, at c. 68 °S off the Antarctic Peninsula, and Convey et al. (2018) report a longer delay in spring warming (a period when ground temperatures remain close to 0 °C during spring) on Signy than at other Antarctic sites, both in the maritime and continental Antarctic. Adverse temperature or moisture conditions can be alleviated to some extent through microhabitat selection, and there is clear evidence from other terrestrial invertebrates on Signy (Collembola and Acari) that different thermal- and hygro-preferences reduce stress exposure (Hayward et al., 2001, 2003). However, in polar environments there is typically limited refuge from environmental extremes, so the resident invertebrate fauna has

had to evolve a range of stress response mechanisms. Amongst invertebrates there are two basic physiological strategies to cope with desiccation stress: 1. Prevent water loss by being desiccation resistant, or 2. Tolerate the loss of water from the body by being desiccation tolerant (Danks, 1999; Everatt et al., 2015). Previous work on the desiccation and heat tolerance of *E. murphyi* 4th instar larvae found that they are desiccation tolerant, and able to tolerate up to 46.7% water loss over 12 d, with little effect on survival (Everatt et al., 2014c). Furthermore, larvae can withstand temperatures of 39 °C for up to 1 h (Everatt et al., 2014b), Whilst the larvae have received increasing research attention (Everatt et al., 2012, 2014a, b, c, 2015; Hughes et al., 2013), the eggs have been largely overlooked with no studies on any element of their physiology in over 25 years (Convey, 1992). In this context, eggs are thought to only be laid during a short period in the brief Antarctic summer and, while sub-zero temperatures can still be experienced during this period, it is thought that low relative humidity or high microhabitat temperature extremes pose a greater risk to egg survival.

3.2.3 Egg physiology and oviposition strategies

Eretmoptera murphyi reproduces parthenogenetically, laying single batches of 48-85 individual eggs within a large spherical hygroscopic gelatinous matrix, or egg sac, that has a water content of 96% when fully hydrated (Bartlett et al., 2018a/ Chapter 2; Cranston, 1985; Convey, 1992). Eggs take approximately 30 days to develop under summer field conditions on Signy Island (Bartlett et al., 2018a/ Chapter 2). This differs from *B. antarctica* the closely related endemic Antarctic midge, which produce 41 (median) eggs per batch, laid in a 'ribbon' arrangement, and which take 16 days to hatch at 4 °C (Harada et al., 2014). The investment in the single egg sac for *E. murphyi* is large, twice the dry mass of the post-oviposition female (Convey, 1992) and may be the consequence of not adopting the multiple-oviposition strategy that typifies many terrestrial Orthocladiinae (Armitage et al., 1995; Nolte, 1993). Producing a single egg sac appears a high-risk strategy, and so the hatching success rate of each sac might be expected to be high to compensate. However, under naturally fluctuating summer field conditions in the habitats to which it has been introduced on Signy Island, mean hatching success is just 35% (Bartlett et al., 2018a/ Chapter 2). Similarly, low success rates were also recorded for *B. antarctica* at 4 °C under laboratory conditions (Harada et al., 2014). It is possible,

though untested, that this may be a result of low fertilization rates in *B. antarctica*, although this cannot explain low hatching success in *E. murphyi*, which is parthenogenic. Furthermore, Frouz (1997) notes that in chironomids the selection of a suitable oviposition site "affects the reproductive success of the whole next generation", and thus could be strong selector on the survival of the eggs and, hence, the growth of the population.

Egg sacs of *E. murphyi* have previously been found in the surface vegetation layer in the vicinity of Signy Island Research Station (Convey and Block, 1996). Whilst often highly saturated, this habitat is also prone to extremes in temperature and desiccation (Walton, 1982). Considering the desiccation risk of such a habitat, Convey (1992) investigated the dehydration and rehydration tolerances of egg sacs, and found them able to tolerate short periods of extreme desiccation (26 h at 35% RH) by forming a 'skin' around the gelatinous matrix. Convey (1992) also noted that eggs tolerated temperatures above 10 °C and developed faster with increasing temperatures (over the range 2 – 12 °C). This is perhaps unsurprising given that chironomids typically have a development rate positively correlated to temperature, within optimal temperature boundaries following a hyperbolic law (Armitage et al., 1995; Frouz et al., 2002; Oliver, 1971; Stratman et al., 2014). However, the temperature tolerance limits of *E. murphyi* eggs remain untested.

One strategy that can be adopted to reduce the impacts of temperature and desiccation stress in such habitats is to reduce the overall surface area to volume ratio of the egg sac, which can be achieved by producing eggs in clusters as well as by aggregating multiple egg sacs together. This latter strategy has been recorded in at least four species of Orthocladiinae as well as in many species of Chironomidae overall, including intertidal and alpine species (Armitage et al., 1995). However, communal oviposition is still apparently uncommon across the Chironomidae (Nolte, 1993), particularly in terrestrial species that cannot rely on water currents to distribute hatched larvae and reduce intraspecific competition (Frouz, 1997, Juliano et al., 2002). For brachypterous species, such as *E. murphyi*, adult dispersal ranges are certainly limited. In addition, parthenogenesis removes the need to seek out a mate. The challenge then becomes selecting oviposition sites in a landscape with a

highly patchy distribution of favourable microhabitat conditions, while also limiting subsequent larval competition linked to high population densities.

3.2.4 Aims of this study

Recent field observations made on Signy have confirmed that *E. murphyi* lays egg sacs both singly and in small aggregations (Bartlett et al. 2018a/ Chapter 2), but the oviposition sites have not been studied in detail. Thus, a primary objective of the current study is to determine if there is any evidence of microhabitat preference for oviposition sites in this species. While the stress physiology of *E. murphyi* larval stages is well characterised, only one study to date has explored the stress physiology of eggs (Convey, 1992). Here we examine heat and desiccation tolerance limits of single egg sacs, as well as clusters of sacs, and place these in the context of microhabitat conditions encountered during summer on Signy Island. Finally, we correlate egg survival with environmental conditions experienced in different microhabitats and discuss the implications for the continued range expansion of this invading species under climate change.

3.3 Materials and methods

3.3.1 Sample collection and processing

All experiments were conducted in laboratories at the British Antarctic Survey's Signy Island Research Station South Orkney Islands, maritime Antarctic, (Section 1.2.2.1, Fig. 1.1 and 1.2) during January 2017, using recently laid egg sacs that had been collected from moss banks surrounding the research station. Egg sacs were obtained from the substrates with a Pasteur pipette and/or a paintbrush in order to minimize risk of damage to the sac and eggs contained therein. All eggs within the sacs were identified to be at the first (opal) developmental stage (Bartlett et al.,2018a/ Chapter 2; Harada et al., 2014) using a dissecting microscope (Leica EZ4). If any eggs showed signs of yellowing or embryonic development the whole egg sac was discarded and not used in this study.

3.3.2 Environmental data collection and substrate cores

Temperature and humidity loggers (Tinytag Plus II) were placed as a single logger 'station' at 5 cm below the surface ('soil'), on the surface ('ground') and suspended 10 cm above the ground surface ('air'). The loggers were programmed to collect data from the 16th December 2017 until the 21st February 2017 (66 d), recording every 30 min. However, data logger failures meant that air temperature was recorded for 41 d, and ground temperature for 30 d, in total. Data presented (Table 3.1 and Appendix III) are from 12th Jan to 21st February 2017 when all loggers were working. To verify how close substrate saturation was to measured humidity, soil cores (n = 5) were taken weekly for seven weeks from the 23rd January 2017, to a maximum depth of 10 cm (where the underlying rock surface allowed) from a 1 m^2 quadrat surrounding the temperature logger station. Cores were taken using a steel soil auger 2.5 x 10 cm, placed in individual sterile sample bags and immediately returned intact to the research station, where they were then separated into surface vegetation and peat/soil substratum layers. Total numbers of egg sacs were recorded for each layer and they were removed. Stones were also removed prior to the wet mass of each layer being recorded using a precision microbalance (Sartorius E - 6202). Substrates were then dried at 60 °C to constant mass and weighed again to obtain dry mass. Water content was then calculated gravimetrically, as the percentage difference between fresh (wet) mass and dry mass.

Table 3.1. *Mean, maximum and minimum air (at 10 cm), ground surface and soil (5 cm depth) temperature (°C), and mean, maximum and minimum relative humidity (% RH) at all locations. Also shown: Mean % water content (WC) relative to dry mass, and hours above experimentally relevant temperatures (30, 20 and 5 °C) for each 'substrate' layer.*

Substrate layer	*n*	Mean temp °C (±SEM)	Max. temp °C	Min. temp °C	Mean %RH (±SEM)	Max. %RH	Min. %RH	Mean % WC (±SEM)	Hours > 30°C (% of total data)	Hours > 20°C (% of total data)	Hours > 5°C (% of total data)
Air (10 cm)	1376	5.2±0.06	22.6	3.4	93±0.4	100	0	-	0	1 (0.1%)	250 (36%)
Ground surface	1471	3.5±0.15	31.2	-4.3	81±0.59	100	0	93.2% ±0.5	2 (0.2%)	20.5 (3%)	176.5 (24%)
Soil (5 cm deep)	3102	2.5±0.03	11.2	0.4	17±0.1	29	0.1	71.1% ±5.5	0	0	150 (9.7%)

3.3.3 Egg tolerance to heat exposure.

To ensure that heat stress was not combined with desiccation stress, egg sacs were kept fully immersed in a medium similar to wet peat and moss turf. Immersion was not considered to be a stress as *E. murphyi* eggs are highly hygroscopic (Bartlett et al., 2018a/ Chapter 2; Convey, 1992). 'Field water' was created by adding local substrate to deionised water at a 1:3 ratio. This mixture was then left for one week at 5 °C in the dark, to replicate the organic matter composition of the environment and provide the egg sacs with a relevant hydration medium that would provide a more even heat distribution than the use of soil substrate alone. The field water had a pH of 5.3 and a salinity of 221 µS, (comparable to measures taken from the moss bank the eggs were collected from – Bartlett et al., 2018a/Chapter 2). Egg sacs containing only healthy 'opal' eggs were placed in groups of 10 into three 3 ml Eppendorf tubes with 1ml of field water, giving a total sample size of 30 egg sacs per treatment. Tubes containing the egg sacs were placed into a water bath (Lauda C6) set to either 20 °C or 30 °C, and survival assessed after exposures of 2, 12, 24, 48, 96 or 192 h. Controls were held inside a 5 °C incubator. After exposure, egg sacs were assessed immediately for any signs of hatching, and then moved into separate Petri dishes per treatment (no pooling), with local substrate that was saturated with field water. Petri dishes containing the exposed egg sacs were then kept at control conditions of 5 °C in constant darkness. Survival was re-assessed at 1, 3, 7, 14, 28 and 35 days post-exposure, using a dissecting microscope. Survival was quantified as the number of eggs that hatched successfully. After 35 days the egg sacs were dissected and the total numbers of hatched and unhatched eggs, and the developmental stages reached by those that did not hatch, were recorded.

3.3.4 Desiccation tolerance and water loss

Egg sacs were exposed to 100%, 98.2% or 75% relative humidity (RH), at 5 °C, as either a group of 10 sacs (three replicates), or as a single sac (five replicates), to determine if clustering sacs together reduced rates of water loss. Prior to the experiment, the egg sacs (singly or as clusters) were weighed to the nearest 0.01 mg using a microbalance (Sartorius micro M3P). Egg sacs were then placed in Petri dishes that were suspended above a solution of deionised water and NaCl using a nylon mesh

fixed to the edges of the containers. The containers used were 2 L airtight plastic boxes, with one box per treatment. A relative humidity of 100% was obtained using pure deionised water (DH_20), 98.2% RH with 31.6g NaCl/L DH_20, and 75% RH with saturated NaCl (Bayley and Holmstrup, 1999). Temperature and relative humidity levels were monitored using data loggers (TinyTag Plus II) prior to each experiment's initiation. Once humidity had stabilised at the desired RH, the egg sacs were placed into the container. All experiments were conducted within a 5 °C dark incubator. Egg sacs were exposed for 2, 12, 24, 48, 96 or 192 h and then removed, reweighed to measure water loss, and placed into control conditions, with subsequent survival assessed as described above.

3.3.5 Statistical analyses

The relationships between time, survival and water loss in experimental studies, as well as microhabitat temperature and % RH data collected from environmental loggers across the summer season, were assessed using Spearman's rank-order correlation, followed by further interrogation using Kruskal-Wallis tests. Spearman's correlation was chosen over Pearson's as the data were not normally distributed (D'Agostino and Pearson normality test) and environmental data, in particular, were deemed to be data with a monotonic relationship. Linear and Gaussian regression models were used to reflect the linear or non-linear relationships, respectively, at 95% confidence. In order to determine any difference in oviposition site choice between soil and moss layer over time, a Wilcoxon matched pairs test was conducted after a normality test (as before). A Mann-Whitney test (unpaired) was used to compare the overall effects of egg sac clustering on water loss and % survival. The time at which the sample experienced 50% mortality is expressed as LT_{50}. Following Suemoto et al. (2004), the LT_{50}, water loss at 50% survival (WL_{50}) and water loss rate (WLR) values were used as "measures of desiccation tolerance, dehydration tolerance, and dehydration resistance, respectively". All calculations were conducted using the statistical software Prism 7.03 (GraphPad Software, La Jolla California USA). All survival data were corrected to the control data using Abbot's Correction prior to analysis (Abbott, 1925).

3.4 Results

3.4.1 Environmental data

The temperature and % RH profiles of each substrate layer were significantly different (Appendix III) (temperature profiles, Kruskal-Wallis, H = 286, $p < 0.0001$; humidity profiles, Kruskal Wallis, H = 4217, $p < 0.0001$). The highest temperature recorded was a ground surface temperature (GST) of 31.2 °C on the 12th January. Temperatures exceeding 30 °C accounted for 2 h of total recorded time (0.3%) (Table 3.1). GSTs exceeding 20 °C accounted for over 20 h of recorded temperatures (3%), with eight consecutive hours above 20 °C occurring on 12th January 2017. The lowest recorded GST was -4.3 °C, on January 9th 2017. Figure 3.1 shows the overall time (h) spent within temperature brackets from 30 °C to -5 °C, for all substrate layers.

Air and ground surface % RH declined significantly with increasing temperature (Spearman's rank correlation, $r_S = -0.54$, $n = 1376$, $p < 0.0001$ and $r_S = -0.46$, $n = 1471$, $p < 0.0001$ respectively; Fig. 3.2). Within the soil substrate, the relationship between % RH and temperature was non-linear (Fig. 3.2) increasing with temperature up to approximately 5 °C and then declining with any further increases in temperature ($r_S = 0.33$, $n = 3102$, $p < 0.0001$).

Measurement of substrate water content over seven weeks showed that the surface substrate, which is comprised of mostly moss turf, had a stable water content of between 90% and 97% saturation, with a mean of 93.2 ± 0.5% SEM ($n = 7$) (Table 3.1). The soil layer had a more variable water content of between 46.8% and 87.1% with a mean of 71.1 ± 5.5% SEM ($n = 7$).

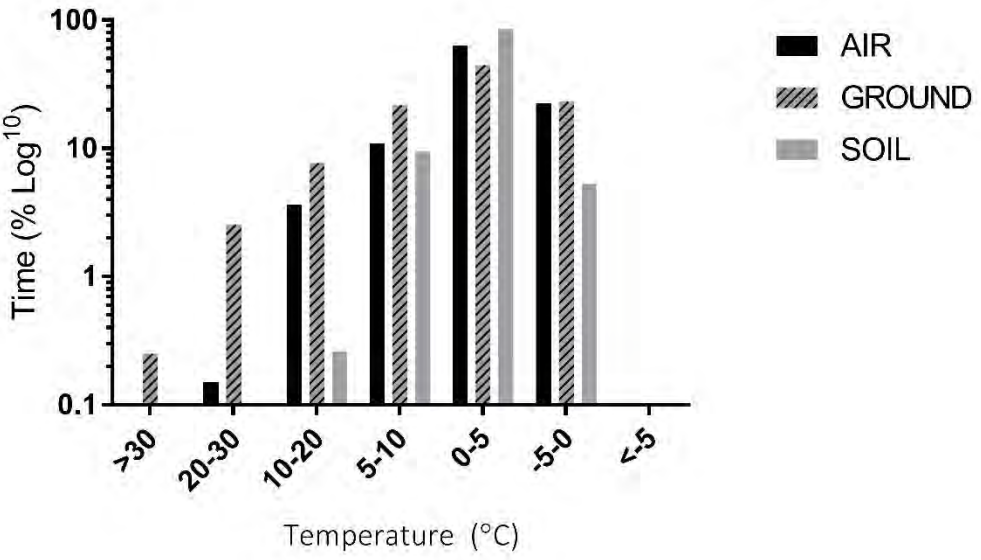


Figure 3.1. *Percentage of time (log_{10} scale), that each habitat (air, ground and soil) spent within different temperature ranges. Dates surveyed for each habitat are as follows: 'Air' = 40 d, from 12th January – 21st February 2017; 'Ground' = 30 d, from 26th December 2016 – 25th January 2017; 'Soil' = 91 d, from 18th December 2016 – 21st February 2017. No habitat experienced temperatures below -5°C.*

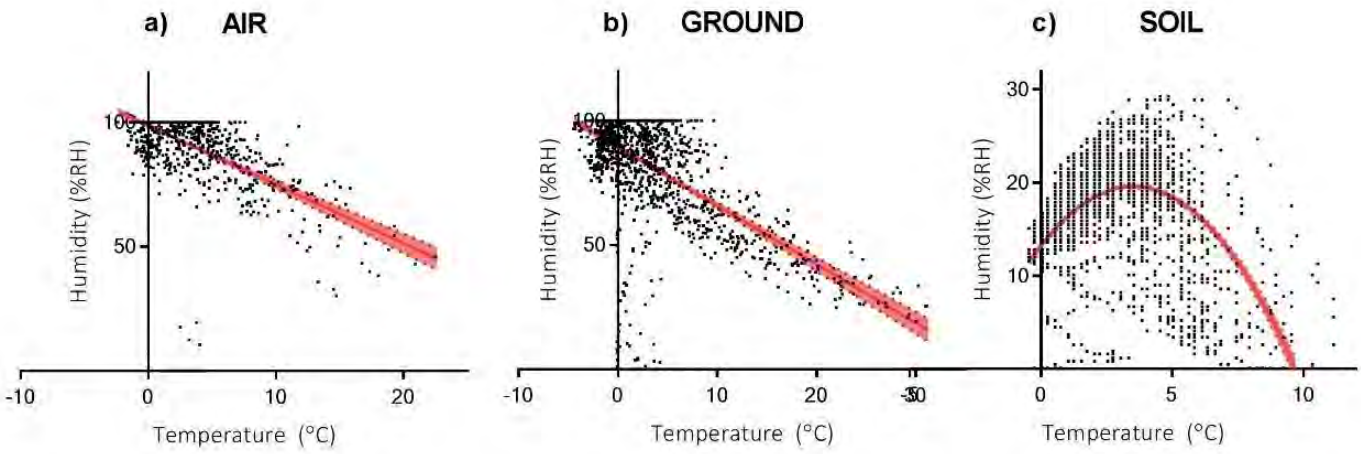


Figure 3.2 *Temperature vs. relative humidity for* ***(a)*** *air, ground* ***(b)*** *and soil* ***(c)*** *microhabitats (see Fig. 2 for dates). Linear regression plotted for air and ground; non-linear (quadratic) plot for soil (all shown in red with 95% CI).*

3.4.2 Oviposition sites

Egg sacs were laid in both the ground surface vegetation and the soil substratum throughout the seven-week sampling period, 23rd January 2017 – 6th March 2017 (Fig. 3.3). There was no strong preference for either substrate as an oviposition site (Wilcoxon matched-pairs test, $p = 0.95$), although there were initially more egg sacs in the soil at the beginning of the sampling period.

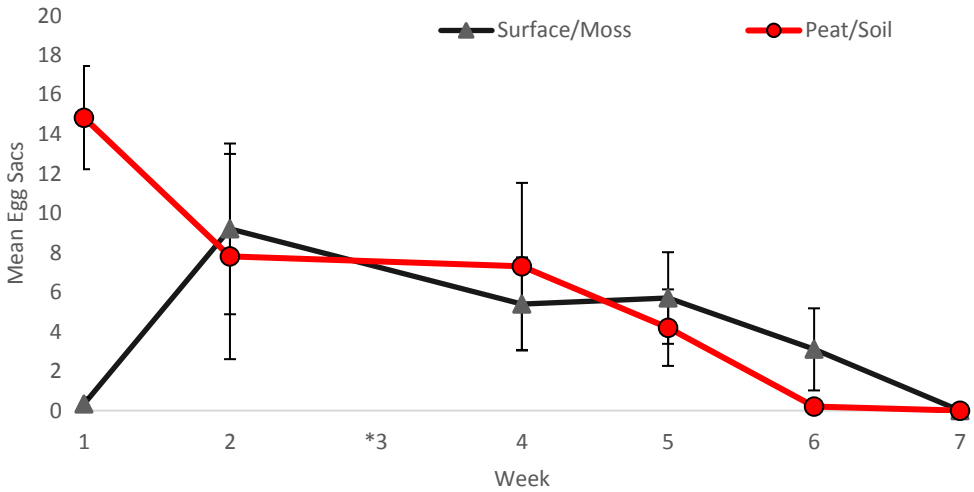


Figure 3.3. ***(a)*** *Single* E. murphyi *egg sac laid on exposed surface vegetation. Image taken in situ, Signy Is. January 2017.* ***(b)*** *Mean ± SEM egg sacs found in each substrate layer over 7 weeks (n = 5 replicate cores), with week 3 (6th Feb) not shown as the cores were compromised prior to processing. No difference between the mean egg sacs oviposited in the two substrates over the period (Wilcoxon, p = 0.95).*

3.4.3 Egg tolerance to heat exposure

A lower proportion of egg sacs produced hatchlings when exposed to the treatments of 20 °C and 30 °C compared to the control of 5 °C, with hatchling proportion decreasing over time (Fig. 3.4a). Overall, the difference between the three temperature exposures was significant (Kruskal-Wallis H = 9.9, $p = 0.034$) with the effect of 30 °C being the greatest (Linear regression, 30 °C = -0.5419 ± 0.1448, $F_{(1,5)} = 9.6$, $p = 0.013$; 20 °C = -0.06471 ± 0.03011, $F_{(1,5)} = 4.62$, $p = 0.08$; 5 °C = 0.0006213 ± 0.008082, $F_{(1,5)} = 0.005$, $p = 0.94$).

Individual egg survival at 20 °C and 30 °C was significantly lower than for eggs maintained at 5 °C (Fig. 3.4b, Kruskal-Wallis, H = 11.94, $p = 0.0004$). Survival decreased rapidly at 30 °C dropping to 60% within 12 h and reaching the LT_{50} between 12 h and 24 h. Survival was higher at 20 °C, and the LT_{50} was not reached over the 192 h exposure period.

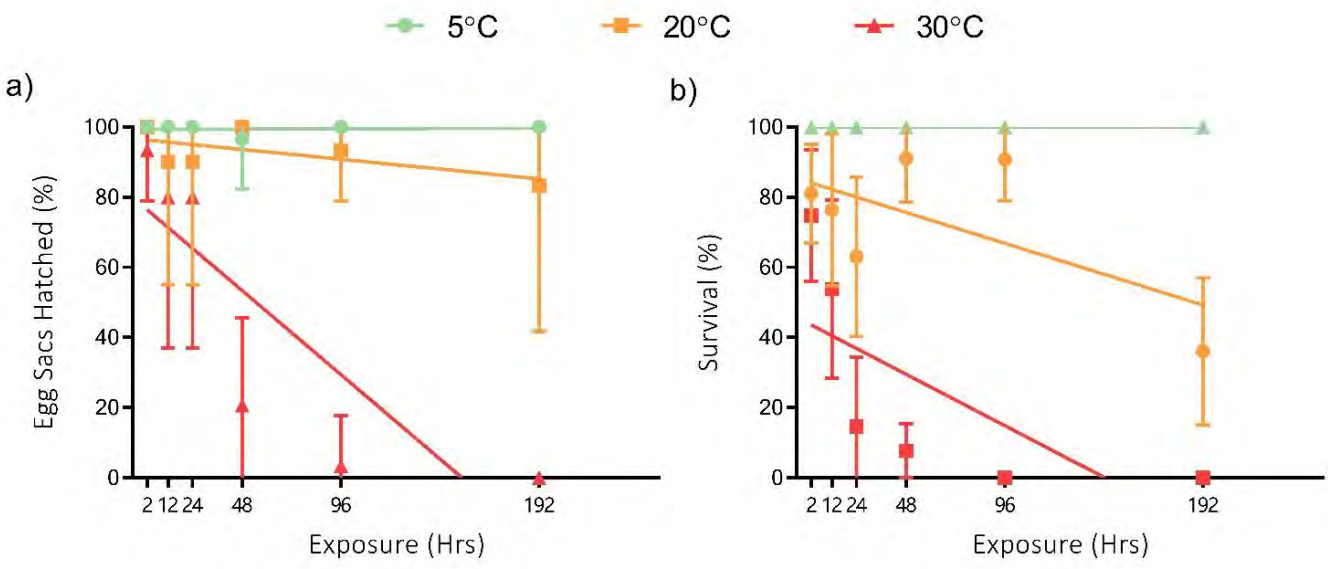


Figure 3.4 ***(a)*** *Mean ± 95% CI % egg sacs (n = 30) that produced hatchlings after exposure to temperatures of either 5°C, 20°C or 30°C, over periods of 2-192 hours.* ***(b)*** *Mean ± 95% CI % survival of individual eggs within each egg sac. Lethal time threshold for 50% of population (LT_{50}) highlighted with a dotted line. Three replicates of 10 eggs sacs were used for each time point at each treatment giving a mean value of: n= 175 ± 28 SD individual eggs for 5°C; n = 177 ± 20 SD for 20°C; n = 187 ± 16 SD for 30°C. Friedmans ANOVA, $F_{(9.3)}$ shows $p < 0.005$ significance between the control (5°C and the treatments of 20°C and 30°C).*

3.4.4 Egg sac desiccation rate and dehydration tolerance

All treatments resulted in a significant change in water content over time (Table 3.2) with an overall significant difference in water loss between single and clustered egg sacs (Mann-Whitney U = 1462, $p < 0.001$). Desiccation rates for single egg sacs were fastest at 75% RH, with 50% water loss within the first 2 h, and > 85% water loss after just 2 days (Fig. 3.5a). Water loss rates were slower (and roughly equivalent) at 98.2% and 100% RH, not reaching ~85% water loss until around day 4 (Fig. 3.5a). Desiccation rates for clustered egg sacs were again fastest at 75% RH, and without the initial rapid loss compared to single egg sacs, there was a much clearer distinction between rates of water loss at 98.2% *vs*. 100% RH (Fig. 3.5b). Clustered egg sacs did not reach 85% water loss until day 2 at 75% RH, after which water content stabilised (Fig. 3.5b). Water loss at 98.2% RH reached 60% around day 2, but never reached more than 70% across the entire 8-day experiment. Apart from one anomaly at 12 h (mean 27% ±7.5 SEM, $n = 10$), water loss in the 100% RH clustered samples never exceeded 30% (Fig. 3.5b).

Table 3.2. *Spearman's rank correlation matrix comparing Time (h), % water loss (WL), and % survival. Rs values < 0.40 (moderate – strong correlations) are highlighted in bold. Significance of correlation denoted with asterisk: * p < 0.05. **p < 0.01. ***p < 0.001. ****p < 0.0001.*

Correlation factors	Egg sac clustering	100%RH	98.2%RH	75%RH
TIME *vs* %WL	Single	**0.87****	**0.74****	**0.52****
	Grouped	**0.65****	**0.77****	**0.96****
% SURVIVAL *vs* TIME	Single	0	-0.10	**-0.42***
	Grouped	0	-0.04	-0.09
% SURVIVAL *vs* %WL	Single	0	-0.22	-0.28
	Grouped	0	-0.35	-0.16

The correlation of survival with time in single egg sacs was influenced by decreasing humidity, with the strongest relationship shown at 75% (Table 3.2). Single eggs sacs showed an overall decline in survival at all treatment humidities, but only reached LT_{50} between 96 h and 192 h at 75% RH (Fig. 6c). Survival for clustered egg sacs did not demonstrate any overall declining trend across all humidity treatments (Table 3.2), but both treatments expressed lower survival overall (Fig. 3.5d)

Despite an overall significant difference in water loss between clustered *vs*. single egg sacs (Mann-Whitney U = 1462, $p < 0.0001$) this was not reflected in overall % survival difference (Mann-Whitney U = 5001, $p = 0.07$). However, at the maximum treatment time of 192 h there was a difference in survival between 100% RH and 75% RH for both clustered and single egg sacs (Fig. 3.5e) (Kruskal Wallis, H = 10.2, $p = 0.005$ for single; H = 10.2 $p = 0.01$ for clustered), as a result of the increased water loss for clustered egg sacs at 75% RH (Fig. 6f) (Kruskal Wallis, H = 7.2, $p = 0.02$).

3.5 Discussion

Terrestrial microhabitat conditions recorded on Signy during this study clearly indicate that the invertebrate fauna can experience temperatures in excess of 20 °C, as well potentially desiccating relative humidity conditions, during a typical summer. Whilst extreme temperature events are still rare on Signy, it is likely that they will increase in frequency under current global warming scenarios. The highest standard meteorological air temperature in Antarctica, 19.8 °C observed on 30 January 1982 (King et al., 2017), was recorded on Signy. This event came about as the result of an unusual band of high pressure from the South Atlantic Sector combined with the more commonly occurring Foehn winds that flow over Signy from the adjacent Coronation Island and have a warming effect. Foehn winds not only bring warm air, but also decrease the humidity (Spiers et al., 2010). Our microhabitat correlations between temperature and humidity (Fig. 3.2a, b) suggest that even slight increases in mean temperature could dramatically reduce air and ground surface humidity conditions, and moisture availability has been demonstrated as a key driver influencing terrestrial communities on Signy (Block and Harrisson, 1995; Convey et al., 2003; Convey and Smith, 2004; Kennedy, 1995). Interestingly, soil moisture content/humidity also appear to be highest at around 3-5 °C (approximately the current summer mean soil temperature on Signy), but declines rapidly as temperatures increase towards 10 °C (Fig. 3.2c).

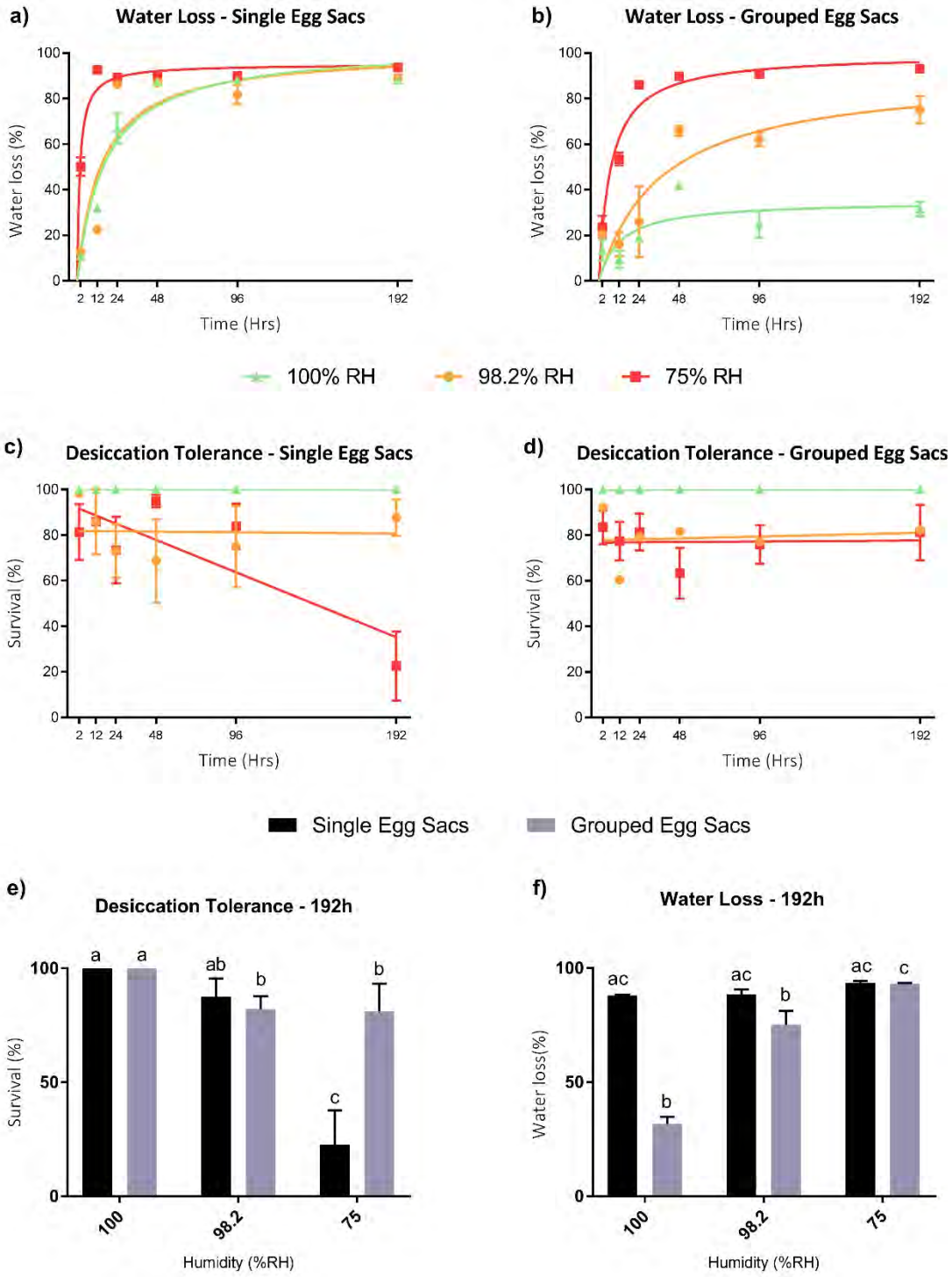


Figure 3.5. ***(a)*** *Mean water loss (WL - % relative to fresh mass) ± 95% CI of single egg sacs held under 100%, 98.2% and 75% relative humidity conditions for 2-192 hours (h)* ***(b)*** *The same data for n = 10 grouped egg sacs.* ***c & d)*** *Mean % survival ± 95% CI of eggs under same treatments with single and grouped egg sacs respectively.* ***(e & f)*** *Mean water loss (%) and survival (%) ± 95% CI, at the maximum exposure time of 192 h. Graphs show the different response to humidity treatments between single and grouped egg sacs. Treated and examined eggs sample size - Single egg sacs n = 5 (1 x 5 replicates), with a mean number of individual eggs at each exposure time point of: n = 255 ± 65 SD for 100% RH; n = 241 ± 46 SD for 98.2% RH; n = 237 ± 65 SD for75% RH. Grouped egg sacs, n = 30 (10 x 3 replicates) exposed at every variable X time-point, n = 12 of those randomly selected for dissection and examination. Of those 12: n = 864 ± 69 SD for 100% RH; n = 841 ± 74SD for 98.2% RH; n = 807 ± 61 SD for 75% RH.*

Mid-range climate forecasts for the Antarctic Peninsula and Scotia Arc region, including Signy and the South Orkney Islands, predict an average 1.5-2 °C warming by 2100 (Collins et al., 2013; Turner et al., 2014), and here we discuss the potential impact of these warming trends on egg survival of the invasive species *E. murphyi*.

Eggs exposed to 20 °C did not experience any significant declines in survival, even after 192 h exposure (Fig. 3.4). This implies that the current warmest conditions on Signy (GST at or above 20 °C not exceeding 8 h) should not be detrimental to egg survival. Convey (1992) found that egg development rates in *E. murphyi* were positively correlated with temperature in experiments testing temperature regimes up to 12 °C but did not investigate specifically whether increasing temperature systematically influenced hatching success. Based on our laboratory data, *E. murphyi* eggs will also be able to tolerate the acute extreme events of > 30 °C GST recorded during the 2016/17 season, which lasted less than 1 h.

Climate forecasts for the region (Collins et al., 2013) suggest mid-term temperature increases are unlikely to affect egg survival. As noted in other studies, the Antarctic terrestrial fauna is generally well adapted to the wide variation in temperature that often characterises their microhabitats and hence is not likely to be challenged – and may even benefit – from mean temperature increases of the scale predicted over the next century (Convey, 1996a, 2011). However, the forecast increase in the strength and frequency of Foehn winds throughout the whole Antarctic Peninsula sector (Cape et al., 2015; Turton, 2017), and an increasing incidence of high pressure events from the South Atlantic sector (Turney et al., 2016), may result in more frequent and severe extreme surface warming events affecting locations such as Signy Island, with negative consequences for the terrestrial fauna, including an increased risk of desiccation. Temperature variation between the air and ground surface is dependent on infrared absorbance, with bright, clear days producing peaks in GST as sunlight heats the ground surface, which can be considered 'extreme events' influencing the contained biota. Overcast days, which are typical of the northern maritime Antarctic, especially the South Orkney and South Shetland Islands, may have warmer air temperatures but do not result in peaks in GST which, as found here, could have a lethal effect on egg development. Guglielmin et al. (2008), in a full year

study covering a range of sites with multiple aspects on Signy, reported that sites with a northern aspect, such as the Backslope where *E. murphyi* is present, are more influenced by incoming radiation in the summer than the air temperature, thus more susceptible than other aspects to extreme surface warming. These features are evident in the data obtained here, with GST spiking above 30 °C on occasion and spending extended periods above 20 °C, even though 'air' temperature was at least 10 °C lower during these events. This temperature discrepancy was found even though the 'air' measurements presented here were taken within the GST boundary layer as defined in standard meteorological assessments (WMO, 2008), and a more standard measurement of air temperature (at a height of 1.25-2 m) would show cooler temperatures and a larger discrepancy still (Walton, 1982) but would be less applicable to the scale of *E. murphyi*. Convey et al. (2018) further highlight the importance of studies of GST and low-level air temperature when studying polar environments.

Relative humidity (RH) conditions recorded in terrestrial habitats on Signy spanned < 10% to 100% (Table 3.1) and, although conditions of 75-100% RH predominated at and above ground surface, the soil substratum peaked only at 29% RH (Appendix III, Table 3.1). However, average available water content (WC) was measured at 71% and 93% of dry mass for the soil and surface substratum respectively, suggesting that gravimetric assessment is a more accurate reflection of microhabitat saturation, at a scale relevant to invertebrates. We infer that air pockets surrounding the humidity sensor as a result of product design may artificially depress the relative humidity of the immediate environment or be too large to measure experienced humidity at a scale relevant to *E. murphyi*. Previous studies on Signy have deployed various techniques to measure substrate moisture with limited success. Using volumetric measurements, Bokhorst et al. (2007c) recorded soil moisture levels not exceeding 0.1%, whilst relative water content measurements of 100% were recorded by Royles et al. (2013a) in comparable moss layers. Worland and Block (1986) reported RH readings of 37-100% in similar substrates, which are more consistent with results discussed here. Methodology aside, this study did find a high level of patchiness in soil saturation compared to the ground surface vegetation layer, with variability through time as well as across replicate substrate cores. Despite the ground surface vegetation layer acting as a sponge, and typically preventing the evapotranspiration of water

from the soil layer below (Tenhunen et al., 1992), the gradient of the Backslope site combined with the permeable frost-shattered rock beneath (Matthews and Marling, 1967) may drive the loss of water in the soil substrate. We suggest that this variance in soil saturation could underlie the patchy distribution of *E. murphyi* larvae as reported by Hughes and Worland (2010) and Bartlett et al. (2018a/ Chapter 2) and even the varied reports in previous substrate moisture assessments (Bokhorst et al., 2007c; Royles et al., 2013a; Worland and Block, 1986).

Previous research on soil invertebrates has clearly demonstrated that conditions as high as 98.2% RH, close to the wilting point of plants, can result in desiccation of species with permeable integuments (Bayley and Holmstrup, 1999). This is certainly true of *E. murphyi* and *B. antarctica* larvae (Everatt et al., 2014c; Hayward et al. 2007). Importantly, however, the slower desiccation rates afforded by these RH conditions permit survival of > 75% water loss in *B. antarctica* and 46.5% loss in *E. murphyi* larvae (Benoit et al., 2007; Everatt et al., 2014c; Hayward et al., 2007). We find here that *E. murphyi* egg sacs also have poor desiccation resistance and lose water rapidly from the gelatinous matrix surrounding the eggs (Fig. 3.5a) but can be highly dehydration tolerant if rates of water loss are slowed, such as through sac aggregation (Fig. 3.5b)

Our data demonstrate that egg sacs oviposited in isolation experience much higher rates of desiccation, losing 50% of their water content in just 2 h at 75% RH, compared to around 20% water loss for clustered egg sacs over the same period (Fig. 3.5). This in turn affects survival, which fell below 50% within 96-192 h for single egg sacs, while no clustered egg samples reached 50% mortality even after 8 days at 75 % RH (Fig. 3.5 c, d). Water loss at 100% RH suggests that, in order to maintain maximum water content, egg sacs potentially need to be periodically submerged, or in contact with wet substrate. Whilst *E. murphyi* is not considered an aquatic species, its larvae do have the capacity to respire underwater and tolerate prolonged submergence (Everatt et al., 2014a). Thus, it could be argued that they are semi-aquatic/terrestrial given the saturated moss banks in which they often reside (Convey, 1992). This dependency on access to water for egg sacs is reduced however, if they are clustered together - presumably because a reduced surface area slows water loss rates from the sac matrix. Clustering of egg sacs from multiple females during oviposition could,

therefore, be a behavioural adaptation which has facilitated the transition of *E. murphyi* to a more terrestrial lifestyle

Whilst the compounding effects and potential role of cross-tolerance between heat and desiccation stress responses has not been explored in this study, previous work on polar chironomids has produced varied results. Everatt et al. (2014b) reported that prior exposure to desiccation at 98.2% RH had no effect on the heat tolerance of *E. murphyi* larvae at 30-40 ºC. Pre-exposure to 0, 75 and 98.2% RH, in contrast, improved the heat tolerance of *B. antarctica* larvae at 30 ºC (Benoit et al. 2009). There is also evidence that desiccation improves survivorship at low temperatures in not only *E. murphyi* (Everatt et al., 2014c) and *B. antarctica* (Benoit et al., 2009), but also other polar invertebrates such as the dipteran *Heleomyza borealis* and the springtails *Folsomia candida* (Holmstrup et al., 2002) and *Cryptopygus antarcticus* (Elnitsky et al., 2008; Everatt et al., 2013b). This positive relationship is the consequence of similar injuries resulting from low-temperature and low-water availability, and the same physiological processes being activated in response (Bayley et al., 2001). Such features being expressed in eggs would allow them to continue hatching after a sudden summer freeze event where both water availability and low temperatures are experienced. But the combined effects of drought and increased temperatures may be detrimental considering the findings of dual stress response in *E. murphyi* larvae (Everatt et al., 2014b,c).

Mean %RH for different *E. murphyi* oviposition sites, i.e. surface vegetation and within the soil (Table 3.2), suggest that the latter environment would expose single egg sacs to conditions that impact on survival. The relationship between soil humidity and temperature (Fig. 3.2c) also illustrates that the soil substrate is susceptible to desiccation under both cooling (< 3 °C) and warming (> 5 °C), whereas the surface vegetation is consistently wetter/has higher %RH at lower temperatures (Fig. 3b). Bokhorst et al. (2007a), also found a non-linear relationship between soil moisture and temperature, and that small increases in temperature led to rapid decreases in soil moisture. This makes the soil habitat a highly variable environment, with less favourable RH conditions than the surface vegetation that is dominated by water-retentive moss turf, and where GST conditions afford higher thermal budgets for development without risking survival. The disadvantage of clustered oviposition could be

increased competition for resources once eggs hatch. In this regard, it is interesting to note that chironomid midges do not generally produce egg batches or sacs in a clustered fashion, particularly terrestrial species (Armitage et al., 1995; Nolte, 1993). However, there is currently no suggestion of a food shortage for detritivorous *E. murphyi* larvae within their habitats on Signy Island, and aggregation likely generates more favourable conditions, as has also been proposed for micro-arthropods in the maritime Antarctic (Cannon and Block, 1988; Block and Convey, 1995; Hayward et al., 2004; Schulte et al., 2008)

3.6 Conclusions

Oviposition site selection, whether in soil or on the surface vegetation layer, has important implications for environmental conditions experienced by eggs - with pros and cons for each microhabitat: in the soil, whilst measurable %RH is typically low at the spatial scale of our logging equipment, actual water content can be high within the substrate itself, albeit with a patchy distribution depending on topography and underlying geology. Soil temperatures on Signy are typically cool and stable, but the thin soils mean small changes to this can greatly affect water content. Within the north-facing site where *E. murphyi* were sampled, the ground surface layer is much more sensitive to irradiation and experiences large spikes in temperature, but also has higher mean %RH than within the soil. Given these microhabitat differences, and the vulnerability of egg sacs laid singly to desiccation, we conclude that oviposition within the soil is a higher risk strategy unless in direct contact with a continuously saturated substrate. This may explain why soil oviposition was higher early in the season where it would coincide with spring thaw. It is unusual for terrestrial chironomids to lay egg sacs in aggregations, however *E. murphyi* does employ this strategy, and we have shown that egg sac clusters are able to survive in both soil and surface vegetation habitats, and that aggregated oviposition could be an advantageous behavioural strategy. Current temperature patterns and extremes on Signy Island are unlikely to affect this species' survival regardless of oviposition site, but predicted changes particularly in the frequency and duration of extreme events with

continued climate warming and changing precipitation patterns, are likely to challenge egg batch survival.

Chapter transition

Understanding the life history and phenology of *E. murphyi*, raises questions about the ability of juvenile life stages to survive overwinter. The following chapter explores the cold tolerance of the life stages of *E. murphyi* in relation to local microclimate data throughout an Antarctic winter on Signy.

Chapter 4: Surviving the Antarctic winter - Life stage cold tolerance and ice entrapment survival in the invasive chironomid midge *Eretmoptera murphyi*.

4.1 Abstract

An insect's ability to tolerate winter conditions is a critical determinant of its success. This is true for both native and invasive species, and especially so in harsh polar environments. The midge *Eretmoptera murphyi* (Diptera, Chironomidae) is invasive to maritime Antarctic Signy Island, where it thrives. It is hypothesised that the ability of fourth instar larvae to tolerate freezing would allow the species' to extend its range further south, if given the opportunity. However, no detailed assessment of stress tolerance in other life stages has yet been conducted. Here, we find that although larvae, pupae and adults all have supercooling points (SCP) of around -5 ºC, only the larvae are freeze tolerant, and that cold-hardiness increases with larval maturity. Eggs are freeze-avoiding and have an SCP of around -17ºC. At -3.34 ºC, the CTmin activity thresholds of adults are close to their SCP of -5ºC, and they are likely chill-susceptible . Larvae could not withstand the anoxic conditions of ice entrapment or submergence in water beyond 28 d. The data obtained here indicate that the cold-tolerance characteristics of this invasive midge would permit it to colonise areas further south, including much of the western coast of the Antarctic Peninsula.

4.2 Introduction

Survival in the polar regions requires the ability to be able to tolerate conditions that regularly fall below freezing, sometimes for months at a time. As poikilothermic ectotherms, terrestrial invertebrates do not have the ability to thermoregulate, and are thus particularly vulnerable to such temperatures. Antarctic winters are long and harsh, and even in the warmer maritime Antarctic where two species of insect are native, temperatures remain below freezing for several months of the year (Convey et al., 2018). They, like any terrestrial invertebrate that lives in the polar regions, are at risk of their body fluids freezing, causing potentially fatal injury (Mazur, 1977). To survive these

conditions, invertebrates have developed physiological and behavioural cold-tolerance strategies that allow them to either tolerate freezing or avoid it (Bale, 2002; Block 1980; Cannon and Block, 1988; Convey, 1996a; Storey and Storey, 1988; Zachariassen, 1985). These cold-tolerance adaptations will also determine the success of any ingressing or introduced species to the polar regions. Thus, understanding how an invasive species survives polar winter conditions is crucial in understanding its potential to establish and spread.

Convey et al. (2018) highlight the importance of understanding the heterogeneous features of polar soils at small and biologically relevant physical scales, and how this influences patterns in microhabitat temperatures experienced by soil biota. Terrestrial invertebrates in these microhabitats may also be vulnerable to flooding and ice entrapment, resulting from snow melt in the summer and subsequent freeze in the winter, especially in soils and vegetation underlain by permafrost. This creates a challenging environment where invertebrates are at risk of low temperature stress, as well as ice entrapment and associated anoxic conditions (Sømme and Block, 1982). Foraging, mating and overall movement are also affected by low temperatures: The point at which an invertebrate loses neuromuscular coordination is defined as the Critical Thermal Minimum (CT_{min}) and if temperatures continue to decline beyond this, then movement will cease altogether and the invertebrate will enter a 'Chill Coma' (Hazell and Bale, 2011). To ameliorate these risks, some chironomids will migrate to find suitable microhabitats (Frouz et al., 2003). Similar behaviour is seen in Antarctic Collembola and Acari, which seek thermally preferential microhabitats, and in the instance of the mite *Alaskozetes antarcticus*, will choose a lower temperature microhabitat in order to maintain a cold-hardened state (Hayward et al., 2003).

As mean monthly temperatures in Antarctica remain below 0 ºC for many months, even with snow cover buffering temperature extremes (Convey et al., 2018; Davey et al., 1990; Kim and Singh, 2014), soil invertebrates must deal with the threat of freezing temperatures. To do this they use one of two general strategies: freeze-tolerance or freeze-avoidance (Bale, 1993, 1996; Cannon and Block, 1988). Freeze-avoiding species, such as the native Antarctic springtail *Crytopygus antarcticus* and the mite *Alaskozetes antarcticus* (Hayward et al., 2003), cannot survive internal ice formation, but can survive

sudden cold shocks and low temperatures to their supercooling point (Bale, 1996). The supercooling point (SCP) of an organism is the temperature at which ice formation starts to occur in its body. Broadly, where the SCP is below the lower lethal temperature (LLT) the species is classed as freeze-avoiding. In the alternative strategy, freeze-tolerance, individuals can survive the formation of ice in body fluids (but not generally within cells) *via* the accumulation of ice nucleating agents to control the formation of ice crystals (Worland and Block, 1999). The LLT of freeze-tolerant species is typically below their SCP. Relatively few species of terrestrial Antarctic invertebrate are freezing tolerant, but confirmed examples include larvae of endemic maritime Antarctic chironomid midge *Belgica antarctica* (Baust and Edwards, 1979), and the fourth instar larvae of the endemic sub-Antarctic and invasive maritime Antarctic midge, *Eretmoptera murphyi* (Worland, 2010).

Eretmoptera murphyi was introduced to Signy Island in the maritime Antarctic (60 ºS, 45ºW) (Fig. 1.3), in the 1960s during a plant transplant experiment (Block et al, 1984). The midge is native to sub-Antarctic South Georgia (55ºS, 45ºW), where it is a palaeoendemic species (Allegrucci et al., 2006; 2012). It has a life cycle of two years, spending most of that as one of four larval instars (Bartlett et al., 2018a/ Chapter 2). It is known to have pre-adaptations enabling survival of temperatures experienced in the maritime Antarctic, and the fourth instar larvae are freezing-tolerant (Worland, 2010), desiccation tolerant (Everatt et al., 2014c), and can rapidly cold harden after short periods of acclimation (Everatt et al., 2012). As *E. murphyi* is resident in water-logged moss banks, the risk of summer flooding and winter ice-entrapment may result in oxygen depleted habitats that may persist for months (Hodkinson and Bird, 2004; Sømme and Block, 1982). *Eretmoptera murphyi'*s ability to respire underwater for up to 28 days will ameliorate the stress of prolonged submergence, and it has been found to endure short periods entrapped in ice where such anoxia tolerance is necessary (Everatt et al., 2014a). However, its ability to potentially survive a winter entrapped in ice, where temperatures will not rise above freezing for months (Convey, 1996a; Walton et al., 1982), remains unknown. Survival for a winter in ice would imply a remarkably high level of anoxic tolerance, whilst the ability to survive submergence beyond 28 days would question the terrestrial classification of *E. murphyi*.

As with studies of the related *B. antarctica* (e.g. Benoit et al., 2008; Elnitsky et al., 2009; Hayward et al., 2007), most physiological examinations of *E. murphyi* have been conducted on mature third and/or fourth instar larvae - with the exception of two studies that examined desiccation and heat tolerance in eggs (Bartlett et al., 2018b/ Chapter 3; Convey, 1992). Thus, knowledge of the cold tolerance abilities of different life stages remains limited. However, differences in cold tolerance between life stages of an invasive insect can be a particularly important factor in assessing their range potential in environmental niche models (White et al., 2018), particularly for non-diapausing insects that may overwinter in more than one life stage (Knight and Croft, 1986; Tauber and Tauber, 1976). As Chironomid midges overwinter exclusively as larvae, enhanced cold tolerance is therefore common in the larval stages (Danks, 1971; Lencioni, 2004; Scholander et al., 1953; Tokeshi, 1995), and typically is limited in adults (Bouchard et al., 2006). However, to date few studies have compared cold tolerance between the larval instars or different life stages of a single chironomid species. Studies of other dipterans have reported a range of tolerances across larval, pupal and adult stages, within the same species (Bouchard et al., 2006; Lee and Denlinger, 2008). Variation in the levels of cold tolerance between different larval instars have also been found in the moths *Epiphyas postvittana* (Burgi and Mills, 2010) and *Spilonota ocellana* (Swain et al., 2017), as well as in mites (White et al., 2018).

As the predicted warming of the polar regions gains pace (IPCC, 2014), more species are expected to be able to colonise higher latitude environments (Bebber et al., 2013; Walther et al., 2002), and predicting which species are able to cope with the challenging environment is essential to understanding their invasion ecology (e.g. Lehmann et al., 2015; Pertierra et al., 2017). A successful species in the polar regions must be able to develop, feed and reproduce in the short available growth seasons (Chown and Gaston, 1999; Convey, 1996b), as well as be able to overwinter successfully (Bale and Hayward, 2010; Cannon and Block, 1988; Sømme, 1982). In the last few decades there has been a move to highlight the importance of ecologically relevant assessments of a species thermal tolerance (e.g., Bale, 2002; Worland and Convey, 2001), both to accurately reflect drivers of thermal physiology for species' of interest, but also as a key factor in establishing the risk of an invasive

species. It is insufficient alone to establish the boundaries of lethal temperatures and broad cold-tolerance strategies. Rather the duration, intensity and pattern of cold stress exposure must also be considered (Rezendez et al., 2014), and experimental assessments should be relevant to the conditions experienced in natural field conditions by the species in question (Terblanche et al., 2011).

4.2.1 Aims of this study

This chapter aims to examine the cold tolerance abilities and strategies of all life stages of the invasive Antarctic midge, *E. murphyi*. Building on the work of Everatt et al. (2014b) we also assess the ability of larvae to survive prolonged periods of ice entrapment and subsequent anoxia tolerance, and concurrently investigate the ability of the midge to survive more than 28 days underwater. Results are placed in the context of winter microclimate data relevant to *E. murphyi* habitats on Signy Island, and this species invasive potential further south is discussed.

4.3 Materials and methods

4.3.1 Study site and sample collection

All experiments, apart from those on ice-entrapment and water submergence, were conducted in laboratories at the British Antarctic Survey's Signy Island Research Station, South Orkney Islands, maritime Antarctic, (Section 1.2.2.1, Fig. 1.3) during the 2016/2017 austral summer season and samples collected as previously described (Section 2.3.1 and 3.3.1). All eggs used were confirmed to be at the first (opal) developmental stage (Bartlett et al., 2018a/ Chapter 2) using a dissecting microscope (Leica EZ4). If any eggs showed signs of yellowing or embryonic development the entire egg sac was discarded. Individual eggs were removed from the egg sacs through microscopic dissection, with care taken to not damage them. Other live *E. murphyi* samples used in the ice-entrapment and water submergence experiments were collected during the 2014/15 austral summer by BAS station staff on Signy Island, and were returned to the United Kingdom by ship in refrigerated

(+4-5 °C) cold storage (10 weeks) and then maintained on their native substrate at +5 °C at the University of Birmingham until use in late 2015 and early 2016.

4.3.2 Overwintering environmental data

In order to determine winter microhabitat conditions on Signy Island, three temperature loggers (Tinytag Plus II TGP-4500) were placed below ground within the top 5 cm of the soil profile which is where the larvae are known to reside (Bartlett et al., 2018a/ Chapter 2). This site was ~10 m a.s.l, behind the research station in a moss bank. Dataloggers were programmed to collect data from 12th March 2017 until 1st February 2018, recording every 2 h with a manufacturer's statement of accuracy ± 0.01 °C (Gemini data sheet, 2018).

4.3.4 Cold-tolerance ability of *E. murphyi*

4.3.4.1 Measurement of supercooling points

Super-cooling points (SCP) were assessed for each life stage as they became available: adults (n = 20), pupae (n = 6 – low availability), all four larval instars (L1, n = 22; L2, n = 22; L3, n = 23; L4, n = 22), eggs (n = 15), and the entire lipid egg sac (n = 32). Some adults (n = 5) were obtained from field-collected pupae that eclosed in the laboratory, the remainder (n = 15) were obtained direct from the field. These were recorded separately, and their SCP's noted, to determine whether 'laboratory raised' adults produced different results from 'field collected' adults. The SCPs of individuals of each life stage were determined by cooling slowly from +5 °C to -25 °C at 0.2 °C min^{-1} in an alcohol bath (Haake Phoenix II C50P, Thermo Electron Corporation) (Everatt et al., 2013b). Each individual was placed in contact with a thermocouple using Oecotak, in the following groupings: n = 1 per thermocouple for egg sacs, adults, pupae, L4 and L3 larvae, n = 2 for L2, n = 5 for L1, n = 10 for individual eggs. Thermocouples were placed within an Eppendorf tube, and inserted into the bottom of a test tube that was two-thirds submerged in the alcohol bath cooling fluid. The temperature of the individuals was recorded using Picolog Recorder Software and a TC-08 Multichannel data logger (Pico Technology Limited, UK) and the SCP was defined as the onset of the freezing exotherm.

4.3.4.2 Cold tolerance strategy of juvenile life stages

The cold tolerance strategy (either freeze-tolerant or freeze-avoiding) of all juvenile life stages was assessed by exposing them to temperatures of either -5, -10, -15, -20, -25, or -30 °C, with a +5 °C control. Temperature was reduced from the control temperature of +5 °C, to the target temperature at a rate of 0.2 °C min^{-1}, and once the target temperature was reached samples were moved immediately. For each temperature exposure, four replicates of five individual larvae (n = 20), and ten replicates of a single egg sac, were place in a sealed Eppendorf tube with a thermocouple wire threaded through a small hole in the lid. The 10 egg sacs contained n = 630 eggs between them (Based on Bartlett et al., 2019a). After each treatment, individuals were removed and placed in a Petri dish containing moist Signy soil substrate and kept at control conditions in a dark refrigerator. Larval survival was determined 72 h after exposure by assessing movement or reaction to stimulation from a fine paintbrush. Egg survival was assessed after 35 d, by dissecting egg sacs and determining the proportion of hatched *vs* unhatched eggs.

4.3.4.3 Lower thermal activity thresholds of adults

Adult *E. murphyi* were chosen for lower thermal activity threshold assessment as they are the most mobile life stage. All adults were field collected and kept at +5 °C for 24 h prior to experimentation. These experiments were conducted in an aluminium block arena (see Hazell et al. 2008), the temperature of which was regulated using an alcohol bath (Haake Phoenix II C50P, Thermo Electron Corporation). A thermocouple wire attached to a digital thermometer was inserted into the arena in order to monitor and record the temperature experienced. Adult activity was monitored using a digital video camera with a macro lens (Lumenera Infinity 2). Six individuals were placed within the +5 °C arena for 1 h before recording to allow acclimation. The video and temperature data were captured using Studio Capture DT software (Studio86Designs, Lutterworth, UK) as the temperature was reduced from +5 °C to -20 °C at a rate of 0.2 °C min^{-1}. The temperatures at which each individual last performed a coordinated movement (CT_{min}) and the final involuntary movement of either legs or antenna (chill coma) were recorded.

4.3.5 Overwintering in ice, and prolonged submergence

4.3.5.1 Field vs. laboratory water

In order to first ascertain the experimental impact of using different water types in freezing experiments (cf. Everatt et al., 2014a), we measured the freezing point of 18 MΩcm deionised water (DIW) and Signy field water (FW). Signy field water was prepared as previously described in Sections 2.3.1 and 3.3.3. Seven 1.5 mL Eppendorf tubes containing each water type were placed in an alcohol bath (as above) and taken from +5 °C down to -10 °C at a rate of 0.2 °C min^{-1}, and the freezing point recorded.

Three groups of n = 5 L4 larvae were placed in either DIW or FW and exposed to -3 °C for either 1, 3, or 7 d. Upon ending of each experiment, it was noted that DIW treatments did not freeze. Survival was assessed 72 h post exposure, as previously described.

4.3.5.2 Long-term ice entrapment

The ability to survive extended periods of ice-entrapment, and the assumed anoxia that results, was assessed by experimentally entrapping L4 larvae in FW ice for 28, 42, or 63 days at -3 °C. Larvae were collected during the 2014/15 Signy field season and kept at +5 °C on moist Signy soil substrate until use. Prior to the experimental treatments, larvae were either first winter acclimated ('A') by keeping samples at 0 °C in soil conditions for one week, or they were non-acclimated ('NA') and taken directly from storage conditions (+5 °C in soil). Two controls were used: Control 1 ('C1') tested the effect of submergence in the treatment water at +4 °C, and Control 2 ('C2') was a standard +5 °C soil control with no submergence stress. In all instances three groups of n = 10 larvae were used for each time point. Survival after treatment (including controls) was assessed after 28 days as above. All experiments were conducted in the dark in a bench freezer (Fryka B3-20) set to either +5 °C, or -3 °C. The freezer unit was calibrated using digital thermometers and the temperature throughout the experiment recorded using a data logger (Tinytag Transit). Due to the length of the post-exposure analysis, soil used in the control and recovery conditions was initially prepared by baking at 60 ºC for 24 h, in order to inhibit any fungi that may have been present.

4.3.6 Statistical analysis

All data were tested for normality prior to further analysis using a Shapiro-Wilk test (alpha = 0.05). The quality control assessments of the effect of field *vs.* laboratory water were analysed with t-tests. All further data were non-parametric and were analysed with Mann-Whitney U or Kruskal-Wallis tests, with or without Dunn's multiple comparisons. Ice entrapment data were first analysed and plotted with a least-squares linear regression (Tellinghuisen, 2008), then assessed for multiple comparisons with a Kruskal-Wallis test with Dunn's multiple comparisons.

4.4 Results

4.4.1 Supercooling and activity thresholds

There was no difference in the SCPs measured in the four larval instars (Kruskal-Wallis, H = 4.3, p = 0.22, n = 89), with all mean values between -5.25 ºC (L1 and L4) and -5.59 ºC (L2). Pupae had a mean SCP of -5.81 ± 0.36 ºC SEM (n = 6). Overall, there were no significant differences in the SCPs of the different larval instars and pupae (Kruskal-Wallis, H = 6.6, p = 0.24, n = 115). Entire egg sacs had an SCP of -2.45 ± 0.12 ºC SEM (n = 32), whilst individual eggs had an SCP of -17.58 ±1.37 ºC SEM (n = 15) (Fig. 4.1).

Adults had an SCP of -5.07 ± 0.6 ºC SEM (n = 20), with no difference between the SCPs of 'lab' *vs*. 'field' adults (Mann Whitney U = 23, p = 0.23, n = 5 'lab', n = 15 'field'). Adult *E. murphyi* CT_{min} was -3.34 ± 0.7 ºC (n = 6), whilst the chill coma was -5.44 ± 1.17 ºC SEM (n = 5) (Fig. 4.2)

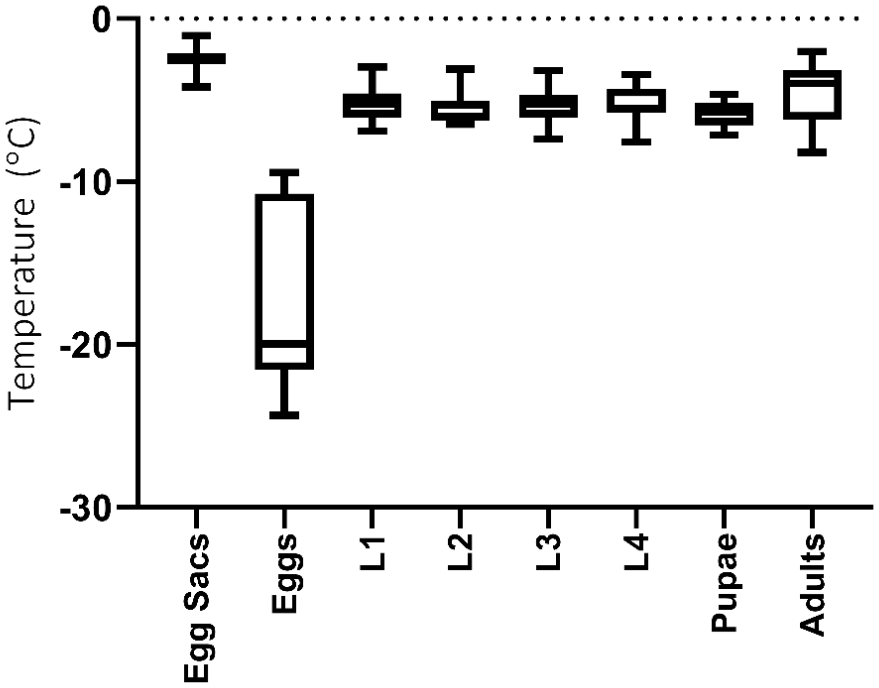


Figure 4.1. *Supercooling points of all life stages, Shown as mean SCP ± SEM. Egg sacs, n = 32; individual eggs, n = 15; pupae, n = 6; adults, n = 20; the larval instars: L1 n = 22; L2, n = 22; L3, n = 23; L4, n = 22.*

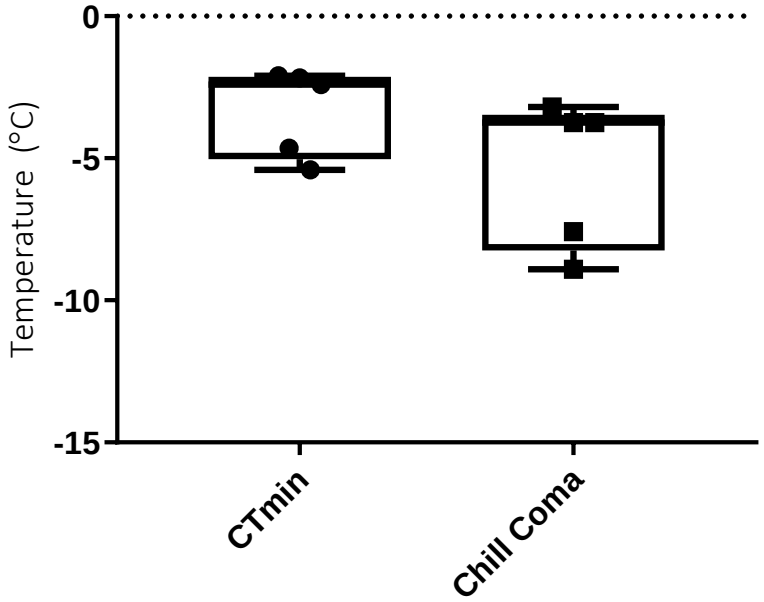


Figure 4.2. *Mean adult activity thresholds ± SEM, showing CT_{min} and chill coma (n = 5).*

4.4.2 Cold tolerance strategy of life stages

Survival of all life stages at sub-zero temperatures was significantly lower than that of of the +5 ºC control (Kruskal-Wallis, with Dunn's multiple comparisons, H = 28, $p < 0.0001$, $n = 35$) (Table 4.1). This was driven by a difference in the LLT between the instars and eggs below -10 ºC: the LLTs of the earlier instars (L1 and L2) were between -10 ºC and -15 ºC, decreasing to -20 ºC for L3 and L4, and -25 ºC for eggs (Fig. 4.3)

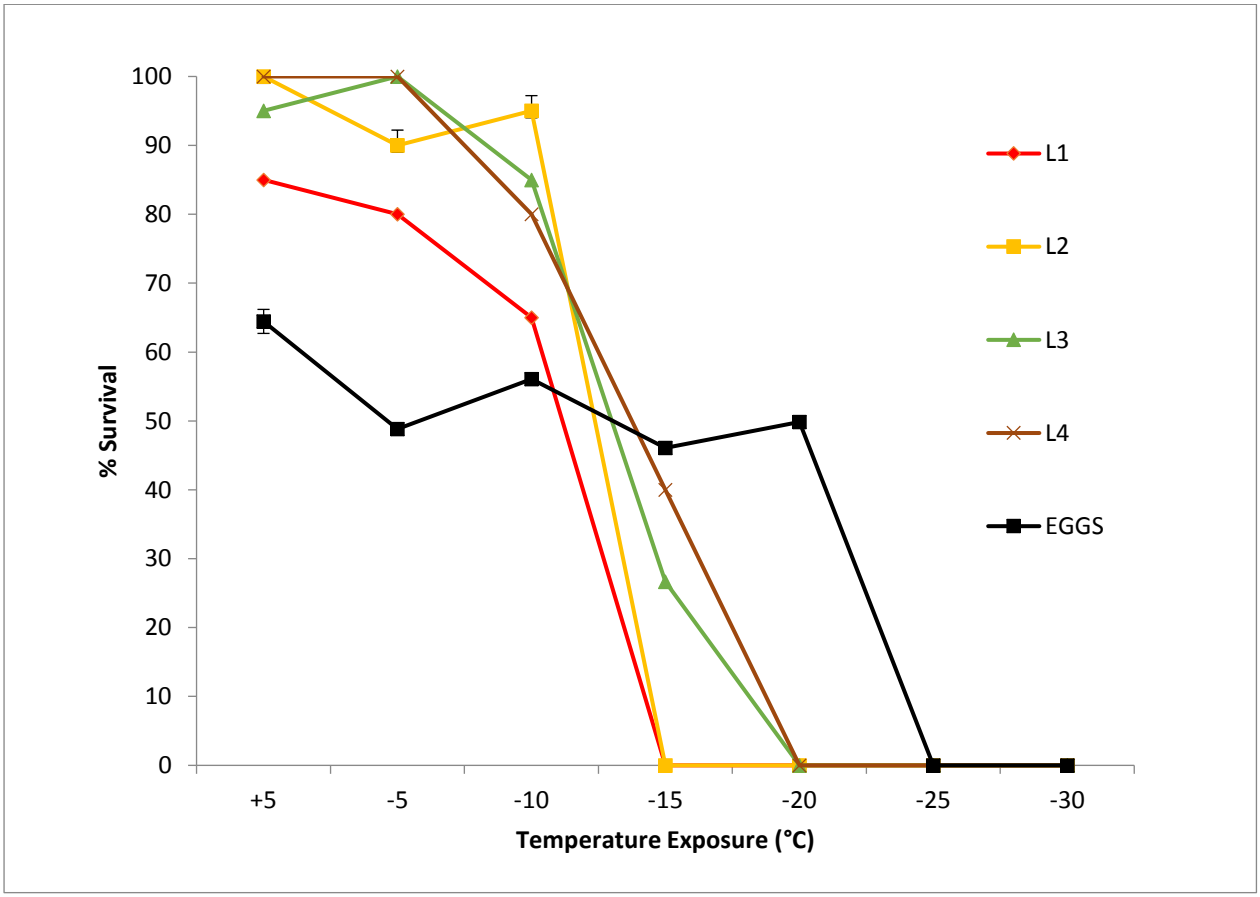


Figure 4.3. *Mean survival ± 95% CI survival of* E. murphyi *larval instars (L1-L4; n = 20 for each instar) and eggs (n = 630 individual eggs from n = 10 egg sacs), after exposure to declining temperatures (discrete treatments).*

Table 4.1. *Results of Kruskal-Wallis test with Dunn's multiple comparisons for the influence of temperature on life stage survival, against the control (+5 ºC). Overall interaction of temperature with control: H = 28.85, p < 0.0001.*

Dunn's multiple comparisons test (°C)	Mean rank diff.	Significant?	Adjusted P value
5 *vs.* -5	1.300	No	>0.999
5 *vs.* -10	3.700	No	>0.999
5 *vs.* -15	15.40	No	0.074
5 *vs.* -20	18.60	*	0.015
5 *vs.* -25	21.10	**	0.003
5 *vs.* -30	21.10	**	0.003

4.4.3 Overwintering in ice, and prolonged submergence

4.4.3.1 Field vs. *laboratory water freezing point*

FW had an SCP of -3.06 ± 0.1 °C, significantly higher than that of DIW at -4.31 ± 0.5 °C (Unpaired t-test (12), $t = 3.9$, $p = 0.002$). These differences were linked with a decline in larvae survival when the different water types were used during ice-entrapment experiments at -3 °C (cf. Everatt at al., 2014b), as DIW did not freeze resulting in lower survival in FW after 7 days as ice entrapment had not been experienced in the DIW condition (Unpaired t-test (4), $t = 5.5$, $p = 0.005$).

4.4.3.2 Prolonged ice entrapment and submergence

Only after 63 days exposure did all the treatments differ significantly from the +4 ºC soil control (Kruskal-Wallis test, overall interaction of conditions with control: 28 d, H = 5.5, $p = 0.13$; 42 d, H = 13.2, $p = 0.004$; 63 d, H = 18.3, $p < 0.001$) (see Table 4.2 for multiple comparisons). At 42 days there was only a difference between the submergence treatment and the soil control, whilst at 28 days there was no difference between any of the treatments or the soil control (Table 4.2). Figure 4.4 shows the least-squares linear regression of the treatments through time.

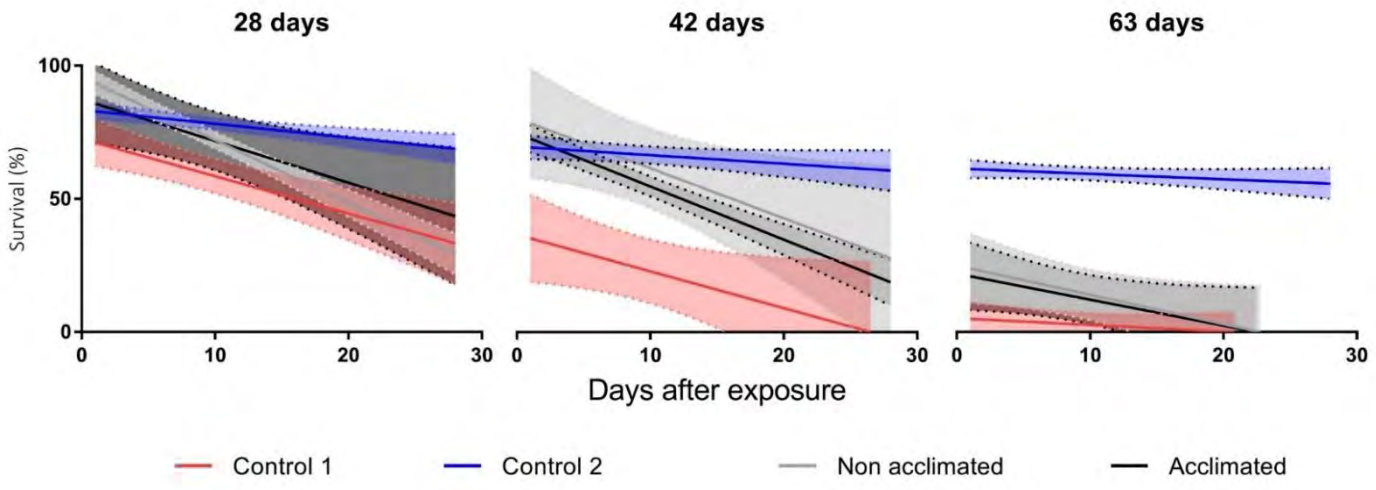


Figure 4.1. *Long-term survival of fourth instar* E. murphyi *larvae following exposure to 28, 42, and 63 days entrapped in ice at -3ºC. Control 1 = +4ºC submerged in field water. Control 2 = +4ºC soil control. Winter acclimated samples were kept at 0 ºC in soil conditions for one week prior to exposure treatment. Non-acclimated samples were taken straight from control conditions (+4 ºC soil). N = 10 x 3 replicates.*

Table 4.2. *Results of Kruskal-Wallis test with Dunn's multiple comparisons, for the influence of ice entrapment (NA/A), or submergence (C1) on L4 survival, against the soil control (C2). Overall interaction of conditions with control: 28 d, H = 5.5, p = 0.13; 42 d, H = 13.2, p = 0.004; 63 d, H = 18.3, p < 0.001.*

Exposure (days)	Dunn's multiple comparisons test	Mean rank diff.	Significant?	Adjusted P value
28	C2 *vs*. C1	9.143	No	0.109
	C 2 *vs*. NA	0.8571	No	>0.999
	C2 *vs*. A	1.714	No	>0.999
42	C2 *vs*. C1	14.5	**	0.002
	C2 *vs*. NA	1.643	No	>0.999
	C2 *vs*. A	4.143	No	>0.999
63	C2 *vs*. C1	18.21	****	<0.0001
	C2 *vs*. NA	11.43	*	0.026
	C2 *vs*. A	12.36	*	0.013

4.4.4 Overwintering environmental data

Only data collected prior to 22nd January 2018 were used due to faults encountered with data loggers. Total recording time was therefore from 12th March 2017 to 21st January 2018 (inclusive).

Belowground monthly temperatures in the period March 2017 to January 2018 averaged between +2.39 ± 0.09 ºC SEM in January and -6.32 ± 0.16 ºC SEM in July. The coldest temperature recorded by any logger was -20.27 ºC, on 23rd July 2017, in the middle of the coldest period recorded in the

dataset – four consecutive days below -10 ºC between 20th and 24th July 2017. The warmest temperature recorded was 16.9 ºC on the 9th December 2017 (Fig. 4.5). Seven months had mean temperatures below 0 ºC, four of which were below the SCP for field water (-3.05 ºC), with two months below the SCP for all *E. murphyi* larval instars (-5.35 ºC). The LLT for eggs (-20 ºC) was not reached, however the LLT of L3/L4 was met for a total of 49 h over a 7-day period. The longest period at temperatures below L3/L4 LLT was 37 consecutive hours between 20th and 23rd July 2017. L1/L2 LLTs were exceeded for 154 h over 28 d. The longest period spent at or below L1/L2 LLTs was 25 h between 26th and 28th May 2017 (Table 4.1, Fig. 4.5).

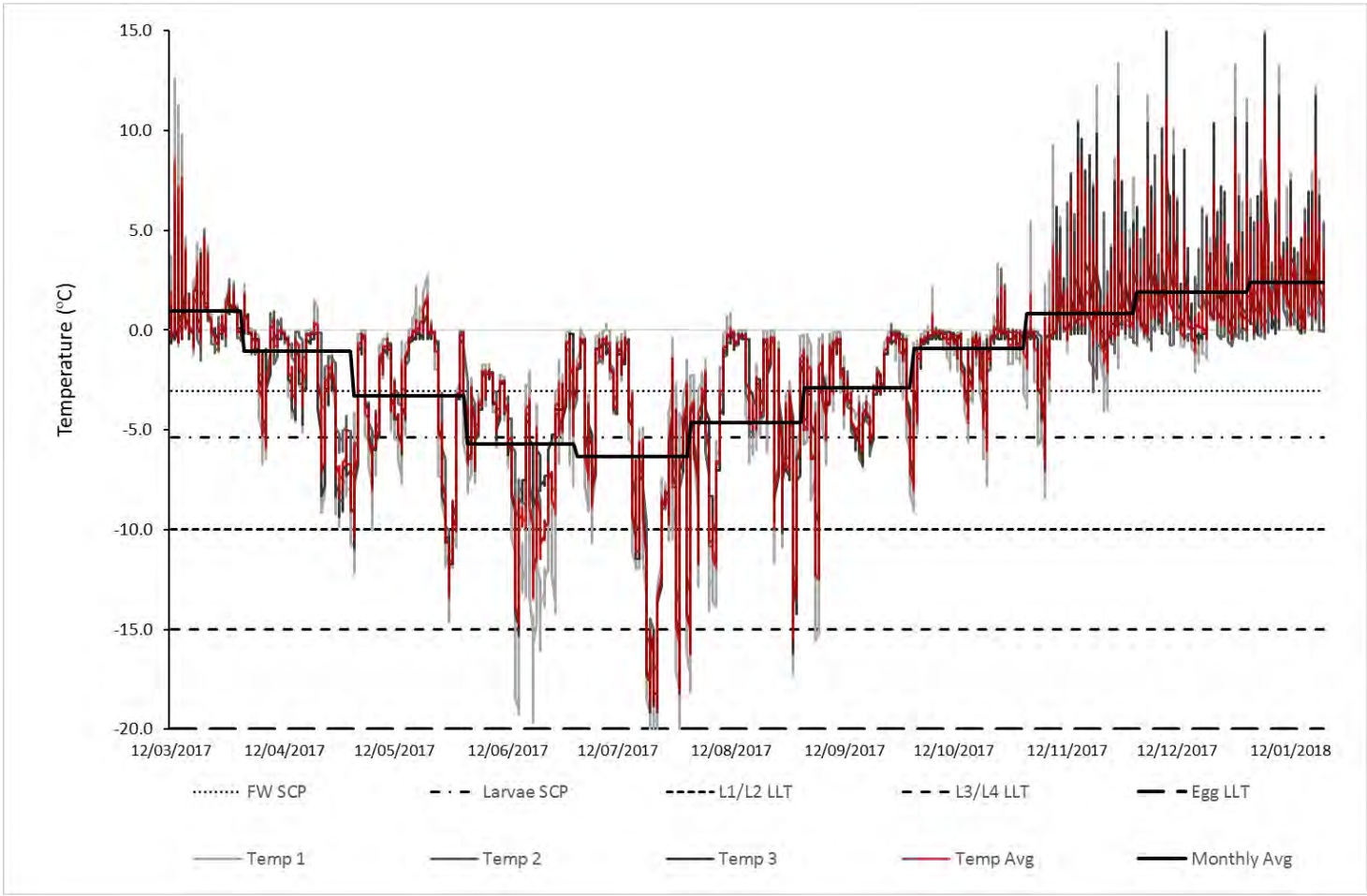


Figure 4.2. *Soil temperature on Signy Island from 12th March 2017 – 21st st Jan 2018 (inclusive). Individual data loggers (Temp 1-3); the average between loggers (Temp Avg); and monthly average are shown. Mean SCP for field-water and* E. murphyi *larval instars are highlighted as well as mean LLTs for different life stages.*

4.5 Discussion

The characteristically low temperatures of the polar regions mean that the land is typically covered in snow and ice, with air temperatures under 0 ºC for much of the year (Meltofte et al., 2013). Yet within the top layers of sub-surface soil, temperatures experienced by resident flora and fauna may differ from the air temperature, as factors such as vegetation and snow cover will act as insulators (Convey et al., 2018; Davey et al., 1992). Previously, microhabitat assessments on Signy Island have recorded a minimum belowground temperature of -14.8 ºC in July 1987, 3 cm below the surface and located adjacent to the Research Station ~10 m a.s.l in a similar location to this study – the same study also reported a minimum surface temperature of -17.1 ºC at the same site (Davey et al., 1992). The minimum belowground temperature reached in this study was -20.27 ºC in July 2017, and in the middle of the coldest period recorded in the dataset – four consecutive days below -10 ºC. It is worth noting that Convey et al. (2018) also recorded microhabitat temperatures on Signy in recent years, but at 150 m a.s.l at Jane Col - an exposed hill site. They recorded a minimum winter temperature in 2009 of -8.7 ºC, significantly warmer than both this study and that of Davey et al. (1992), despite a minimum air temperature of -30.1 ºC recorded the same year. Much of this variation may be the result of snow cover, as the effect of snow as an insulator is well documented at micro and macro-scales (Convey et al., 2014b, 2018; Cooper, 2015). At the time of Davey et al's (1992) recorded minimum, snow cover was 6 cm, whilst Jane Col is usually covered by deep (> 0.5 m) snow drifts between June and November (Marshall, 1996), which would explain the warmer temperature reported by Convey et al. (2018).

Data in this study were collected from three sites within 3 m of one another. As *E. murphyi* has a distribution of at least 35,000 m^2 over an undulating landscape (Hughes and Worland, 2010), the environmental conditions described here may not be representative of the entire distribution range due to likely variation in snow depth, as exemplified by the difference in minimum temperatures between this study and that of Davey et al. (1992) despite the same locale. It is worth noting, however, that the location of the temperature loggers in this study is associated with a high level of *E. murphyi*

abundance (Bartlett et al., in review/ Chapter 5), thus previous levels of snow depth here are not prohibitive to *E. murphyi* success.

The most striking difference in the cold tolerance strategies of the life stages of *E. murphyi* was the ability of eggs to supercool to temperatures of -17.58 ºC, compared to larval instars, and pupae and adults, which all had SCP's around -5 to -6 ºC. All larval instars were freeze-tolerant, consistent with the findings of Worland (2010) and Everatt et al. (2012) yet cold-hardiness increased with larval maturity. Eggs had both a low SCP, and good survival down to -20°C suggesting that they are freeze-avoidant (cf. Bale 1996). Indeed, eggs showed better survival at -15 and -20°C than all other life stages, with an LLT of -25°C (Fig. 4.3). Despite this level of cold tolerance, the midge is not thought to overwinter in the egg stage (Bartlett et al., 2018a/ Chapter 2; Convey, 1992; Cranston, 1984). High cold tolerance in the egg stages is seen across insect groups globally, with many species demonstrating lower egg SCP than their other life stages (e.g. temperate and sub-tropical Diptera (Ceratopogonidae): McDermott et al., 2017; North American Lepidoptera: Uelmen et al., 2016; Fennoscandinavian Lepidoptera: Tenow and Nilssen, 1990; temperate Hemiptera: Bale et al., 1988; Strathdee et al., 1995; temperate Nematocera: Hanson, 1991). This level of cold tolerance in eggs is therefore not unique and may be the consequence of egg characteristics such as higher fat content and sclerotization of the eggshell providing a physical barrier to nucleators, which is found in other Nematocera (Kreß et al., 2016), the sub-order in which Chironomidae reside.

Environmental data presented here would suggest that, if necessary, eggs could survive the temperatures experienced in the soil in winter on Signy. Based on these data alone, it may not be possible for some larvae to overwinter, particularly the earlier instars (Fig. 4.5). However, juvenile and mature *E. murphyi* larvae are capable of rapidly cold hardening, during which they can lower their LLT by up 6.5 ºC for juvenile larvae, and 2.5 ºC for mature larvae (Everatt et al., 2012).Worland (2010) also reported that mature larvae could survive temperatures as low as -20 ºC after 4 days acclimation to -4 ºC. In both studies it was considered that acclimation to such low temperatures may be unnecessary, as Signy winter conditions were thought to be milder. But here we show that acclimation may be a necessary process at least in some microhabitats, as a result of variation in snow

cover. Based on the data obtained in the current study, a decrease in LLT of 6.5 ºC for L1/L2 larvae would reduce it to -16.5 ºC. This would reduce their exposure to the LLT to just 31 h over 5 days in July, compared to 154 h over several months. For mature larvae the lower LLT would similarly move from -15 ºC to -17.5 ºC, reducing exposure to LLT from 49 h over two months to 20 h over just 4 d.

It has previously been suggested that the L2 and L4 instars are the only stages of *E. murphyi* that overwinter (Bartlett et al., 2018a/ Chapter 2; Convey and Block, 1996; Hughes and Worland, 2013). Considering the SCP, LLT and acclimation potential of the larvae, all instars appear to have the physiological capacity to overwinter, as is seen in *B. antarctica* (Harada et al., 2014; Suggs et al., 1983). In order to verify this, further studies on the acclimation capabilities of early instars, the long-term freeze tolerance of all instars at temperatures relevant to Signy and confirmation of which instars overwinter, are required. Pupae are not thought to overwinter (Bartlett et al., 2018a/Chapter 2), and their ability to tolerate cold remains untested. However, with SCPs similar to larvae (-5.81 ± 0.36 ºC), there is little risk to pupal survival during the summer months.

Adult CT_{min} and chill coma values were both close to their SCP, around -5 ºC. Given chilling injury begins soon after chill coma (Hayward et al 2014), this life stage is likely the most vulnerable to temperature and is perhaps best classified as chill-susceptible (Bale et al., 1996). A study on the adults of *E. murphyi's* closest relative, *B. antarctica*, found that they are also freeze-intolerant and were unable to rapidly cold-harden to temperatures below -5 ºC (Lee et al., 2006). The lack of cold-hardiness in adults of both species is unsurprising as neither survives long in adult form during summer months, generally emerging and only active on warm days (Bartlett et al., 2018a/ Chapter 2; Harada et al., 2014; Sugg et al., 1983).

The winter microclimate data obtained here indicate that soil conditions on Signy are below the SCP of field water for a maximum of four months (May-Aug) (Fig. 4.5). It is possible that *E. murphyi* habitat will be frozen for this time, but whether this extends to complete ice entrapment of the larvae for the entire duration is unknown, as this would rely on a prior flood event or very high levels of substrate water saturation immediately prior to the freeze event. The data presented here extend to 63

days and suggest ice entrapment of 28 continuous days would not affect L4 survival, but that survival after 42 days of ice entrapment decreases significantly, and by 63 days, ice entrapment is lethal. The results from the long-term ice entrapment suggest that, whilst temperatures are cold enough for long enough during the winter months to result in ice entrapment, this is clearly not the predominant experience of overwintering larvae given that the midge is still highly abundant and thriving in the environment (Hughes and Worland, 2010). It is possible that larvae avoid prolonged ice entrapment through microhabitat choice, and like *B. antarctica* and the mite *A. antarcticus*, will seek drier microhabitats to avoid inoculative freezing (Hayward et al., 2001; Teets et al., 2011). Both Bartlett et al. (2018a/ Chapter 2) and Hughes and Worland (2010) posited that patchy water content of Signy substrates drove patchiness in *E. murphyi* distributions in the studied summer seasons, so it is possible that similar habitat choices are made in the winter. Any short-term flooding and freezing events that do entrap *E. murphyi* larvae, such as the freeze-thaw cycles of summer and the shoulder seasons (Convey et al., 2018), are not likely to result in any significant mortality if they do not exceed 28 d.

During summer, however, it is possible for habitats on Signy to experience prolonged flooding as a result of snow melt, leaving the terrestrial fauna exposed to potential anoxic conditions. In their study of the ability of *E. murphyi* to survive in a submerged environment, Everatt et al. (2014b), found that the midge could survive up to 28 days by respiring underwater – a unique quality in any terrestrial midge studied to date. Eltinsky et al. (2009) also reported that *B. antarctica* was able to tolerate submergence in field water for up to 10 d, although capacity to respire was not studied. Here, beyond 28 days, survival was greatly reduced, and 63 days submergence was lethal to the whole population. However, the ability to tolerate submergence for up to 28 d, possibly longer, means that spring deluges and rainfall that saturate the moss banks, are unlikely to prove detrimental to the population in its current location on a slope with easy drainage (Hughes and Worland, 2010; Matthews and Maling, 1967). However, as the polar regions are affected by climate change, precipitation events are expected to increase (Convey et al., 2018; Royles et al., 2014). It is therefore likely that flooding events will increase in frequency, but whether they will extend to weeklong deluges that will affect *E. murphyi*, remains to be seen.

Typically, survival after a stress response is measured within 72 h post exposure (e.g. Everatt et al., 2012, 2014a, b; Lee et al., 2006; Hayward et al., 2007). In the current study, a 72 h assessment did not reflect sub-lethal effects of the treatment and the resulting long-term declines in survival. For instance, at 72 h post exposure, survival at 63 days was 30% for both ice entrapment treatments, but after one-month mortality was 100%. Ecologically, a 30 day timeframe post stress exposure is a more significant measure of survival and crucially, longevity and potential fecundity (see also Renault, 2011b).

4.5.1 Implications for *E. murphyi* as an invasive species

The ability to survive prolonged submergence does imply flexibility in habitat choice: in its native range on South Georgia, *E. murphyi* is found on the edge of streams (Cranston, 1985) and currently the midge is resident on Signy in well-drained moss banks. Survival up to 28 days fully submerged suggests that the species could establish in less steep ground that experiences periods of standing water. With a physiology similar to that of *B. antarctica*, Everatt et al. (2012) suggested that there was no thermal limitation on *E. murphyi's* ability to colonise areas that extend to a similar latitude, with *B. antarctica* range extending to 68 ºS (Hughes et al., 2013; Usher and Edwards, 1984). Findings here further support this suggestion, particularly as all instars and even the eggs have the physiological capacity to survive temperatures found at much higher latitudes than Signy: the LLTs of all juvenile life stages, and L3, L4 and eggs are within the minimum winter recorded ground temperature for Anchorage Island at 68 ºS (Convey et al., 2018).

4.6 Conclusions

The invasive midge *E. murphyi* switches cold-tolerance strategies within its life cycle. Larval instars are all freeze-tolerant, increasing in cold-hardiness with maturity. Eggs, however, appear to be freeze-avoiding with a much lower SCP than all the other life stages. Adults seem the least cold-tolerant of the life stages and are perhaps best classified as chill tolerant or chill susceptible, much like adults of

the related *B. antarctica*. All juvenile stages have the physiological capacity to survive temperatures typically experienced overwinter. Long-term (> 28 d) ice entrapment and submergence is lethal to *E. murphyi*, but such prolonged anoxic conditions are unlikely to be experienced on Signy under present conditions. The success of the midge and its ability to overwinter is the result of its ability to acclimate and small-scale differences in habitats that influence whether it would be exposed to fatal conditions. These physiological features do not limit the potential of *E. murphyi* as an invasive species on Signy, or its ability to colonise areas even further south.

Chapter Transition

The ability of *E. murphyi* to tolerate a range of environmental conditions has highlighted its success as an invasive species. The following chapter surveys the midge's current distribution on Signy Island, updating previous distribution surveys. The potential for the midge to spread to other areas of the island is modelled and high-risk areas identified.

Chapter 5: An insect invasion of Antarctica - the past, present and future distribution of *Eretmoptera murphyi* on Signy Island.

The work presented in this chapter is currently in review with Insect Conservation and Diversity as: Bartlett JC, Convey P, Pertierra LR, Hayward SAL (2019) An insect invasion of Antarctica: the past, present and future distribution of *Eretmoptera murphyi* (Diptera, Chironomidae) on Signy Island.

5.1 Abstract

Increasing human activity in Antarctica, and the continued warming of the polar climate, means the risk of non-native terrestrial species colonising and establishing in its biodiversity- and nutrient-poor ecosystems is increasing. Of the five non-native invertebrate species in terrestrial Antarctica today, the flightless midge *Eretmoptera murphyi* is perhaps the most persistent insect invader. Accidentally introduced to Signy Island (60 °S) in the 1960s from sub-Antarctic South Georgia (54 °S), *E. murphyi* has steadily increased its distribution, however, its status has not been reassessed for a decade. Here we update the distribution of *E. murphyi* on Signy, specifically assessing whether 'footpaths' to regularly visited research sites represent dispersal corridors. Both the abundance and range of *E. murphyi* have increased significantly since 2009, particularly along paths leading away from the original introduction site, and the species is now on the cusp of moving into new valley systems. We identify a moderate association with soil/substrate and vegetation types and build Maximum Entropy (MaxEnt) models to predict areas of the island that may be at highest risk of future colonisation. As a detritivore with no competitors or predators, *E. murphyi* may have a major impact. For example, accelerating nutrient cycling which may have wider impacts on all levels of biodiversity. This study highlights the need for an assessment of current biosecurity protocols applied within the Antarctic Treaty System and the need for systematic and regular monitoring of introduced and invasive species in Antarctica.

5.2 Introduction

The Antarctic region is the least biologically invaded area in the world, in part thanks to its geographic isolation, harsh climate and limited history of human activity. As a result, few terrestrial species have established naturally in the area south of 60 °S latitude since the last glacial maximum (Frenot et al., 2005; Hughes et al., 2015). However, increasing human activity in the region in recent decades – primarily in the forms of tourism and scientific research – has diminished the isolation of Antarctica, particularly within the maritime Antarctic, a region encompassing much of the Antarctic Peninsula and the archipelagos of the Scotia Arc. Most recent arrivals of non-indigenous species in the Antarctic region have occurred because of accidental introductions by humans and, as the human footprint has increased (Pertierra et al., 2017), so records of non-indigenous species becoming established have risen, with the South Shetland Islands and northern Antarctic Peninsula being especially affected (e.g., Greenslade et al., 2012; Molina-Montenegro et al., 2012; Volonterio et al,. 2013). To date, most invertebrate colonisation events have happened in the sub-Antarctic islands (Convey and Lebouvier, 2009; Frenot et al., 2005). The few terrestrial introductions that have occurred below 60° S latitude have been in or close to research stations (Hughes et al., 2014; Molina-Montenegro et al., 2010). For example, the introduction of *Lycoriella* sp. (Diptera, Sciaridae), *via* imported fresh vegetables, to Casey Station in the eastern continental Antarctic (Anon, 2002). Further colonisation of non-indigenous species is likely, given the continuing increase in activity around Antarctica as new research stations are established, and land-based exploration as well as tourism expands. Establishment is then facilitated by predicted changes that an altered and ameliorated climate may bring (Chown et al., 2012b; Convey, 2011; McGeoch et al., 2015; Turney et al., 2016). Understanding the biology of established non-indigenous species and monitoring their distributions will be key to understanding how best to control and mitigate this challenge. Only with deeper knowledge of species' physiology, life history, dispersal mechanisms and rate of population expansion, can management and governance processes in the Antarctic be effective in preventing further introductions, as well as minimising the risks of species invasions that have already occurred (Chown et al., 2012a, 2016; Hughes et al., 2010; Hughes and Pertierra, 2016).

Eretmoptera murphyi is an established non-indigenous species on Signy Island (Section 1.2.2.1, Figs. 1.1, 1.2). Native to sub-Antarctic South Georgia, *E. murphyi* is thought to have been accidentally introduced to Signy during plant transplant experiments conducted in the 1960s (Block et al., 1984; Burn, 1982). Since its initial discovery on Signy in 1980, the species has progressively expanded its range from the original introduction site immediately adjacent to the research station (Block et al., 1984; Burn, 1982; Dózsa-Farkas and Convey, 1997; Hughes and Worland, 2010; Smith, 1996). At some sites, the midge is now found at biomass densities exceeding that of the entire native micro-arthropod fauna at those locations and, consequently, is influencing litter turnover, with implications for the terrestrial ecosystem and native biodiversity (Hughes et al., 2013). The species is currently considered a 'persistent alien', in the absence of direct evidence of impacts on native species which would lead to it being classified as an 'invasive alien' (Frenot et al., 2005).

Eretmoptera murphyi has several physiological traits that have allowed it to succeed in the more extreme maritime Antarctic in comparison to the milder climate of South Georgia. Its larvae have appropriate cold tolerance and can rapidly cold-harden (Block et al., 1983; Everatt et al., 2012, 2015; Worland 2010), as well as an ability to respire in water and withstand ice entrapment (Everatt et al. 2014a). Both eggs and larvae are also desiccation tolerant (Bartlett et al., 2018a/ Chapter 2; Everatt et al., 2014c). The species has a 2-year life cycle and is parthenogenetic – as a result it does not require synchronous adult emergence and subsequent mating. This contrasts with its closest relative the sexually-reproducing chironomid and only higher insect endemic to Antarctica, *B. antarctica* (Bartlett et al., 2018a/ Chapter 2). This means that *E. murphyi* can have an extended emergence period across the whole summer season (Bartlett et al., 2018a/ Chapter 2), making the most of environmental windows of suitability, and potentially using this flexibility to increase its distribution range *via* the more mobile, and more-readily wind dispersed, surface-dwelling adults.

Studies of the distribution and abundance of *E. murphyi* on Signy have only recently begun in earnest, with the benchmark survey of Hughes and Worland (2010) taking place in 2007-2009, c. 40 years after the suspected introduction, and 25 years following its initial discovery (Burn, 1982). Hughes and Worland (2010) provided the first comprehensive assessment of the spread of *E. murphyi* around its

original introduction site and found densities as high as 150,000 larvae m^2, with evidence of the midge being more prevalent along paths leading away from this area. These data suggested that, whilst flightless adults may be capable of dispersal to some extent, it is the mechanical process of human footfall and disturbance of larvae in the soil that is a key factor facilitating its spread – as has also been noted for non-indigenous plant species in Antarctica (Molina-Montenegro et al., 2012). While Hughes and Worland (2010) did not identify the limit of the species' distribution, their study confirmed that *E. murphyi* had spread to cover an area of at least 35,000 m^2, doubling the previous estimate made in 1995 (Dozsa-Farkas and Convey, 1997). The survey noted a highly patchy distribution pattern, with *E. murphyi* typically associated with dead organic matter, rather than live moss or inorganic substrates such as gravel and rock. This is consistent with *E. murphyi* being a detritivore (Cranston, 1985; Hughes et al., 2013). However, as yet, no assessment has been made of the availability and extent of suitable habitats for *E. murphyi* around the island, or how the species' abundance changes across different substrate types, i.e. evidence of substrate preference.

Species distributions and relationships with associated habitats have typically been analysed using correlative models that use linear and/or logistical regressions to establish the main drivers of species abundance (Wiens et al., 2009; Porfirio et al., 2014). More recent developments of open source software, using geographical information, now allow ecologists to use known species presence data in combination with environmental niche predictors to more accurately model likely changes in distribution (Rushton et al., 2004; Raghavan et al., 2016). Such models can aid understanding of species responses to climate change (Wang et al., 2018), help assess extinction rates (Thomas et al., 2004), identify habitat loss scenarios (Angileri et al., 2016), and help prioritise biological conservation efforts (Pyke et al., 2005). They can also be used to forecast suitable areas for the spread of an invasive species (e.g. Pertierra et al., 2017; West et al., 2016) through estimations of 'probability of presence' given a series of biotic and/or abiotic variables such as terrain, habitat classification, temperature or dispersal vectors.

Antarctica is a globally important reservoir of unique ecology and biodiversity, and Signy Island provides one of the best examples of terrestrial biodiversity in the region (Smith, 1990). As a non-

native detritivore in such high abundance, *E. murphyi* may alter the Signy Island ecosystem, and areas beyond if given the opportunity to disperse. Few studies to date have examined Antarctic invertebrate invasions in depth, with no regular monitoring of long-term abundance and distribution changes of those species that have colonised.

5.2.1 Aims of this study

In this study we aim to update the work of Hughes and Worland (2010) and attempt to document the full extent of *E. murphyi's* distribution on Signy Island. We will establish any association with preferred environments through physical study of its habitat and, using Maximum Entropy (MaxEnt) species distribution modelling, aim to highlight any areas at risk of further colonisation across the island.

5.3 Materials and methods

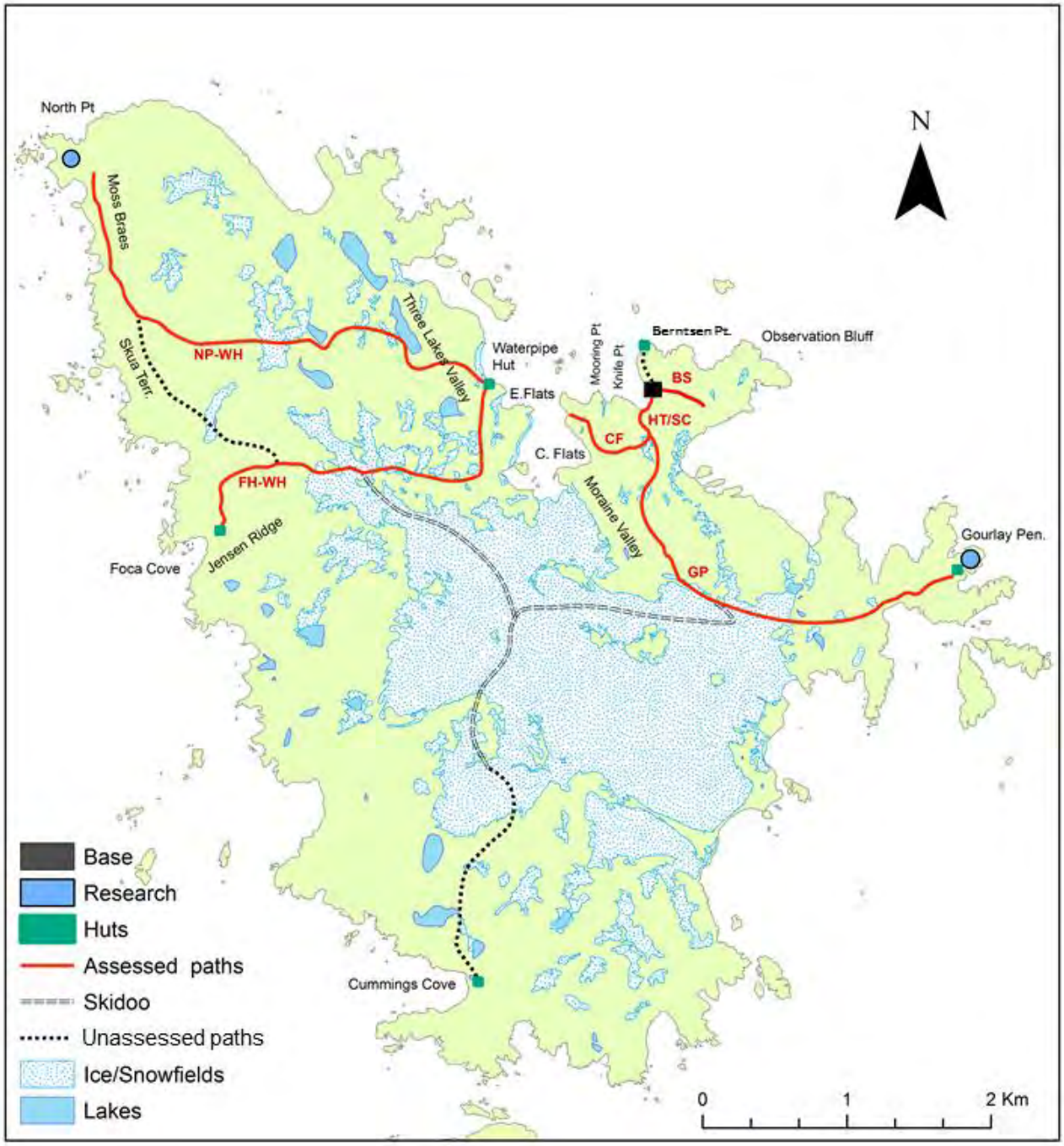
Signy Island is located in the South Orkney Islands archipelago in the maritime Antarctic, (Section 1.2.2.1, Fig. 1.2 and 1.3). The island is 6 x 5 km, with a maximum elevation of 288 m a.s.l across a small mountain range that holds the island's large ice cap. Signy experiences positive temperatures for most of the austral summer (December to March) with an average summer temperature of +4 ºC. During winter, the island is surrounded by sea ice extending from the Antarctic continent and temperatures remain well below freezing, with a winter average of -10 ºC. Rising temperatures throughout the Antarctic means that the island is de-glaciating and consequently much ice-free land is experiencing primary succession.

5.3.1 Extent of *E. murphyi* distribution

Distribution surveys were primarily conducted along frequently used paths from the British Antarctic Survey (BAS) research station on Signy Island. In total, 14 'stop-sites' were sampled along the 'High-tide' (HT) and 'Stonechute' (SC) paths (Fig. 5.1), with soil cores taken at 1 m and 5 m distances

perpendicular to the path, every 20 m +/- 2 m (measured using a tape reel) in order to evaluating any radiating dispersal affects from the paths. Where the path broadened away from the sea, samples were taken on either side of it at both 1 m and 5 m. Otherwise all samples were taken to the inland side of the path, with the final 'stop site' at the summit of the SC. Fourteen 'stop sites' were also sampled along the 'Backslope' (BS) path (Figs. 5.1, 5.2), following the same procedure, and sampling on both sides of the path, with the final 'stop site' at the summit plateau between Factory and Observation Bluffs. 'Whole island' survey routes targeted the likely habitat of *E. murphyi* (moss patches or banks) at *c*. 50 m intervals (closer if habitat irregular), taking cores from each site. These routes followed the frequently used paths from the top of the SC to the huts on the Gourlay Peninsula (GP trail) where regular penguin colony monitoring has been conducted for 22 years (14 sites sampled), and from the top of the SC to the Cemetery Flats (CF) crossing which is the main access route to freshwater lakes and other parts of the north and west of the island (7 sites sampled). Further routes were surveyed from Waterpipe Hut (WH) over Jane Col to Foca Hut (FH) on the west coast of the island (16 sites sampled), and from North Point (NP), where penguin and bird survey work is currently conducted several times a season, over Spindrift Col and down to WH (Fig. 5.1, 10 sites sampled). These latter routes reflect historically well-used paths around the island. Focus was given to sampling close to the field huts established on the island (see (Appendix V).

In addition to the survey routes, a grid of points 100 m apart was plotted over the entire area adjacent to the research station, covering an area of 490,000 m^2, in order to establish the extent of *E. murphyi's* distribution (Fig. 5.3). This 'edge of extent' grid incorporated Berntsen Point at its northern-most point, extended several meters beyond the summit of the SC path at its southern-most point, Observation Bluff and Gash Cove to the east, and as far as the gully between Mooring and Knife Points to the west. As previously, soil cores were sampled at each grid point and analysed for presence of *E. murphyi*.


North Pt
Moss Braes
Skua Terr.
NP-WH
Three Lakes Valley
Waterpipe Hut
E.Flats
Mooring Pt
Knife Pt
Berntsen Pt.
Observation Bluff
BS
HT/SC
CF
C. Flats
Moraine Valley
GP
Gourlay Pen.
FH-WH
Jensen Ridge
Foca Cove
Cummings Cove
N
Base
Research
Huts
Assessed paths
Skidoo
Unassessed paths
Ice/Snowfields
Lakes
0
1
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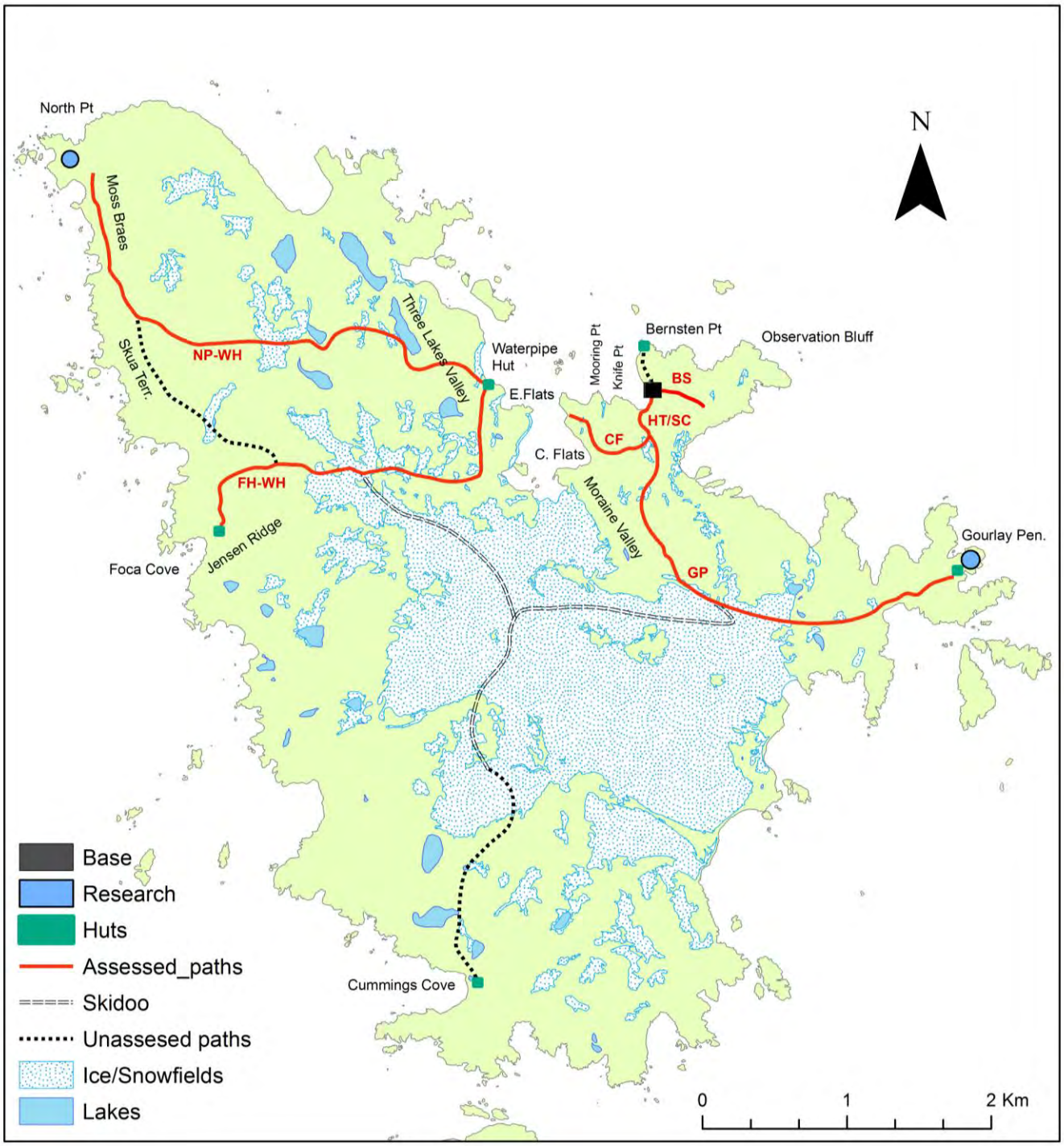

Figure 5.1. *Map of survey routes across Signy Island, shown with regular research sites, shelter huts and the research station. Discussed locations annotated in black, and abbreviations for survey routes shown in red (see text for full names).*

5.3.2 Sample collection and processing

Soil cores (n = 3) were taken from each designated sampling point, using a steel 2.5 cm ø soil corer, with all cores returned to the Signy laboratory in individual sterile sealed bags. Samples were collected during the 2016/17 austral summer and returned to the United Kingdom by ship in +4 °C cold storage (10 weeks), and then maintained at +4 °C at the University of Birmingham until analysis. All samples from areas of known/likely distribution (HT, SC, BS and Edge of Extent Grid), were analysed within six months of initial collection. Further 'Whole Island' routes were analysed within one year of collection. Individual larvae that were visible by eye (L2-L4) and egg sacs were extracted from the substrate by washing through stacked sieves (2, 0.5, 0.25 mm mesh sizes) and hand-picked from the remaining soil on the 0.25 mm sieve, with the 0.5 mm sieve checked for any further individuals. L1 larvae were not included in this survey due to their very small size. Prior to washing, any clumps of moss or peat substrate were teased apart with tweezers, freeing any individuals that may have been amongst the fibres.

The constituent parts of each core's upper and lower dominant substrate components were noted for subsequent correlation analysis with *E. murphyi* abundance. Substrates were divided into 10 sub-types, six for the upper substrates and four for the lower (Table 5.1). Larval densities were expressed as the mean for each set of three cores. Egg sacs were also included, with their larval potential calculated using known egg hatching success rates (cf. Bartlett et al., 2018a/ Chapter 2) (Equation 1). Densities per m^2 were estimated by calculating the surface area of the corer and scaling up (cf. Hughes and Worland, 2010). This gives a multiple of 1,949 to apply to the combined larval (L) counts and larvae from egg sacs (E_L), to obtain a count of larvae per m^2 (L/m^2).

$$E_L = \left(E_g \times E_s\right) \times E_h$$

Equation 5.1.*Calculation of larvae produced from egg sacs (E_L): Where,* E_g *= the mean egg sac count for three cores,* E_s *= mean eggs per egg sac (48*),* E_h *= % eggs that hatch (35%*) *after Bartlett et al. (2018a/ Chapter 2).*

5.3.3 Statistical analyses and data visualisation

In order determine any correlation between substrate type (see Table 5.1) and the abundance of *E. murphyi*, two regression methods were employed. Firstly, mean abundance per m^2 (for BS and HT/SC paths) were log_{10} transformed and plotted against the binary presence/absence data for each substrate type. They were then investigated for significant substrate types associated with *E. murphyi* abundance using an ordinal logit regression (OLR) (XLStat, verified with SPSS) by categorising the population abundance per m^2 into the following categories: zero, low (1-1000), medium (1001-10,000), and high (10,001 – 100,000). Whilst this provided a good model of what were the significant relationships, further detail was required, so a general linear model (GLM) was run on the log_{10} transformed data (XLStat) to obtain a true R^2 value for substrate type – abundance relationships. Substrate type (see Table 5.1) upper inorganic (Ui) had zero observations, whilst upper vascular (Uv) and upper algae (Ua) had just one each, so were removed from the following analysis. Both methods produced the same significant substrate type-abundance relationship, validating one another and the decision to take the significant substrates forward into the maximum entropy model.

Table 5.1. *Substrate type for upper (Ux) and lower (Lx) core constituents.*

	Soil	**Peat**	**Moss**	**Vascular**	**Algae**	**Inorganic**
Lower	Ls	Lp	Lm	*n/a*	*n/a*	Li
Upper	Us	Up	Um	Uv	Ua	Ui

5.3.4 MaxEnt modelling for whole island colonisation risk.

Maximum entropy species distribution modelling (MaxEnt) (Phillips et al., 2006) is a common technique used to model a species' potential distribution based on the known geographic distribution and associated environmental background information. Its application here allows identification of similar areas to those already occupied by *E. murphyi,* indicating the relative potential for establishment. MaxEnt v3.4.1 (http://biodiversityinformatics.amnh.org/open_source/maxent/) was used to model colonisation risk for areas of the island with input layers created as follows: using a

digital elevation model (DEM) of Signy Island, GIS raster layers with a cell size of 5 m^2 were generated for aspect, slope and altitude. Using this topographical information, the programme outputs preferential habitats for *E. murphyi* presence across the island, based on the locations of its current distribution. This layer of topographical preference for *E. murphyi* occurrence was then combined with other raster layers to account for human activity around the island; preferred substrate type (i.e. findings from abundance-substrate type correlations set out above), and distance from the current distribution of *E. murphyi*. All raster layers were created in either ArcMap 10.4.1 or QGIS v2.18 'Las Palmas' (QGIS Development Team 2016. QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org). Human footprint was classified into nine categories from very high to infrequent/nil (Appendix IV), based on a subjective assessment of the level of activity in an area in any one season on the island, derived from discussions with, and observations of Signy Island research personnel during the 2016/17 season. The distance layer was created using Euclidean distance interpolation, radiating from the area of current *E. murphyi* presence as defined in this study. The substrate type layer was constructed based on the results of the OLR/GLM modelling and presence/absence of the substrates preferred by *E. murphyi*. Terrain models selected environments of similar aspect, slope and altitude to those where *E. murphyi* is verified to be present in order to predict further 'at risk' areas. The final distribution map was then created in ArcMap 10.4.1, with added landscape features and sites of significance. Areas of the island currently covered in ice or permanent water bodies were discounted as areas at risk and masked out accordingly.

As "true absence" data (whole island transects) has been recorded, the need to generate pseudo-absence points to calibrate the SDM model is removed: *Eretmoptera murphyi* is flightless, has a small activity window (a few months), and has only been active on the island for ~50-60 years, thus we can be confident that any absence data represent true absences. We accept that we cannot say that the entire Signy I. area was systematically sampled as a condition to select our background, but we do have a high degree of certainty regarding the species extent, and consequently conclude that there is no sampling bias.

5.4 Results

5.4.1 Local distribution and abundance of *E. murphyi*

Fig. 5.2a shows that the distribution of *E. murphyi* now extends well beyond the immediate area of the research station (RS), and it can be found in densities of up to 12,000 L/m^2 close to the summit of the SC path, 240 m beyond the RS. Abundance lower down the path is equally high, with several points exceeding 10,000 L/m^2. The highest densities were found on the BS path with two instances, at 40 m and 60 m, of densities exceeding 50,000 L/m^2, the highest being 98,241 L/m^2 at 40 m. Densities reduced further up the path, although still sporadically with densities >10,000 L/m^2 after ~100 m. The mean densities along the HT/SC and BS paths were not significantly different (HT/SC, mean 3,317 L/m^2 ± 671 SEM, n = 32; BS mean 4,904 L/m^2 ± 2,031 SEM, n = 56; p = 0.56, unpaired t-test). Combining the data across both paths, 28% of sample points gave zero counts, 27% low counts (1-1,000), 35% medium counts (10,001-50,000) and 10% high counts (50,001-100,000).

E. murphyi population density did not differ significantly with increasing distance from the path (p = 0.31, Mann-Whitney). However, there was a small (but non-significant) decline in density further from the introduction site (BS - r = 0.09, p = 0.2; HT/SC - r= 0.03, p = 0.5) (Fig. 5.2b).

5.4.2 Edge of distribution and whole island survey

The 100 x 100 m edge of extent grid (Fig. 5.3) found evidence of *E. murphyi* along both currently used and historic paths, with a small number of larvae found on a ridge south-east of the old study site path, over 300 m from the original introduction site. Surveys of other paths around the island found no evidence of *E. murphyi* presence (see Appendix V)

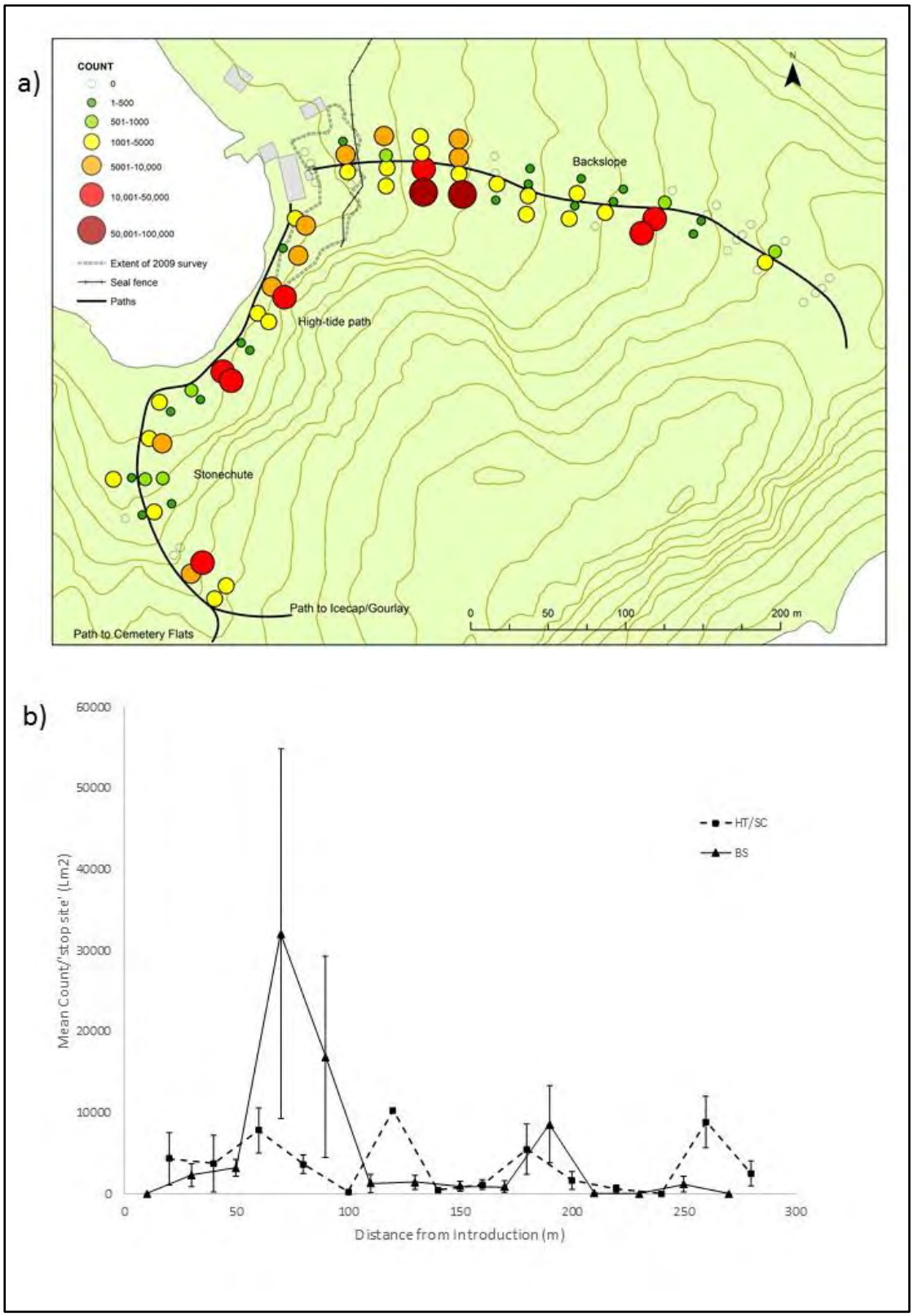

Figure 5.2. ***(a)*** *Abundance and distribution of* E. murphyi *in the 2016/17 season along and adjacent to the Backslope (BS) path and the High-tide (HT)/Stonechute (SC) paths. Shown with the outline of 2009 survey (Hughes and Worland 2010). Circles represent the mean number of larvae per m*^{2}*.* ***(b)*** *Mean abundance (± SEM) per 'stop site' along each of the two routes (BS and HT/SC), and their approximate distance from the original introduction site (adjacent to the research station).*

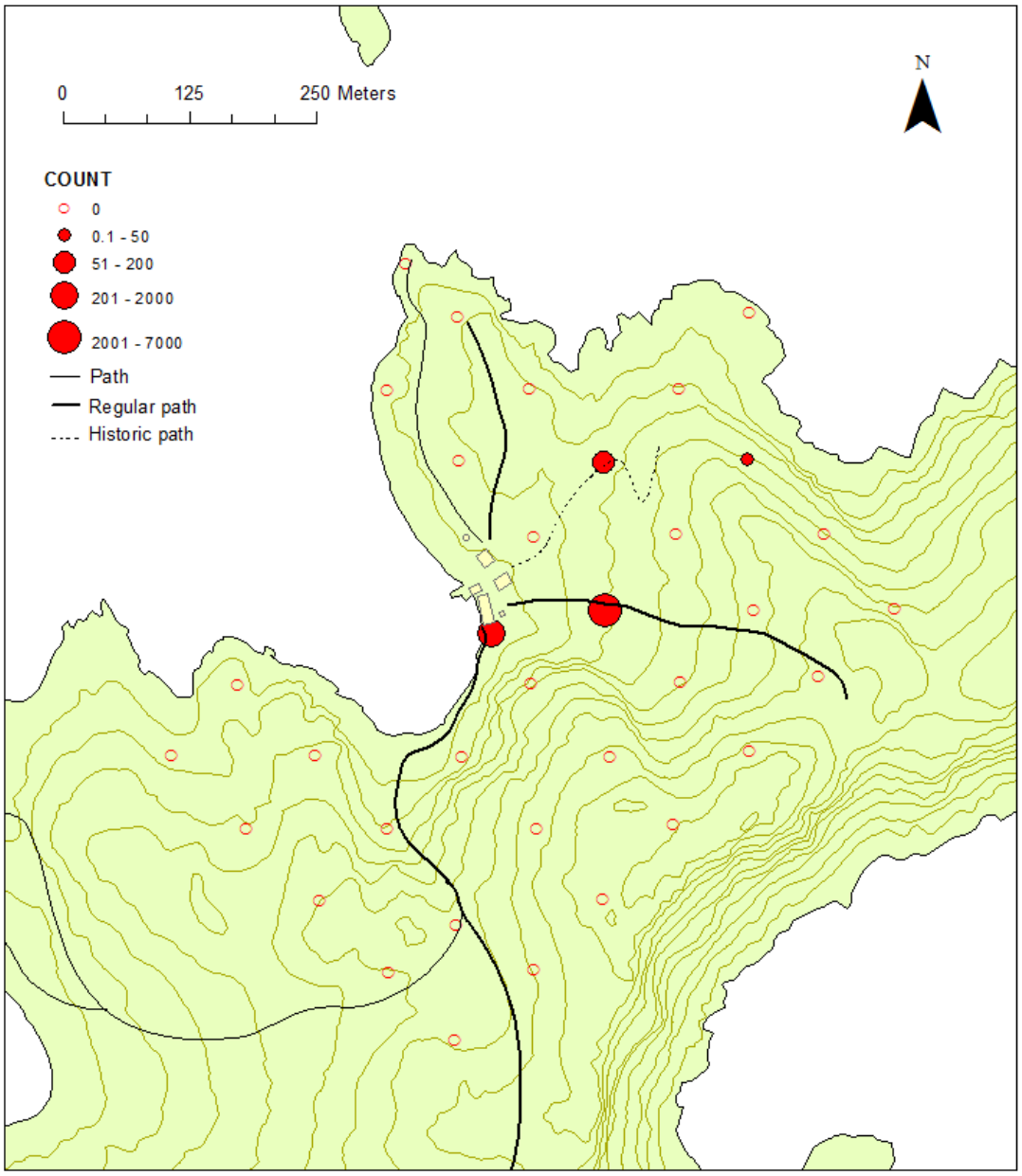

Figure 5.3. *Map showing the 'edge of extent' grid and associated abundance. Filled red circles represent the mean number of larvae per m².*

5.4.3 Substrate type distribution and *E. murphyi* substrate preference

The frequency of different substrate types (Table 5.1) differed significantly across collection sites ($p >$ 0.001, Kruskal Wallis). This was largely driven by the dominance of moss in the upper part of substrate cores (Um = 43%) and soil in the lower core (Ls = 30%). The most common habitats were those of moss banks: Um/Ls (55%) and Um/Lp (14%), whilst the least observed combinations were those of bare ground/peat (Table 5.2). Results for the OLR found that only soil and peat in the lower

core (Ls and Lp respectively) had significant positive associations with *E. murphyi* density (Ls, p = 0.032; Lp, p = 0.024) (Fig. 5.4). These two substrates explained 10% of density variability through a GLM (r^2 = 0.10, p = 0.011). Additional results from the GLM and associated correlation matrix indicated that Ls was the most significant of the two substrate types (p = 0.003). Further analysis of substrates along footpaths around the island found suitable habitats (moss banks with soils and peat in the lower core: Um/Ls or Um/Lp,) for *E. murphyi* colonisation around Cemetery Flats, the Gourlay Peninsula and Three Lakes Valley in particular.

Table 5.2. *Combination of occurrences for each substrate type (see Table 5.1 for acronyms) in the upper and lower core, with the most positive (in bold) or most negative (italics) associations with* E. murphyi *abundance (R2) shown along with related significance (*=p<0.01).*

Substrate combination	Habitat description	% Occurrence (BS & HT/SC combined)
Us/Lm	-	0
Up/Lm	-	0
Us/Lp	Soil on peat – bare ground/peat	1.15
Up/Ls	Peat on soil - bare ground/peat	1.15
Up/Lp	Peat core – bare peat	2.3
Up/Li	Peat on stone – bare peat	2.3
Us/Ls	Soil core – bare ground	3.45
Us/Li	Soil on stone – bare ground/fellfield	3.45
Um/Li	Live moss on stone – recent succession	6.9
Um/*Lm*	Live moss bank core – established moss peat bank	9.2
Um/Lp	Live moss on peat – moss peat bank	15
Um/Ls*	Live moss on soil – recent succession/ organic breakdown	55.1

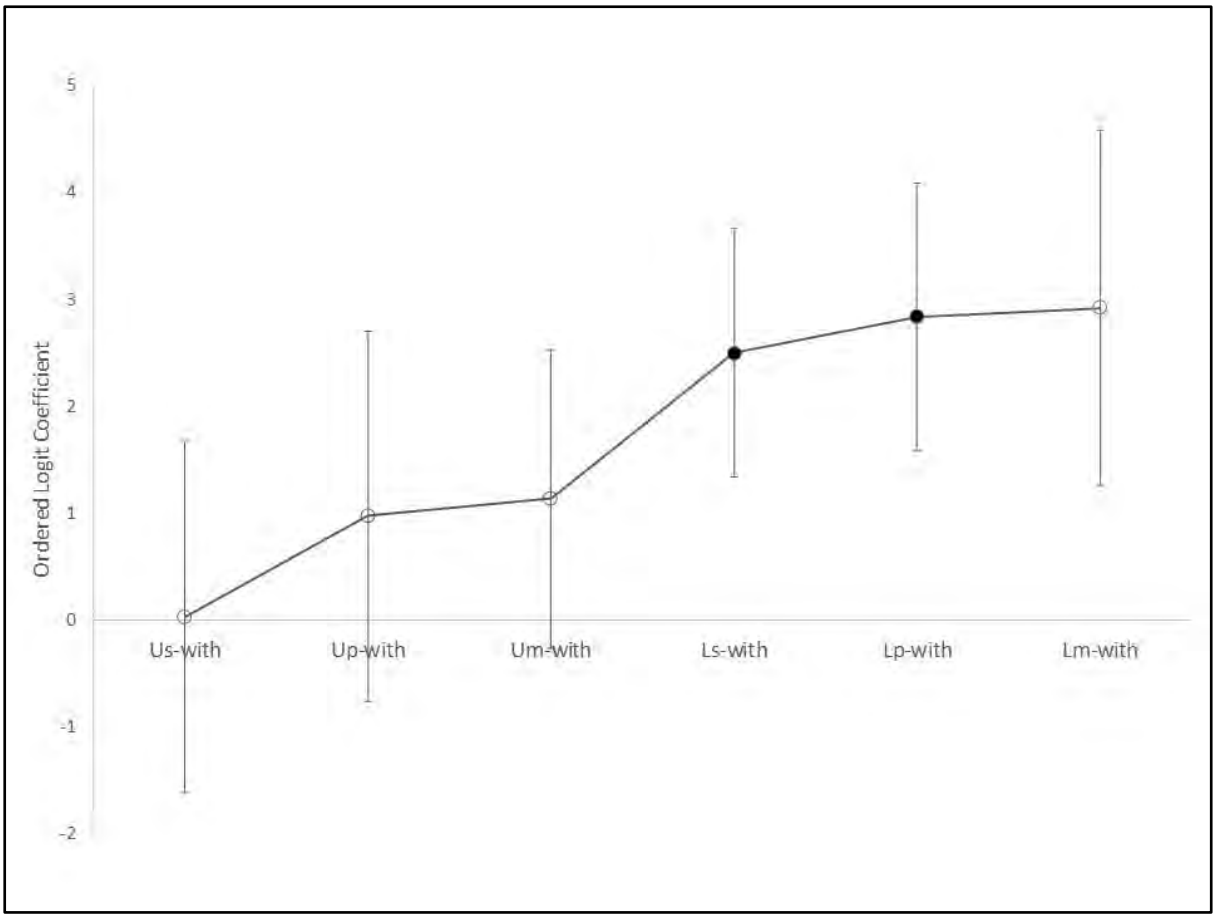

Figure 5.4. *Results of ordinal logit regression (OLR) – key substrate types (Table 5.1) as values of ordered logit coefficient, plotted in order of least to largest influence on* E. murphyi *abundance (± SEM). Substrate types testing significant through the OLR shown as solid fill circles = $p < 0.01$. All variables > 0 plotted.*

5.4.4 MaxEnt modelling for whole island colonisation risk.

Results from the MaxEnt model (Fig. 5.5 and Appendix VI) indicated that, beyond the area of known distribution, the areas of highest overall risk were Berntsen Point, Observation Bluff, Cemetery Flats, parts of Mooring and Knife Points, and the trail through Moraine Valley. Medium - high risk areas included parts of the Gourlay Peninsula, Elephant Flats, Waterpipe Hut and Three Lakes Valley. Areas at a medium risk of establishment were largely found in patches around Moss Braes near North Point, parts of Skua Terrace, Jensen Ridge and Foca Cove, and areas close to Cummings Hut. Of the environmental variables taken from the DEM, aspect explained 65% of the known distribution envelope of *E. murphyi,* with slope 25% and altitude 10%.

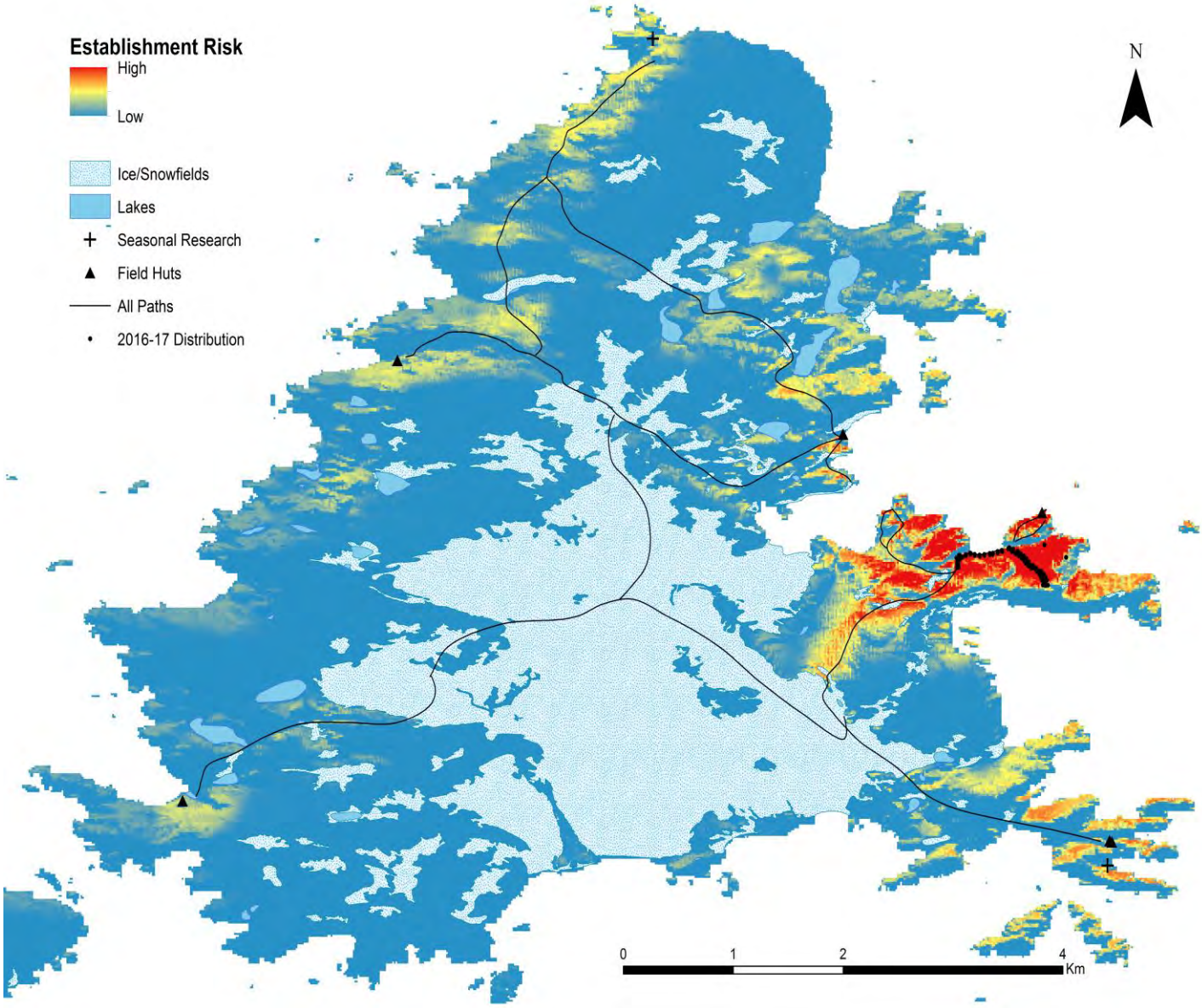

Figure 5.5. *Results of MaxEnt model showing the establishment risk of* E. murphyi *around Signy, based on its 2016/17 distribution, available habitat and human footfall around the island. Also shown are: all paths (including skidoo routes across the ice cap), field huts and seasonal research sites. Model visualised using ArcMap 10.4.1 with raster symbology using* n=*6 SD and Gamma stretch = 1.5.*

5.5 Discussion

With the increasing risk of non-native species colonising Antarctic terrestrial habitats, there is an urgent need to regularly assess the rate at which successful invaders can extend their distribution, whilst also assessing their preference for available habitats in order to best forecast other areas that may be at risk. This study represents the most comprehensive survey of the flightless midge *E. murphyi* on Signy Island to date. The species has extended 85,000 m^2 beyond its presumed introduction site in the 1960s, and we report a distribution increase of 50,000 m^2 beyond that of the last survey in 2009 (Hughes and Worland, 2010) (Fig. 5.6). Thus, while its spread began slowly, it has clearly accelerated in recent years. The importance of human footfall along routes to regularly visited research sites is further supported as a likely means of dispersal.

This survey doubles the known range of *E. murphyi*. The highest population densities were found on the Backslope path immediately behind the Research Station (Fig. 5.2a, b), with a steady but small decline after this point with distance up the path. Densities along the High-tide/Stonechute path were patchier, but densities of > 1,000 L/m^2 persisted along this route. There was no discernible difference in the substrate types between the two paths, but the High-tide/Stonechute path is used by personnel on a more frequent basis than the Backslope, which may account for the higher abundance further along this path.

The combination of methods used here: targeted surveying (path transects) and systematic surveying (extent grid), illustrates the value of both techniques, with the targeted survey highlighting details of density patterns, and the systematic grid locating outlying populations. We recommend that both methods are used in future monitoring as a minimum requirement in assessing changes in abundance and distribution over the largest area possible. Such combined approaches to non-indigenous species monitoring are also recommended as best practice by Rew et al. (2006) in their comprehensive analysis of survey methods used for the monitoring of invasive plant species in the United States. Furthermore, they recommend that all monitoring of species distribution and abundance coincides with the collection of habitat data, such as we have demonstrated here in characterising substrate type

and topography, to "improve our understanding of factors affecting NIS (*sic*, non-indigenous species) occurrence, and to produce probability occurrence maps of target species".

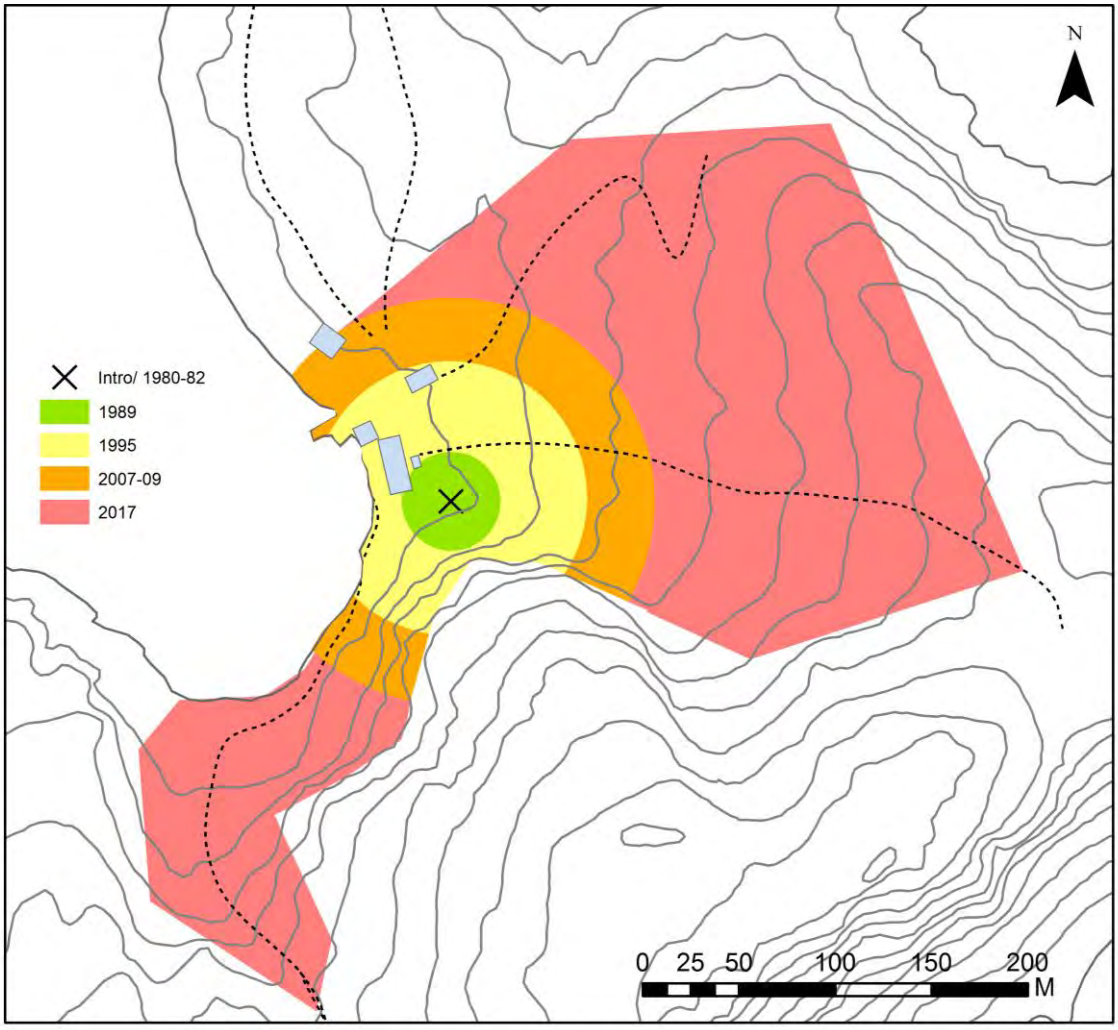

Figure 5.6. *Spread of* E. murphyi *over time: introduction site and initial recordings of presence (Burns 1982; Block et al. 1984); 1989 distribution ~200 m² (Smith 1996); 1995 distribution ~16,000 m² (Dozsa-Farkas and Convey 1997 – calculating as radius); 2007-09 distribution ~35,000 m² (Hughes and Worland, 2010 – calculating as radius); 2017 distribution, this study ~ 85,000 m² calculated as exact polygon from areas of known presence.*

Regression analyses identified that, whilst live moss in the upper part of the sampled cores (Um) and soil in the lower part (Ls) were the most abundant substrates, it was the presence of soil and peat in the lower part of the cores that had the strongest correlation to *E. murphyi* population density. This is consistent with *E. murphyi* being a detritivore. Hughes and Worland's (2010) data also support the negative association of *E. murphyi* with both live moss and inorganic substances such as stones and gravel in the lower core identified in this study. However, substrate type explained only 10% of

variation in density, making it likely that other factors are primary drivers of population density and distribution. It also remains unclear if the association of *E. murphyi* with soil and peat is because of preference for this habitat, or because the midge is generating the soil substrate type and aiding decomposition of the existing moss and vegetation.

Population densities over 12,000 L/m^2 close to the summit of the Stonechute path, and of 1,000 L/m^2 one metre from the path at the summit (Fig. 5.2b), suggest that this species could be on the verge of entering into new valley systems. This is of concern given the availability of prime habitat leading down to Cemetery Flats. If able to establish in the Cemetery Flats area, the risk of wider spread will be increased by the seal populations there. Fur seals in particular are very mobile, disrupting the moss bank surface as they move (Favero-Longo et al., 2011), and there is the potential that larvae and eggs may be carried in the fur of the animals, particularly the egg sacs which have a sticky gelatinous outer membrane (Bartlett et al., 2018a/ Chapter 2). Results from the MaxEnt model suggest that combinations of suitable habitat and human footfall are frequent through the ice-free areas of the island, but that primarily the areas of Cemetery Flats and the Gourlay Peninsula are most at risk of further colonisation, largely because of their proximity to the existing distribution of *E. murphyi* and high footfall of visiting personelle.

Older and less used paths have no trace of *E. murphyi* on them at present (Appendix V) yet do have suitable habitats as identified by both MaxEnt modelling and along-route sampling. The moss and peat banks of Skua Terrace and Moss Braes in particular present a large area of potential habitat, (Cannone et al., 2017), especially where live moss does not extend too deeply (Fig 5.1). In addition, if larvae or eggs could survive a short low-tide crossing from Cemetery Flats – where researchers quickly move through shallow sea water and pools to access the northern part of the island – then much of the area around Waterpipe hut and Three Lakes Valley also becomes accessible for colonisation, with Elephant Flats and soils directly surrounding Waterpipe Hut at particular risk.

Within the wider region of the maritime Antarctic, favourable climate and habitats for *E. murphyi* have been identified in the South Shetland Islands and along the western coast of the Antarctic

Peninsula, with islands in Marguerite Bay close to Rothera Research Station at particular risk (Hughes et al., 2013). The indigenous and endemic chironomid *B. antarctica,* which already occurs across this region, favours similar habitats to *E. murphyi* and has generally similar physiological capabilities. Therefore, any expansion of *E. murphyi* into this region could result in impacts on, or even displacement of *B. antarctica*, particularly given the possible advantage gained from *E. murphyi*'s more flexible parthenogenetic life history strategy (Bartlett et al. 2018a/ Chapter 2).

Hughes et al. (2013) concluded that *E. murphyi* was significantly affecting litter turnover on Signy Island. With a biomass at some sites of 2-5 times that of the estimated indigenous micro-arthropod and micro-invertebrate community (mites, springtails, tardigrades, nematodes and rotifers), a mean *E. murphyi* population of 21,000 L/m^2 could increase litter turnover by as much as 66.51 g dry mass m^{-2} per annum. This is almost a full order of magnitude (9.3 times) the consumption rate of the native Signy community (*cf*. Davis, 1981). In an analogous study, Hänel and Chown (1998) found that another invasive chironomid, *Limnophyes minimus,* on sub-Antarctic Marion Island, was also increasing litter turnover by 6-10 times the rate of the larvae of the native moth *Pringleophaga marioni*, superseding it as a major contributor to the island's nutrient cycling. As *E. murphyi* is the only macro-invertebrate on Signy Island, and has no predators or competitors, its impact on the terrestrial ecosystem is likely to be unimpeded. Furthermore, as *E. murphyi* develops greater population densities (Hänel and Chown, 1998; Hughes and Worland, 2010; this study) and is physically larger than *L. minimus* (Mercer et al., 2001), it is likely to be one of the most significant introductions to a sub-or maritime Antarctic terrestrial ecosystem yet recorded.

Whilst the impacts of *E. murphyi*'s introduction on native fauna, soil biogeochemistry, substrate formation and vegetation development remain to be fully established, this study has shown that *E. murphyi* is found in association with soil substrates and anecdotally it is reported that the substrates on Signy where *E. murphyi* is present have become more humus-dominated than peat (pers. comm., P. Convey and Signy Station Leader, M. Jobson). Ultimately, faster litter turnover aided by *E. murphyi* (Hughes et al., 2013) will result in a richer, more humus-textured soil, which could benefit other

native and non-native fauna (Gabriel et al., 2001) and flora (Zefferman et al., 2015), and lead to further changes in terrestrial community structure.

The accidental introduction of *E. murphyi* to Signy Island is not a unique case for Antarctica but does represent perhaps the most successful and best studied invertebrate introduction outside of the sub-Antarctic region to date. Introduced to Signy with *E. murphyi* was the enchytraeid worm, *Christensenidrilus blocki* (Block et al., 1984), which remains persistent but far less abundant or widely distributed (Hughes and Worland, 2010). In neither instance was eradication attempted, with initial studies instead focusing on the physiology of *E. murphyi* (Block et al., 1984), rather than the risks it might pose as an invasive species. Now the distribution of *E. murphyi* is so extensive that eradication is not feasible without considerable and unacceptable damage to the larger ecosystem. Within the maritime Antarctic, another dipteran species, *Trichocera maculipennis*, was recently accidentally introduced to King George Island in the South Shetland Islands – this species was first discovered around the Uruguayan Artigas Base in 2006, and previously had only been recorded in the Northern Hemisphere (Volonterio et al., 2013). Eradication of this species from the affected sewage system has been attempted on more than one occasion but has not been successful (Uruguayan Antarctic Institute 2008; Volonterio et al., 2013). Introductions of Collembola (Greenslade, 1995; Greenslade et al., 2012; Greenslade and Wise, 1984; Wise, 1971), Acari, and spiders (Pugh, 1994, 2004) have also been recorded throughout the Antarctic, with 11 species of Collembola now naturalised on various sub-Antarctic islands (Greenslade et al., 2012), and a further 18 species on the verge of invading South Georgia where three exotic species are already present (Greenslade and Convey, 2012). Whilst these introductions are increasingly frequent (Chown et al., 2012 a, b), it remains the case that, to date, only *E. murphyi*, *C. blocki* and *T. maculipennis* have successfully established within the maritime Antarctic region for any duration.

The in-depth monitoring of *E. murphyi*'s spread within the current study has identified a potentially imminent risk to other valley systems on Signy Island. However, such monitoring of non-native invertebrate species is not currently present either as an advisory or a mandated requirement with either the Antarctic Treaty area or the various sub-Antarctic islands. Transfer in soil and associated

vegetation is a known means of anthropogenic transfer of *E. murphyi*, with such an event already having taken place from the species' native South Georgia to Rothera Research Station at 68 °S on the Antarctic Peninsula, *via* the treads of industrial plant equipment (Hughes et al., 2010). Current measures used to prevent the transfer of biological material into the Antarctic region and between different Antarctic locations, primarily rely on manual scrubbing of boots and equipment, and a boot wash containing the antimicrobial/viral disinfectant Virkon-S (SCAR, 2009; IAATO Guidelines, 2018; BAS Biosecurity Handbook, 2016). The efficacy of these measures in preventing invertebrate transfer however is unknown, and Virkon-S has never been tested as an insecticide. We suggest that it would be prudent to re-evaluate existing biosecurity procedures, such as recommended by Hughes et al. (2019), Hughes and Pertierra (2016) and a subsequent ATCM proposal (United Kingdom and Spain, 2018). A further species that would benefit from such regular monitoring would be the invasive predatory beetle *Trechisibus antarcticus* on South Georgia which, ironically, may have drastically reduced *E. murphyi* populations in its native range, along with other endemic invertebrate species (Convey et al., 2011; Ernsting, 1995). Its current distribution, and thus the magnitude of its impact on South Georgia's terrestrial invertebrate communities, remains unknown and unchallenged.

5.6 Conclusions

Eretmoptera murphyi has significantly expanded its range on Signy Island over the last decade, and this appears to be in direct association with routes of human movement across the island. The species' preferred habitat is moss bank with soil and peat substratum layers, largely on a northerly aspect, although this preference only explains a small proportion of variation in its population density. It is also unclear if *E. murphyi* is selecting peat substrates, or actively driving the production of peaty soils. A pre-adapted physiology to survive maritime Antarctic conditions and life history features that permit a flexible and opportunistic phenology, combined with anthropogenic intervention, has enabled *E. murphyi* to thrive, with likely further expansion to new areas on Signy in coming decades. This species potentially has a major ecosystem influence by opening nutrient cycling bottlenecks, and its full (negative or positive) impact on the native ecosystem and communities remains to be evaluated. Thus, there is an urgent need to investigate its role in wider ecosystem processes, as well as ensure

appropriate biosecurity protocols are in place to minimise both the rate of population expansion on Signy Island as well as prevent colonisation of other areas in the maritime Antarctic.

Chapter Transition

The increasing distribution of *E. murphyi* will have some level of impact on the terrestrial ecosystem on Signy Island. The next chapter evaluates these impacts through wide ranging baseline surveys, placed in the context of the island's overall patterns of nutrient cycling.

Chapter 6: Ecological consequences of a single introduced species to the Antarctic: Terrestrial impacts of the invasive midge *Eretmoptera murphyi* on Signy Island.

6.1 Abstract

As simple nutrient-poor ecosystems, the terrestrial soils of Antarctica are sensitive to change. Yet an increase in anthropogenic introductions means understanding the impact from non-native species is pertinent, and essential in developing future risk assessments. This study explores the impacts that have resulted from the introduction of the chironomid midge, *Eretmoptera murphyi,* to Signy Island, through comparative baseline assessments of vegetation, microbes, soil biochemistry, substrate composition, and micro-arthropod abundance. The key findings show that the introduction of *E. murphyi* to Signy Island has resulted in an increase in nitrogen availability within the nutrient-poor soils. We found that the invasive midge is increasing levels of available nitrates in the soils by three- to five-fold, and the addition may be impacting the ecosystem through changes in decomposition rates, with potential effects on the micro-arthropod community. We also measured the levels of nitrogen in habitats occupied by native vertebrate wildlife aggregations around the island and found the increase in nitrogen availability associated with *E. murphyi* is akin to deposition from seals. This is the first study in the Antarctic region to assess the impact of a non-native invertebrate introduction across trophic levels and adds to knowledge of maritime Antarctic terrestrial ecology. We suggest that these changes will only have greater impacts over time, potentially benefiting currently limited vascular plants and altering the vegetation community.

6.2 Introduction

The establishment of non-native species is considered one of the greatest threats to global biodiversity. With a changing climate likely to assist further species introductions in many regions, and an increase in anthropogenic dispersal, establishing the impact of existing invasions will be crucial in identifying high-risk species in the future (Chown et al., 2012a; Convey, 2011; Duffy et al.,

2017; Turney et al., 2016). Over the last few decades there have been several introductions, and subsequent establishments, of non-native invertebrate species in the Antarctic region, particularly in association with human activity (Hughes et al., 2015; Molina-Montenegro et al., 2010), yet little is understood of the ecological consequences that result. The simple terrestrial ecosystems of the polar regions have limited biodiversity and are characterised by low levels of soil nutrients and slow litter turnover (Beyer and Bölter, 2002; Smith, 2007). Energy and nutrient cycling are primarily driven by microbes and micro-arthropod detritivores within the soil community, and any alterations to the nutrient budget can have large consequences for ecosystem functioning (Davis, 1981; Koltz et al., 2017; Smith, 2007). In the future, as climate change drives its own impacts on terrestrial ecosystems, it may become harder to separate the impact of incoming invasive species from the effects of warming. Thus, regular impact assessments are required across a time-series of climate change to better understand how the two may interact (Yang and Gratton, 2014). Soil invertebrates are "underappreciated contributors to global ecosystems through their ecological interactions" (Schmitz et al., 2014), and understanding the manner in which they, whether native or invasive, interact with abiotic variables and affect ecosystem processes is a key frontier in ecology, and vital for predicting future change in terrestrial ecosystems (Yang and Gratton, 2014).

The polar regions are typically detritivore-dominated ecosystems, with most carbon processed from detrital sources, known as 'brown' energy (Koltz et al., 2017). This is especially true in terrestrial ecosystems that lack predatory or herbivorous species (Convey and McInnes, 2005; Davis, 1981; Hogg et al., 2006), and particularly so on the Antarctic continent where microbes are often at the top of the food chain and drive much of the nitrogen and carbon cycling (Cowan et al., 2010). In more fertile and temperate habitats, soil bacteria are typically more influential in mineralising carbon and nitrogen, whilst soil fungi are more important in nutrient-poor systems such as polar habitats, as they are more capable of breaking down tougher substrates such as slow-growing mosses (Wookey et al., 2009). In the maritime Antarctic, and additional to microbial activity, nutrient and litter turnover is also achieved by micro-arthropod groups such as Collembola and Acari (mites). There is also input from Chironomidae, which are present on the western Antarctic Peninsula and are the only native

higher terrestrial invertebrates found in the Antarctic region (Chown and Convey, 2016; Convey and Block, 1996; Convey, 2017).

The harsh environment of Antarctica combined with slow-growing vegetation and low biodiversity mean that decomposition is slow, and nutrient processing limited. Terrestrial Antarctic ecosystems have very low amounts of nutrients, with 0.05% nitrogen in the Antarctic Vestfold Hills for example (Leishman and Wild, 2001), compared to 1-5% in a temperate habitat (Polish calciferous grassland - Bednarek and Tkaczyk, 2002). Higher levels of soil nitrogen in Antarctic substrates are usually the result of direct or wind-blown guano, with penguins, other seabirds and seals being key contributors to soil nutrients throughout the Antarctic region (Ryan and Watkins, 1989; Simas et al., 2007; Speir and Cowling, 1984; Zhu et al., 2009). Orthinogenic deposition from birds is higher in ammonium than nitrates, a result of the uric acid content of bird guano, which may be beneficial to native plants. Rabert et al. (2017) found that the Antarctic grass *Deschampsia antarctica* increased biomass by 6.5-fold with ammonium fertilisation, and 4-fold with nitrate. However, seabird guano rich in ammonium has no, or even detrimental, effects on temperate plant growth, unless supplemented with nitrate (Szpak et al., 2012). In addition, microbial activity is higher in ornithogenic soils than in non-ornithogenic soils, which leads to faster decomposition of substrates (Roser et al., 1993; Tscherko et al., 2003; Zdanowski et al., 2005). Any alterations in nitrogen levels within nutrient-poor ecosystems, whether as a result of species introduction or significant environmental change, can therefore result in cascading effects through the soil into the vegetation. For example, a change in the size of chironomid swarms, deriving from an aquatic 'brown' food web, caused long term changes in terrestrial plant communities in the Arctic, purely by raising nitrate levels from carcass deposition (Dreyer et al., 2015). Plant biomass increased and graminoids slowly took over from dwarf shrubs, changing the floral community.

Changes in soil biochemistry and pH will also impact the microbial community, which plays a key role in nutrient cycling (e.g. Rousk et al., 2009; Zhang et al., 2017). Soil bacteria are essential nitrogen cyclers and fix atmospheric nitrogen into plant available forms. Other bacteria are crucial for

nitrification or denitrification, converting ammonia to nitrate, and nitrate to gaseous nitrogen, respectively. Similarly, fungi are essential in degrading the organic matter in the first instance. Capable of breaking down cellulose, starch and lignin-rich detritus such as is found at high latitudes (Robinson et al., 2002), they are often the starting point in the decomposition process, especially in ecosystems lacking in soil arthropods (Stehouwer, 2004). Where soil arthropods are present, accelerated litter turnover will initially benefit soil bacteria, that will consequently have greater access to organic compounds (Stehouwer, 2004).

Where soil invertebrates are introduced, particularly in ecosystems with low biodiversity, the impacts can affect the native flora and fauna community. For example, on the sub-Antarctic island of South Georgia, the introduction of two carabid species resulted in a decline in native detritivores, but an increase in the body size of one native herbivorous invertebrate, ultimately leading to negative impacts on local vegetation (Brandjes et al., 1999; Convey et al., 2011; Ernsting et al., 1995, 1999). In the Arctic, the accidental introduction of two species of Collembola is thought to present a threat to native Collembola species that are considered "characteristic of the Svalbard environment" (Coulson, 2015). In northern temperate ecosystems the invasion of earthworm species into forests that lack native earthworms has resulted in substantial changes to forest soils through alteration of nutrient turnover and storage, soil structure and composition (Alban and Berry, 1994). Because of the important role of earthworms in soil ecosystems, the invasion has resulted in cascading effects on soil flora and fauna, microbes, and even affects understory plant communities (Bohlen et al., 2004).

Signy Island (60 °S,45 °W, Fig 1.2) is typical of maritime Antarctic terrestrial ecosystems, with habitats dominated by cryptogams – primarily carpet and turf forming mosses – lichens and algae. Antarctica's two native vascular plant species, *D. antarctica* and *Colobanthus quitensis,* also occur on Signy Island, but are comparatively restricted in relation to the cryptogam community (Cannone et al., 2017; Smith, 1972, 1990). Signy's brown food web is of limited diversity, and like many maritime Antarctic systems is dominated by few Collembola and Acari species, as well as Nematoda, Tardigrada and Rotifera (Maslen and Convey, 2006; McInnes, 1995; Velasco-Castrillón et al., 2014).

The decomposition rate is limited by this lack of significant detritivore activity, but primary drivers are abiotic factors such as the cold climate, depth of the permafrost active layer, and high precipitation rates (Roads et al., 2014). These factors help to create large moss banks that are some of the best examples of their kind in the Southern Ocean area (Ochyra et al., 2008; Smith, 1972, 1990), and changes to the rate of decomposition may result in changes to this vegetation community (Britton et al., 2018).

The introduction of the parthenogenetic midge *E. murphyi* (Chironomidae, Orthocladiinae) to Signy Island in the 1960s has made it the largest terrestrial animal on the island and the only macro-detritivore. Since its introduction, the midge has significantly expanded its range around the Research Station (Bartlett et al., in review/ Chapter 5). *Eretmoptera murphyi* is locally highly abundant, outweighing the biomass of native micro-arthropods where they co-occur (Hughes et al., 2010, 2012). Understanding that this detritivore would likely affect litter turnover, Hughes et al. (2012) experimentally assessed its biomass and feeding rate compared to that of the native micro-arthropod community, finding that the midge was likely to be increasing decomposition by almost an order of magnitude. However, no studies have examined this in the field, or looked at the range of potential ecological impacts as a result of this introduction. Furthermore, few studies have examined terrestrial trophic relationships in the maritime Antarctic, whether from native or introduced species (Bokhorst and Convey, 2016). To our knowledge, only one study exists of the impact of terrestrial Chironomidae on ecosystem function, at any latitude, that of another invasive species in the sub-Antarctic (*L. minimus*) and its role in decomposition (Hänel and Chown, 1998). All other studies to date focus on aquatic species, usually in a public health context (e.g. Failla et al., 2015)

6.2.1 Aims of this study

This study aims to examine the impact of the invasive terrestrial chironomid *E. murphyi* on Signy and provide baseline data on interactions between the midge and the following variables: vegetation and micro-arthropod community, substrate composition, microbial abundance, and soil biochemistry in the form of soil organic carbon and soil nitrogen. This information can be used to build a greater

understanding of terrestrial Antarctic ecosystem functioning and the effects that can result from a single species introduction.

6.3 Materials and methods

All fieldwork was conducted on Signy Island during the 2016/2017 austral summer season. Laboratory work was conducted at the University of Birmingham, with the exception of the microbiology which was undertaken at the British Antarctic Survey headquarters in Cambridge.

6.3.1 Sampling grid

To establish the effect of *E. murphyi* abundance on the Signy Island terrestrial ecosystem, a grid of 4 x 4 points, 10 m apart was plotted using bamboo stakes on the 'Backslope' behind the British Antarctic Survey Research Station, in an area of known *E. murphyi* distribution (Bartlett et al., in review/ Chapter 5) (Fig. 6.1). Multiple biological and environmental variables were assessed in relation to *E. murphyi* abundance across the grid, and if relevant, also to one another.

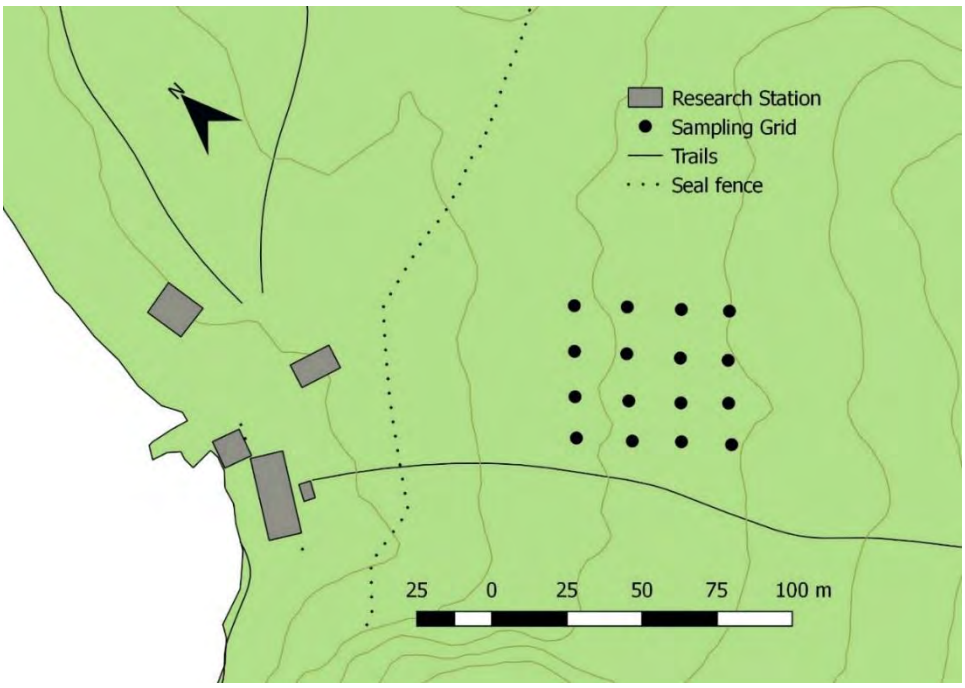

Figure 6.1. *Map of the 'backslope' area behind Signy Research Station, showing the location of the 'Sampling Grid', the seal fence and main footpaths/trails.*

6.3.1.1 Sample collection

Three substrate cores were collected from every grid point with a steel 2.5 cm ø soil corer, for the following analyses: invertebrate abundance, substrate composition (i.e. soil, peat, moss, inorganic), soil organic carbon (SOC), microbial abundance and pH. All cores were returned to the Signy laboratory in individual sterile sealed bags. Cores for invertebrate and substrate analysis were processed immediately. Cores for microbial analysis were frozen at -20 °C and returned to the United Kingdom (UK) by ship (10 weeks), and then stored at the University of Birmingham until analysis, remaining at -20 °C for the duration. Cores used for SOC analysis were also transported back to the UK as above but were kept at +4 °C. Appendix VII illustrates an example sampling regime at one grid point.

6.3.1.2 Invertebrate abundance

In order to ascertain *E. murphyi* and micro-arthropod abundance within the sampling grid, all invertebrates were extracted from the collected substrate cores. Individual *E. murphyi* larvae (instars L2-L4), *E. murphyi* egg sacs, Collembola and Acari were extracted from the cores by washing through stacked sieves (2, 0.5, 0.25 mm mesh sizes). All three invertebrate groups were then hand-picked from the remaining soil on the 0.25 mm sieve, with the 0.5 mm sieve checked for any further individuals. Prior to washing, clumps of moss or peat substrates were teased apart with tweezers and checked for any invertebrates prior to agitation and washing with the rest of the soil core.

Abundances of Acari, Collembola and *E. murphyi* larvae were averaged between the three replicate cores. In addition, for *E. murphyi*, the larval potential of the egg sacs was also calculated (cf. Bartlett et al., in review/ Chapter 5). Densities m^{-2} were estimated by calculating the surface area of the corer and scaling up (cf. Bartlett et al., in review/ Chapter 5, Section 5.3.3; Hughes and Worland, 2010).

6.3.1.3 Substrate composition

As it is suspected that *E. murphyi* may be influencing litter turnover and decomposition (Hughes et al., 2012), substrate composition was recorded at each grid point. Substrate cores (n = 3) were separated out into their constituent parts of live moss, peat, and soil. Stones and inorganic material

were removed, and each part/substrate type was weighed, dried to a constant mass at 60 °C and weighed again. The percentage of soil in the core was calculated from the subsequent dry mass. Substrate depth to the rock/gravel bed was measured *in situ* with a bamboo cane, marking the ground surface before removing and measuring the depth. This was repeated three times at each grid point, with the results averaged to give depth to the nearest 0.5 cm at that site.

6.3.1.4 Soil biochemistry

Detritivores such as terrestrial Chironomidae are noted influencers of nutrient cycling (Armitage et al., 1995; Dreyer et al., 2015; Schmitz et al., 2014), and the influence *E. murphyi* may have on such processes on Signy remains to be established. To assess this, available soil nitrogen was measured *in situ* with cation (CEM) and anion (AEM) ion-exchange membranes (AMI/CMI-7001, Membranes International, Ringwood USA). Strips of membrane were cut to 10 cm x 2.5 cm, and a hole punched through the top into which a string was tied to aid retrieval, with a knot tied in the CEM string to identify from AEM. Preparation and elution protocols followed Clark et al. (2011) and Sherrod et al. (2003), respectively as follows. Prior to installation in the field the membrane strips were washed with deionised water (dH_20 hereafter) to remove impurities and preconditioned as follows: CEM strips were saturated with H^+ by leaving overnight in 2M HCl; AEM strips were saturated with HCO_3^- by shaking overnight in 1 M $NaHCO_3$. In both instances, 100 mL per membrane strip was used. Membranes were then rinsed with dH_20, and CEM rinsed for a further 12 h in frequently changed dH_20 to remove traces of acid. The strips were inserted into the field sites in $n = 5$ pairs, where one pair is x1 AEM and x1 CEM strip side by side. All pairs were placed within 0.5 m of each other (Appendix VII). Care was taken to ensure the whole strip was submerged in substrate, with only strings remaining visible. They were left *in situ* for 14 d.

Upon retrieval, membranes were cleaned of substrate with dH_20, and ions eluted. Membranes were soaked for 3 h on a stirring plate, in either 0.5 M HCl (CEM) or 1 M NaCl (AEM), with 50 mL used per membrane (max 250 mL per set). The AEM/CEM solution for each replicate pair was then transferred into a sterile pre-labelled 50 mL centrifuge tube and frozen at -80 °C to be transported

back to the UK by ship (10 weeks), before transfer to the University of Birmingham and continued storage at -80 °C until analysis.

Prior to analysis, the eluted ions were thawed overnight at room temperature and then analysed immediately with a Skalar Continuous Flow Analyser (Chemistry unit SA3000 and interface SA8505). At the time of use the phosphate line was faulty, so only ammonia (NH_3), and nitrate (NO_3^-) analyses were conducted. The Skalar was run on mixed NH_3 and NO_3^- standards (0.2, 0.5, 0.8, 1.2, 3, 4 mgL^{-1}), using NH_4Cl and $NaNO_3$ respectively. The CEM and AEM sample solutions were pooled into one sample vial per replicate pair: 1 mL of each ion solution, with 1 mL of deionised water into a 3 mL vial. A 'drift analysis' (0.5 mg/L standard) was run every 10 samples to check the accuracy of the results, followed by a wash cycle before auto-sampling resumed sample processing. The following reagents were used for NH_3 analysis (Krom, 1980) (methods for all reagent and standard preparation in Appendix VIII): buffer solution – potassium sodium tartarate/tri-sodium citrate/Brij 35/HCl, sodium salicylate solution, sodium nitroprusside solution, sodium dichlororisocyanurate solution. These reagents were used for NO_3 analysis (Gal et al., 2004): buffer solution – Ammonium chloride/Ammonium hydroxide/ Brij 35, o-phosphoric acid colour reagent, distilled water and Brij 35. Final results were expressed as NH_3 and NO_3^- mgL^{-1}, and results were averaged per site to give total nitrogen (TN), NH_3, NO_3^- per grid point.

6.3.1.5 Soil organic carbon and C:N ratio calculations

Calculations of carbon to nitrogen ratios (C:N) are used as a proxy for decomposition rates of local substrates (Enríquez et al., 1993). Soil organic carbon (SOC) was calculated through loss of ignition analysis (LOI) (Ball, 1964; Heiri et al., 2001; MICCI, 2019). Approximately 5 g of soil from each core was placed in a pre-weighed ceramic crucible and oven dried at 50 ºC for 24 h. The dried soil was crushed to powder form and reweighed to the nearest 0.001 g. Powdered soil within the crucible was then heated to 110 ºC for 5 min to drive water off, before being placed in a muffle furnace at 550 ºC. After the final burn, the crucibles and soil therein were weighed again and SOC calculated (Eq. 1). An average %SOC was calculated between the three replicate cores.

$$\%\text{SOC} = 100 - \left\{\frac{(Wa - Wc)}{(Wb - Wc)}\right\} \times 100$$

Equation 6.1. *Calculation of soil organic carbon (SOC) as a percentage of soil mass. Where: Wa = Weight after burn; Wc = crucible weight; Wb = weight before burn (Ball, 1964).*

Calculations for the C:N ratio required the transformation of TN data from Section 6.3.1.4, and %SOC to both be in the same units. The simplest transformation was to convert to g/m^2 for both. This was done for nitrogen (mg/L) by first converting to g/L and then calculating the surface area of the ion-exchange membranes (2.5 cm x 10 cm). This was multiplied by two to reflect the pair of AEM and CEM and gave nitrogen in g/cm^2, from which g/m^2 could be calculated. Carbon was calculated through bulk density (BD) assessment of the soil analysed giving g/cm^3, which could be converted to g/m^2. SOC was calculated per gram and multiplied by the BD to give SOC g/m^2.

6.3.1.6 Habitat and vegetation survey

As changes to decomposition and soil nutrients may result in changes to the vegetation community (Dreyer et al., 2015; Stehouwer, 2004), vegetation surveys were also conducted at each grid point. Vegetation cover was surveyed using a 1 m^2 quadrat, with the bamboo grid point marker as the bottom left of the survey quadrat (Appendix VII) and using the classifications in Table 6.1. As *Polytrichum* spp. and other acrocarpous mosses compete (Juutinen et al. 2015), these were quantified separately. Although rare, the two vascular plant species, *D. antarctica* and *C. quitentis* do occur in the area so were included in the survey, as any changes in the abundance of vascular plants may be associated with changes in available nitrogen (Hill et al., 2011; Rabert et al., 2017). Overall habitat type (i.e. fellfield, moss bank, stream etc.) at each quadrat was also noted as an additional correlate. All cover results are expressed as a percentage of the entire quadrat.

Table 6.1. *Habitat type and field layer classifications used in the vegetation surveys. Shown with ID code used for surveys and analysis.*

Habitat type		Field Layer	
Type	**ID**	**Type**	**ID**
Stream	sm	*Deschampsia antarctica*	hr
standing water	sw	*Colobanthus quientis*	pw
Ridge	ri	*Polytrichum* spp.	pt
snow-bed	sb	Acrocarpous moss spp. (exc. pt)	ac
moss bank	mb	Polycarpus moss spp.	po
Meadow	me	*Prasiola crispa*	pc
rock outcrop	ro	Liverwort species	lw
Scree	sc	Lichen species	li
fjell field	ff	Mineral soil/bare ground	mi

6.3.1.7 Microbial abundance

Microbial abundance can have a strong influence on decomposition and is key in Antarctic systems (Barrett et al., 2006; Cowan et al., 2010). Microbes are also inextricably associated with soil invertebrates (Karsten and Drake, 1995), and form part of the diet and/or the gut microbiota of *E. murphyi* larvae (Bridge and Denton, 2006). In order to establish any relationship between *E. murphyi* and microbial abundance in the soils, the following work was conducted in laboratories of the British Antarctic Survey. In all instances sterile technique was followed and all work with samples, media and consumables conducted in a laminar flow cabinet using pre-sealed, or alcohol-flamed, sterile tools. The microbial load of the soil cores from each grid point was assessed by plating, incubating and enumerating colony forming units of both fungi and bacteria (CFUs). Each grid point had $n = 3$ replicate cores, and for the purposes of this experiment, each replicate was treated as a separate sample, with $n = 3$ of the following dilutions made for each replicate core. This gave a total of 144 plates between the 16 sample sites.

Soil cores were removed from the -20 ºC storage but kept in their respective sealed bags and left to thaw overnight at +15 ºC, after which 1 g of soil was shaved off into a 15 mL Falcon tube. Sterile water (100 mL) was added, and the solution vortexed into suspension for 10 min. Before the solution settled, 1 mL of the suspension was transferred to a new 15 mL sterile Falcon tube, from which serial

dilutions were then taken. Dilutions of 10^{-1} and 10^{-2} were used for the final enumerations, with appropriate calculations taken and final results all reported as 10^{0}. Initially two types of media were used in the trials: Czapek Dox (CD) agar with added Rose-Bengal for fungal growth (Hughes et al., 2003b), and 10% Tryptic Soy Agar (TSA) for bacterial growth (Goordial et al., 2016). However, CD did not work well over a 14 day incubation whilst TSA produced both fungal and bacterial growth, therefore only TSA was used in the final experiments. The TSA agar was made to 10% as follows: 500 mL of sterile water was mixed with 2 g TSA and 6.75 g of bacteriological agar (Agar No. 1). Sterile agar was plated into single-vented Petri dishes, and 100 µL of the soil dilution plated with a sterile L-shaped spreader, with each dilution from each replicate on a separate labelled plate. Plates were stored upside-down and incubated for 14 day at 9 ºC.

After the incubation period, CFUs for bacterial and fungal colonies from each plate were enumerated. CFUs were averaged between each of the three replicates to obtain an average per core, and then again between the cores to obtain an average per site. The remaining soil samples were returned to the University of Birmingham at +4 ºC, where pH analysis was conducted.

6.3.2 Local and island-wide nitrogen levels

To ascertain background levels of soil nitrogen, several sites from the area local to current *E. murphyi* distribution were sampled. A grid of 35 points 100 m apart was plotted over the entire area adjacent to the research station, covering an area of 490,000 m^2, and incorporating the entirety of the known *E. murphyi* distribution (cf. Bartlett et al., in review/ Chapter 5) (Appendix IX). These points were assigned numbers, of which 12 were randomly selected using a number generator. Selected sites were then analysed for soil biogeochemistry using ion-exchange membranes as described in Section 6.3.1.4. Proximity of these sites to wildlife activity was noted, along with habitat type and general site characteristics (Table 6.2). Sites with no relationship to either *E. murphyi* distributions or wildlife colonies in this area were considered ‘control’ sites.

Additional to local background data, ion-exchange membranes were also deployed to key wildlife sites, and moss banks similar to *E. murphyi* habitats, around Signy. This provided assessment of the scale of any measured change associated with *E. murphyi* abundance, in relation to the regular deposition seen from native marine vertebrate wildlife colonies and concentrations that are the current major contributors to most Antarctic terrestrial ecosystems (e.g. Zhu et al., 2009). Surveys of uncolonized habitats served as additional 'control' sites far removed from both *E. murphyi* and wildlife activity (Table 6.2). One site on Cummings Ridge was considered a 'mixed control' due to the proximity of seabirds and a substantial seal presence upwind. The protocol was as above, with n = 5 pairs of membranes inserted into substrates within the colony/ habitat (Table 6.2).

Table 6.2. *Background samples sites, both the 'local' grid and 'wildlife/habitat' sites with site name (either geographical or allocated if from the 100 m grid), classification of wildlife colony or control and latitude and longitude information. Description and relevant* E. murphyi *distribution from Bartlett et al. (In review/Chapter 5).*

Site	Classification	Description	Latitude	Longitude
Cryptogam Ridge	Control mixed	Moss bank, adjacent sea birds (< 5 m)	-60.7264	-45.6659
Elephant Seals	Elephant seal	Bare ground, adjacent seals (< 5 m)	-60.7038	-45.609
Strombus Ridge	Giant petrels	Moss bank, in colony area	-60.6975	-45.648
Moss Braes	Control	Moss bank, pristine	-60.679	-45.629
North Point	Penguins	Bare ground, in colony area	-60.6749	-45.6248
Gourlay Peninsular	Fur seals	Bare ground, in colony area	-60.7284	-45.5903
1-100	Wildlife mixed	Rocky outcrop, regular activity from fur seals and sea birds	-60.7062	-45.5923
2-100	Fur seals	Bare ground, in colony area	-60.7067	-45.594
4-100	Control	Fellfield, gully	-60.7076	-45.6012
9-100	Control	Moss bank, irregular fur seal activity	-60.7085	-45.5994
10-100	Wildlife Mixed	Moss bank, adj. seals (< 5 m). *E. murphyi* area – 1000 L/m^2	-60.7088	-45.5958
13-100	Control	Moss bank, pristine	-60.7085	-45.5905
17-100	Control Mixed	Moss bank under sea bird cliff. *E. murphyi* area – no presence	-60.7094	-45.5977
33-100	Control	Fellfield, ridge	-60.7111	-45.5941
35-100	Control	Moss bank, ridge	-60.7111	-45.5906

6.3.3 Statistical analyses

Data from the 'Sampling Grid' were examined for outliers using Grubbs analysis (Grubbs, 1950) with an 0.05 alpha, which were then removed before plotting into regression charts and analysed for significant correlations with a Spearman's correlation matrix. Differences between nitrogen levels of sites were explored with either a Mann-Whitney U test, or a Kruskal-Wallis test, after a Shapiro-Wilkes normality test (alpha = 0.05). Analyses were carried out with a combination of XLSTAT and Prism 7.0.

6.4 Results

6.4.1 Sampling grid

Overall, habitat type varied little from 'moss bank' so was not considered further. Vegetation types from the vegetation quadrat survey that resulted in either nil or very low results were excluded from further analysis. These included: polycarpous moss spp., liverworts, *Prasiola crispa*, *D. antarctica* and *C. quientis*. Correlations between the remaining variables surveyed in the 'sampling grid' are listed in Table 6.3. *Eretmoptera murphyi* larvae were most abundant at Site 1, with an average of 43 larvae between three cores giving 83,807 larvae m^{-2} (See Methods, Section 6.3.1.2). All data with outliers removed are presented in Appendix X.

The most significant positive associations were between *E. murphyi* and nitrogen measures, in particular total nitrogen (TN) with $r = 0.80$, $p < 0.0001$ (*E. murphyi vs* NO_3^- $r = 0.63$, $p < 0.01$; *E. murphyi vs* NH_3: $r = 0.78$, $p < 0.01$). Figure 6.2 shows the most significant correlations of the surveyed variables with *E. murphyi* abundance. Other strong positive relationships identified by the correlation matrix (Table 6.3) were between Collembola (C) and nitrate and TN (C *vs* NO_3^- $r = 0.62$, $p < 0.05$; C *vs* TN: $r = 0.68$, $p < 0.05$). There was a strong negative relationship between the C:N ratio, Collembola ($r = -0.65$, $p < 0.05$), soil (%) ($r = -0.86$, $p < 0.001$) and also *E. murphyi* ($r = -0.53$, $p = 0.06$), with bacteria and prevalence of bare ground also negatively influencing C:N ($r = -0.48$ and -0.46, respectively). Further significant negative relationships were found between moss cover (%),

lichen and *Polytrichum* spp. (respectively: $r = -0.68$, $p < 0.01$; $r = 0.63$, $p < 0.05$). Bacteria had a negative association with SOC ($r = -0.67$, $p < 0.05$), whilst fungi had a positive relationship with SOC ($r = 0.62$, $p < 0.05$) (Fig. 6.3c). Results for pH had little variability (4.54 ± 0.2) and were initially only recorded for reference to bacterial and fungal abundance, on which it had no effect (linear regression, bacteria *vs* pH: $F_{(1,12)} = 0.03$, $r^2 = 0.002$, $p = 0.86$; fungi *vs* pH: $F_{(1,12)} = 0.98$, $r^2 = 0.07$, $p = 0.34$).

Table 6.3. *Mean, standard deviation and results from Spearman correlation of variables from the 'sampling grid'. Shaded values are different from 0 with a significance level alpha = 0.05. Values with an additional asterisk are significant to alpha = 0.01. Em =* E. murphyi*; Tot N = total nitrogen; Coll. = Collembola; Tot. Inv = Collembola and Acari combined; Moss% = non-*Polytrichum *moss spp; Polyt. =* Polytrichum *moss spp; Min. Soil = mineral soil/bare ground; Bact. = bacteria abundance.*

	Variable	M	SD	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	Em	7.06	12.10	-															
2	NH3	0.87	0.37	**0.78 ***															
3	NO3	2.27	2.21	**0.63 ***	0.61														
4	Tot. N	3.29	2.60	**0.80 ***	**0.77 ***	**0.94 ***													
5	SOC	65.20	22.59	-0.26	0.09	-0.06	-0.21												
6	Acari	0.86	1.01	0.17	0.09	0.18	0.24	-0.09											
7	Coll.	12.94	12.29	0.23	0.37	**0.62**	**0.69**	-0.17	0.51										
8	Tot. Inv.	14.19	13.56	0.31	0.38	0.58	**0.68**	-0.26	0.60	**0.97 ***									
9	Moss	85.00	24.15	-0.11	0.13	0.16	0.09	-0.01	0.48	0.38	0.25								
10	Soil	12.93	15.76	0.50	0.46	0.19	0.42	-0.57	-0.02	0.44	0.44	0.05							
11	Depth	82.23	30.71	-0.19	-0.11	-0.30	-0.31	0.26	-0.23	-0.43	-0.42	-0.17	-0.54						
12	Polyt.	6.46	13.59	-0.15	-0.09	-0.27	-0.26	0.16	-0.55	-0.57	**-0.61**	**-0.63**	-0.22	0.40					
13	Lichen	4.54	13.73	0.37	0.33	-0.08	0.07	-0.01	-0.09	-0.26	-0.09	**-0.68 ***	0.00	0.13	0.21				
14	Min. Soil	3.08	11.09	-0.08	0.04	-0.31	-0.13	-0.48	-0.36	-0.04	-0.04	-0.46	0.46	-0.04	0.37	0.21			
15	Bact.	7350.85	5943	0.12	-0.10	-0.17	0.05	**-0.67**	0.24	0.18	0.32	-0.26	0.48	-0.17	0.00	0.37	0.46		
16	Fungi	700.85	429.98	0.03	0.35	0.09	0.04	**0.62**	-0.26	-0.27	-0.25	-0.37	-0.25	0.20	0.19	0.55	0.00	-0.18	
17	C:N	216.43	236.68	-0.53	-0.38	-0.48	**-0.64**	**0.59**	-0.11	**-0.65**	**-0.66**	0.09	**-0.86**	0.53	0.21	0.02	-0.46	-0.48	0.30

Further interrogation of the abundance data found that low abundances of *E. murphyi* were associated with low TN, with the five lowest midge counts also associated with the five lowest TN levels, whilst this was not true of Collembola abundance, which had a count of 24,037 at the site with the 2nd lowest TN level (Appendix XI). This suggests that midge abundance is more influential on soil nitrogen levels than Collembola abundance.

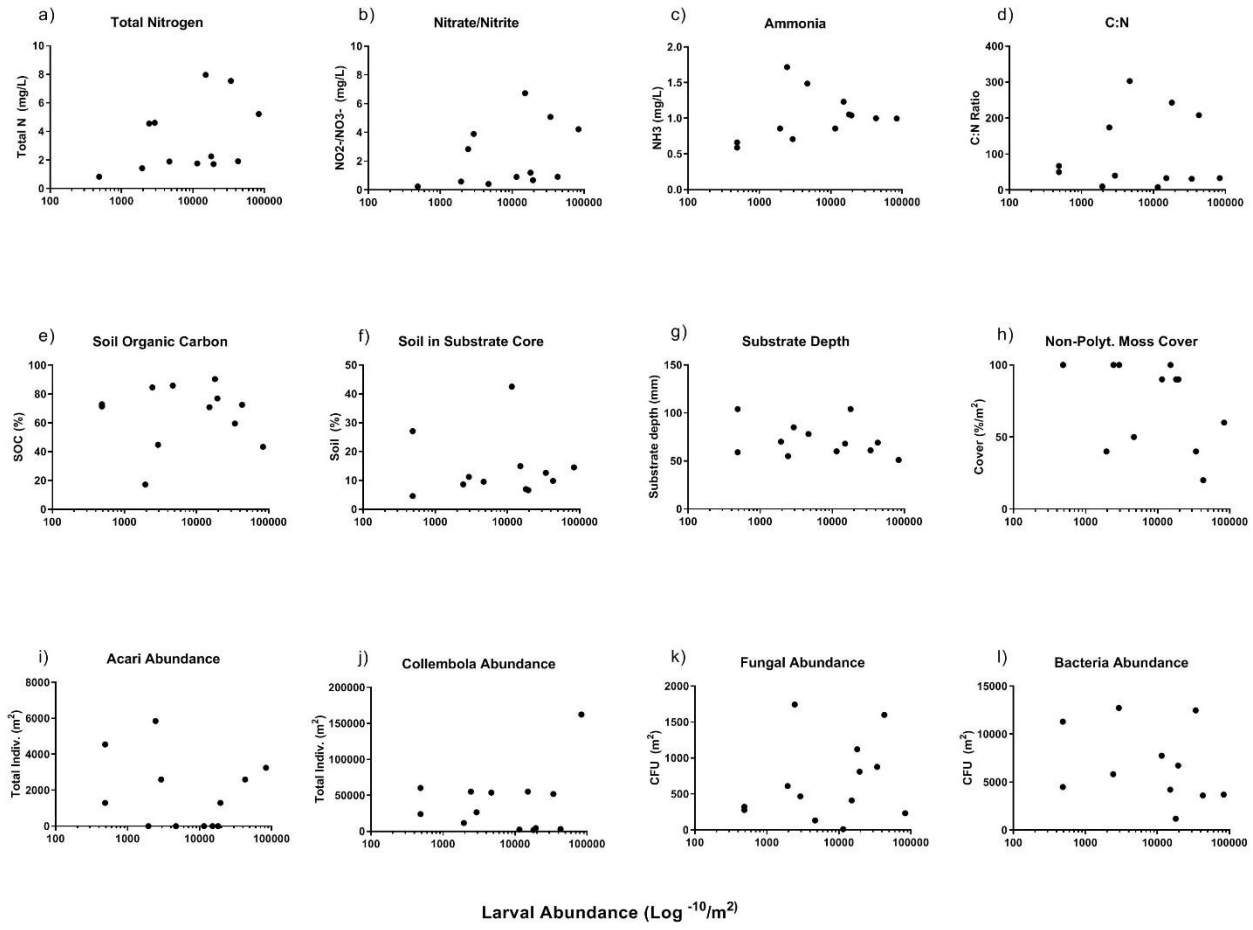

Figure 6.2. *Scatterplots and associated trends of* E. murphyi *abundance and surveyed variables from the sampling grid. n = 16 sites sampled with n = 3 cores from each site assessed for larvae abundance. Max abundance 83,807 larvae/m*^{2}.

6.4.2 Local and island-wide nitrogen levels

All sites with *E. murphyi* activity were pooled together (including the sampling grid points), as were wildlife colonies (i.e. all seal sites pooled), and control sites from around the island. Soil biochemical analyses from the different sites identified that penguin colonies had the highest level of nitrogen deposition, primarily driven by higher levels of ammonia, which were between 2.5 and 5 times as high as other seabird sites (Fig. 6.4 and 6.5). Consequently, penguins differed significantly from giant petrel and seal colonies, across all nitrogen measures (Kruskal-Wallis: TN, $H = 8.9$, $p = 0.01$; NH_3, $H = 8.6$, $p = 0.01$; NO_3^-, $H = 9.2$, $p < 0.01$) (Fig. 6.5 a-c). As penguin colonies differed so much from other wildlife sites, they were left out of comparisons with *E. murphyi* in further analyses. In comparison to the giant petrel and seal colonies, *E. murphyi* sites (including those from the sampling grid) were only significantly different in ammonia levels (Kruskal-Wallis: NH_3, $H = 13.19$, $p = 0.001$).

There was no difference in the levels of nitrate between *E. murphyi* and these wildlife colonies (NO_3^-, $H = 1.0$, $p = 0.5$), and consequently, no difference in overall TN levels (TN, $H = 2.5$, $p = 0.2$) (Fig 6.5 d-f). Sites with *E. murphyi* presence had significantly higher levels of TN compared to controls (MWU = 72, $p < 0.001$) as a result of an increase in nitrate availability (NO_3^- MWU = 101, $p < 0.0001$). There was no difference in ammonia levels between control sites and *E. murphyi* sites (NH_3, MWU = 228, $p = 0.8$) (Fig. 6.5 g-i).

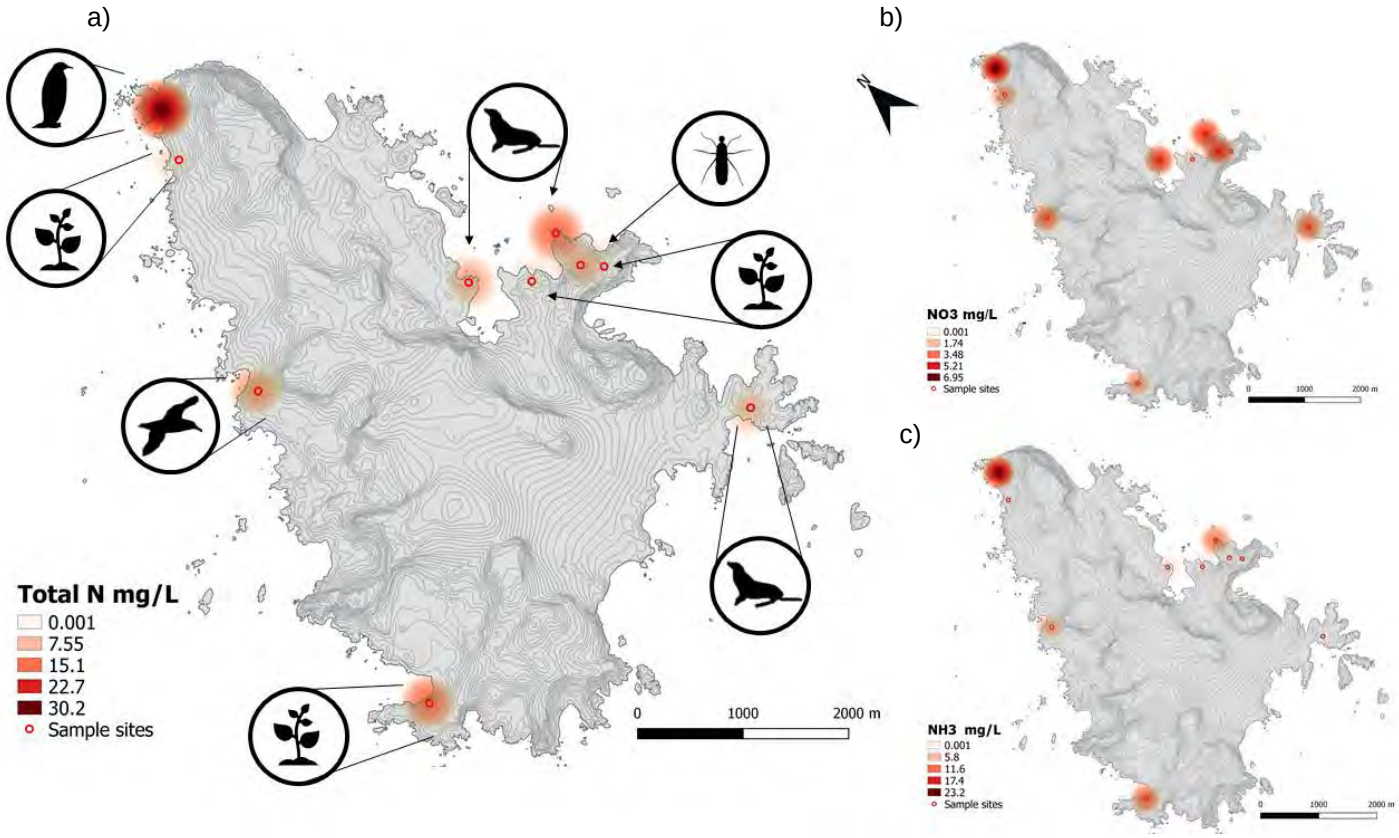

Figure 6.3. *Map of Signy Island sites sampled: penguin colonies, giant petrel (GP) colonies, Seal concentrations,* E. murphyi *location, and background habitats of 'pristine sites' and sites with some sea bird (GP) activity. Soil nitrogen represented relative to one another as red 'clouds'. a) Total nitrogen of sites, b) Nitrate,(NO_3) c) Ammonia (NH_3).*

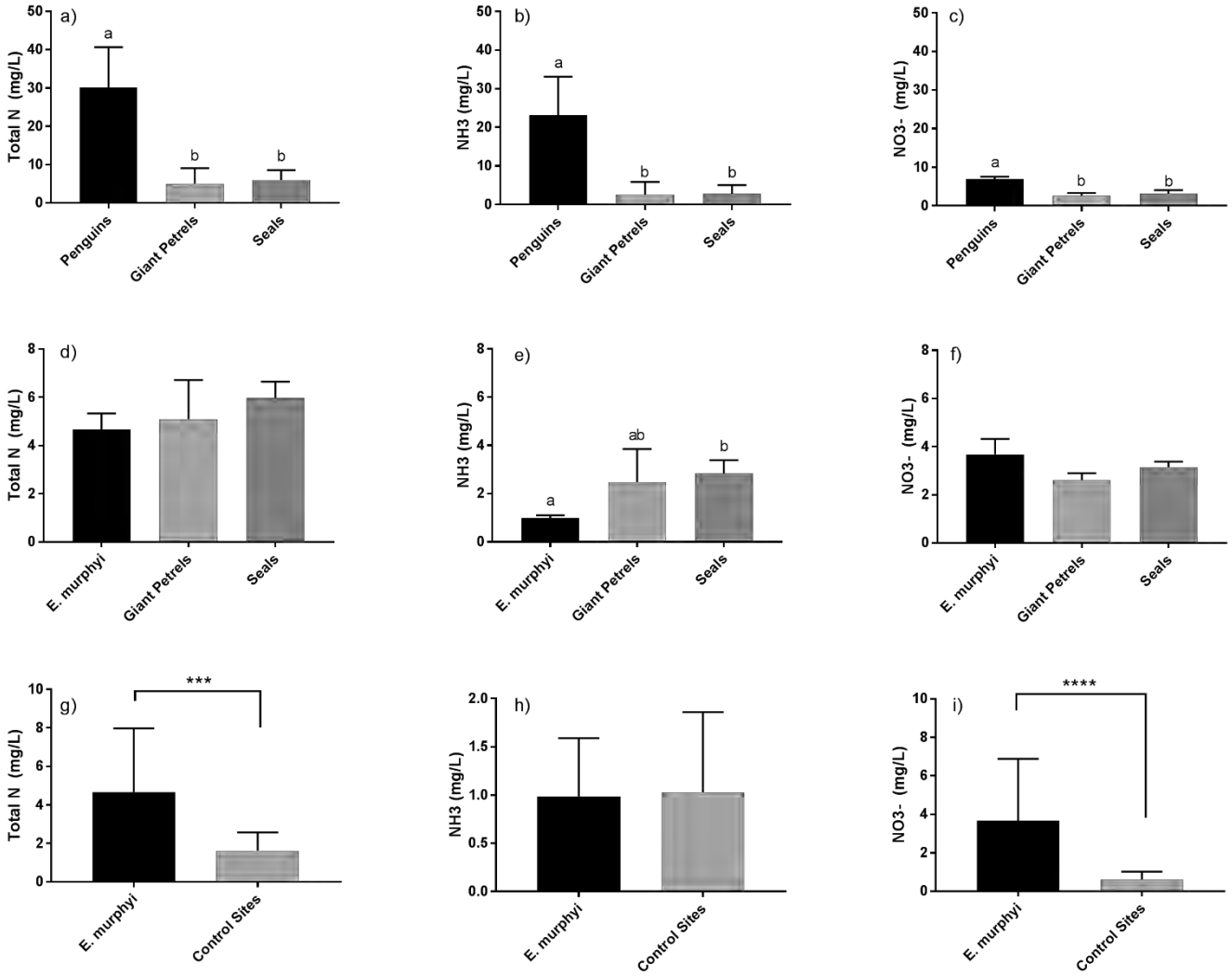

Figure 6.4. *Charts a-c, amongst wildlife colonies, Kruskal-Wallis: Total N, H = 8.9, p = 0.01; NH_3, H = 8.6, p = 0.01; NO_3: H = 9.2, p < 0.01 Charts d-f, amongst* E. murphyi *and key wildlife colonies, Kruskal-Wallis: Total N, H = 2.5, p = 0.2; NH_3, H = 13.19, p = 0.001; NO_3, H = 1.0, p = 0.5; NO_3, H = 9.2, p = 0.009. Charts g-i,* E. murphyi vs *Control (non-wildlife), Mann-Whitney U test: Total N, MWU= 72, p < 0.001; NO_3, MWU = 101, p < 0.0001.*

6.5 Discussion

The key findings from this study show that the presence of *E. murphyi* within the terrestrial Antarctic ecosystem of Signy Island is associated with an increase in nitrogen availability in the nutrient-poor

soils. Crucially, the increase in nitrogen is in the form of nitrates (NO_3) (Fig. 6.5i), which can be readily taken up by plants, to be utilised in the production of chlorophyll and plant growth (Bijlsma et al., 2000). The scale of nitrogen fertilisation associated with *E. murphyi* was similar to that around giant petrel and seal colonies, but lower than that associated with penguin colonies, which had significantly higher ammonia levels (Fig. 6.5 b, e). Ammonia levels in *E. murphyi* areas were roughly equivalent to that of control sites (Fig. 6.5h). The lower levels of ammonia seen in association with *E. murphyi* indicate that the nitrogen is not a result of wind dispersal, or even direct deposition from neighbouring seal or seabird concentrations that are found in Factory Cove and on Factory Bluffs, respectively, ~150-200 m away. This is exemplified by the low nitrogen levels found in areas directly underneath the Factory Bluff cliffs, which is closer to both seal and seabird activity (Appendix IX). Overall, nitrate availability increased by three-five times in association with the presence of *E. murphyi*. As a soil detritivore, an increase of nitrogen deposition is not surprising as this group of invertebrates are known to influence terrestrial nutrient cycling (Yang and Gratton, 2014). It is notable that the midge is raising available nitrate levels to a level comparable to those found in vertebrate colonies, on an island of limited nutrient resource. Dreyer et al. (2015) found that chironomid carcass deposition alone was enough to increase available nitrogen, also by three- to five-fold, in an Arctic ecosystem. In an analogous fashion, the introduction and/or range expansions of earthworms in the Northern Hemisphere is significantly increasing available soil nitrogen, with cascading effects on terrestrial ecosystems (Bohlen et al., 2004; Ozawa et al., 2005).

Eretmoptera murphyi abundance was positively associated with micro-arthropod abundance, notably Collembola, with Acari having much lower abundance overall. Both *E. murphyi* and Collembola can influence available soil nitrogen, and thus were negatively associated with the C:N ratio (Fig 6.3b). However, on interrogation of the raw data a case can be made for the midge having a stronger influence on soil nitrogen levels: the five lowest TN levels in the 16-point sampling grid include the four lowest *E. murphyi* counts, but the 6th, 8th and 9th highest Collembola counts. Furthermore, a count of over 24,000 m^{-2} Collembola was associated with the 2nd lowest TN level (Appendix XI). Whilst midge egg batches were included in the counts, eggs of micro-arthropods were not due to their small

size (i.e. not easily visible), so numbers of Collembola could have been underestimated at some sites. However, only two *E. murphyi* eggs sacs were collected across the 16 sites and will not have affected the correlations identified. The positive relationship between the midge and Collembola may be explained by recent research conducted on Signy Island micro-arthropod populations. Bokhorst and Convey (2016) reported that abundances of some Collembola and Acari are positively correlated to nitrogen concentrations in cryptogam species on the island. They found that nitrogen originating from penguin colonies was taken up by moss and passed to the micro-arthropods through the brown food web. Collembola are omnivorous detritivores and will consume dead organic matter, algae, lichens and microbes that have, in turn, assimilated available nitrogen (Block, 1985; Bokhorst et al., 2007b; Broady, 1979; Cannon, 1986). They will therefore associate with higher quality litter, aiding decomposition in the process. Furthermore, in temperate systems Collembola are attracted to earthworm excreta, depending on the nitrogen contained therein, with those of lower ammonia content and higher nitrates proving more favourable (Gutiérrez-López et al., 2011). Therefore, *E. murphyi* frass may be attracting native micro-arthropods, through the increases in nitrogen, to the brown food web and in particular increasing the level of favourable nitrates, hence the strong correlation of Collembola and *E. murphyi* with both increasing nitrogen and decreasing C:N.

Whilst no significant correlations were found between microbial abundance and *E. murphyi* abundance, there was a strong and differing effect of bacteria and fungi on SOC, which in turn could influence the C:N ratio. Soil bacterial loads were negatively associated with SOC (and subsequently C:N), whilst fungi were positively associated. This has also been reported in alpine ecosystems, where the divergence in roles is considered as a potentially stabilising effect on soil biochemical process, particularly those under thermal stress (Zhang et al, 2017). In these ecosystems, a high C:N ratio is the result of low-quality litter from slow growing mosses that retain carbon, low nitrogen levels, and the subsequent low rates of nutrient turnover - a framework that is associated with fungal-dominated food webs and their consumers, Collembola and Acari (Bardgett, 2005). These features describe the terrestrial ecosystem of Signy Island well. Whilst results from this investigation did not find a significant change in either bacterial or fungal load in association with increased midge abundance,

the relationship requires further exploration, particularly as fungi have been found in the guts of *E. murphyi* (Bridge and Denton, 2006). Furthermore, it is worth considering the role of nitrogen-fixing bacterial communities in association with *E. murphyi* abundance. For example, earthworms are found to increase soil nitrogen levels, in part because their gut provides a favourable habitat for nitrogen-fixing bacteria which are then excreted into the soil (Karsten and Drake, 1995, 1997). It remains unclear if *E. murphyi* has a distinct diet compared to the resident Collembola or Acari, which might explain why their abundance is more strongly associated with increases in N, or whether the midge is processing the same diet in a slightly different way, for instance as a result of different microbiomes.

Fragmentation of substrates through detritivory releases stored carbon, some of which will be assimilated by the invertebrate or microbial decomposers, and some which will be washed away as particulate matter (Wardle and Lavelle, 1997; Wookey et al., 2009). Combined with increased nutrient deposition from excreta, detritivory can alter the C:N ratio, which is a useful proxy for decomposition (Enríquez et al., 1993). Where *E. murphyi* occurs, the associated increase in N and reduction in SOC reduces the C:N ratio and attracts other micro-arthropods which may also affect those values, amplifying the influence of the midge. The C:N ratio in places with no midge activity was very high at 600:1. This was greatly reduced to 8:1 in places with high *E. murphyi* abundance, highlighting their potential role in accelerating decomposition. This supports the experimental findings for *E. murphyi* of Hughes et al. (2012) who found that, based on the midge's feeding rate, it is capable of increasing litter turnover by almost an order of magnitude compared to Signy's micro-arthropod community alone. Chironomid larvae typically feed on dead organic matter, but like Collembola have been found to also feed on lichen, so it is possible that they share food resources when occupying the same area (Bokhorst et al., 2007b; Delettre, 2000). However, at certain sites, *E. murphyi* biomass can exceed that of all other micro-arthropods and micro-invertebrates combined (Collembola, Acari, nematodes, rotifers, tardigrades and protozoa) by two to five-fold (Hughes et al., 2012). It is therefore likely that it is the size, and appetite, of the invasive detritivore driving these changes in decomposition and nutrient cycling.

Another invasive chironomid, *Limnophyes minimus* is also a significant detritivore on sub-Antarctic Marion Island (Hänel and Chown, 1998; Smith, 2007). It is also capable of increasing litter-turnover by an order of magnitude more than the next most influential species on the island, a native moth, and is considered a keystone species in the terrestrial ecosystem (Klok and Chown, 1997). The correlation of increased *E. murphyi* abundance and higher levels of soil and decreased moss bank depth, could be indicative of the midge accelerating decomposition and altering substrate composition. This would suggest that, as the midges are turning over the litter, they are reducing the volume of the moss banks and producing a more nutrient-rich soil in place of the dead moss. In a study of the potential effects of introduced detritivores such as earthworms, isopods and millipedes in a high-latitude ecosystem, van Geffen et al. (2011) found that decomposition was significantly stimulated. They concluded that potential range expansion of detritivores, as a result of climate change at high latitudes, will result in accelerated decomposition and an increase in the carbon flux. This in turn can lead to new habitats, and new niches for species, both native and non-native, to fill.

A C:N ratio above 35 will slow any decomposition and one as high as 600, as was found in this study, would indicate a composting rate akin to fresh wood (Tramoy et al., 2017). Polar regions are large sinks of atmospheric carbon as they respire less through decomposition than is gained through photosynthesis (Jonasson et al., 2001), and decomposition rates are constrained by low temperatures, water saturation and anoxic site conditions, substrate acidity and high concentrations of secondary compounds, such as lignin (Robinson, 2002). Consequently, Arctic and Antarctic terrestrial ecosystems tend to have a high C:N ratio. Furthermore, as nitrogen concentration in cryptogams decreases with an increase in latitude (Speed et al., 2009), individual tundra species in the Arctic and Antarctic also have a medium to high C:N ratio: mosses between 50 and 100 and lichens in excess of 100, occasionally as high as 200 (Lee et al., 2009). These factors combined with Signy's low background nitrogen levels, and environmental conditions that allow carbon to remain stored in moss that is periodically frozen for long stretches of time, or even preserved in permafrost (Roads et al., 2014), may explain the exceptionally high C:N ratio found here. Where moss banks on Signy are drier, decomposition is slower still with site moisture speeding up vegetation decomposition (Davis,

1980). At the other end of the hydration spectrum, permanent or frequent inundation to form saturated mires will also slow decomposition (Moore, 2002). Thus, faster decomposition is likely a factor of both increased detritivore activity, and moist but neither saturated nor dry, substrate conditions. It also bears consideration that such conditions may also be what attracts the midge in the first instance, as both Bartlett et al. (2018a) and Hughes et al. (2010) suggest a positive relationship between *E. murphyi* and substrate moisture as a possible driver of patchy distribution.

There may be a tipping point in midge abundance that leads to a rapid decline in non-*Polytrichum* moss cover (Fig. 6.2h). Changes in decomposition rates, in combination with higher levels of available nitrogen, are likely to affect local vegetation in the long term. Britton et al. (2018) found that increasing nitrogen in an alpine environment decreased the C:N ratio, which in turn enhanced decomposition. This change in soil biochemistry depleted moss cover, but favoured graminoids resulting in changes to the vegetation community. Similarly, long term nitrogen increases in both Arctic and alpine ecosystems resulted in declines in all cryptogams evaluated (Brancaleoni et al., 2009; Chapin et al., 1995; Nilsson et al., 2002). Both lichen and *Polytrichum* cover were negatively associated with non-*Polytrichum* moss cover, suggesting that they may also be drivers of cryptogam community structure . It is worth noting that *Polytrichum* moss species can benefit from increased nitrogen, unlike some other bryophytes, and thus will have a competitive advantage over other moss species over time, in high-nitrogen areas (Juutinen et al., 2015). The moss community on Signy Island is presently dominated by carpet-forming species such as *Andreaea depressinervis*, and previous work has found that *Polytrichum alpestre*, a turf forming moss on Signy, creates a more aerobic and warmer below-ground environment (Davey et al., 1992) – an increase in the latter over the former may therefore further affect rates of decomposition which is accelerated by warming, and also the microhabitat experienced by invertebrates (Bartlett et al., 2018b/ Chapter 3; Hayward et al., 2003; Yergeau et al., 2012).

Whilst the bulk of Signy's vegetation is in the form of moss banks (Smith, 1972, 1990; Ochyra et al., 2008), fellfield is the dominant habitat with the graminoid *D. antarctica* and pearlwort *C. quitensis*

the only representatives of vascular flora (Cannone et al., 2017; Smith, 1972, 1990;). Rabert et al., (2017) found that these two species do benefit from additional nitrogen, with increases in biomass recorded during experimental fertilisation of both ammonia and nitrates. Neither of the vascular plant species were found in our surveys yet they are known to occur in the same area and would likely benefit from the association with *E. murphyi* and nitrogen increase (Bokhorst et al., 2007a). Studies of nitrogen fertilisation in northern tundra ecosystems find that graminoids, and particularly *Deschampsia* species, are likely to out-compete cryptogams, herbaceous plants and even shrubs, if given extra nitrogen (e.g. Gasarch and Seastedt, 2015; Dreyer et al., 2015). In Antarctica, Hill et al. (2011) reported that *D. antarctica* can acquire available nitrogen 160 times faster than the Antarctic mosses that it competes with. Furthermore, they suggest that climate warming will accelerate the spread of *D. antarctica* as more nitrogen becomes available through enhanced decomposition. Consequently, long-term nitrogen addition to Antarctic terrestrial ecosystems, combined with warming, could result in changes to the floral community with graminoids dominating over mosses. These changes may also make it easier for non-native vascular species to colonise (Pertierra et al., 2017), and facilitate the establishment of not-native species in general.

As climate change is predicted to warm the polar regions faster than anywhere else, the combined influence on invading species and warming cannot go unremarked (Convey et al., 2009a; Vaughan et al., 2003). Alien invertebrate species are likely to perform better under climate change than local species, and so their impact on ecosystems will be further accelerated in a warming climate (Janion-Scheepers et al., 2018). Even without the introduction of a disruptive invertebrate it is likely that decomposition rates will accelerate with increasing temperatures, as more water is made available and microbial activity enhanced by increasingly favourable conditions (Bokhorst et al., 2011; Yergeau et al., 2012). Furthermore, Bokhorst et al. (2011) reported that, in the maritime Antarctic, decomposition under a warming climate is especially influenced by increased soil nitrogen availability. A warming Antarctic may also result in changes to the vegetation community, as vascular plants can take advantage of longer growing seasons, milder conditions and expand both their biomass potential and range of distribution (Bokhorst et al., 2007a; Green et al., 2011). Climate change will increase ice-free

habitats for native and introduced species to expand into and increase the chances of successful colonisation, particularly of alien invertebrates (Frenot et al., 2005; Lee et al., 2017; Philips et al., 2017; Pertierra et al., In review. – Appendix XII).

6.6 Conclusions

This study marks only the second investigation of a terrestrial chironomid on ecosystem function and the first study to address associated impacts on nitrogen availability. It is also the most comprehensive investigation in the maritime Antarctic to assess the impact of a non-native invertebrate introduction. *Eretmoptera murphyi*'s success on Signy Island has provided an opportunity to consider the impacts of an alien invertebrate species on vulnerable Antarctic ecosystems, and through this baseline study we conclude that it is significantly increasing levels of available nitrogen, primarily through changes in nitrate levels. The increase is akin to the levels of input seen from seals on the island, and the increase in nitrate where *E. murphyi* is present is a strong potential explanation for associated increases in micro-arthropod abundance, which could further increase decomposition rates. Over time, the increased nitrogen availability has the potential to change the bryophyte community and benefit the native vascular plants *D. antarctica* and *C. quitensis* or indeed any newly colonising plant species. As this study was planned as an initial assessment of the impact of *E. murphyi* on Signy Island, further evaluation of the fate of the nitrogen as well as the resulting community changes, particularly in moss, micro-arthropod and microbiological diversity, is required to fully understand the scale and mechanisms of impacts on the terrestrial community.

Chapter Transition

Considering the physiological capabilities of *E. murphyi*, its potential to spread around Signy Island, and the potential for ecological change, it is pertinent to consider the midge's ability to spread from

Signy. The following chapter explores the efficacy of current biosecurity measures on Signy Island, and the potential for *E. murphyi* to survive oceanic dispersal.

Chapter 7: The effectiveness of Virkon® S disinfectant against the invasive chironomid *Eretmoptera murphyi* and implications for Antarctic biosecurity practices.

7.1 Abstract

The flightless midge *Eretmoptera murphyi* is a non-native species that has been introduced to Signy Island, Antarctica, and where continued distribution is thought to be aided through human movement, *via* the treads of boots. Current biosecurity protocols for boot cleaning applied in the Antarctic region primarily rely on boot washes using a microbial biocide, usually Virkon® S. As no pesticides are currently permitted in the Antarctic Treaty area (the area south of latitude 60 °S), we investigated the efficacy of Virkon® S in controlling the spread of *E. murphyi* using boot-wash simulations. We found it to be ineffective against *E. murphyi* larvae at 4 °C and at the recommended 1% concentration. Rather, *E. murphyi* could tolerate submergence in 1% Virkon® S at both 4 °C and 20 °C for over 8 h. Application of higher concentrations increased effectiveness, but larvae still exhibited > 50% survival after 5 h of submergence in 10% Virkon® S at 20 °C. However, larvae exposed to 50 °C water for just 10 s (0% Virkon® S) as part of an alternative boot-wash simulation experienced 100% mortality, but showed complete survival at 45 °C. The study also explored the tolerance of *E. murphyi* to sea water, both as an alternative to Virkon® S, as well as assessing the species potential for marine dispersal. Fourth instar larvae tolerated 100% sea water submergence for up to 21 d, but successful hatching of eggs was reduced at even 25% sea water concentration. We show that the boot-wash protocol alone is ineffective for the control of this invasive insect species, but that water of at least 50 °C is lethal to mature larvae. We find that the midge could potentially survive at sea for up to 21 d, and that sea water would not make a suitable alternative to Virkon® S, except for the treatment of L1 larvae.

7.2 Introduction

Throughout history, humans have acted as an agent of change in ecological systems through the deliberate or unintentional introduction of species to different areas. Antarctica's geographical isolation and challenging environmental conditions have, to date, acted as barriers to non-native species dispersal and establishment, thereby minimising non-native species impacts on the continent itself (Frenot et al., 2005; Hemmings, 2007; Hughes et al., 2015). In 1959 the Antarctic Treaty was signed, establishing the Antarctic Treaty area as all land and ice shelves south of 60 °S latitude. This was later followed by the Protocol on Environmental Protection to the Antarctic Treaty in 1991, that asserted the Antarctic Treaty area's natural ecosystems must be protected and declared them a "natural reserve, devoted to peace and science" (Secretariat to the Antarctic Treaty, 2011; Grant et al., 2012). The remote lower latitude sub-Antarctic islands are closely linked to the Antarctic Treaty area in biological terms, and similarly are of high conservation value, but are regulated under national sovereignty. However, in recent decades, increasing levels of human activity are progressively breaking down the geographical barriers between Antarctica and the rest of the world, with tourist numbers rising in particular (Duffy et al., 2017; Frenot et al., 2005; Hughes et al., 2015; Tin et al., 2009). Human activity has already led to c. 200 species of non-native animals and plants successfully establishing in the Antarctic and sub-Antarctic regions, the majority of these being in the sub-Antarctic, but with increasing numbers recorded from the maritime Antarctic (Frenot et al., 2005; Hughes et al., 2015). Introductions of Acari, Collembola, Diptera and even spiders (Greenslade et al., 2012; Greenslade and Wise, 1984; Pugh, 1994, 2004) have also been recorded throughout the Antarctic. Furthermore, the transfer of pathogens may risk disease in local wildlife populations that may be "immunologically naïve" due to evolution in microbial isolation (Grimaldi et al., 2014; Kerry and Riddle, 2009).

Contemporary and predicted scenarios of climate change are also having a negative impact on Antarctic conservation efforts. Through increased liquid water availability, and ice-free habitat, with a reducing the number of extreme cold events and extending growing seasons, previously unsuitable areas of Antarctica are becoming available to both native and non-native species alike (Convey et al.,

2009c; Lee et al., 2017). Impacts of species introductions can have significant consequences within the simple terrestrial ecosystems of the Antarctic regions. The introduction of a single detritivore to the maritime Antarctic, *Eretmoptera murphyi*, has been found to increase litter turnover within the local environment by almost an order of magnitude (Hughes et al., 2013) and significantly change nutrient cycling processes (Chapter 6). Whilst, in the sub-Antarctic, a new guild of predator can lead to the near wipe-out of many elements of native terrestrial invertebrate communities (Lebouvier et al., 2012). All Parties to the Antarctic Treaty are therefore responsible for developing and enacting measures to prevent or minimise the introduction of non-native species, to control and, if feasible, eradicate any that have established (Hughes and Convey, 2014; Hughes and Pertierra, 2016). Available practical response measures are limited by the requirement to keep collateral damage to native habitats and species to a minimum, simple cost and logistic practicability, and the sometimes contradictory, existing legislation. For instance, Article 7 of Annex III Waste Disposal and Management bans the use of pesticides within Antarctica, unless under certain necessary circumstance (Hughes et al., 2015), thus broadly, the traditional and most widely used insecticides (pyrethroids, neonicotinoids and insect growth regulators (IGR's)) are not an option for use in Antarctica. Disinfectants, however, are permitted and are routinely deployed to destroy microbial pathogens and prevent their spread (Curry et al., 2002).

The Virkon® S range of disinfectants is currently listed as a recommended disinfectant by the Council of Managers of National Antarctic Program (COMNAP) and the International Association of Antarctica Tour Operators (IAATO), as an approved biocide (COMNAP, 2010; IAATO Guidelines, 2018). These products are also marketed in the UK as DEFRA-approved virucides for farms (DEFRA, 2018), and claim effectiveness through oxidation against bacteria, viruses and certain strains of fungi at temperatures as low as 4 °C (Gasparini et al., 1995; Hernandez et al., 2000). Virkon ® S powder is easy to transport and has low dermal toxicity, does not give off toxic vapour and, should it end up in an aqueous environment, will decompose over time into a harmless mixture of non-toxic salts (Curry et al., 2005). The efficacy and low toxicity of Virkon® S has led to its application in Antarctica, where it has proven effective at preventing the spread of microbial

pathogens under ambient (4 °C) conditions when used to wash equipment or footwear (Curry et al., 2005).

The convenience of Virkon® S products has prompted toxicity testing against higher order organisms beyond its intended use against microbial pathogens, in particular against invasive marine species within aquatic environments that are more vulnerable to the off-target effects of harsher chemicals (Stockton-Fitti and Moffit, 2017). Tests on the New Zealand mud snail, *Potamopyrgus antipodarum*, found that 20 min exposure to 2% Virkon® S solution resulted in 100% mortality at 15 and 22 °C, but that a 1% solution achieved total mortality only at the lower temperature (Stockton-Fiti and Moffit, 2017). In the same study 2% Virkon® S solution was highly effective against Quagga mussels, *Dreissena rostriformis bugensis*, whilst *Ciona intestinalis*, an invasive tunicate that affects mussel farming in Canada, has also been found to be vulnerable to Virkon ® S at 1% concentration (Paetzold and Davidson, 2011). However, the faucet snail, *Bithynia tentaculate,* proved to be resistant at 20 – 23 °C over 1 – 24 h, in dilutions of 1% and 2% (Mitchell and Cole, 2008). Its efficacy against higher order insects is untested, though soaking eggs of the yellow mealworm *Tenebrio molitor* in 1% Virkon ® S for 10 min did not prevent hatching (Li et al., 2016) and mixing it with certain insecticides reduces the efficacy of the insecticide against the house fly *Musca domestica* (Watson et al., 2008).

The flightless chironomid *E. murphyi* is endemic to the sub-Antarctic island of South Georgia (54 °S, 36 °W, Fig. 1.1), but was discovered in 1980 in the maritime Antarctic on Signy Island (South Orkney Islands, 60 °S, 45 °W , Fig. 1.2 and 1.3) at the site of previous plant transplantation studies (Burn, 1982). Originally reported to be restricted to a 1 m^2 'introduction' site, the midge has since colonised an area of ~85,000 m^2 and can be found along footpaths regularly used by staff and visitors at the research station, and is now on the verge of entering into new valley systems (Bartlett et al., in review/ Chapter 5; Hughes and Worland, 2010). The midge is, therefore, likely to spread to other areas of the island, with the north-western coast that hosts some of the best examples of Antarctic moss banks in the Southern Ocean, at a medium risk of establishment (Bartlett et al., in review/ Chapter 5; Canonne et al., 2017). The northern part of the island is typically accessed by traversing a single littoral

zone/intertidally exposed crossing, immediately entering areas identified as medium – high risk for establishment (Bartlett et al., in review/ Chapter 5). While no studies to date have explored saltwater tolerance in *E. murphyi*, its nearest relative, the endemic maritime Antarctic chironomid *Belgica antarctica* is capable of tolerating a 10 day hyperosmotic seawater exposure (Elnitsky et al., 2009). Furthermore, the current distribution of *E. murphyi* on Signy Island is largely close to the coast, including supralittoral areas. Loose substrate containing *E. murphyi* can easily be moved to the shoreline in surface run-off, especially after snow melt, storms and downpours, and the presence of seabirds and seals in known *E. murphyi* habitats will also increase the possibility for the midge to be transferred into the sea via epizoochry. If able to survive for a period in sea water, then the possibility exists for transfer to other beaches on the island or on to adjacent islands, *via* natural animal vectors or local marine currents.

At present, anthropogenic transfer of *E. murphyi* is the greatest known introduction risk in Antarctica. In 2005 a British Antarctic Survey (BAS) vessel carried construction vehicles contaminated with soil containing various invertebrate species including *E. murphyi* from South Georgia to Rothera Research Station on Adelaide Island, off the Antarctic Peninsula (68 °S) (Hughes et al., 2010). In this instance no establishment has been detected, probably due to lack of suitable habitat, but many suitable locations across the maritime Antarctic are at risk, with the South Shetland Islands a particularly suitable candidate region, and a major logistical hub for the northern Antarctic Peninsula (Hughes et al., 2013). Current biosecurity measures employed by BAS encompass the whole supply chain and include the cleaning of containers and cargo, where pyrethrum-based insecticides may be used to fumigate shipping containers prior to transportation to Antarctica. Relevant to Signy Island and *E. murphyi*, BAS biosecurity regulations require the cleaning of soil from equipment, boots and clothing, and the use of Virkon ® S products at a 1% dilution in boot wash baths prior to entry and exit from the island (BAS, 2018). However, Virkon ® S is primarily an anti-microbial agent and its effectiveness is limited without physical brushing or scrubbing of any contaminated surfaces (i.e. boot soles, etc.).

Whilst chironomids are usually ideal candidates for chemical control, this is typically in freshwater habitats and locations that do not have the same restrictions of chemical use as Antarctica. Similarly, often used biocontrol methods, such as the introduction of a lethal bacterium, are not viable as this would contravene Antarctica Treaty legislation (Craggs et al., 2005; Stevens et al., 2013). This leaves only existing approved protocols, naturally occurring materials, or cultural control as the available methods of controlling *E. murphyi*'s spread.

7.2.1 Aims of this study

We investigate whether use of Virkon® S is effective against *E. murphyi* and evaluate whether current boot wash protocols are an effective biosecurity measure against the midge. We also examine *E. murphyi*'s tolerance to seawater immersion – the only natural barrier to the midge's physical dispersal and discuss the use of seawater as a potential alternative to chemical control.

7.3 Materials and methods

7.3.1 Sample collection

Eretmoptera murphyi larvae were collected in soil on Signy Island (Fig. 1.3) behind the British Antarctic Survey's Signy Research Station, during the 2016/17 austral summer. Samples were collected, shipped and maintained on soil substrates from the site of collection, which is the species' natural habitat and source of food, and returned to the United Kingdom by ship in +4 °C cold storage (10 weeks). Samples were then maintained on the soil substratum under controlled conditions at +4 °C at the University of Birmingham. Individual larvae were extracted by breaking apart soil substrate with a fine brush and tweezers or by washing through stacked 2 mm and 0.5 mm mesh sized sieves – in the latter instance, all larvae were 'rested' in a Petri dish in control conditions for 48 h after extraction, to ensure that the extraction process was not a mitigating stressor. All larvae were subsequently assigned to instars based on size (Bartlett et al., 2018a/ Chapter 2). If necessary, soil containing larvae was kept moist using "field water" (water from a 3:1 mix of deionised water and

Signy soil) and kept at +4 °C until use to imitate the natural environment of the midge. All experiments using eggs were conducted in laboratories at the Signy Research Station during January 2017, using recently laid egg sacs collected from moss banks surrounding the research station. Egg sacs were removed from the substrates as described in previous chapters (Section 2.3.1 and 3.3.1).

7.3.2 Efficacy of the disinfectant Virkon ® S and use of boot wash protocols

Correspondence with the manufacturers of Virkon ® S (Lanxess, Germany, sourced from Fisher Scientific UK Ltd, Loughborough, UK) stated that Virkon® S begins to degrade at temperatures above 40 ºC and that, while a 10% Virkon® S solution can be prepared under laboratory conditions the maximum recommended concentration of Virkon® S for practical use is 5% at room temperature. Therefore, all treatments including Virkon® S dilutions took place at room temperature or below. As it was our intention to use a 10% dilution, dilutions were measured using a colourimeter, and it was found that we had exceeded the maximum 5% and were able to mix a 10% dilution if used immediately, prior to re-granulation.

7.3.2.1 Boot wash modelling (short-term exposure experiments)

Distinct life stages of insects have previously been found to have different tolerances of the same pesticide (e.g. Athanassiou et al., 2012). It was therefore important to measure any difference in the boot wash effects between the different instars of *E. murphyi*: Virkon® S solutions were made up in concentrations of 0% (control) 0.1%, 1%, and 10% with deionised water and stored at +4 °C. Twenty mL of 0.1% Virkon® S was measured using a graduated syringe and deposited into a 100 mL beaker. Under cold conditions of +4 ºC, three groups of either L4, L3 or L2 larvae (n = 8) were placed on a 250 μm nylon net, which was folded and gathered together in hand so that the larvae were together at the base. The net and larvae were then completely submerged in a Virkon® S dilution of either 0% (control), 0.1%, 1% or 10%, for 10 s. The net containing the larvae was then blotted on tissue paper to remove excess Virkon® S, and the larvae quickly removed to control conditions (+4 °C) in a Petri dish containing moist soil substrate. Survival was assessed after 72 h as described previously (Section 3.3.3).

In order to establish the potential for hot water boot washes to act as an effective biosecurity measure against *E. murphyi*, the above 'net and dip' method was used on three groups of $n = 5$ L4 larvae. A 28 mL test tube containing ~15 mL of 'field water' was placed in an alcohol bath (Haake Phoenix II C50P) and heated to either 40, 45 or 50 ºC. A minimum of 40 ºC was chosen as *E. murphyi* larvae are known to survive temperatures up to 39 ºC (Everatt et al., 2014b). Once placed in the 'dipping net', larvae were submerged in the heated water for 10 s, immediately removed to control conditions, and survival assessed as above, immediately after exposure and then again at 24 h and 72 h.

7.3.2.2 Long-term exposures

To test for any benefits of warming Virkon® S and/or an increase in exposure time, Virkon® S solutions of 0% (control), 1%, 4%, and 10% were prepared and stored at either +4 °C or 20 °C. Three groups of $n = 10$ mixed L3/L4 instar larvae were placed in a Petri dish for each dilution, at each temperature (no soil) and then exposed to 2 mL of one of the Virkon® S dilutions. The Petri dishes were kept at either +4 °C or 20 °C and survival assessed every hour for 8 h. The time taken for half (median Lethal Time, LT_{50}) or all (100% Lethal Time, LT_{100}) individuals to die was noted. Based on the results from the 8 h experiments, hourly assessments were repeated at only +4 °C for all dilutions, for a duration of 18 h, then left overnight and assessed again at 27 h, in order to find the LT_{100} for each dilution.

7.3.3 Salinity exposures

To assess the ability of *E. murphyi* to withstand immersion in seawater for both biocontrol and dispersal, we exposed both larvae and egg sacs to a range of salinity dilutions. For experiments on eggs conducted on Signy Island, seawater was collected locally. Experiments on the larvae, conducted at the University of Birmingham, also used Antarctic seawater from stocks at the British Antarctic Survey. In all instances and for all dilutions, pH and salinity (µS) were measured (Hanna combi meter-HI-98129). All eggs within the egg sacs were identified to be at the first (opal) developmental stage (Bartlett et al. 2018a/ Chapter 2). If any eggs showed signs of yellowing or embryonic development the whole egg sac was discarded and not used in this study.

7.3.3.1 Effect of long-term immersion

To establish the potential for marine dispersal, long term submersion in sea water was assessed. Three groups of n = 10 L4 larvae were submerged in either 100% sea water, 0% (field/freshwater), or placed in a soil control. Additionally, to provide a comparison with *B. antarctica* (Elnitsky et al., 2009) a 200% salinity treatment was used. For this, 100 mL of sea water was evaporated to salt crystals and those salt crystals dissolved in a further 100 mL of sea water. Survival of the immersed larvae was monitored after 2, 7, 14, and 21 day of continuous immersion, as described above.

7.3.3.2 Effect of salinity dilution

In order to establish the point at which salinity is lethal to juvenile life stages, we assessed the tolerance of eggs and larvae to seawater concentration gradients. Three groups of n = 10 egg sacs were submerged for 35 days at +4 ºC in either a soil control, 0% (field/fresh water), 25, 50, 75, or 100% sea water. At the end of the treatment period, the egg sacs were carefully dissected and the percentage of eggs that had hatched recorded. The same dilution experiment was conducted on three groups of n = 10 L4 larvae that were kept submerged for 7 days. After treatment the larvae were returned to soil control conditions and survival assessed after 72 h, as described above.

7.4 Results

7.4.1 Efficacy of the disinfectant Virkon® S and use of boot wash protocols

7.4.1.1 Boot wash modelling (short-term exposure)

Short (10 s) exposure to all concentrations of Virkon® S solution resulted in 0% mortality, in both L4 and L2 larvae. Only one death was observed among L3 larvae. However, a 10 s exposure to 50 ºC water (0% Virkon® S) did result in 100% mortality in L4 larvae. Immediately post exposure, all larvae were in a heat coma, but at both 40 ºC and 45 ºC, they fully recovered to 100% survival within 24 h.

7.4.1.2 Long-term exposure time experiments

Immersion of larvae in 0% Virkon® S (control) over 8 h resulted in no mortality at either 4 ºC or 20 ºC. There was a difference in the response to temperature at 8 h over the dilution treatments, with mortality significantly higher at 20 ºC compared to control dilutions (Kruskal Wallis, H = 9.9, $p < 0.0001$), than those treatments at 4 ºC (Kruskal Wallis, H = 8.2, $p = 0.01$). Within the 1% Virkon® S solution, some mortality was apparent after 4 h exposure, with no significant difference between the two tested temperatures at 8 h (Mann Whitney U = 3, $p = 0.7$) and survival remaining above 50%. When larvae were immersed in a 4% Virkon® S solution there was a marked decline in survival in the 20 ºC treatment, with LT_{50} observed after 5 h exposure, but the LT_{50} was not reached until after 8 h at 4 ºC. Mortality occurred after 3 h at 20 ºC in the 10% Virkon® S solution, reaching LT_{50} at 5 h. Unlike the lower concentration treatments, a sharp decline in survival at 4 ºC was also observed, with the LT_{50} reached after 8 h. (Fig. 7.1).

When the experiment was repeated at 4 ºC for up to 18 h, greater levels of larval mortality were observed. At 4 ºC the LT_{100} was 13 h in larvae exposed to 10% Virkon® S, and 14 h for 4% Virkon® S. Larvae exposed to 1% Virkon® S had > 50% survival even after 18 h of exposure, although longer exposure (27 h) led to only 10-20% survival. No deaths were observed in the control treatments over the same periods (Fig. 7.1).

Each of the different exposure concentrations exhibited a pattern of mortality over time that was sigmoidal, showing that while larvae were able to tolerate Virkon® S for a period of time, there was a threshold after which survival rapidly declined.

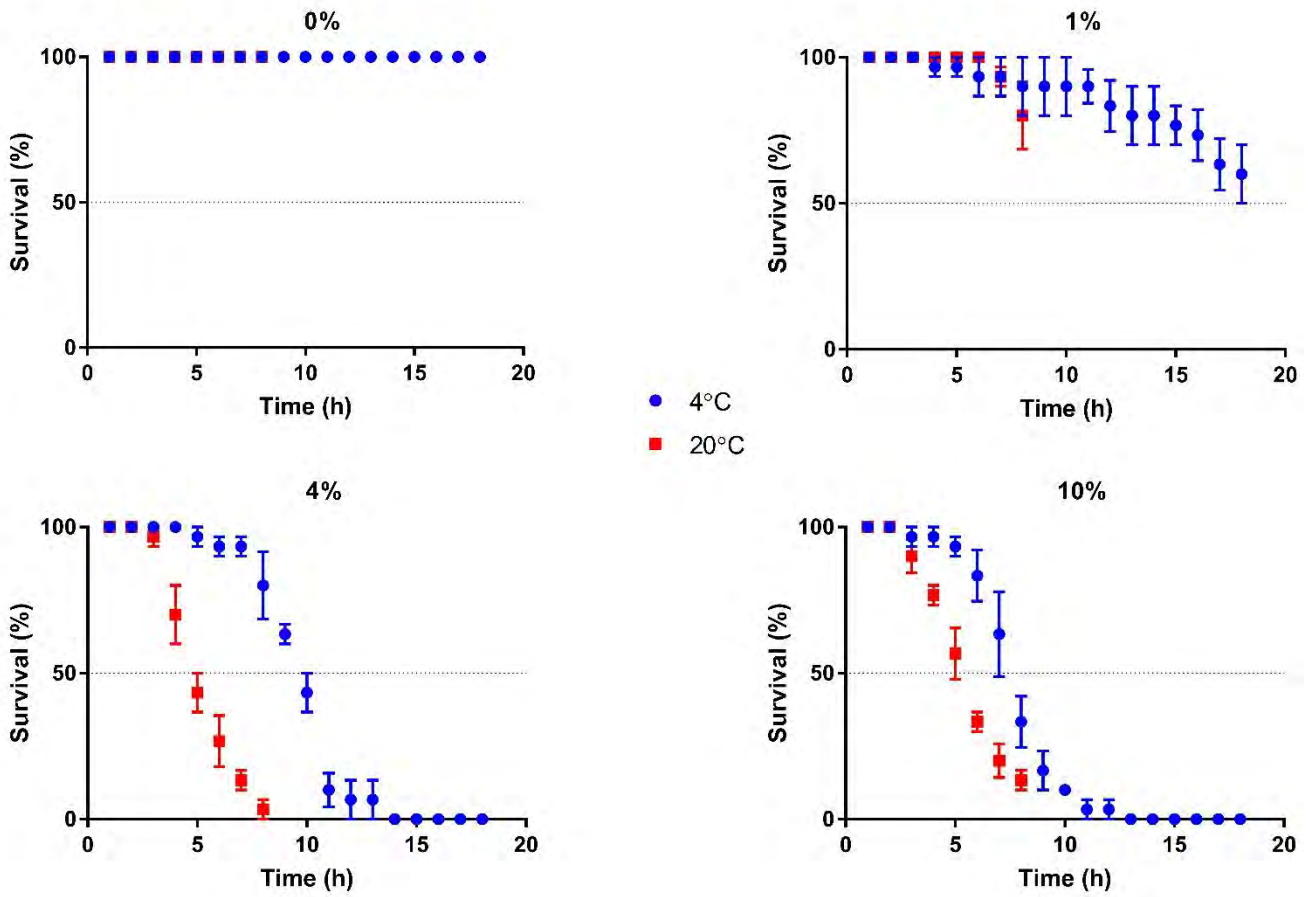

Figure 7.1 *Virkon-S exposures of control (0%) 1%, 4% and 10% dilutions at either 4 ºC or 20 ºC, with three groups of n = 10 larvae exposed for 1-18 h (4 ºC) or 1-8 h (20 ºC). Shown as mean survival at each timepoint ± SEM.*

7.4.2 Salinity exposure

There were no significant differences between the pH or the salinity of laboratory stored (used on larvae) *vs*. field collected sea water (used on egg sacs) (pH: Mann-Whitney U = 7, $p = 0.3$; µS: Mann-Whitney U = 12, $p > 0.99$).

7.4.2.1 Effect of long-term submergence

There was no difference in survival between larvae submerged in soil, 0% or 100% sea water for up to 21 days (Kruskal-Wallis, H = 0.9, $p = 0.65$); however, an overall difference between results was driven by the influence of 200% sea water, where larval survival was reduced to just 26% after 2 days (Fig. 7.2).

7.4.2.2 Effect of salinity

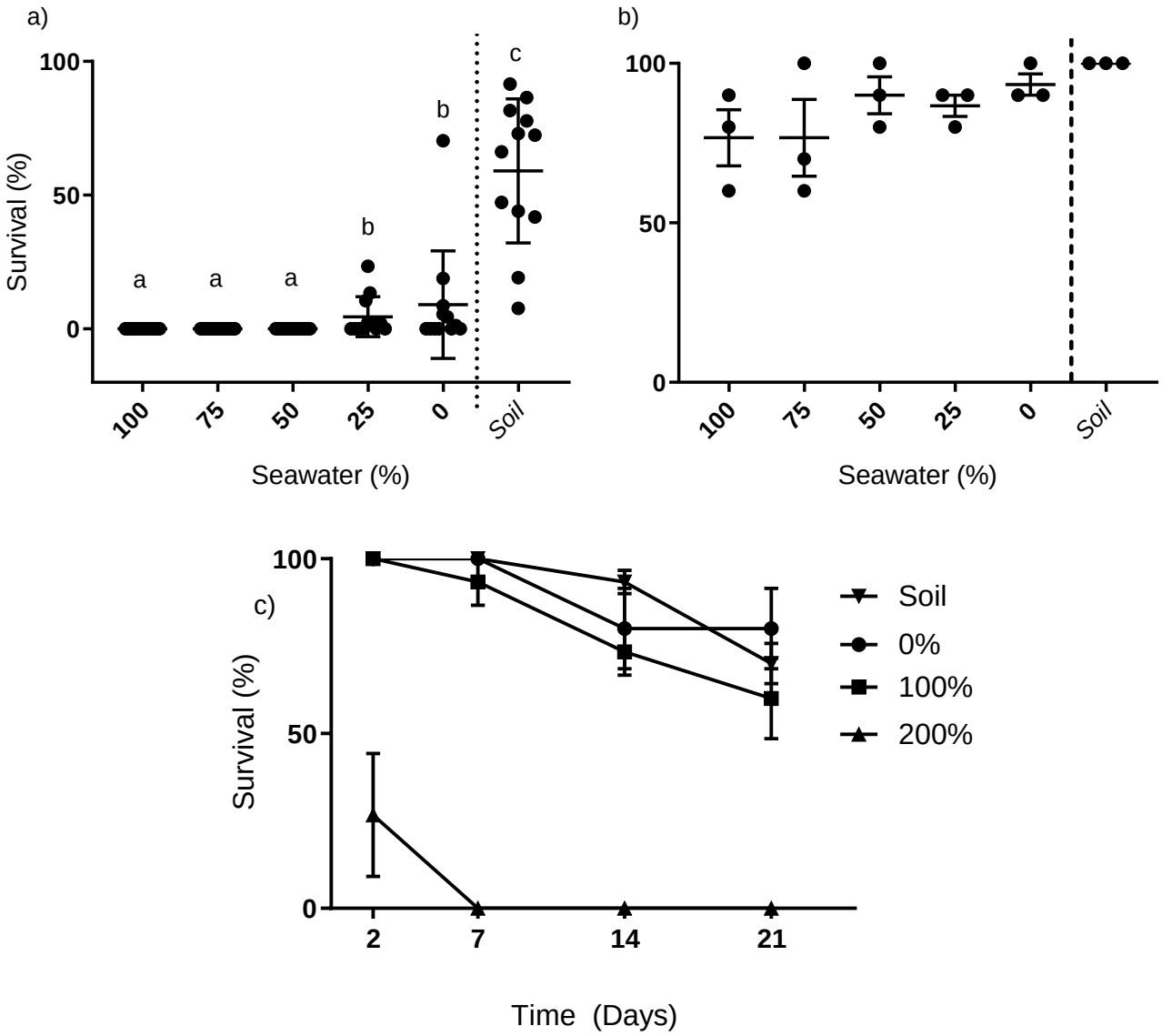

Figure 7.2 ***(a)*** *Mean ± SEM tolerance of eggs to seawater dilutions and a soil control after exposure for the whole gestation period (35 d). Three groups of n = 10 egg sacs, with ~70 eggs in each sac. Dilutions with the same letter are not significantly different* ***(b)*** *Mean ± SEM tolerance of L4 larvae to sea water dilutions after 7 d continuous exposure with a soil control.* ***(c)*** *Mean ± SEM tolerance of L4 larvae to long term (up to 21 d) exposure to either soil, 0%, 100% or 200% sea water. Three groups of n = 10 larvae.*

Our experiments showed no significant difference in larval survival when submerged in 0, 25, 50, 75 or 100% seawater, or with a non-submerged soil control (Kruskal-Wallis, H = 7, $p = 0.17$), although there was a slight and non-significant declining trend in survival with increasing salinity. In contrast, in egg sacs exposed to the same salinity range for their entire development period of 35 d, the proportion of eggs hatching was greatly reduced even at low salinity (amongst all treatments:

Kruskal-Wallis, H = 50.5, $p < 0.001$; multiple comparisons between treatments: 100%, 75% and 50% *vs* soil, $p < 0.0001$; 25% and 0% *vs* soil, $p < 0.001$). No eggs hatched under 50, 75 and 100% seawater treatments. Exposure to 25% seawater or to field/fresh water led to hatching success of 4.5% ± 2.1 (SEM) and 9.1% ± 5.8 (SEM), respectively, while hatching success in the soil control was 59% ± 7.7 (SEM). Observations made throughout the 35 days exposure period confirmed that the eggs developed within the egg sacs as described in previous studies (Bartlett et al., 2018a,b/ Chapter 2, 3), but that in all saline exposures development slowed at maturation and, of the few eggs that did hatch, the L1 hatchlings did not survive and often did not fully escape from the egg casings within the egg sac.

7.5 Discussion

The primary aim of this study was to assess the potential effectiveness of the biocide Virkon® S, currently in wide use as a boot sterilising agent in Antarctica, in preventing the further spread of the non-native midge *E. murphyi*. Secondary to this was the exploration of the midge's ability to tolerate seawater exposure, as a further potential biosecurity approach and also a barrier to dispersal. This insect is already established on Signy Island in the maritime Antarctic and there are concerns that transfer to other sites could be facilitated by human activity, particularly *via* soil and associated peat or vegetation carried on footwear or equipment (Bartlett et al. in review/ Chapter 5; Hughes et al., 2013; Hughes and Pertierra, 2016). Our data demonstrate that Virkon® S in its currently used form is not an effective biosecurity measure, and that larvae can survive seawater exposure for one month. Thus neither, alone, are effective agents.

Current biosecurity protocols in Antarctica primarily focus on reducing the risk of microbial transfer, with only boot scrubbing used to directly eliminate any macro biology (COMNAP 2011; IAATO, 2018). However, in practice this may consist of dipping footwear into baths containing a 1% Virkon® S solution for only a few seconds. Our approach to modelling these procedures and their impact on *E. murphyi* showed that larvae can survive direct exposure to the standard 1% Virkon® S solution, with

no soil load, at typical temperatures experienced on Signy Island for a duration comparable to that of a typical boot wash (c. 10 seconds). The chemical treatment component of the protocol currently in use is therefore ineffective for this purpose of controlling movement of this insect but is a proven biocide against microorganisms (Curry et al., 2005).

All instars of *E. murphyi* demonstrated considerable tolerance to Virkon® S in all tested concentrations, and a survival rate of 100% was achieved in all boot wash simulations. In contrast, in previous studies of organisms sensitive to Virkon® S, a 5 min contact time with a 1% Virkon® S solution was sufficient to achieve a 99.99% reduction in *Escherichia coli* and other bacteria or cause 78% mortality in the New Zealand mud-snail at room temperature (Hernandez et al., 2000; Stockton-Fiti and Moffitt, 2017). Clearly both the lack of susceptibility of *E. murphyi* to Virkon® S over the short contact time in a typical boot washing process, means that this procedure alone is not of use in controlling the potential dispersal of the midge. Any success achieved in stopping the spread of *E. murphyi* to other sites may be attributed to any initial brushing required before footwear is dipped in the chemical which would remove attached soil and attached midge life cycle stages. Whilst boot scrubbing, as well as the boot-wash dips are both mandatory by BAS (BAS, 2018), human error may lead to ineffective or missed scrubs, leaving the Virkon® S boot wash as the only the barrier. Organic loads have been found to significantly decrease the efficacy of Virkon® S as a biocide (Guan et al., 2013), so it is critical to its function against microbial pathogens that boots are scrubbed prior to disinfecting.

Experiments subjecting larvae to Virkon® S at various concentrations for longer exposure periods, while demonstrating increased mortality, indicated that the time required to kill a significant proportion of the population (LT_{50}, at least 8 h) was impractical for boot wash procedures. Previous work (Everatt et al., 2014b) has shown that air temperatures of 15 °C can stress *E. murphyi* sufficiently to cause mortality in an otherwise favourable environment, but only after several months of continuous exposure. In this study, soil control conditions at an air temperature of 20 °C did not induce mortality within the length of the experiments, although this temperature did reduce the LT_{50} for progressively increasing concentrations of Virkon® S (see Figure 7.1). While these data do

confirm that Virkon® S is toxic to *E. murphyi* at ambient Antarctic temperatures, the contact times required are far too long for practical use in biosecurity protocols in the field. However, our assessment of Virkon's effect on *E. murphyi* is limited to binary survival outcomes and we have not assessed any potential long-term effects to the larvae, such as reproductive capacities.

The degradation of Virkon® S at temperatures above 40 ºC, its ineffectiveness as an insecticide at lower temperatures, stronger dilutions and longer exposures, combined with *E. murphyi*'s known heat-shock responses (Everatt et al., 2014b), led us to investigate hot water alone as a potential biocontrol. Everatt et al. (2014) showed that *E. murphyi* larvae enter heat coma at 31°C, and a few individuals can survive air temperatures up to 39 °C for 1 h. We found that very short exposures (10 s) to high temperature water, whilst inducing heat coma above 40 °C, were not lethal below 50 °C. Thus, hot water in a boot wash or scrub, must exceed 50 °C to be effective at killing *E. murphyi* larvae.

Larval survival in 100% seawater for as much as 21 days indicates that the short intertidal crossing between Cemetery Flats and Elephant Flats on Signy Island would not present a barrier to larval dispersal around the island. However, if microscopic L1 larvae were transferred, it is unlikely that they would survive as they seem to have little tolerance to even dilute seawater, dying within moments after emergence from egg casings. Most larval dipterans cannot tolerate prolonged seawater submergence (Bayly, 1972) but larvae of *B. antarctica*, Antarctica's only endemic chironomid and *E. murphyi's* closest relative, can withstand extensive osmotic dehydration (Elnitsky et al. 2009) This study identified almost 50% survival of a 10 days exposure to 100% seawater, in which the total body water content of the larvae reduced by 30%. L4 larvae also showed no difference in survival between 100% sea water and the freshwater or soil controls over 21 d, but were not able to withstand prolonged periods of exposure to hyperosmotic seawater (200%), with the LT_{50} reached within just 2 d. Likewise, *B. antarctica* survival also declined rapidly during exposure to higher saltwater concentrations, but 25% of larvae survived 6 days submerged in the equivalent of 200% seawater (Elnitsky et al., 2009).

The ability of larvae to survive prolonged periods in seawater also raises the possibility of oceanic dispersal to adjacent islands, either through rafting, such as is seen in a related South American chironomid, *Halirytus magellanicus* (F. Simoes pers. comm.) and Antarctic and Arctic Collembola (Coulson et al., 2000; Hawes et al., 2008), or *via* zoochoric association with seabirds or seals. *Eretmoptera murphyi* is found in areas of Signy Island with considerable elephant and fur seal activity (Bartlett et al., in review/ Chapter 5) as well as various nesting seabirds, and the association of species with footpaths raises the likelihood of animal transport. Fur seals are very mobile, disrupting moss bank surfaces, *E. murphyi* habitat, as they move (Favero-Longo et al., 2011). There is the potential that larvae and eggs may be carried in the fur of the animals, especially egg sacs which have a sticky outer membrane (Bartlett et al., 2018a/ Chapter 2) and enter the ocean this way. Whilst prolonged attachment and exposure to seawater would limit successful hatching, in principle more mature larvae could spend as much as three weeks at sea.

Based on the data obtained in this study, we suggest that the use of hot water (>50 ºC) to scrub soil containing invertebrates off contaminated items, followed by a Virkon® S wash as a microbial biocide on the 'clean' boots, would provide the most effective control measures against *E. murphyi* whilst not sacrificing the benefits of Virkon® S as a microbicide/virucide. The addition of hot water ensuring the death of any remaining *E. murphyi* after scrubbing. This could be implemented at existing boot wash stations both prior to arrival and on departure from islands. To mitigate the further spread of *E. murphyi* around Signy Island, ideal scenarios would include a new scrub station at the edge of the known *E. murphyi* distribution, such as is depicted in Figure. 7.3.

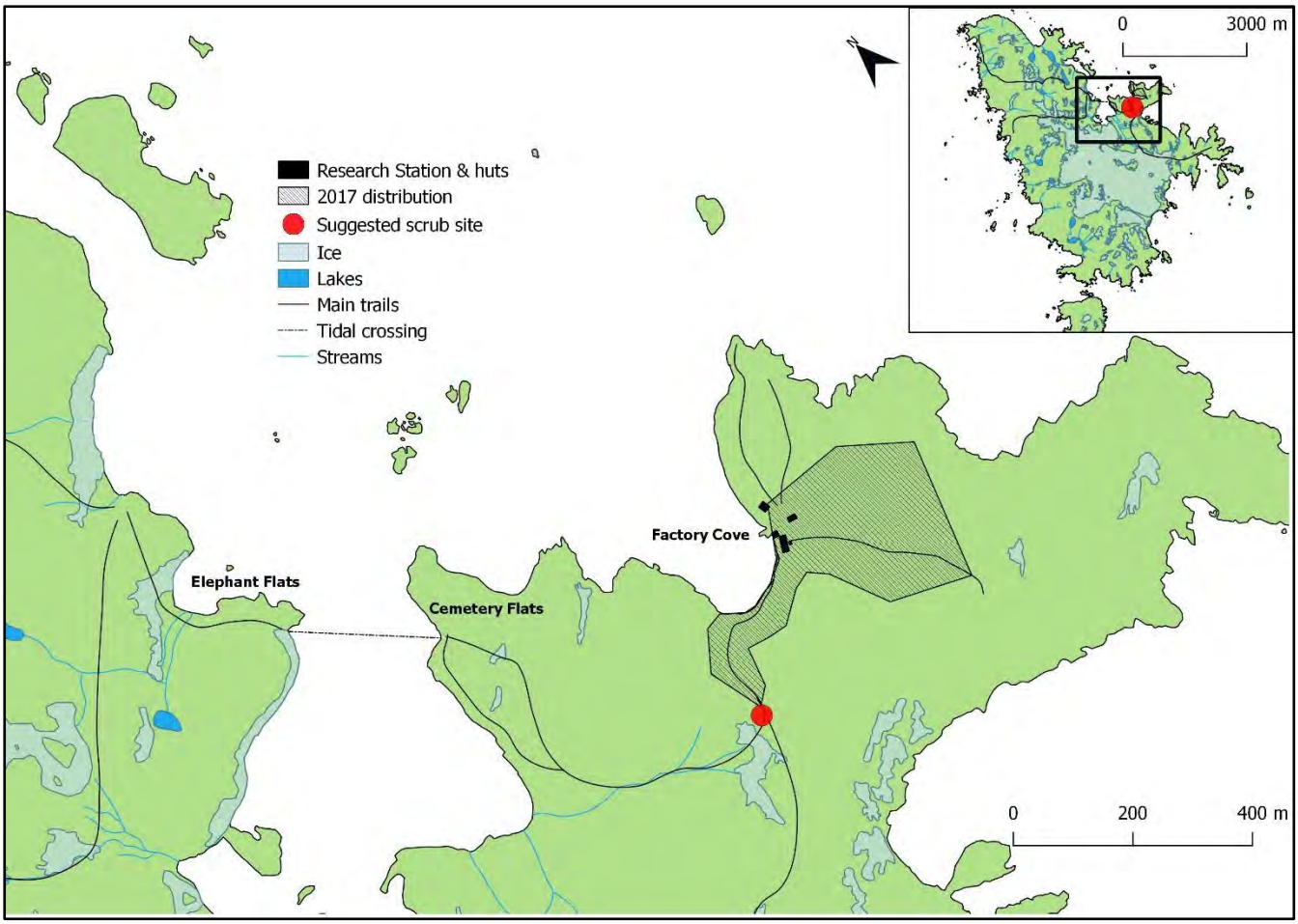

Figure 7.3. *Map showing suggested site for scrub station on Signy Island at the summit of the Stone Chute along paths likely to lead to the spread of* E. murphyi *to other areas of the island. Includes most recent distribution polygon (Bartlett et al. in review/ Chapter 5).*

7.6 Conclusion

Whilst the focus of this study has been on the invasive midge *E. murphyi* on Signy Island, the findings and suggested additions to the existing protocols may be relevant to all areas of Antarctica that are vulnerable to invasive invertebrates or that have already been colonised. *Eretmoptera murphyi* is not a unique example in the Antarctic region, but as a flightless species it is reliant on mechanical, or potentially oceanic, methods of dispersal to increase its range. Within the maritime Antarctic, another dipteran species, *Trichocera maculipennis*, was recently introduced to King George Island (South Shetland Islands) (Potocka and Krzeminska, 2018; Volonterio et al., 2013). Although most attention has been given to observations of this species having colonised research station sewage systems, it is

thought that it may well be established in the local natural environment (Potocka and Krzeminska, 2018; Uruguayan Antarctic Institute 2008; Volonterio et al., 2013). As adults can fly it is capable of greater natural dispersal than *E. murphyi*, but soil-dwelling life stages could be dispersed through similar mechanisms as *E. murphyi*.

The combination of increasing human activity and ongoing regional climate change will likely facilitate further colonisation events, both in the continental and maritime Antarctic. Without realistic reviews of existing biosecurity protocols, such as those recommended by Hughes and Pertierra (2016) and the subsequent ATCM proposal (United Kingdom and Spain, 2017), it is likely that anthropogenic introductions will continue. Prevention of the transfer of soil organisms contained therein needs to be a priority action for all stakeholders involved in the protection of Antarctica. Here, using *E. murphyi* as a model species, we have demonstrated important limitations in probably the most widely implemented biosecurity measure, and suggest alternative actions that could potentially be used to reduce the spread of non-native invertebrate species which, if left unchecked, have the potential to disrupt Antarctica's fragile ecosystems.

Chapter Transition

Gaining understanding of the impacts of *E. murphyi* has highlighted its capacity as an influential invasive species in terrestrial Antarctic ecosystems, and provides an example of how a single species introduction can affect change. But findings from this thesis, and previous work, indicate this species is pre-adapted to spread beyond Signy and is unlikely to be much affected by forecast climate change. The following discussion will provide a synthesis of all current information to look at the potential future of *E. murphyi*: how it could spread onto the Antarctic Peninsula and survive there, and how climate change may affect this trajectory.

Chapter 8: General Discussion

The planet's 'last remaining wilderness' is no longer untouched by human activity. Over a century of exploration, and more recently tourism, has slowly introduced new species to the Antarctic region, with consequences for both terrestrial and marine ecosystems. The region is also now facing rapid climate change, exposing virgin ground beneath the ice that is ripe for colonisation (Lee et al., 2017). The oceans are warming, and circulations changing, bringing new species to the Southern Ocean, and disrupting globally important food chains (Duffy et al., 2017; Fraser et al., 2018). Antarctica is rapidly changing and will change much more by the end of this century (Larsen et al., 2014; Turner et al., 2014; Fig. 8. 2). Humanity has a responsibility to demonstrate that we can do better by this continent than we have with all others that we have already colonised, and to do that we must first understand it, and crucially, understand the consequences of our actions upon it.

Biologically unique, Antarctica hosts 16 distinct biogeographical zones (Terauds and Lee, 2016), and a variety of zoological and botanical life: from microbes to insects, algae to flowering plants. These organisms must endure the range of physical stresses that partner with some of harshest environments on the planet. Thus, whether native or invading, any organism must be able to withstand these conditions and thrive regardless if it is to succeed. Understanding how a successful invasive species does this, helps to identify other potential colonisers, and builds the foundation upon which we can create accurate risk assessments. From here we can monitor and control the spread of potential colonisers, predict their potential roles within invaded ecosystems, and crucially take action against ourselves as the largest vector for species introductions.

The work presented within this thesis has built upon that of my predecessors and explores multiple facets of the biology and ecology of the invasive chironomid, *E. murphyi*. Building up from the basics of the species' life history and phenology in Chapter 2, through its tolerance to stressors faced in Antarctica across multiple life stages in Chapter 3 and 4, to its expanding distribution and impact on terrestrial ecosystems in Chapters 5 and 6. From here I have endeavoured to highlight the importance of effective biosecurity in Chapter 7, and the risk of the midge to broader areas in Antarctica if these

lapse. This body of research therefore encompasses work ranging from whole-organism biology, to ecosystem function and then into policy recommendations, all through a single species. Now, using *E. murphyi* as a model invasive organism, and Signy as a model ecosystem, I will expand upon each chapter, and draw the thesis together by taking the findings one scale further – to regional impacts considering both current, and future climates and an increasing human presence in the Antarctic.

8.1 Life cycles and phenology

Eretmoptera murphyi is representative of many polar invertebrates with an extended life cycle, compared to temperate species (Convey, 1996b; Chapter 2). For any introduced species to succeed in Antarctica, they must have a sufficiently flexible life-history strategy to cope with these adverse challenges, and *E. murphyi* demonstrates this by being able to emerge throughout the available summer season and successfully endure the spikes in GST (Chapter 3), as well as potentially overwinter in more than one life stage (Chapter 2 and 4). It's suite of pre-adaptations to the cold climate of Signy Island (Everatt et al., 2012; Worland, 2010) has allowed it to successfully established itself as the island's only terrestrial higher invertebrate.

As the climate warms in the Antarctic region, and for the maritime Antarctic in particular (Larsen et al., 2007), temperature and drought stressors on native and invading species will ease, opening new areas for colonisation, and/or extending the active seasons of invertebrates. Initial studies of *E. murphyi's* life cycle reported that the species may have a single mass emergence and reproductive effort, akin to their cousins on the Antarctic Peninsula, *B. antarctica* (Convey, 1992; Cranston et al., 1985; Sugg et al., 1983). However, in Chapter 2 we found that the midge has an advantageously flexible life history utilising an entire season of positive temperatures, meaning it is therefore likely to take advantage of longer summers as a result of climate change, with adult emergence and subsequent egg laying able to occur throughout the season, rather than as one single, environmentally-cued event (Fig. 8.1). Furthermore, we confirm parthenogenesis in *E. murphyi*, the first instance of this in a maritime Antarctic insect. Whilst this is not rare in polar invertebrates, this does make *E. murphyi* the only higher insect in the Antarctic region to reproduce in this manner (Chown and Convey, 2016). A

prolonged emergence season combined with parthenogenesis primes *E. murphyi* to become a successful invader in regions with a favourable climate. The studies conducted in Chapter 2 further highlight this by demonstrating that even with several points in the life cycle where survival or successful oviposition are below, or close to 50%, ~13 eggs will still successfully hatch for every adult midge. Consequently, the species is capable of doubling its population size after completion of each two-year lifecycle and could well colonise another area with just a single individual. However, despite findings of an average of 48 eggs per sac in Chapter 2 (n = 30), the following chapter found an average of 63 eggs per sac (n = 10). Whilst the original study found a higher average still, with 85 eggs per sac (n = 30) (Convey, 1992). Thus, the figure of 48 eggs is conservative, and therefore, so is the estimation of a doubling in population each lifecycle. Given the highest figure from Convey (1992), populations have the potential to increase by as much as five-fold in two years.

Whilst physiological pre-adaptation and an advantageous life history strategy has enabled *E. murphyi* to successfully invade Signy, it still has a low reproductive output for a chironomid, even a polar species (Armitage et al., 1995; Nolte, 1993). Eggs are laid in a similar manner to that of its cousin *B. antarctica* (Harada et al., 2014; Suggs, 1983), further supporting suggestions that *E. murphyi* and *B. antarctica* are con-generic (Allegruci et al., 2006, 2012). To understand environmental factors that may affect reproductive output of this tenacious invader, we must look to the microhabitats and microclimates that will influence the most crucial juvenile stages, and thus understand any constraints on the midge's ultimate success. The question of diapause remains unanswered but given the broad period during which pupae were found in the field, and the staggered emergence of adults, there doesn't appear to be a definitive environmental cue for either pupation or eclosion.

8.2 The importance of microhabitats

Microhabitat selection by invertebrates in Antarctica is a balance between maximising heat for development and mitigating the risks of desiccation or freezing injuries (Hayward et al., 2003). The size of terrestrial invertebrates such as *E. murphyi*, means that air temperature is not always a useful proxy for the microhabitat temperatures experienced. Variations in air, ground, and below-ground

conditions are largely a result of air temperature as well as infrared radiation, insolation or insulating effects such as snow cover (Convey et al., 2018; Davey et al., 1992; Guglielmin et al., 2008). For example, ground surface substrates can absorb heat, with as much as 21.8 ◦C diurnal fluctuation in the summer (Walton, 1982) (Fig. 8.1). In the winter, air temperatures have been known to drop as low as -40 ◦C on Signy, yet temperatures beneath the snow are mitigated meaning that they rarely drop far beyond freezing (Davey et al., 1992) (Fig. 8.1). Inevitably, the conditions experienced by terrestrial invertebrates residing in these habitats will vary depending on where they are in the substrate profile (Convey et al., 2018; Hayward et al., 2001, 2003). But understanding how they will respond to the variety of conditions at the corresponding points in their life cycle requires knowledge of both their life history and their physiology, as well as information on year-round microhabitat conditions and the potential for these to alter under climate change.

Chapters 3 and 4 evaluate the physiological responses of *E. murphyi* life stages to summer and winter conditions with consideration of experienced microclimate data. Eggs, which only occur in summer (Chapter 2), were studied in response to primarily heat and desiccation stress in Chapter 3. All other life stages, particularly the overwintering larval stages, were assessed in response to cold and ice-entrapment in Chapter 4.

8.3 Life stage physiology

8.3.1 Heat and desiccation in eggs

Whilst there is variability within habitats, there is still limited refuge in Antarctic terrestrial ecosystems to avoid environmental extremes. Variation within the same habitats as a result of water content or substrate type, mean that often only pockets of suitable conditions are available. In Chapter 2 we hypothesised, in line with Hughes and Worland (2010), that the patchiness in substrate water content may be driving the observed patchiness in *E. murphyi* distribution on Signy. Closer analysis of the substrates in Chapter 3 found that there are indeed large differences through the substrate profile, with vegetation layers retaining more water, and the soil layers beneath being friable. Soil in particular was the most variable, with a lower overall water content and humidity, and is likely to

drain faster depending on local topography and geology. Temperature differences were also found between the air, ground surface and below-ground habitats, with the ground surface layers experiencing highs in excess of 20 ºC for several hours at a time, peaking up to 30 ºC, whilst the air temperature could be as much as 10 ºC lower (Fig. 8.1). For an organism that is pre-adapted to the cold such as *E. murphyi* (Everatt et al., 2012; Worland, 2010), exposure to these temperatures could be detrimental, and the sites and manner in which they choose to oviposit may determine the fate of the eggs.

Within studies contained in Chapter 2, it was observed that *E. murphyi* lays eggs throughout the substrate profile in the summer months, including on the ground surface where temperatures are higher (Fig. 8.1). Current temperature and humidity extremes recorded in Chapter 3 are unlikely to affect egg survival, furthermore, the egg sacs are likely to hatch in either substrate habitat, especially if the individual egg sacs are aggregated together. The eggs had a high tolerance to the temperature fluctuations experienced on Signy, with desiccation a more influential stressor on survival, especially if an egg sac is laid singly in the dryer soil layer. However, whilst current environmental and habitat conditions on Signy are not an impediment to the egg development stage of *E. murphyi*'s life cycle, this may not be the case in the future, as the frequency and duration of extreme heat and desiccation events increases under climate change (Turner et al., 2014). In addition, the stresses of summer heat are only one extreme endured during the two-year life cycle of the midge, it also has to survive winter.

8.3.2 Cold and anoxia

Cold-tolerance adaptations will determine the success of any colonising species in the polar regions, with low-temperature being a prime stressor on any organism, native or introduced. The majority of work on *E. murphyi* to date has focused on its low-temperature biology (Block et al., 1984; Everatt et al., 2012, 2015; Worland, 2010), finding that the fourth instar has the advantage of pre-adaptation to cool environments (Worland, 2010) and can rapidly cold-harden and acclimate to reducing temperatures (Everatt et al., 2012).

Chapter 4 expanded on this work by reassessing the cold-tolerance of fourth instar larvae, as well as all juvenile life stages (eggs, L1-L4) and adults. This included collecting data on the SCPs of all life stages for the first time. Eggs were freeze-avoiding with the lowest SCP of all life-stages at -17 ºC. Larvae, pupae and adults had SCP's around -5 ºC, and larvae were freeze-tolerant, with increasing cold-hardiness with each instar, whilst adults were deemed chill-susceptible (Fig 8.1). Analysis of microclimate data suggests that, despite having all hatched well before the onset of winter (Chapter 2), eggs are capable of surviving the temperature experienced in the soil in winter on Signy.

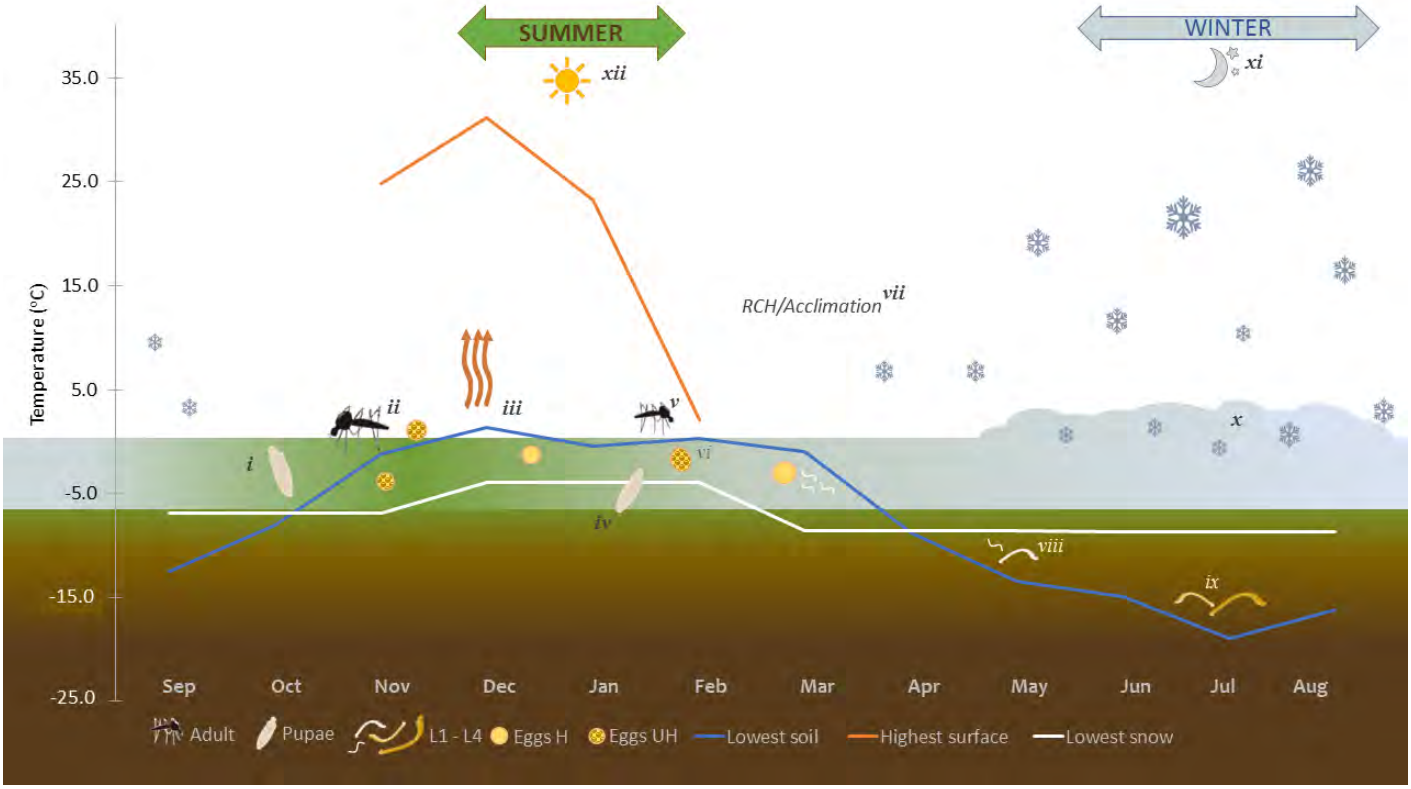

Figure 8.1. *Schematic of a year in the life of* E. murphyi. *Lowest recorded soil temperatures each month from 2017-2018, logger was placed in the top 5 cm of the soil layer on Signy Island (Chapter 4). Highest recorded surface temperatures for the summer months taken from data loggers on the vegetation surface (Chapter 3). Lowest recorded temperatures beneath a snow profile, from Convey et al. (2018), where only one record for the three months of each season is reflected. i) Basal snow melt (Guglielmin et al., 2008) and the first pupae soon after. ii) adult midge emerge three weeks after pupation and are largely surface active through the summer. iii) infrared radiation is absorbed by the dark moss and soil/peat surface layer causing a spike in ground surface temperatures, as high as 31 ºC at the height of summer. Static life stages such as unhatched eggs laid on the surface, will be subjected to these high peaks that are detrimental to survival if more than an hour long (Chapter 3). iv) The last pupae are seen on the 3rd January 2017. v) The last adult is seen on the 19th January 2017. vi) The last unhatched egg sac is seen on the 30th January. As temperatures drop, larval instars are subjected to temperatures below their SCP of around -5 ºC, vii) and it is now that they must rapidly cold-harden after Worland (2010) and Everatt et al. (2012), as temperatures will drop below their LLT if there is minimal snow cover (Chapter 4). viii) for L1 and L2 larvae their LLT is -10 ºC, ix) whilst for the more mature L3 and L4 instars it is -15 ºC. x) snow acts as a buffer on the harshest of air temperatures and combined with acclimation, is why this midge can survive the winter. xi) The winter solstice at the end of June marks the beginning of coldest and darkest month of the year, July. xii) The summer solstice in December.*

Aside from the hazards of physical injury from low temperature, the nature of the midge's habitat, and their preference for high water content substrates, means that there is a risk of entrapment in ice each winter. Furthermore, the freeze-thaw cycles at the onset and retreat of winter, mean that both ice entrapment and prolonged submergence are possible. Everatt et al. (2014b) briefly investigated the midge's response to this over a month of ice-entrapment, and in Chapter 4 we extended this to a whole winter scenario of up to 64 days in ice, finding that the midge cannot withstand more than 28 days entrapment. Microclimate data from the 2016/17 winter on Signy suggest that it is likely that the habitat of *E. murphyi* will be frozen for the duration of winter but are inconclusive as to whether this would extend to complete ice entrapment of the midge. Because entrapment beyond 28 days is severely detrimental, yet the midge still highly abundant and expanding its distribution (Chapter 5), we conclude that it is unlikely that widespread ice-entrapment occurs, or drier microhabitats are selected prior to the onset of winter in order to avoid inoculative freezing, as is seen in *B. antarctica* (Teets et al., 2011).

The cold-tolerance of *E. murphyi* across all life stages suggests no inhibition to the midge's ability to survive at higher latitudes under current environmental conditions, and it is likely that as the maritime Antarctic warms, the pressures of the cold will ease. Previous work by Everatt et al. (2012, 2013a), and Worland (2010) concluded that, whilst the larvae can acclimate, this an unnecessary trait based on an assumed winter temperature that did not fall below -10 ºC, and an acclimated LLT down to -19 ºC . The data from Chapter 4 illustrate that temperatures can fall to -20 ºC, below the LLT of all larval instars and at the limit of the LLT for eggs. Thus, acclimation through rapid (or seasonal) cold hardening must be an essential component in the arsenal of cold-physiology employed by *E. murphyi* to survive winter on Signy (Fig. 8.1). Precipitation will also alter temperatures experienced below ground, with snow cover adding several degrees of insulation to subnivean soils beneath (Convey et al., 2018; Davey et al., 1992). Climate change is expected to increase precipitation rates throughout the maritime Antarctic; thus, increased snowfall may benefit *E. murphyi* by raising below-ground temperatures and the potential for the midge to remain active in the subnivean environment during the shoulder seasons. Studies on subnivean or intra-nivean invertebrates have found that overwintering

species can remain active into the winter months, with some Collembola using the intra-nivean habitats to escape waterlogged ground beneath, and thus potentially avoid the risk of ice-entrapment (Aitchison, 2001; Leinaas, 1981). Hågvar and Hågvar (2011) found a Norwegian subnivean habitat to be full of active and feeding invertebrates, including dipteran larvae, through the winter months. Closer examination of microhabitats on Signy, in correlation with both snow depth, and thresholds for *E. murphyi* foraging behaviour would indicate whether there are areas of the midge's territory that would allow similar conditions, and thus year-round foraging. As yet, a state of dormancy has not been confirmed in *E. murphyi* and it is suspected that no such obligate cue is present due to the activity levels witnessed in year-round storage conditions (Chapter 2). Further exploration of the lifecycle could resolve this.

8.4. Current distribution and ecological impacts

The monitoring of an invasive species' distribution is critical in both its management and informing appropriate risk assessments. No formal procedures are currently in place to monitor *E. murphyi*, yet several estimates have been made on its distribution since its discovery on Signy in the 1980s (Block et al., 1984; Burns, 1982; Dozsa-Farkas and Convey, 1997; Hughes and Worland, 2010; Smith, 1996). The first comprehensive survey was conducted in 2007-09 by Hughes and Worland (2010), finding that the midge had spread to occupy 35,000 m^2 in the preceding 40 years. They also suggested that personnel on the island were likely vectors for its spread along pathways. Chapter 5 updated this distribution survey and expanded the areas assessed. It supports the proposal of Hughes and Worland (2010), finding strong associations of *E. murphyi* abundance with footpaths away from the area of introduction, and reports a new distribution area of 85,000 m^2. Whilst the midge was not found anywhere beyond Factory Cove, it is now on the brink of moving into new valley systems and the use of species distribution modelling (MaxEnt) highlights key areas at risk of further colonisation around the island.

Chapter 6 explored how an increasing abundance and distribution of *E. murphyi* impacts the terrestrial ecosystem on Signy, with the key finding being that the midge introduces 3-5 times as much nitrate as

are found in areas without the midge. This raises nitrogen levels to that seen around seal colonies on the island, highlighting the large impact that this one species introduction can have on a nutrient-poor ecosystem. This was discussed in relation to other trophic groups, with the largest changes over time expected from the vegetation layer, hypothesising that as native graminoids like *D. antarctica* benefit from increased nitrogen, they may ultimately outcompete mosses.

Taking the schematic from Yang and Gratton (2014) in Chapter 1 (Fig. 1.6), the new information from Chapter 6 can be used to create a version for the Signy Island terrestrial ecosystem, highlighting the role that *E. murphyi* now plays in the brown food web (Fig. 8.2). Studies from the Arctic show that chironomid carcasses alone can increase nitrogen levels enough to alter the vegetation communities in nutrient-poor tundra systems, thus it is likely that similar processes are happening with *E. murphyi* on Signy (Fig. 8.2 i). Previously, the main nitrogen sources on Signy were atmospheric or guano deposition (Fig. 8.2 iii), but the introduction of *E. murphyi* has provided an additional major source of nitrates to the system (Fig. 8.2 vi). The case could thus be made, as with *L. minimus* on Marion Island in the sub-Antarctic, that *E. murphyi* is now a keystone species in Signy's terrestrial ecosystem (cf. Hänel and Chown, 1998; Mercer et al., 2001). Further work on both the role of the midge in overall nitrogen cycling and the ultimate fate of the additional nitrates through the trophic web, would underpin this hypothesis.

One of the largest side-effects of increasing detritivory and nitrogen availability is the lowering of the C:N ratio, and thus an increase in the quality and decomposition speed of litter. Soil microbes (Fig. 8.2 v) interact differently with litter and soil chemistry to determine soil C and N availability, with fungi responding negatively to increasing N availability over time compared to bacteria (Wallenstein et al., 2006). Findings from Chapter 6 also demonstrate the opposing influence that bacteria and fungi have on SOC in Antarctica (Fig. 8.2 v). Both additional nutrients and climate change drivers such as increasing temperature, are expected to shift soil microbes in favour of more fungal-dominated communities over bacteria-dominated, which may further influence the C:N ratio (e.g. Deslippe et al., 2012; Rinnan et al., 2013). Warming will also affect invertebrate and vegetation communities, particularly at the poles, where longer growing seasons will be advantageous to both native and

introduced flora (Convey et al., 2018; Chapin et al., 1995; Bokhorst et al., 2007a; Pertierra et al., 2016). The combination of a more fertile soil as a result of *E. murphyi* activity, and a less hostile environment as a result of climate change, may therefore lay the foundation for community changes, and provide conditions for the establishment of non-native species that otherwise may not have been able to survive.

8.5. Climate change and the future for *E. murphyi*

As the climate warms, polar invertebrates will broadly benefit as active seasons extend and drought and low-temperature pressures ease, reducing associated physiological stresses on development (e.g. Bokhorst et al., 2008). Whilst this will likely be true also for *E. murphyi* in the short term, long term warming trends may not be so beneficial. Chironomidae species typically have narrow temperature niches, following a negative relationship with long-term temperature increases, a trait that drives the study of climate related paleolimnology (Eggermont and Heiri, 2012; Hoffman, 1988). For example, whilst climate warming studies in the Arctic have found that warming has increased the body size of several invertebrate species (Culler et al., 2015; Høye et al., 2009), it may decrease the size and potentially the abundance of chironomids (Hodkinson et al., 1996; Lackmann et al., 2016; Tixier et al., 2009). The importance of chironomids in Arctic ecosystems means that these changes will result in consequences for brown and green foodwebs alike (Dreyer et al., 2015), with negative implications for the vertebrates that rely on them (Koltz et al., 2018). For *E. murphyi* in the Antarctic, warming may alter its potential range, as the midge is pre-adapted to the cooler climates of the maritime Antarctic (Everatt et al., 2012) and progresses through all life stages most effectively at +4 ºC, which is also near the average summer temperature in most of the maritime Antarctic region (Convey et al., 2018; King et al., 2017; Chapter 2; Appendix XII). *Eretmoptera murphyi* can only survive short periods (hours) at +20 to 30 ºC (Everatt et al., 2014b; Chapter 3) whilst its lower limits extend to to around - 20 ºC (Everatt et al., 2012; Chapter 4).

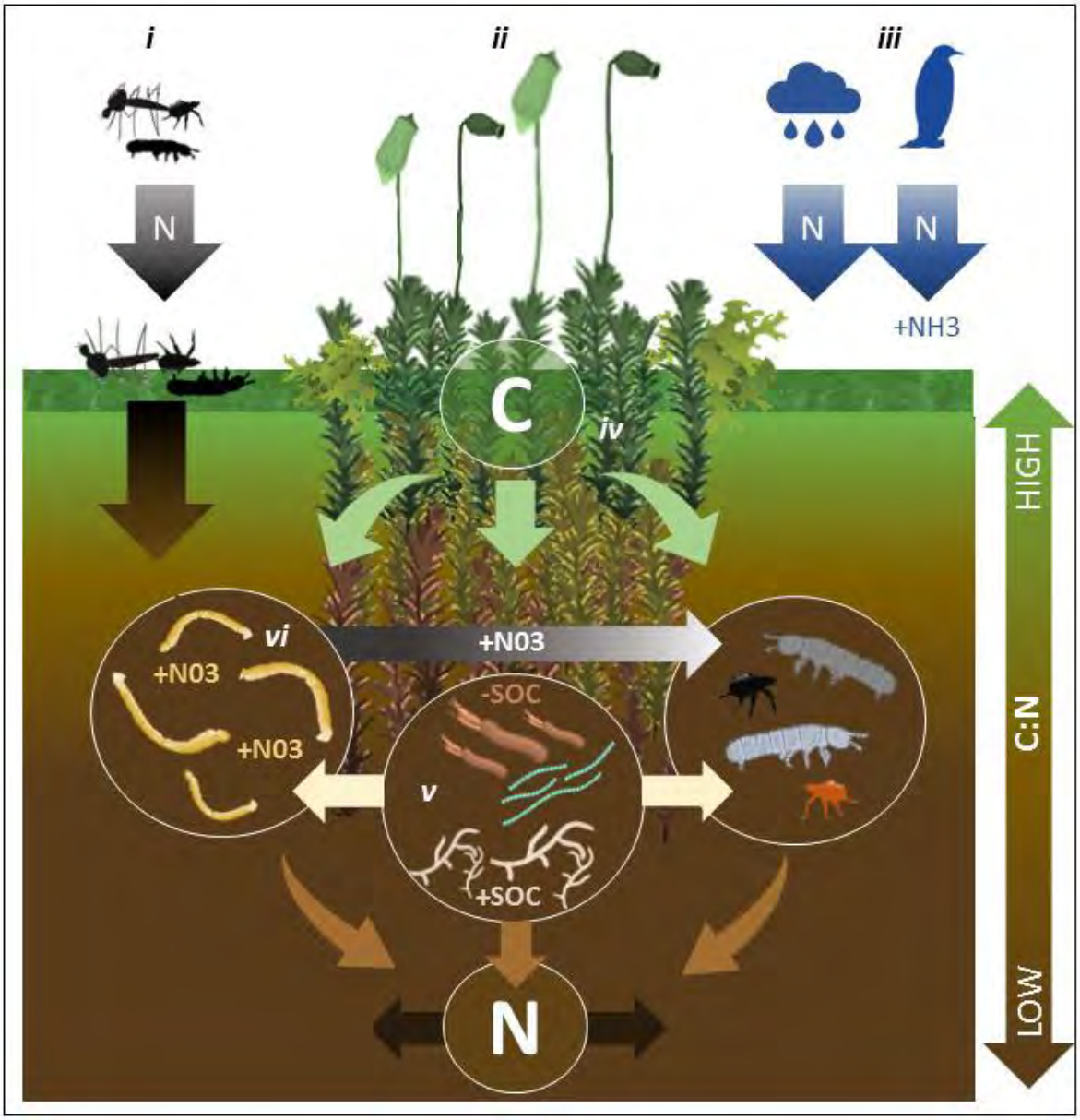

Figure 8.2. *Schematic of terrestrial ecosystem functioning on Signy Island in areas colonised by* E. murphyi. *NH_3 = Ammonia, NO_3 = nitrates, SOC = soil organic carbon, N = nitrogen, C = carbon. i) Direct nitrogen deposition to the detrital pool through the dead biomass of invertebrates, and the additional contribution from* E. murphyi *presence, could introduce "high quality biomass with a low C:N into belowground systems" (Dreyer et al., 2015). ii)* E. murphyi *and micro-arthropods aid decomposition of the floral biomass through fragmentation of the detritus, with effects on the C:N as carbon is released/assimilated, and nitrogen levels increased through excreta and organism death. iii) Deposition from atmospheric, or guano sources that are particularly rich in ammonia, was previously the only major source of nitrogen to the system. iv) Carbon stored in plants is fragmented and either released as particulate matter or assimilated by detritivorous microbes and invertebrates. v) Microbial activity has differing impacts on soil organic carbon (SOC), they also form part of the diet of* E. murphyi *and Collembola (Bokhorst et al., 2007b; Bridge and Denton, 2006) and take up mineralised nutrients through immobilisation. vi)* E. murphyi *is associated with an increase in nitrate levels which may be benefiting the micro-arthropod community (Bokorst and Convey, 2016; Gutiérrez-López et al., 2011) and contributes to the nitrogen pool. The quality and size of the 'brown' food web of Signy, in terms of the key elements carbon and nitrogen, are major determinants of its function. The addition of* E. murphyi *is thus capable of affecting change through the increase in nitrogen to a low-nutrient environment.*

In addition to the work presented within the thesis chapters, I have collaborated on a project exploring the range potential of *E. murphyi* in the wider Antarctic region (Appendix XII). The study finds that expected climate warming (following IPCC RCP 8.5) combined with the climatic envelope most preferential for *E. murphyi*, may push its northern range limit further and further south. This will ultimately drive the species down the Antarctic Peninsula by the end of the century – if it is able to colonise this region in the first instance (Fig. 8.3). As the sub-Antarctic region warms, the midge's native territory of South Georgia, along with the southern regions of South America, are likely to warm enough to negatively impact any midge there. Conditions in these areas become largely unfavourable compared to present (reduction of 60% suitability). Thus *E. murphyi* may struggle in its home territory by 2100 yet find more suitable environments further south on the Antarctic Peninsula (Fig. 8.3 c). As this is a first step in exploring the response of a terrestrial invasive invertebrate's range shift in Antarctica, further experimentation is required over the duration of *E. murphyi's* entire life cycle. The adjunct study assessed life-stage progression at 0, 2, 4, 6 and 8 ºC, with advancement to pupae through to adult only noted at 4 ºC. Whilst this is also in line with what we already know of *E. murphyi's* current inhabited environment, this was a short-term study, and longer-term effects may result in more detailed conclusions.

It is worth noting that with predatory invasive beetles likely affecting the native population on South Georgia (Convey et al., 2011), combined with a warming climate that may adversely affect the midge in its home territory, there is a risk that the *E. murphyi* population on South Georgia may collapse in the future. This would leave the Signy Island population as the only population in existence, and perversely, discussion surrounding its conservation should be considered if this is the case.

The potential range shift of *E. murphyi* towards the continent highlights the pressing need for rigorous biosecurity protocols and risk management around this species. From the same study, Figure 8.4 highlights areas on the Antarctic Peninsula that are at the most risk of invasion based on currently favourable habitats under existing climates, and the level of human activity/routes associated with areas of *E. murphyi* populations (Appendix XII). It also shows that *E. murphyi* can pose a high risk to

several areas, in particular the South Shetland Islands and Rothera on Adelaide Island where there are high levels of human activity, and spatial connectivity between here and South Georgia and Signy Island. This midge, and many other terrestrial species, have already been accidentally moved from South Georgia to Rothera (Hughes et al., 2010). This incident was rectified before any individuals were known to have moved from the offending vehicle, but upcoming development on Signy Island raises this risk once more (BAS, 2019).

In Chapter 4 it was found that the larvae of *E. murphyi* are able to survive in a submerged environment up to 28 d, supporting the work of Everatt et al. (2014a). Survival beyond this is greatly reduced, confirming *E. murphyi* as a terrestrial species, but this does raise questions about the ability of the midge to survive in sea water as well as freshwater, which is a trait seen in *B. antarctica* and explored in *E. murphyi* in Chapter 7. If *E. murphyi* were to be transferred to the Antarctic Peninsula, it would not only risk alteration to the native terrestrial ecosystem as per Chapter 6, but it may also mean that the midge has greater opportunity to successfully disperse *via* the ocean and arrive on other islands or coastal locations.

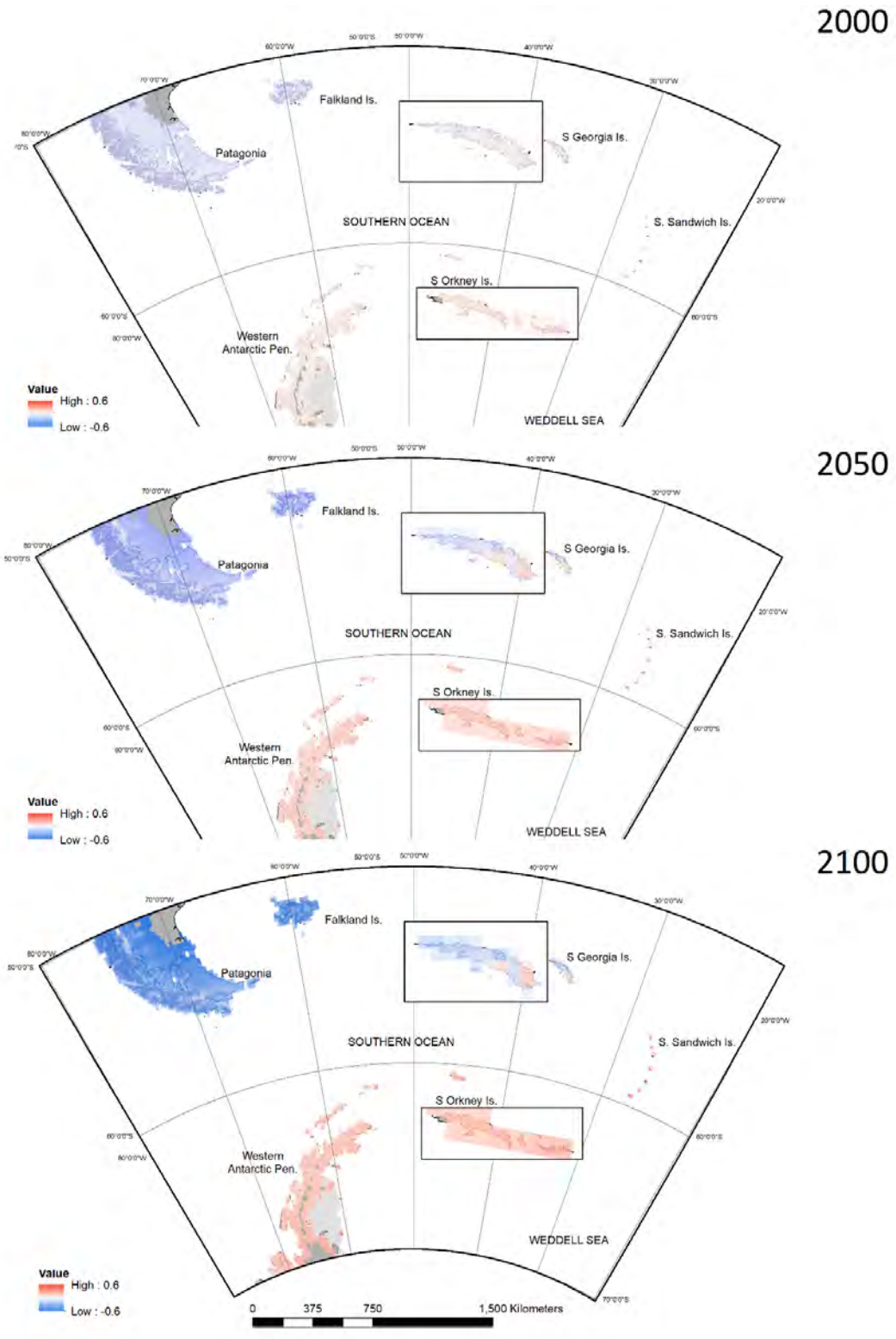

Figure 8.3. *Changes in thermal suitability for larvae survival of* E. murphyi *under (a) present (2000s) and future ((b) 2050s and (c) 2100s) scenarios based on mean temperatures of the warmest quarter obtained in regard to 1950s values. Colour bars denote the difference between the predicted survival rate in 1950 and subsequent decades every 50 years, with warmer colours denoting higher rates of survival. The insets in the maps represents a zoom of the native and currently invaded archipelago (S. Georgia I. / S. Orkney Is). Results from Pertierra, Bartlett et al., submitted. (Full paper in Appendix XII).*

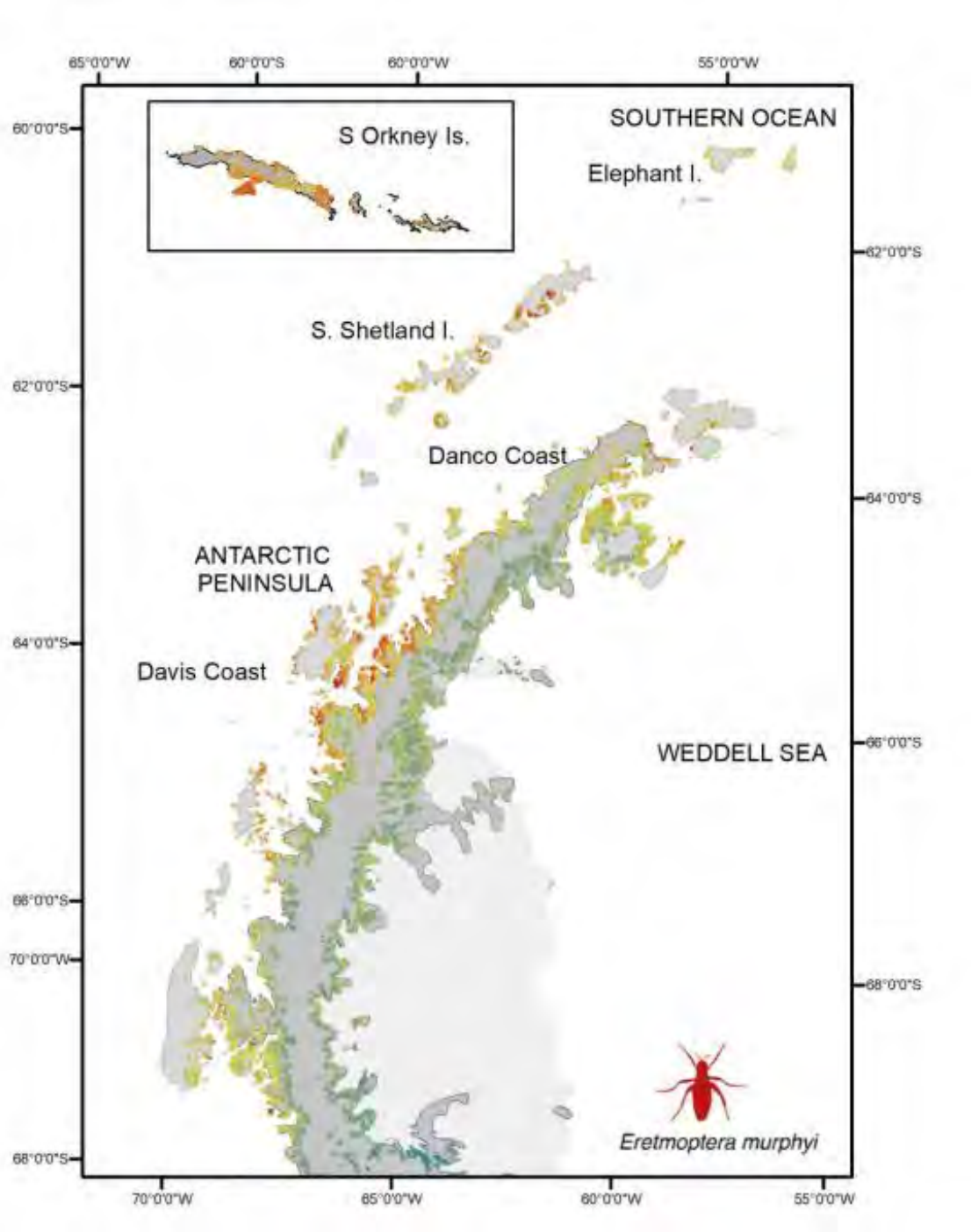

Figure 8.4. *Biosecurity mapping for of the* E. murphyi *in the maritime Antarctic, based on the aggregated risk of establishment with 5 arc-min degrees resolution (WGS84 projection). Aggregated risk values were calculated from the averaged consensus between the correlative and the mechanistic SDM favorability values (S1), combined with human footprint pressure (see methods in Appendix XII). Warmer colours denote higher levels of risk. Results from Pertierra, Bartlett et al., submitted. (Full paper in Appendix XII).*

8.6. Biosecurity and dispersal potential

Chapter 7 highlighted current weaknesses in existing biosecurity protocols regarding control of *E. murphyi*, and also documented that the midge is capable of surviving in the ocean for the entire gestation period of the egg (although L1s would not survive hatching), or 21 days as mature larvae. This result raises questions about the midge's ability for oceanic dispersal. Through analysis of open source data from the National Oceans and Atmospheric Administration, key currents in areas associated with current *E. murphyi* populations (South Orkney plateau), or key ports/infrastructure locations on the Antarctic Peninsula (Rothera and the South Shetland Islands), can be mapped with the potential direction of travel of any drifting midge larvae/eggs. Figure 8.5 highlights the overall trends of several dozen 'drifters', with 10 day intervals shown. Notably, currents crossing the South Orkney plateau are moving north-east out into open ocean (Fig. 8.5 d), posing little risk of transporting the midge beyond the South Orkneys, whilst currents on the Peninsula move towards coastlines and islands (Fig. 8.5 a-c and e-g). If the midge were transferred to Rothera, not only do all currents in that area move towards the mainland of the Peninsular (Fig. 8.5 b, c), but they are also capable of moving at speed, well within the survival times of *E. murphyi* larvae in sea water (Fig. 8.5 a). The South Shetland Islands are also a well frequented area off the Peninsula, with dozens of international research stations across several islands, and King George Island hosting ten different countries alone. The archipelago nature of the South Shetlands means that distances between islands are small, and available coastlines large. Figure 8.5e shows how the midge could potentially disperse from Livingston to Anvers Islands, *via* smaller islands en route. Figure 8.5f highlights the circulatory nature of some currents, whilst Figure 8.5g shows currents moving out towards Elephant Island.

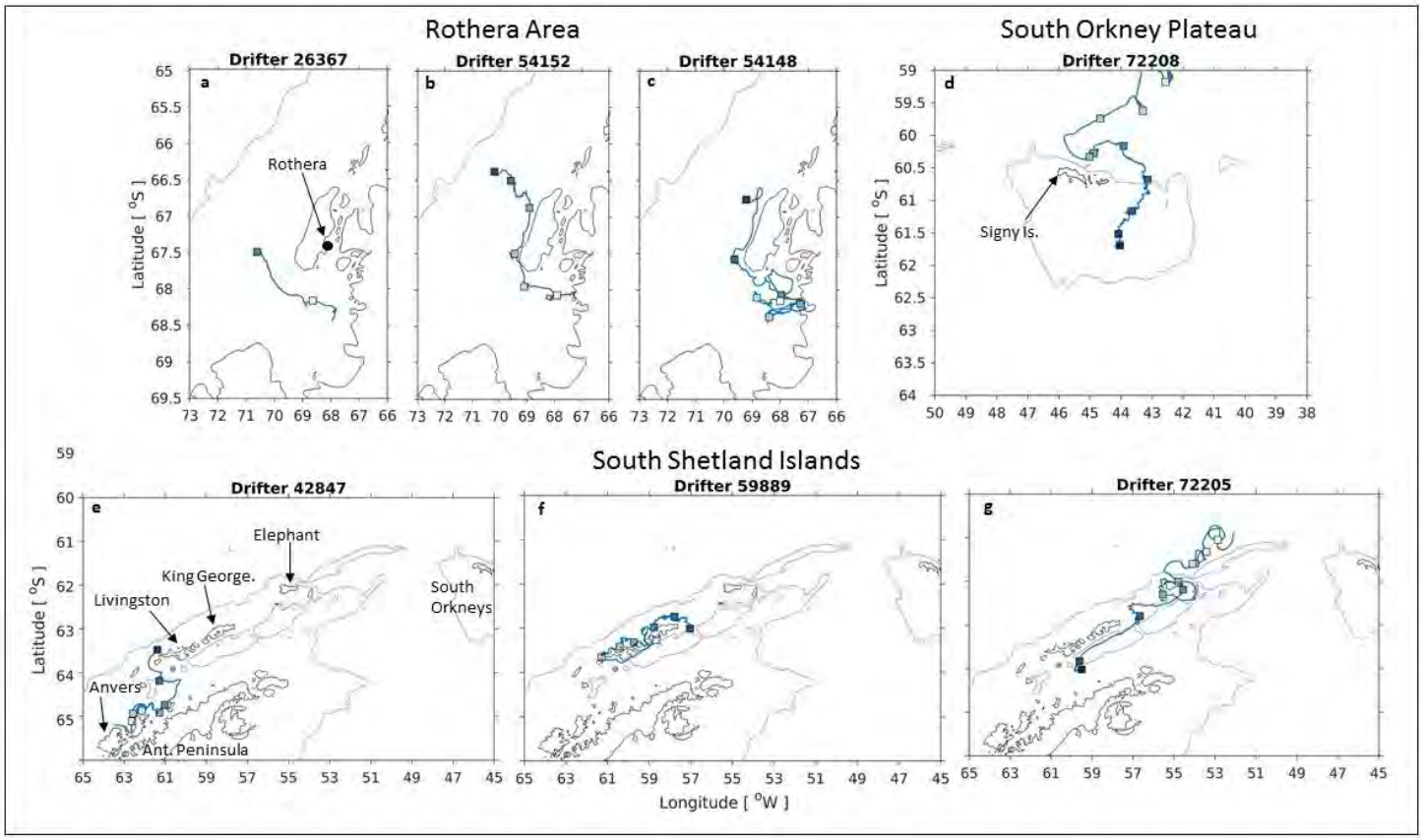

Figure 8.5. *Drifter data from key regions related to* E. murphyi *dispersal. Blue squares mark 10 d intervals that are shaded with time, where the oldest point is darkest. The coastline is marked in black and the 1000 m isobath in grey. Drifters that passed through the following co-ordinates were extracted, in relation to the areas of key interest (i.e. infrastructure/ research stations): Rothera series 68-67°S, 70-65°W, South Orkney Plateau 62-60.25°S, 47-43°W, South Shetland Is. series 63.5-60.5°S, 63-53°W. Key ports/stations and islands of interest are labelled. Data obtained by Sarah Thorpe of the British Antarctic Survey from National Oceanic and Atmospheric Administration (NOAA) http://www.aoml.noaa.gov/phod/gdp/index.php*

If *E. murphyi* were able to colonise areas on the Antarctic Peninsula, it is likely that it would share habitats, and thus directly compete for resources with Antarctica's only native chironomid *B. antarctica*. The two species have similar life cycles, physiological tolerances, and potentially diets (Baust and Edwards, 1979). However, the invading midge is parthenogenic and able to emerge all summer, rather than in one mass event like *B. antarctica* (compare Cranston, 1995 with Sugg et al., 1983; see also Chapter 2). This may give it a competitive advantage and allow it to increase in population faster than the native midge. Similar scenarios in temperate invertebrates have seen parthenogenic species invade twice as fast as sexual species, rapidly expanding populations to outcompete any native organisms they may share a niche with (Caron et al., 2013; Vorburger, 2003).

Taking this to an extreme outcome, the introduction of *E. murphyi* alone, could potentially see a reduction in Antarctica's only native (and endemic) insect *B. antarctica* as it outcompetes it for resources. This could also lead to an increase in available nitrogen that will change the abundance and distributions of other micro-arthropods, and ultimately lead to the dominance of graminoids over the 'forests of Antarctica', the mosses. Aided by climate change, the midge, and the trail of terrestrial change it could leave in its wake, could continue to extend its range south. Assisted by human transport and useful currents, the midge could colonise most of the islands on the Antarctic Peninsula, and spread down the Peninsula itself, taking advantage of the newly available ice-free land as it goes. This is currently all hypothetical, but this thesis demonstrates that it is a plausible scenario if the transfer of soils and whatever they may contain is not checked or taken seriously. Current biosecurity measures are not adequate to prevent the movement of *E. murphyi* around or off Signy Island, and no systematic schemes for invasive species monitoring or management are currently enforced by the Antarctic Treaty, although they are currently suggested, particularly regarding movement between Antarctic ACBRs (Hughes et al. 2019). A recent publication detailed the benefits of a long-term monitoring programme in the South Shetland Islands, and records new species and an increasing rate of introductions during the monitoring period of 2011-2017 (Enriquez et al., 2019).

Previously, Myers et al. (2000) established criteria for the successful eradication of invasive species in temperate systems, that also include the importance of knowledge of both the system invaded and the biology of the invader. These six criteria are:

1. Sufficient funding must be in place to undertake a large-scale project.
2. That authority must be granted agency to allow for the development and maintenance of regulations, treatments and monitoring.
3. For the biology of the invasive organism to be known and specifically for its susceptibility to control measures to be assessed.
4. To prevent reinvasion through effective biosecurity measures
5. To monitor the invading species whilst it is at low densities

6. For restoration and continued management of the invaded environment, particularly if the invader has become a 'keystone' species in the habitat.

Points 1, 2, 4 and 5 are highlighted as actions to be raised with the ATCM through the work presented by Hughes et al. (2019), and this thesis whilst investigating Antarctica's most persistent invading species, *E. murphyi*, has covered point three in Chapters 2, 3, 4 and 7. Chapter 5 has addressed point five by monitoring *E. murphyi'*s current distribution, and Chapter 6 has identified its role in the invaded environment, as a potentially new keystone species. This irrefutably confirms *E. murphyi* as an invading alien under the definitions of Frenot et al. (2005), and one that can affect large scale change in Antarctic terrestrial ecosystems.

8.7. Conclusions

The combination of pre-adaptation to the climate, parthenogenesis, an ineffective biosecurity chemical, the midge's ability to survive an appreciable amount of time in sea water, a changing climate and favourable oceanic currents in key areas, looks like a perfect storm to allow successful colonisation of *E. murphyi* further south. Only one case of human scrubbing error is needed to accidentally introduce *E. murphyi* to the Antarctic Peninsula. From there it could feasibly disperse to the other islands, or the mainland. Over time, conditions will only become more favourable for the midge.

Eretmoptera murphyi is by most measures, an unremarkable detritivore. Because of this, it should be considered as a model terrestrial invasive species, through which we can learn much of the physiology, dispersal and ecology of successful invading invertebrates in Antarctica. Similarly, the simple ecosystem of Signy allows us to unpick the role of a single species, and monitor the interactions therein, whether affected by an invasive species, climate change, or both. The two combined have proved to be an effective model system to highlight the impact of a terrestrial invasion in a polar habitat.

Biological invasions are arguably a larger threat to the native diversity of Antarctica than climate change and knowledge of them is paramount. A new study of the human footprint in Antarctica has shown that we are competing with nature for the increasing slithers of ice-free land in and around the continent, and that the visual human footprint in Antarctica has grown to impact half of all coastal areas (Brooks et al., 2019). As tourism and research continues to bring more people and more infrastructure to this continent, it is the most sensitive terrestrial ice-free areas that are disproportionally affected. Climate change is one huge pressure changing the landscapes and affecting organisms in Antarctica, and the encroachment of humans the other. What we introduce to the Antarctic is a symptom of a larger issue – that humans are the most damaging invasive species of all.

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Appendices

Appendix I – End of Season Adult *E. murphyi* Emergence Data.

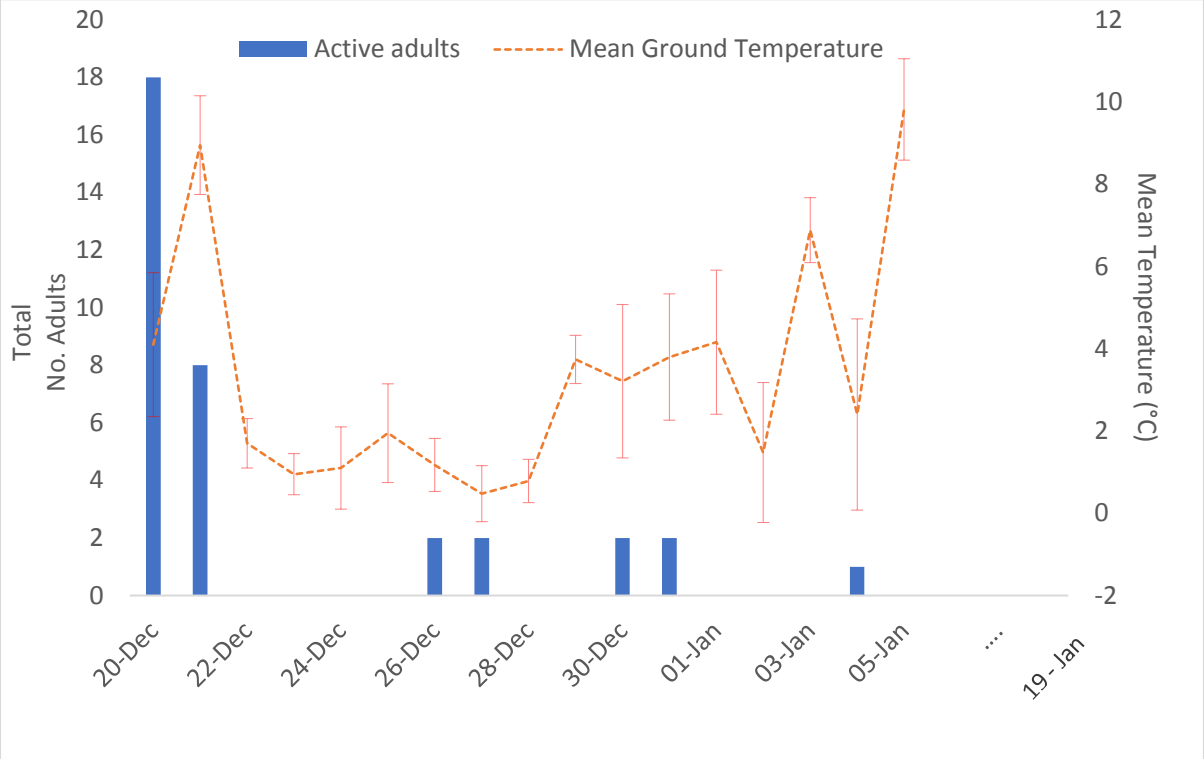

Figure A. I. *Emergence of adults within daily assessed quadrats on Signy Is. from 20th December 2016, until 6th January 2017 when populations had diminished. Plotted alongside mean (± SEM) ground temperature. Last adult seen active (not part of quadrat assessments) on the 19th January 2017.*

Appendix II – Signy Island Summer Ground Surface Temperature 2016-17.

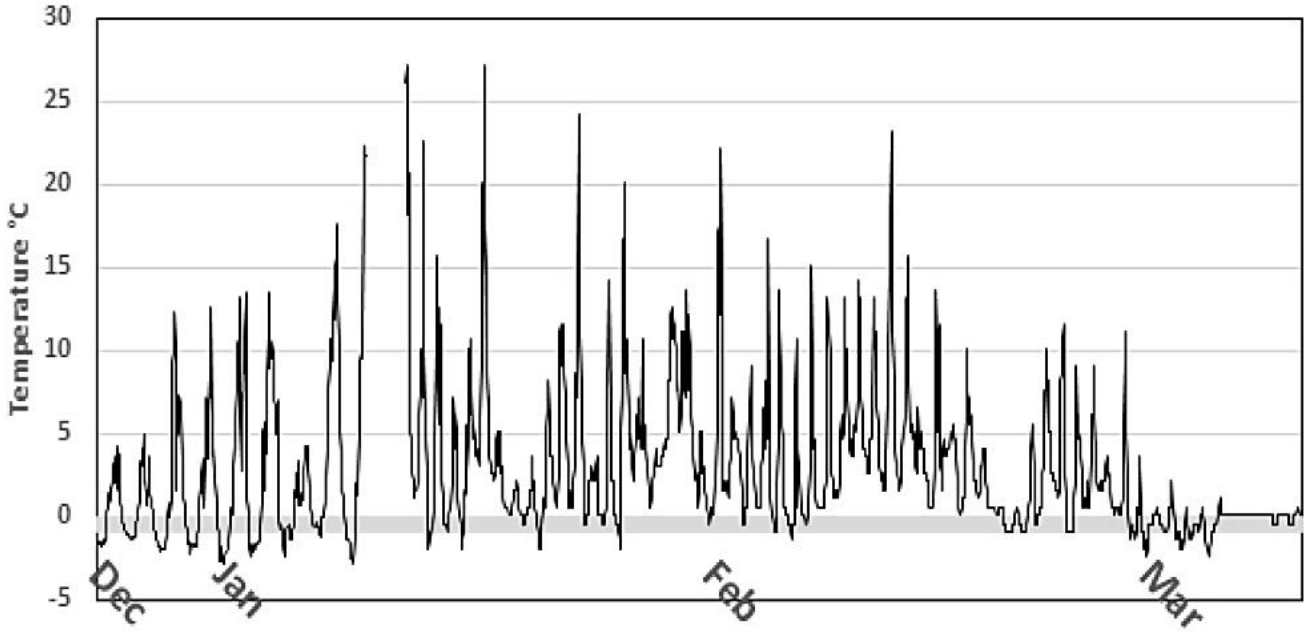

Figure A. II. *Ground surface temperatures recorded on the Signy backslope between the 28th December 2016 and the 10th March 2017. Temperatures logged every 30 minutes. Data from the 6th -12th January missing due to equipment removal by skua. Notably, the diurnal fluctuation on the ground is large, with values on the 5th January spanning from -2.8 ºC at 5am to +22.6 ºC at 3pm. Maximum temperature reached is 27.1 on the 12th January, minimum temperature was -2.8 ºC on the 5th January.*

Appendix III – Signy Island Temperature Station Data, Summer 2016-17.

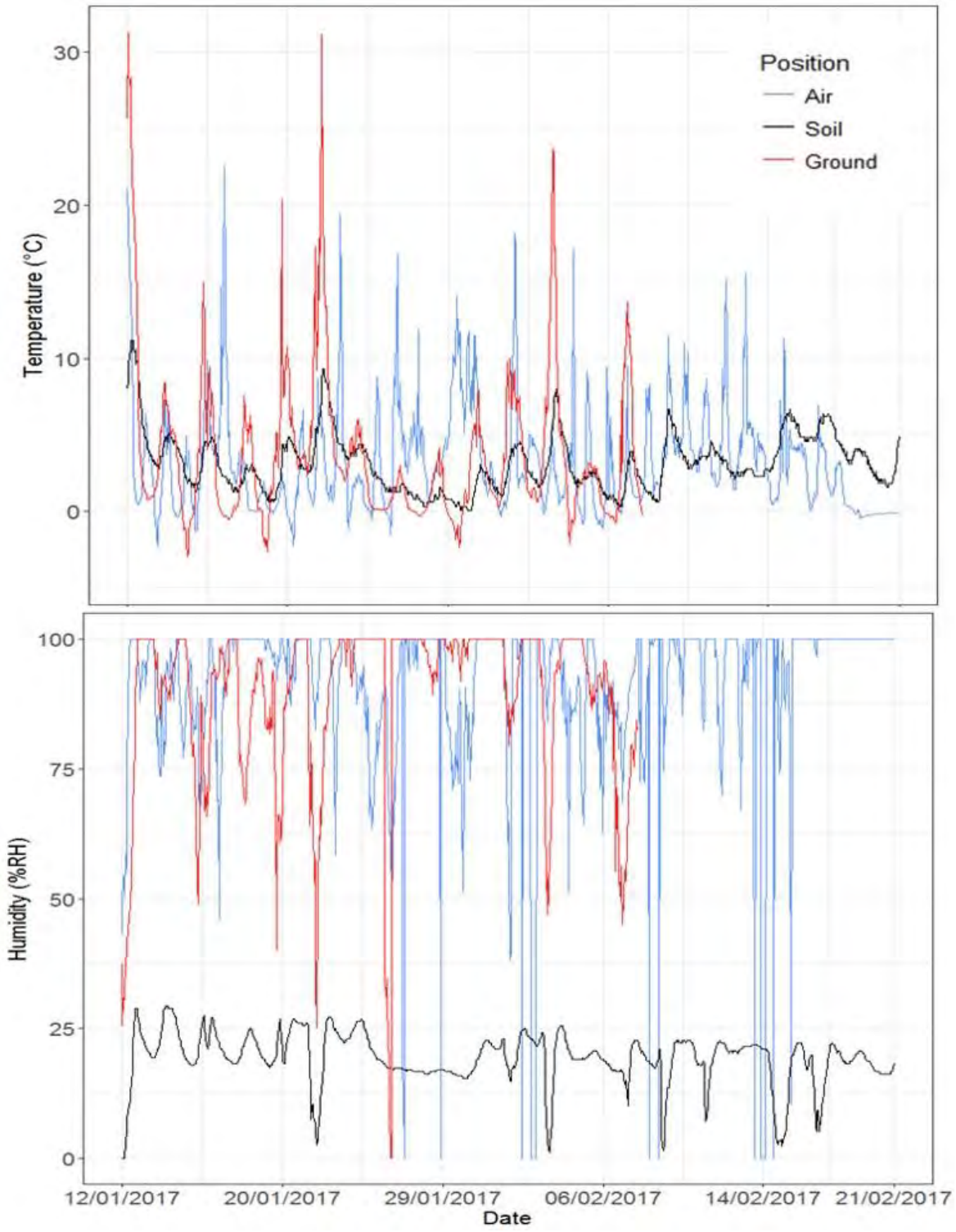

Figure A.III. *Air (10 cm above ground), Soil (5 cm below surface) and Ground/surface, temperatures and relative humidity recorded on Signy Island in* E. murphyi *habitat 12th Jan to 21st Feb (40 days).*

Appendix IV – Classification of Human Footprint.

Table A. IV. *Classification of human footprint (HFP) around Signy Island. See also Appendix VI.*

HFP Classification	Assigned value	HFP level	Example
Nil	0	No human footprint	Inaccessible areas -steep cliffs and glaciers
Infrequent	1	≤ 1 a season	Mountain summits, remote coves
Very Low	2	At least once a season	Annual seal survey routes
Low	3	A few times a season	Cummings Hut
Medium Low	4	Several times a season	Research conducted at Northpoint
Medium	5	Several times a month	Cemetery Flats
Medium High	6	Several times a week	Regular research on Gourlay Peninsular
High	8	Accessed daily	Immediate routes leading from Research Station
Very High	9	Several times a day	Directly around the Research Station

Appendix V – Results of *E. murphyi* Sampling Along Historical Trails.

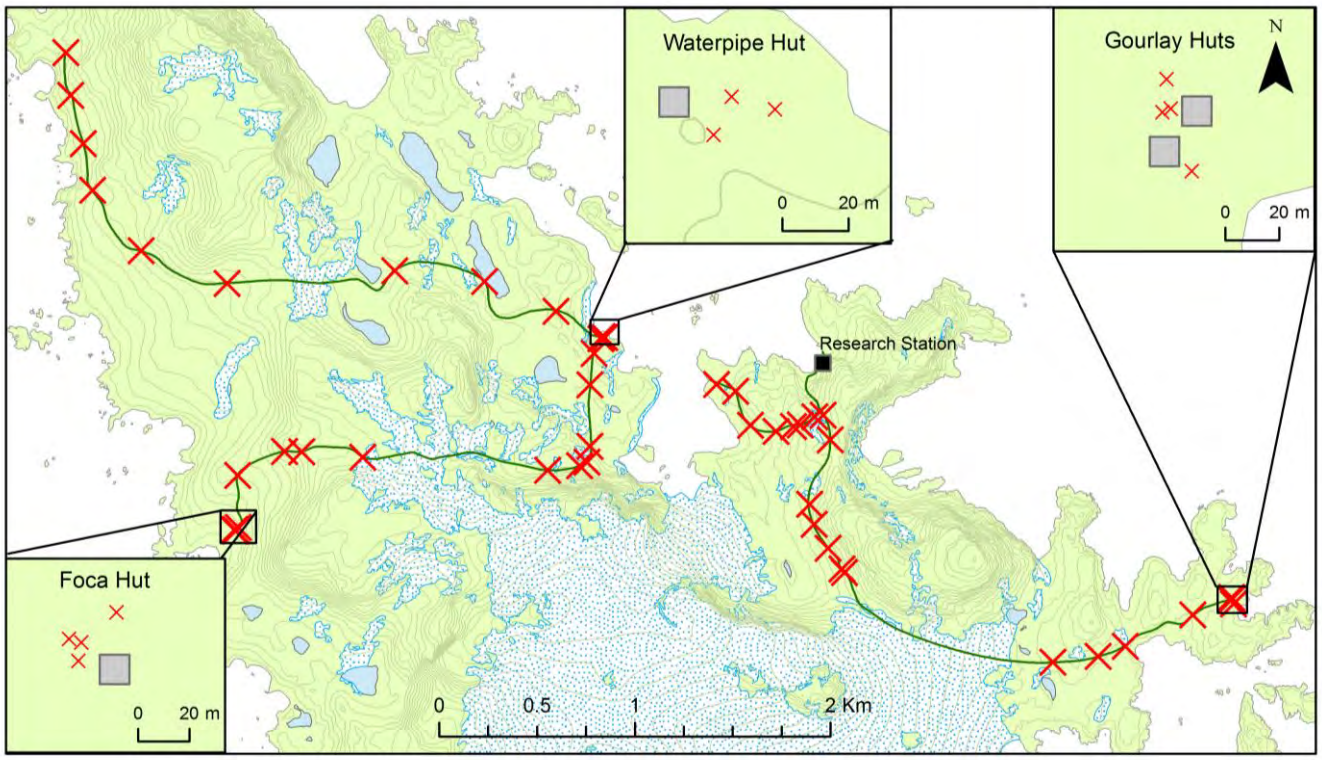

Figure A.V. *Survey routes and sampling locations across Signy Island, with focus on shelter huts of Waterpipe, Gourlay and Foca (insets). All sample sites, shown as 'x' points, found no presence of* E. murphyi. *Created using ArcMap® 10.4.1 software by Esri.*

Appendix VI – Input Layers for MaxEnt Modelling of 'at-risk' Sites.

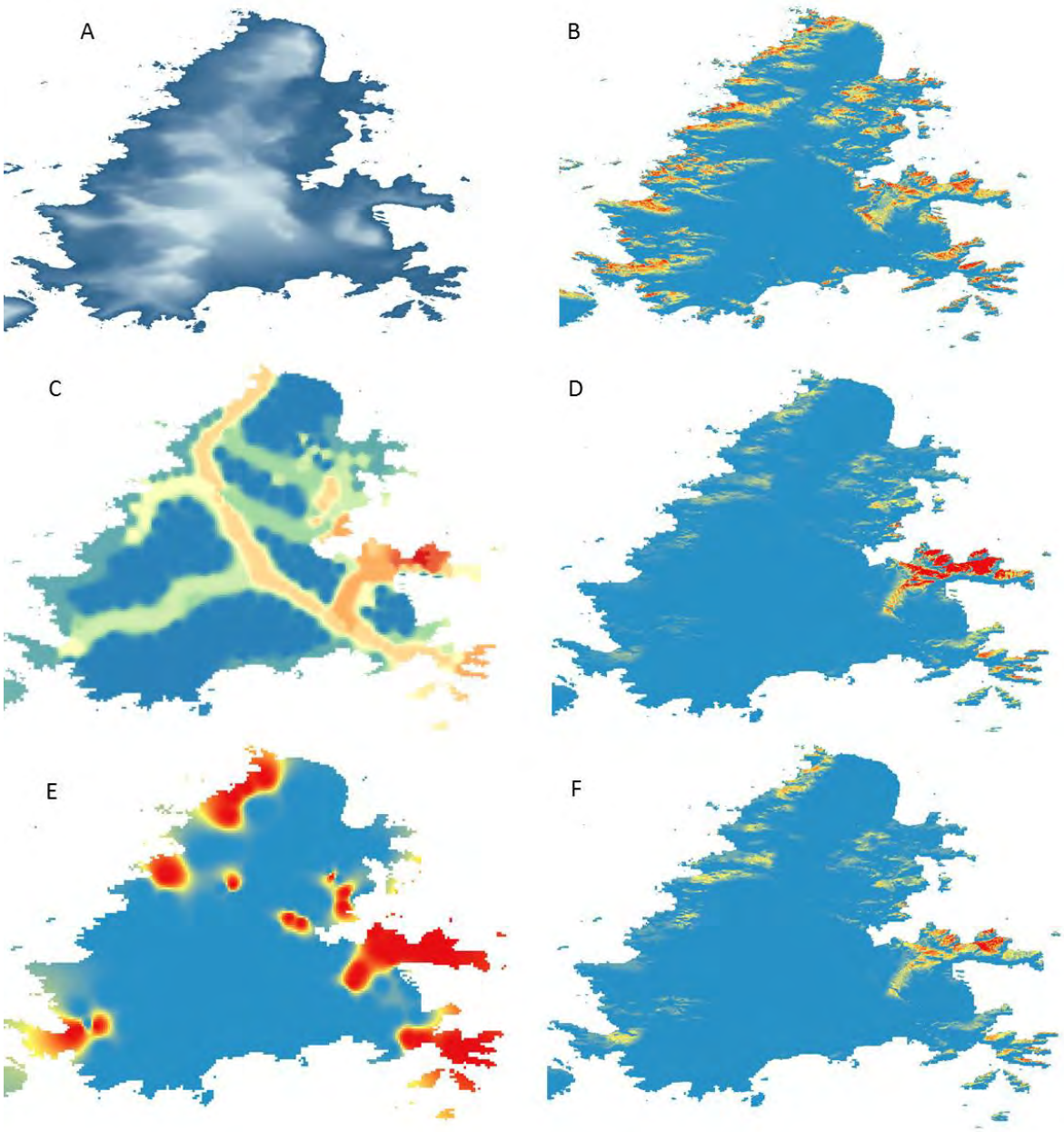

Figure A.VI *Signy Is. raster layer input (left side – a,c,e) and subsequent outputs (right side – b,d,f) from MaxEnt modelling:* ***(a)*** *Signy Island DEM (altitude, aspect, slope), where lighter shades = higher altitude* ***(b)*** *MaxEnt output of potential suitable environments based on digital elevation model and current distribution correlations** ***(c)*** *Human footprint (hfp) (see Fig. A.IV) ** ***(d)*** *MaxEnt output combining suitable environments and human footprint** ***(e)*** *verified presence of preferential substrates** ***(f)*** *with substrates and Euclidean distance from existing* E. murphyi *population considered*. * hot to cold shading, where red = higher values (presence, suitability, hfp, high establishment risk), and blue = lower values (absence, unsuitability, low-zero hfp, low establishment risk). Created using QGIS v2.18 'Las Palmas' (QGIS Development Team 2016. QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org).*

Appendix VII – Image of ‘Sampling Grid’ survey point.

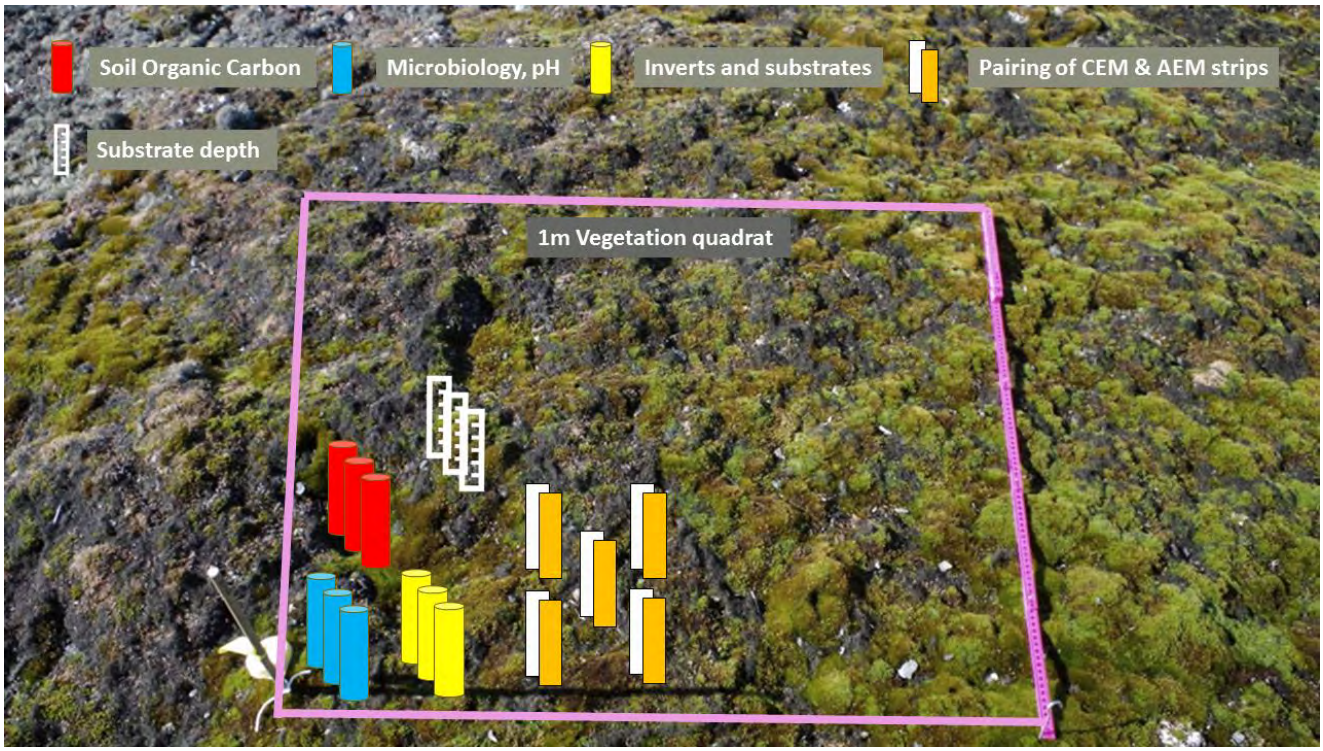

Figure A.VII. *Example of ‘Sampling Grid’ survey point with bamboo point marker, 1 m vegetation quadrat shown with examples of sampling cores (*n = *3 cores per variable), biochemical strips (*n = *5 pairs) and substrate depth (*n = *3).*

Appendix VIII – Biochemistry reagents and standards.

All reagents and standards were made within 48 h of the Skalar runs. All standards and reagents are stable for at least 1 week. Pre-warm the detergent Brij 35 in a water bath until liquid prior to use. Ammonia after Krom, 1980) and nitrate analysis after Gal et al. (2004).

Ammonia analysis

Reagent A: Buffer Solution

Dissolve 33 g of potassium sodium tartrate in approx. 800 mL of distilled water. Add 24 g of tri-sodium citrate and dissolve. Make the solution up to 1 L with distilled water and add 3 mL of Brij 35. Mix well and adjust pH if necessary, with hydrochloric acid to 5.2 ± 0.1.

Reagent B: Sodium salicylate solution

Dissolve 25 g of sodium hydroxide pellets in 50 mL of distilled water. Add 800 mL of distilled water and 80 g of sodium salicylate. Make the solution up to 1 L with distilled water and mix. Store in a dark coloured bottle.

Reagent C: Sodium nitroprusside solution

Dissolve 1 g of sodium nitroprusside in approx. 800 mL of distilled water. Make up to 1 L with distilled water and mix. Store in a dark coloured bottle.

Reagent D: Sodium dichlororisocyanurate solution

Dissolve 2 g of dichlororisocyanurate acid sodium salt in approx. 800 mL of distilled water. Make up to 1 L with distilled water and mix.

Stock standard solution: 100mg/L

Dissolve 381.9 mg of ammonium chloride in approx. 800 mL of distilled water. Make up to 1 L with distilled water and mix.

Nitrate/nitrite analysis

Reagent A: Buffer Solution

Dissolve 50 g ammonium chloride in approx. 800 m of distilled water. Adjust the pH to 8.2 with the ammonium hydroxide solution (25%). Make up to 1L with distilled water and add 3 mL of Brij 35.

Reagent B: Colour reagent

Dilute 150 mL of o-phosphoric acid in approx. 700 mL of distilled water. Add 10 g of sulphanilamide and 0.5 g of N-(1-naphthtyl) ethylenediamine dihydrochloride and dissolve. Make up to 1 L with water and mix. Store in dark coloured bottle.

Reagent C: Predilution agent

Dilute 3 mL of Brij 35 in approx. 800 mL of distilled water. Make up to 1 L with distilled water and mix.

Stock standard solution: 100mg/L

Dissolve 6.068 g sodium nitrate into 800 mL of distilled water. Make up to 1 L with distilled water and mix.

Mixed working standards

Working for a 25 mL standard per concentration following Equation A. VIII:

$$100\ mg/L\ \ stock\ \times x = 0.2\ \mu g\ \times\ 25\ mL$$

$$x = \frac{0.2\ \times 25}{100}$$

$$x = 0.05$$

Equation A. VIII. *$C_1V_1 = C_2V_2$ calculations for working standard concentrations.*

Mix appropriate volume of each of the stock standards (Table A.VIII) and mix together in a 25mL clean flask. Make up to 25 mL with distilled water and mix.

Table A. VIII. *Formulation of final working standard concentrations*

Volume taken from each 100 mg/L stock (µL)	Final concentration in 25 mL (µL)
0.05	0.2
0.125	0.5
0.2	0.8
0.3	1.2
0.75	3
1	4

Appendix IX – Nitrogen levels from *E. murphyi*/research station local area.

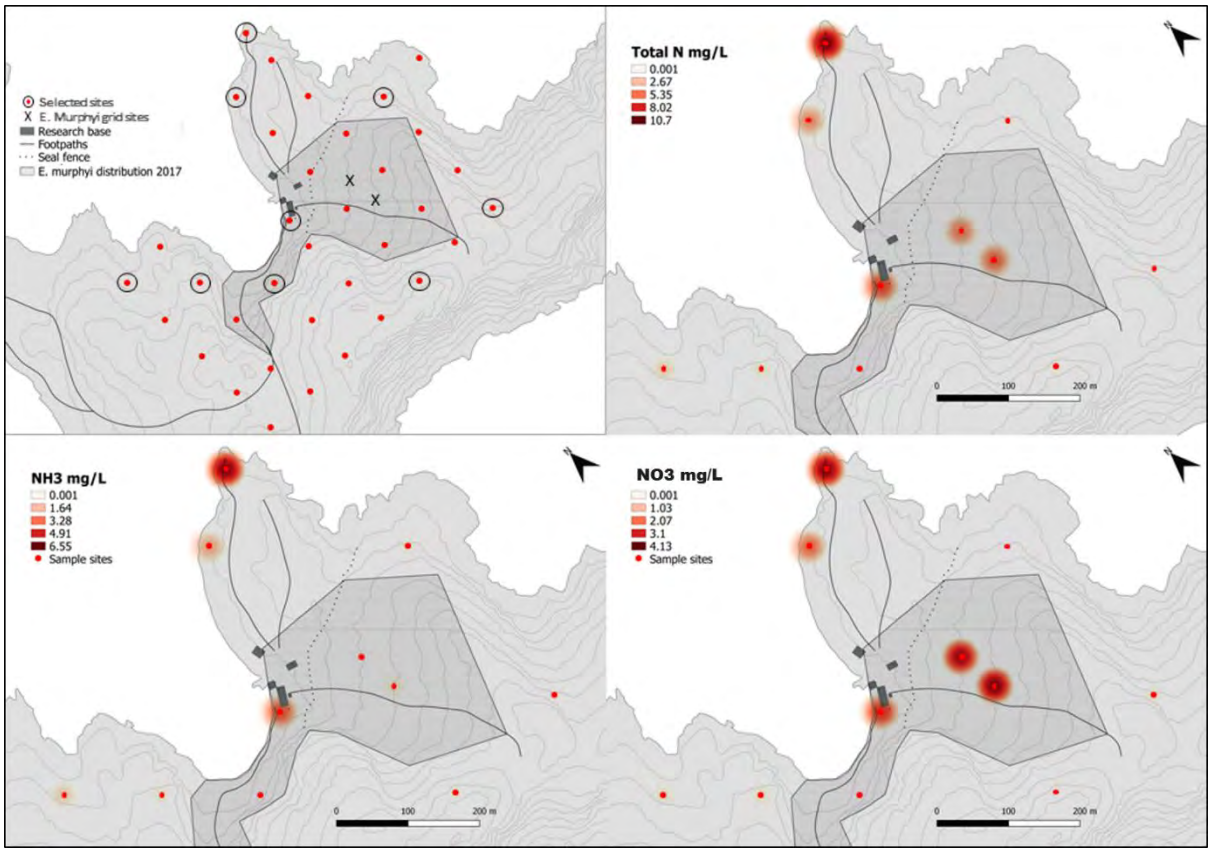

Figure IX. *Soil nitrogen represented relative to one another as red 'clouds.* ***(a)*** *100 x 100 m grid with randomly selected sample sites shown circled. Points from the 'Sampling Grid' marked with an 'X'.* ***(b)*** *Total Nitrogen* ***(c)*** *Ammonia* ***(d)*** *Nitrate. Created using QGIS v2.18 'Las Palmas' (QGIS Development Team 2016. QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org).*

Appendix X – Collected data from sampling grid.

Table A. X. *Original data with abundance levels scaled to m^{-2} (after Bartlett et al., in review/ Chapter 5, Section 5.3.2), and outliers (-) removed after Gubbs analysis (Gubbs, 1950).*

Grid Site	*E. murrphyi* m^2	NH3 mg/L	NO3 mg/L	Total N mg/L	SOC %	Acari m^2	Collem. m^2	Inverts. Tot m^2	Non-poly. moss cover %	Soil %	Depth cm	Polytrichum cover %	Lichen cover %	Mineral soil %	Bacteria CFU m^2	Fungi CFU m^2	C:N
1	83807.00	1.00	4.22	5.22	43.40	3248.33	-	3248.33	100.00	14.52	51.00	0.00	0.00	0.00	3711.11	233.33	32.51
2	34107.50	-	5.08	7.54	59.51	-	51973.33	51973.33	40.00	12.66	61.00	0.00	50.00	0.00	12477.78	877.78	30.83
3	2436.25	1.72	2.84	4.56	84.64	5847.00	55221.67	61068.67	100.00	8.66	55.00	0.00	1.00	0.00	5833.33	1744.44	173.62
4	487.25	0.66	-	-	72.87	4547.67	60419.00	64966.67	100.00	4.61	104.00	0.00	0.00	0.00	4488.89	277.78	67.35
5	42878.00	1.00	0.91	1.91	72.56	2598.67	3248.33	5847.00	20.00	9.84	69.00	10.00	70.00	0.00	3622.22	1600.00	208.06
6	19490.00	1.04	0.68	1.72	76.95	1299.33	4547.67	5847.00	90.00	6.64	165.00	5.00	5.00	0.00	6733.33	811.11	542.10
7	18028.25	1.06	1.20	2.25	90.35	0.00	1949.00	1949.00	90.00	6.99	104.00	10.00	1.00	0.00	1188.89	1122.22	242.84
8	0.00	0.62	0.70	1.33	81.84	0.00	4547.67	4547.67	100.00	1.53	99.00	0.00	0.00	0.00	411.11	1066.67	610.75
9	11499.10	0.86	0.91	1.76	10.78	0.00	2598.67	2598.67	90.00	42.54	60.00	0.00	10.00	0.00	7755.56	11.11	7.59
10	0.00	0.41	0.26	0.67	49.52	649.67	1299.33	1949.00	95.00	0.00	78.00	5.00	1.00	0.00	7633.33	411.11	617.77
11	0.00	0.51	0.84	1.36	90.63	649.67	5197.33	5847.00	50.00	0.00	70.00	50.00	0.00	0.00	2877.78	755.56	362.85
12	487.25	0.59	0.24	0.83	71.40	1299.33	24037.67	25337.00	100.00	27.10	59.00	0.00	0.00	0.00	11300.00	322.22	49.65
13	4677.60	1.49	0.41	1.90	85.90	0.00	53922.33	53922.33	50.00	9.55	78.00	50.00	0.00	0.00	23177.78	133.33	303.01
14	1949.00	0.86	0.58	1.43	17.25	0.00	11694.00	11694.00	40.00	59.24	70.00	10.00	1.00	40.00	21944.44	611.11	10.39
15	15104.75	1.23	6.73	7.96	70.91	0.00	55221.67	55221.67	100.00	14.96	68.00	2.00	0.00	0.00	4222.22	411.11	32.62
16	2923.50	0.71	3.89	4.60	44.81	2598.67	26636.33	29235.00	100.00	11.22	85.00	2.00	0.00	0.00	12733.33	466.67	40.25

Appendix XI – *Eretmoptera murphyi* and Collembola abundance in association with total nitrogen levels.

Table A.X *Data with outliers removed, sorted for increasing TN levels. The six highest values for* E. murphyi *and Collembola abundance are highlighted in bold, whilst the six lowest are shown in red.*

Total N (mg/L)	**Collembola (m^{-2})**	***E. murphyi* (m^{-2})**
0.67	1299	0.00
0.83	**24037**	487.25
1.33	4547	0.00
1.36	5197	0.00
1.43	11694	1949
1.71	4547	**19490**
1.76	2598	11499
1.90	**53922**	4677
1.91	3248	**42878**
2.25	1949	**18028**
4.56	**55221**	2436
4.60	**26636**	2923
5.22	-	**83807**
7.54	**51973**	**34107**
7.96	**55221**	**15104**

Appendix XII – Co-authored Paper submitted to Journal of Biogeography.

At the time of thesis submission, this paper has been submitted to the Journal of Biogeography and is currently accepted with revisions. It is presented here as was submitted and is unamended except for references that directly refer to chapters within this thesis that have been highlighted as such.

Integrating correlative and mechanistic niche models with human pressures to strengthen biosecurity risk assessments under climate change in Antarctica

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Contributions: LRP and PA conceived the study and designed the methodological approach. JB, SH and PC identified analytical parameters. JB, SH and PC conducted the laboratory experiments. MAOT, GCV and GD provided baseline environmental data. LRP, PA conducted the spatial analyses. All authors interpreted and discussed the results. LRP, JB and PA drafted the manuscript. All authors revised the manuscript.

Abstract

Complementarity modelling techniques can offer a valuable contribution to underpin more effective biosecurity provisions in the face of biological invasions, the risk of which is often enhanced under current climate change scenarios. Correlative species distribution models are subject to substantial spatio-temporal limitations since historical occurrence records of data-poor species become progressively obsolete. Mechanistic approaches offer additional knowledge on the underlying processes, but their use is more limited as they rely on the availability of background ecophysiological studies. Here we present a case study of a sub-Antarctic midge, *Eretmoptera murphyi*, introduced to maritime Antarctic Signy Island, to demonstrate how integration of these techniques may help to develop global conservation planning strategies applicable at high latitudes in the Southern Ocean and Antarctica. In this study both correlative and mechanistic models converged to predict high environmental suitability in the native and currently invaded areas. Geographic projections of the mechanistic model under climate change scenarios predicted that suitability's for *E. murphyi* survival will shift towards more southern locations. Increased human activity in the Antarctic region is driving new biological invasions, and our combination of correlative and mechanistic models with human footprint data identified priority gateways for control. Finally, our results also provide a demonstration that climate change studies should integrate long-term predictions and short-term empirical observations, since the impact may vary depending on the area and the timescale considered.

Keywords: Biological invasions, climate change, human footprint, Chironomidae, Southern Ocean, Species distribution models

Introduction

Global environmental change is having a profound impact on species distributions across latitudinal gradients up to the polar regions, causing unprecedented global biodiversity redistributions (Pecl et al., 2017). The polar regions have become sentinels of such biogeographic changes due to their relatively pristine nature and only recent exposure to human disturbances. Biological invasions are a major threat to polar ecosystems due to increased anthropogenic contact between semi-analogous regions in combination with a reduction in climatic barriers. Regional human activities in the Southern Ocean may facilitate inadvertent species dispersal that otherwise would be hampered by geographic or environmental barriers (Chown et al., 2012a; Pertierra et al., 2017a; Hughes et al., 2019). As a result, the often-endemic biota of the Southern Ocean and sub-Antarctic islands as well as the Antarctic continent face the combined effects of climate change and new biotic interactions arising from the arrival of non-indigenous species (e.g., Duffy et al., 2017; Hughes et al., 2015; McGeoch et al., 2015; Volonterio et al., 2013). The impact from either or both processes has led to reports of collapsing Antarctic and Southern Ocean ecosystems (Bergstrom et al., 2015).

To evaluate the extent of changes in potential distribution, driven by environmental changes and biological invasions, the use of correlative species distribution modelling techniques (SDMs) has increased over the past two decades (Guisan and Thuiller, 2005). This methodological framework aims to identify climatically analogous areas to the realised range of a species, based on the idea that these sites potentially have a high risk of colonisation, and so can be targeted as priority sites for active protection and biosecurity control (Jimenez-Valverde et al., 2011). However, classic SDMs are correlative in nature and so cannot easily be used to differentiate cause-and-effect, which weakens the reliability of any spatial prediction (Araujo and Guisan, 2006; Beck et al., 2014). They also rely on the selection of biologically meaningful variables (Fourcada et al., 2017), which is often a difficult or currently impossible task. In addition, potential distribution estimates are inherently restricted by the input data available from the realised distributions which are in practice a subset of the potential distribution (Jimenez-Valverde et al., 2008). These limitations generate challenges in accurately identifying the environmental optima for the pre-selected variables (Liu et al., 2013). Further, when

considering invasive species, the approach is weakened by these species new to an environment and associated abiotic conditions despite this being a general assumption of SDMs (Guisan and Thuiller, 2005). Another common challenge is the need to model and predict environmental suitability's from few species data records in both the native and invaded range for remote islands (Broenniman et al., 2008; Galante et al., 2017). Therefore, interpretation of results can benefit from integrating correlative SDMs with complementary methodologies. In this context, mechanistic models offer an alternative approach that utilize functional parameters of a species' biology to build complementary spatial predictions (Kearney and Porter, 2009). Experimental approaches are a key tool to understand the underlying processes causing biogeographic changes (Acevedo et al., 2017). Therefore, combining spatial and physiological data can strengthen risk assessments for applied purposes (Aragón et al., 2010a; Aragón and Lobo, 2012).

In the present study correlative and mechanistic SDMs are combined to provide integrated spatial predictions on invasion risk by the midge *E. murphyi* in the Antarctic Peninsula and Scotia Arc region. This species is a paradigmatic case of potential range shift towards the relatively undisturbed ecosystems at higher southern latitudes where its relative, *Belgica antarctica*, is native. *E. murphyi is* a flightless Orthocladiinae midge (Chironomidae) endemic to the sub-Antarctic island of South Georgia in the South Atlantic sector of the Southern Ocean (Convey and Block, 1996;). This midge is an invasive species further south on the Scotia Arc in the South Orkney Islands (maritime Antarctic), where it was introduced accidentally to Signy Island (SI), likely in the 1960s in association with plant transplant experiments (Block et al., 1984). In its native range on South Georgia, increasing temperatures since the 1950s, and the arrival of predatory carabid species in the 1980s or 90s have led to suggestions of declining populations (Convey et al., 2011; Ernsting et al., 1995). In contrast, following its introduction to SI, *E. murphyi* has expanded its distribution around the Signy research station area, in particular along well-used path routes suggesting human-assisted dispersal (Bartlett et al., in review/ Chapter 5; Convey, 1992; Convey and Block, 1996;). In addition, this species currently has no competitors or predators on SI. This is a concern since Hughes et al. (2013) noted that, in the area where the species occurs, it increases litter turnover by almost an order of magnitude more than the entire native

invertebrate community (see also Bridge and Denton, 2007; Montiel, 1998), and hence it can be argued to have an important impact on ecosystem functioning in terms of nutrient-cycling. *E. murphyi* has appropriate pre-adaptations to tolerate a range of conditions on SI as well as further south on the Antarctic Peninsula, including cold/freezing (Everatt et al., 2012), meltwater submergence (Everatt et al. 2014a) and desiccation stress (Everatt et al. 2014b, c). For all these reasons and given the ecological proximity of the Antarctic Peninsula and the spatial connectivity between it and the South Orkney Islands, mediated by human activities from Antarctic national operator logistics and tourism (Hughes et al., 2018), this species is an obvious candidate to pose a significant risk of further invasions to the Antarctic Peninsula region. Importantly, *E. murphyi* is not unique in presenting such risks – and thus represents an ideal study system to develop models in order to strengthen biosecurity assessments.

In this study we aim to assess the invasive potential of *E. murphyi* onto islands in the South Atlantic Ocean and ice-free habitats of the Antarctic Peninsula. This is examined as a function of climate suitability and human pressure. Predictions based on recent past, current and future periods are used for testing the hypothesis that changing environmental conditions are driving geographic range shifts of suitable conditions for this species, thus promoting both its invasiveness at higher latitudes and also progressively exposing it to suboptimal conditions in its native range. This information supports the development of climate change conservation planning measures, aimed to anticipate the extent and impacts of any such expansion of its range, as well as to provide robust justification for any need for enhanced biosecurity measures to minimise these risks (Hughes et al., 2015).

Materials and Methods

Study area and climatic profiles

The study area includes land within the limits of 50º to 70ºS latitude and 80ºW to 20ºE longitude, which encompasses southern Patagonia and the archipelagos of the Falkland Islands, South Georgia, South Orkney Islands, South Sandwich Islands and South Shetland Islands (Fig. 1). Temporal trends of temperature data from five meteorological stations representative of the study area were examined. We

performed linear regressions of time series of the annual mean temperatures for the warmest quarter as recorded by weather stations available in https://legacy.bas.ac.uk/met/READER/. These series comprised South Georgia (Grytviten station, 1947-2015), SI (Signy station, 1947-1995), King George Island (Bellingshausen station, 1969-2017) and Adelaide Island (Rothera station, 1978-2017) for the available periods (starting from the first available year in the invaded zone) (see Fig. 1). Additionally, since the available period for the invaded SI is the shortest, we also added data from the weather station from nearest island in the same archipelago (Laurie Island, 1947-2016; see also Royles et al., 2013a). In case of non-significance of linear regressions, we additionally performed Generalized Additive Models (GAMs) with four splines to test for alternative significant non-linear trends (Wood and Augustin, 2002).

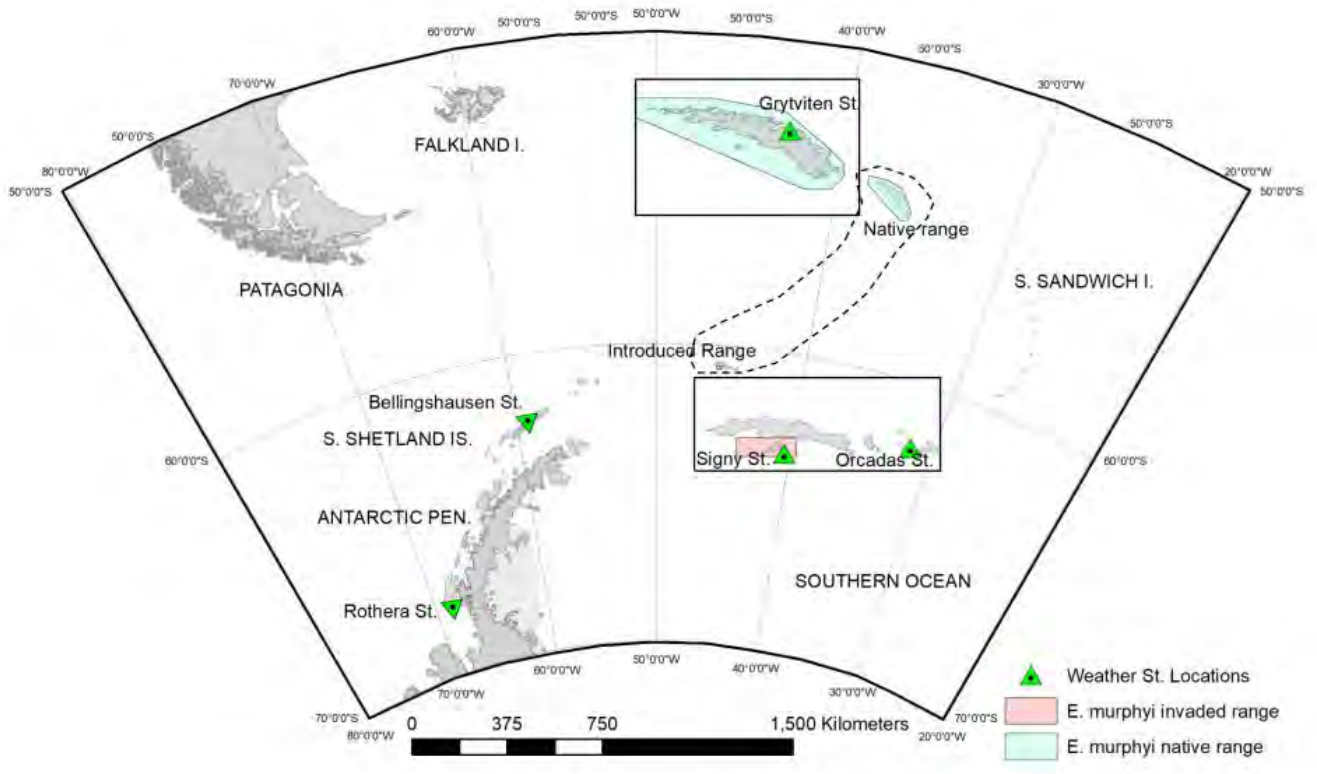

Figure 1. *Known distribution of the sub-Antarctic midge* (Eretmoptera murphyi). *Records are illustrated for the native range in South Georgia (blue) and for the invaded site in Signy Island (red). Weather stations whose data were used in this study in the study area are represented in green dotted triangles.*

Correlative Species Distribution Models.

Exploration on the potential macro-climatic distribution of *E. murphyi* was performed first by applying presence-only SDMs on contemporary spatial occurrence data with reference to macroclimatic thermal variables at 5 arc-min spatial resolution. We selected five thermal WorldClim2 variables (Fick and Hijmans, 2017) after performing a Pearson correlation test. The threshold applied to detect highly correlated variables was based on the correlation coefficient, excluding those showing r <0.9. Thus, five variables (mean diurnal range, isothermality, seasonality, minimum temperature of the coldest month and mean temperature of the warmest quarter) were selected as the predictors. Distributional data for *E. murphyi* in South Georgia were derived from the literature: The species was originally described as widespread (Schaeffer, 1914), with most recent records traceable to Grytviken, Husvik and Bird Island areas (Convey and Block, 1996; Allegrucci et al. 2006). From these sources a total of 15 records, set in a grid-cell at a resolution of 5 arc-min, were assigned at ice-free coastal bays where the species has been recorded historically. One additional record was added to indicate the species' presence on SI following Bartlett et al. (2018a).

The geographic distributions of invasive species are inherently farther from the environmental equilibrium than non-invasive species because there are potentially environmental suitable places where the species has not yet arrived (Acevedo et al., 2016). Thus, the purpose when modelling invasive species is to characterize as accurately as possible the potential distribution rather than the realized one (Jiménez-Valverde et al., 2011). In such cases, profile or geometrical techniques are recommended since they do not rely on information regarding species absences (Jiménez-Valverde et al., 2008, Aragón et al., 2010a). Thus, we obtained Mahalanobis distances (MD) because this procedure takes into account the associations and interactions among predictors (Farber and Kadmon, 2003). We calculated MD from each cell to the mean of the hypervolume of the predictors (i.e. here the species' thermal centroid), based on species occurrences. Predictions were set under the same envelope extent as the mechanistic SDM for comparison purposes.

Physiological experiments and mechanistic species distribution models

We measured the physiological response of *E. murphyi* larvae in terms of different fitness components as a function of temperature. The experimental design was based on both literature and field observations (Block et al., 1984; Hughes et al., 2013; Worland, 2010). For the laboratory experiments, live *E. murphyi* larvae were collected immediately adjacent to the British Antarctic Survey (BAS) research station on SI, during the 2016/17 austral summer season. Samples were returned to the United Kingdom by ship stored at +4 °C for 10 weeks, and then maintained at +4 °C at the University of Birmingham until use. It must be noted that incubator chamber temperatures directly relate to air temperatures in the field allowing for spatial macroscale projections, but actual ground and vegetation surface offer microhabitat conditions typically warmer that will to be accounted for, for example, in biophysical studies (Bartlett et al., 2018b; Convey et al., 2018). The cultures were maintained using Signy peat/soil substrate, which is both the species' habitat and food source. To ensure that environmental conditions deviated as little as possible from the natural environment, the substrate was kept moist with a soil solution comprising 3:1 deionised water to Signy soil. Only final/fourth instar (L4) larvae were used in experiments, selected according to instar class size categories (cf. Bartlett et al. (2018a/ Chapter 2), as this life stage is the best characterised physiologically (Everatt et al., 2014 a, b, c), and larvae within soil are the most likely agents to be dispersed by human movement. Ten individual larvae were placed in each Petri dish containing moistened substrate, with three and seven replicate dishes alternated for each consecutive temperature exposure (due to limited availability of larvae). Larvae were exposed to constant temperatures of either 0 °C, 2 ºC, 4 °C, 6 ºC or 8 °C for 30 days to simulate typical Antarctic and sub-Antarctic austral summer conditions. After 30 d, larvae were removed and three primary fitness components documented: survival, growth and fecundity. Survival was assessed by recording spontaneous movement or independent movement after gentle stimulation with a fine paint brush. Any that continued development through pupation, eclosion to adult or oviposition was also recorded.

To assess the impact of different temperature treatments we performed two Kruskal-Wallis tests (Siegel and Castellan, 1988), with either larval survival or pupation rates as dependent variables, since residuals

were not normally distributed even after data transformation. Then, once significance was detected, we further aimed to characterize whether such variables followed a concave curvilinear pattern reflecting centrality of unimodal preferred temperatures for a given fitness component (*cf.* Lynch and Gabriel, 1987). To this end, we performed multiple regressions where each dependent variable was included both as a simple and quadratic terms. For these analyses, response variables were Box-Cox transformed to reach normality of residuals. The projected envelope based on the larval survival curve was set to the range above 50% larval survival to delimit the envelope from a biologically meaningful criterion.

The number of individuals that completed their life cycle to the adult stage provided insufficient variability to perform any of the above spatial analyses. However, counts of adult emergence and oviposition were combined into a single dependent variable for statistical analysis, which still gave a small dataset, with all occurrences being at the 4 ºC exposure. To examine whether this pattern was statistically significant the proportion of replicates where these events occurred was compared against the remaining levels of the temperature treatment. This test was useful to check whether preferred temperatures match with other variables of the fitness components.

Biosecurity risk mapping and future scenarios

For practical management purposes the human-assisted invasion risk potential for *E. murphyi* in the Antarctic Peninsula and Scotia Arc was assessed by combining human footprint pressure levels with the climatic suitability obtained from the consensus between the correlative and the mechanistic models so as to generate an aggregated biosecurity risk index. The human footprint map, available from Pertierra et al. (2017b), estimates the intensity of local human activities in Antarctica and hence it can be taken as a simplified proxy of the relative dispersal pressures.

Future climate conditions were estimated for 2050 and 2100 at a 15 arc-min spatial resolution under the RCP 8.5 future climate scenario using an ensemble of five climate models from NASA Earth Exchange Global Daily Downscaled Projections (NEXGDDP: nex.nasa.gov/nex/projects/1356/; Thrasher et al., 2012) database following the assembling procedure outlined in Duffy et al. (2017). Here, we compared the shift of predicted larval survival and subsequent suitability though the geographic projection of the mean temperature of the warmest quarter in 2000, 2050 and 2100. This

was relative to the baseline suitability of 1950 prior to the commencement of the recent warming process (Turner et al., 2016). To achieve this, we subtracted the initial suitability at the pre-warming conditions from the different climatic scenarios (recent and future) to identify favourability trends across regions and time. Thus, an increase of suitability in sub-optimal areas would result in higher larvae survival, while a decrease in suitability would cause higher mortality.

Results

Historical distribution of E. murphyi and climatic trends at the Southern Ocean

Figure 1 shows the current native and introduced range of *E. murphyi* across the Southern Ocean islands. The species has an accessible area of 4,000 km^2 in the native range of South Georgia Island and 20 km^2 in the invaded island of Signy. However, roughly half of these islands are permanently covered by ice and the available records are restricted to coastal ice-free locations. Occurrence data are not up to date in the native range, posing uncertainties in the current status of the South Georgia population.

Climatic data have been extensively monitored in the relevant parts of Antarctica and the Southern Ocean archipelagos. There were significant and positive linear trends over recent decades for the recorded mean summer air temperature at South Georgia (r = 0.65, $F_{1,49}$ =37.18, P < 0.0001), SI (r = 0.61, $F_{1,47}$ = 28.89, P < 0.0001), and Laurie Island (r = 0.55, $F_{1,68}$ = 29.69, P < 0.0001; Fig. 2a). In the native species range (South Georgia) mean temperature from the linear regression has risen from c. 4.4 ºC to c. 6 ºC during the 1947-2015 period. The mean summer temperature in the invaded island (Signy) has risen from c. 0.2 ºC to c. 1.4 ºC for the period 1947-1995 (Fig. 2a). Similarly, the closest station to the invaded location with a period comparable to the native range (Laurie Island) showed an increase from 0.1 to 1.3 ºC. In contrast, data analyses from the more southern islands did not show a significant linear trend (King George Island: r = 0.04, $F_{1,47}$ = 0.10, P = 0.75; Adelaide Island r = 0.02, $F_{1,38}$ = 0.02, P = 0.87). The GAMs showed a significant non-linear trend in both King George (R^2 = 36.3, P < 0.0001) and Adelaide Islands (R^2 = 35.3, P < 0.0001), with initial increases of temperature that changed to monotonical decreases in the last decade (Fig. 2b).

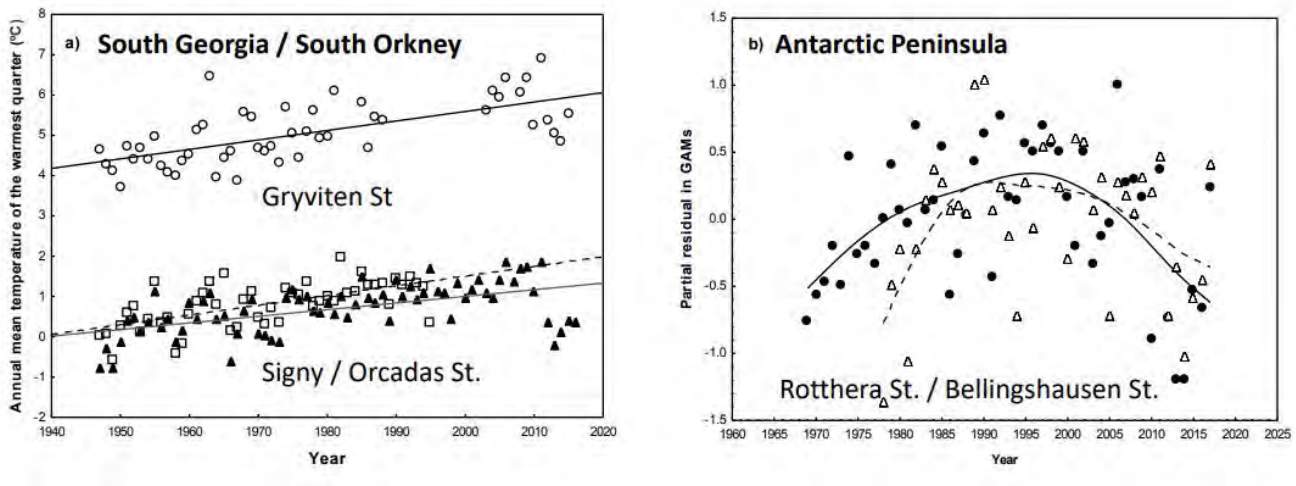

Figure 2. *Temporal trends for the annual mean temperature of the warmest quarter recorded by weather stations on* ***(a)*** *South Georgia (open circles, solid line), Signy (open squares, dashed line) and Laurie (solid triangles, solid grey line) Islands, and* ***(b)*** *King George (solid circles, solid curve) and Adelaide Islands (open triangles, dashed curve). GAMs: General Additive Models*.

Physiological response

Our experimental design included a thermal range encompassing the austral summer conditions in the recent past, present and predicted future trends for the representative locations examined in the Antarctic Peninsula and the Southern Ocean archipelagos (see Figs. 2a and 2b). The analyses showed a significant effect of temperature on L4 larval survival rate (Kruskal-Wallis test: $N = 27$, $H = 12.91$, $P = 0.011$), being greatest (100 %) at 2 and 4 ºC in a centred unimodal fashion (Fig. 3). Consistent with this, the regression analysis including both the simple and the quadratic terms of survival also supported a concave unimodal response curve (multiple regression: $R = 0.64$, $F_{2, 24} = 8.48$, $P = 0.001$), with the partial correlations of both terms being significant (simple term: $t = 3.03$, $P = 0.005$; quadratic term: $t = -3.70$, $P = 0.001$). This result justified a polynomial fit of the survival curve, where the maximum survival was predicted using either the transformed or untransformed data, respectively peaking at 3.3 ºC and 3.4 ºC (Fig. 3 and in Supplementary Material 1). As a result, 3.3 ºC was identified as the preferential temperature for larval survival, with the concave unimodal response curve used to build the mechanistic models. At 0 ºC and 8 ºC treatments had 70% and 40% survival (Fig. 3), respectively, but the mean 50 % survival limits were estimated to occur at approximately -5 ºC and +11 ºC.

The effect of temperature on pupation was marginally significant (Kruskal-Wallis test: $N = 27$, $H = 9.96$, $P = 0.041$, Fig. 3) (see Supplementary Material 1).

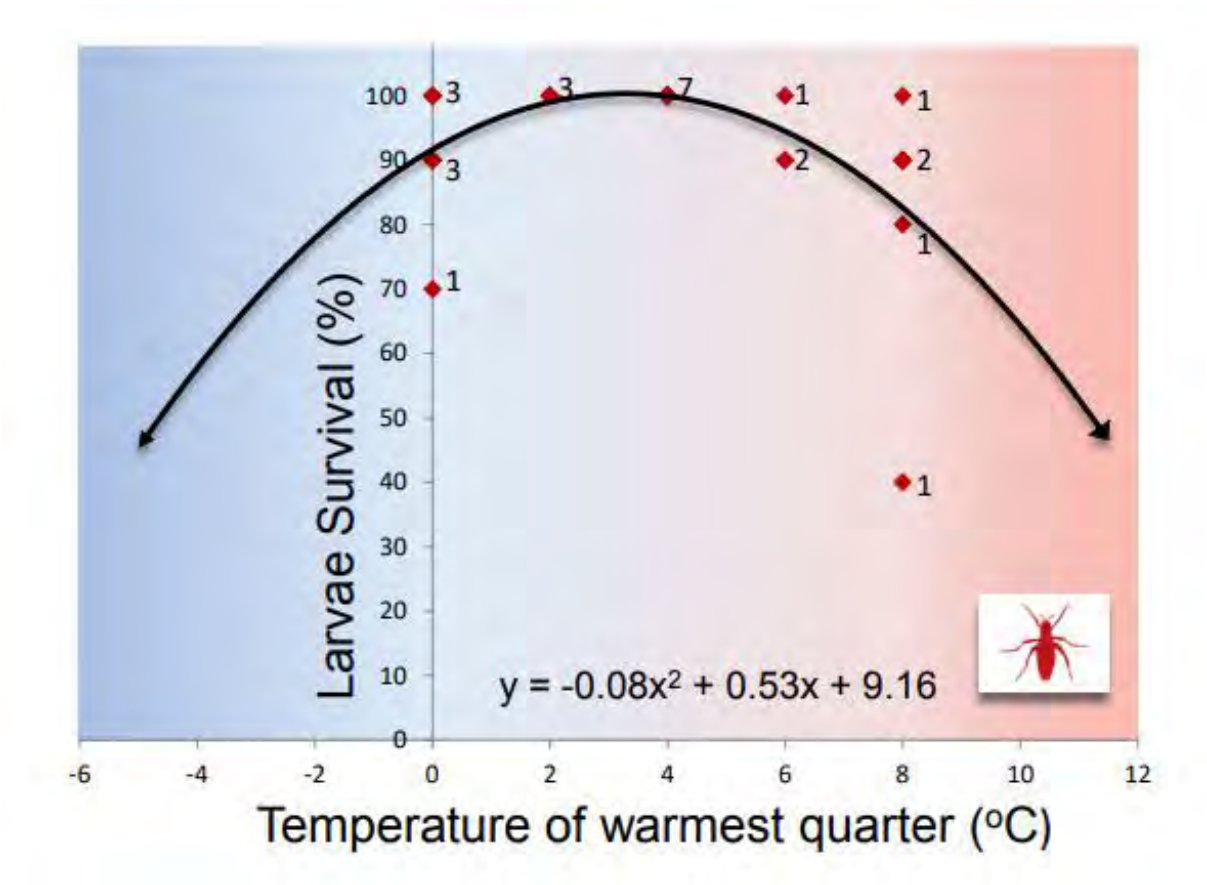

Figure 3. *Polynomial fit of the larval survival response of the* E. murphyi *over 30 days experimental exposure to different temperatures. The response curve is extrapolated to the upper and lower 50 % survival limits. Dots with the numbers of replicates are indicated.*

Geographic predictions from correlative and mechanistic distribution models

Based on correlative data, *E. murphyi* occurrence locations associated to the WorldClim dataset ranged from 0.95 (SI population) to 5.11ºC (S. Georgia population) for the mean temperature of the warmest quarter (4.23 ºC on average). For these occurrence records the mean lowest minimum temperature was -12.3 ºC for the coldest month, the mean diurnal temperature range was 9.3 ºC, and isothermality was 50.8 % (i.e. the diurnal temperature range was approximately half of the annual temperature range). These values are low in comparison to temperate and tropical regions, where the diurnal ranges can

reach 28ºC and the isothermality can be close to 100%. Temperatures for *E. murphyi* occurrences associated to the WorldClim database in both the native and invaded ranges were very similar to those in local automatic weather stations over the 1970-2000 period.

For the correlative SDM, climatic distances to the species niche centroid were obtained to identify areas of climatic suitability in the native sub-Antarctic and the introduced area in the maritime Antarctic (SI), while further examining the potential niche in the Antarctic Peninsula. Figure 4a shows the results of correlative SDMs for the 1970-2000 period on the suitability across sites within the > 50% survival envelope in five quantiles, defined here as very high (0-11: lowest mahalanobis distances), high, moderate, low, and very low suitability (17-21: largest distances). The rectilinear envelope bounded by 50% predicted survival of *E. murphyi* encompasses South Georgia, the South Sandwich and South Orkney Islands, Patagonia Highlands, and the northern and western Antarctic Peninsula. Within this envelope, the predicted species climatic suitability assessed through MDs was highest in the native area (South Georgia) and comparable to that of the South Sandwich Islands. The climatic suitability in the area of invasion (South Orkney Islands) was not lower than in the native range, but higher than most of the western Antarctic Peninsula. The South Shetland Islands had moderate suitability, suggesting additional effects beyond raw temperature gradients across latitudes.

As a complementary approach, a mechanistic model was geographically projected from the parameterized experimental results for larval survival and expressed in five quantiles, where very high suitability corresponds to 90 - 100% survival and very low to < 60% survival (Fig. 4b). The results obtained were similar to the correlative model in that the native range had the highest suitability. However, in this analysis the South Shetland Islands had similar suitability to the rest of the western Antarctic Peninsula and were comparable to the currently invaded area. The South American region included in the analysis had overall lower suitability, but this increased with altitude and reached high suitability in Andean alpine environments.

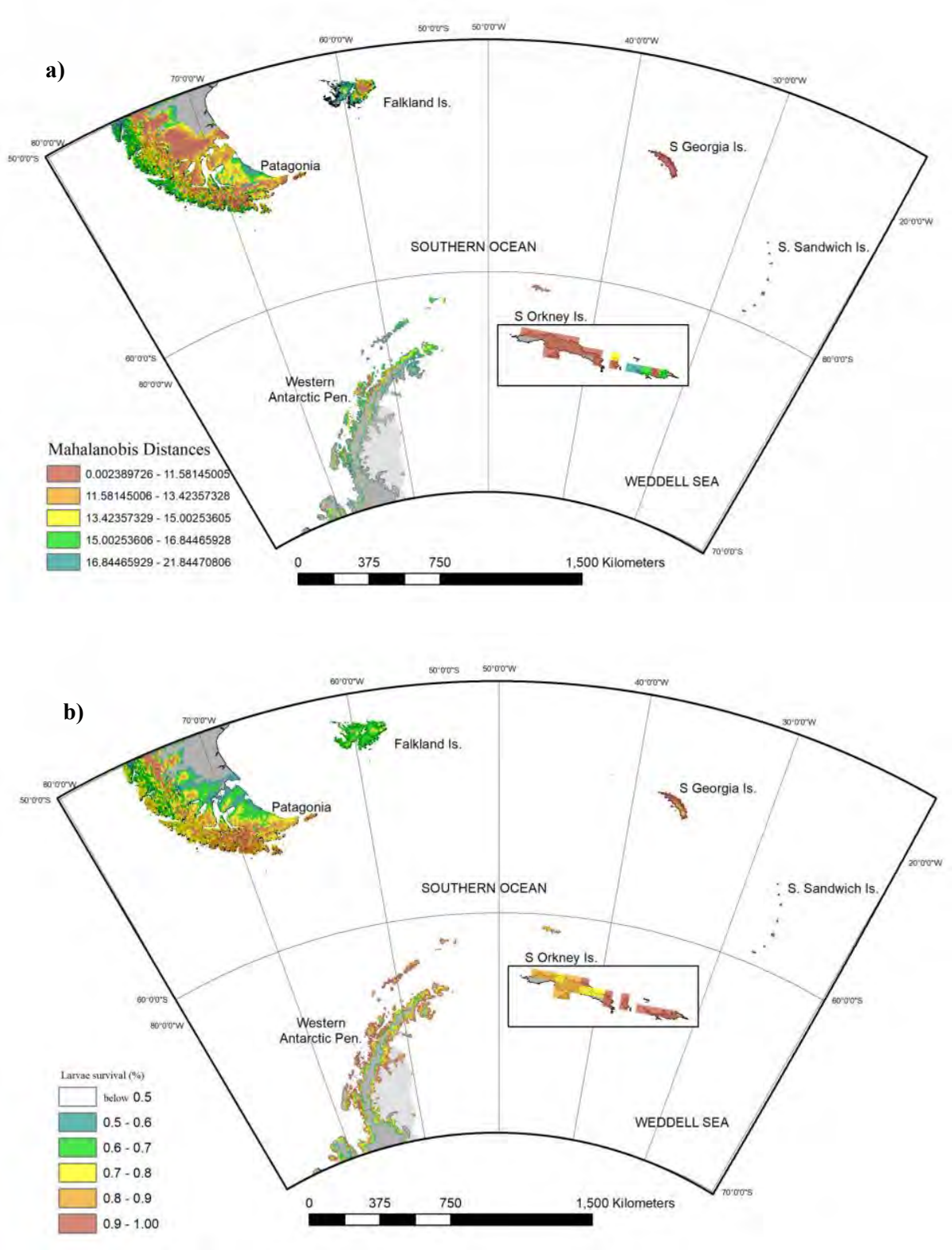

Figure 4. *Potential distribution models based on* ***(a)*** *historical spatial occurrences and* ***(b)*** *larval survival temperature response for the sub-Antarctic midge* (Eretmoptera murphyi) *using the 1970-2000 WorldClim dataset. Smaller Mahalanobis distances indicate closeness to the species' thermal multivariable centroid and thus higher macroclimatic similarity. Larvae survival curve was obtained from physiological experiments. The results are displayed in five quantiles.*

Biosecurity mapping

A biosecurity risk map was created from the combination of SMD outputs and human footprint (Fig. 5). First, the averaged suitability's from the consensus of the two SDMs was calculated as illustrated in Supplementary Material 2. The human footprint levels for the Antarctic Peninsula, taken from Pertierra et al. (2017b) were then incorporated. The final aggregation of climatic suitability's and human pressure is shown in Fig. 5. This mapping reveals several high-risk sites in the South Shetland Islands, as well as sites along the Antarctic Peninsula on Anvers Island, the Graham Land Coast and Davis Coast. In the South Shetland Islands, the three areas of highest vulnerability to invasion are Fildes Peninsula and Admiralty Bay on King George I., and the coastline around Port Foster in Deception Island This largely reflects the high levels of human visitation currently experienced in these localized sites and, thus, the assumed correspondingly high dispersal pressure that results.

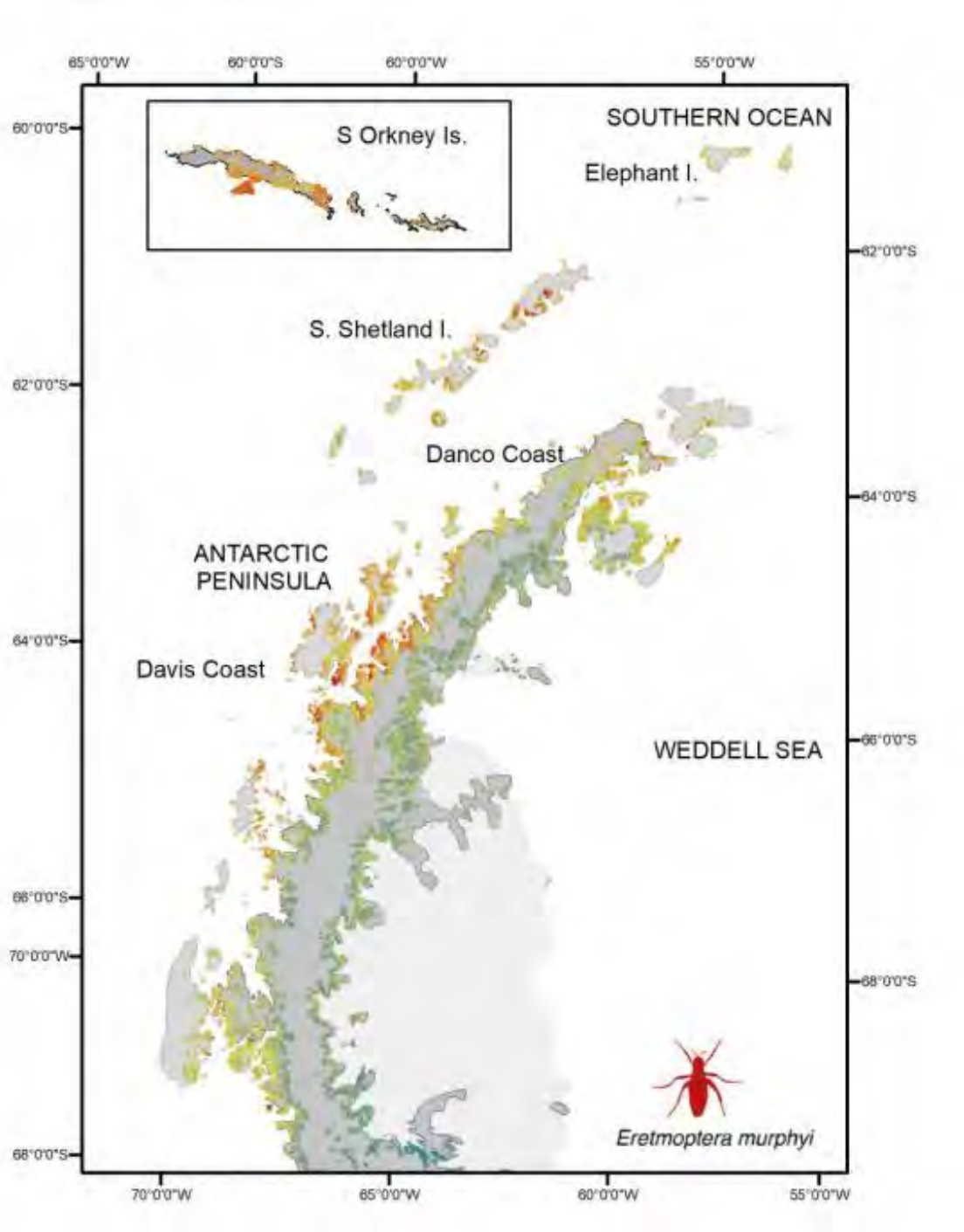

Figure 5. *Biosecurity mapping for of the sub-Antarctic midge* (Eretmoptera murphyi) *in maritime Antarctica based on the aggregated risk of establishment with 5 arc-min degrees resolution (WGS84 projection). Aggregated risk values were calculated from the averaged consensus between the correlative and the mechanistic SDM favourability values (S1), moderated by the human footprint pressure (see Methods).*

Geographic predictions of larval survival under climate change scenarios

Warming temperature trends in the study region are predicted to lead to ice-free areas to become more favourable over time to *E. murphyi* larvae (Fig. 6). In the 2000s these increases have led to a change in predicted larval survival relative to the 1950s (Fig. 6a), with the exception of parts of the South Orkney and South Sandwich Islands, where a small increase was predicted. Some areas of South America show a small decrease in potential habitat suitability.

By 2050 some areas of the South Orkney, South Sandwich and South Shetland Islands, and the Antarctic Peninsula show a moderate increment (5-15%) in predicted larvae survival relative to the 1950s (Figure 6b), while montane sites in southern South America become notably more unfavourable (Fig. 6b) with decreases of up to 30%. Most coastal areas in the native range of South Georgia show a small decrease of favourability for larval survival, except for the eastern end of the island.

By 2100 the suitability in the Antarctic Peninsula increases by 30% in some areas. The conditions in southern South America became largely unfavourable (up to 60% decrease) (Fig. 6c), and South Georgia shows a further decrease in suitability (Fig. 6c), with areas around East Cumberland Bay becoming increasingly unfavourable. However, areas in the eastern part of the island become more favourable.

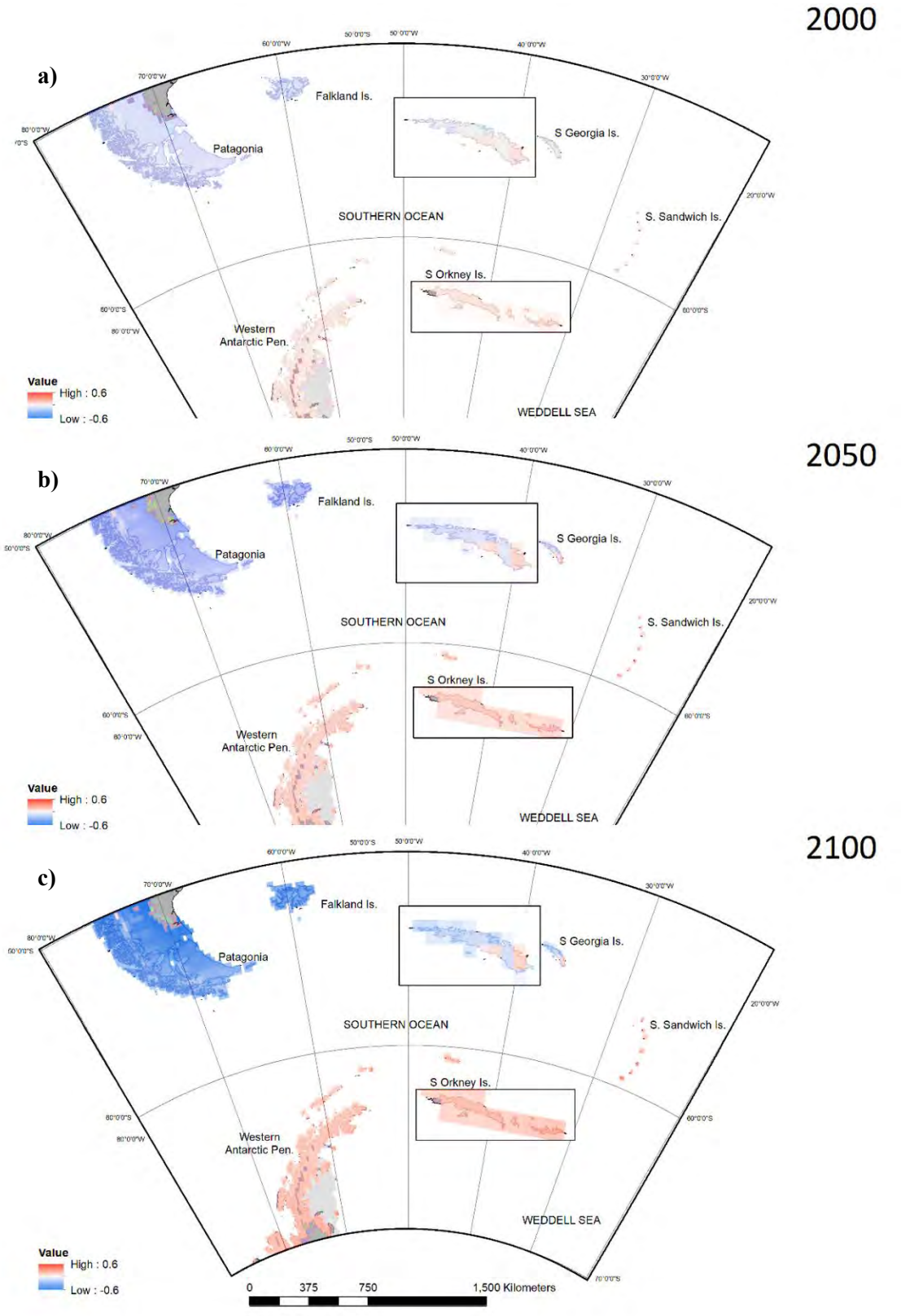

Figure 6. *Decadal changes in thermal suitability for larval survival of the sub-Antarctic midge* (Eretmoptera murphyi) *at present (**(a)** 2000s) and under future **(b)** 2050s and **(c)** 2100s scenarios based on mean temperatures of the warmest quarter obtained relative to 1950s values. Colour bars denote the difference between the predicted survival rate in 1950s and in subsequent decades every 50 years. The insets in the maps give a magnification of the native and currently invaded island groups (South Georgia, South Orkney Is).*

Discussion

The future distribution of *E. murphyi* will be determined by its capability to withstand changes in the environment, and knowledge of the environmental factors affecting fitness components, such as survival, is crucial to understanding the underlying processes. Larval instars dominate this species life cycle spanning almost 2 years and encountering the full range of seasonal conditions. This is contrast to adults, which typically live for just a few weeks, and eggs, with oviposition to hatch periods of around a month. Larvae are also the most likely stage to be transferred by human activity, and so it is their survival capabilities that will ultimately determine the capacity of this species to invade new locations in the Antarctic region. (Everatt et al., 2012, 2014b; Worland, 2010; Bartlett et al., 2018a, in review/ Chapter 2 and 5). It is also larval stages that undertake the role of ecosystem engineers, releasing nutrient bottlenecks in currently nutrient poor habitats, and thus have the biggest impact on the resident fauna and flora (Hughes and Worland, 2010).

Eretmoptera murphyi temperature preferences

Previous studies have investigated the effects of temperature on *E. murphyi* larvae. Everatt et al. (2014b) found that, in exposure to 4 °C, 9 ºC or 15 ºC over 56 d, 4 ºC produced the highest larval survival, consistent with the current study where the preferential temperature for larvae survival was around 3.3 ºC. Importantly, successful development through to oviposition was also only observed at 4 ºC, but not at any of the other temperatures we tested (0, 2, 6 or 8°C). Both this study and that of Everatt et al. (2014b) showed a slightly negative effect on larval survival of exposure to 8 °C or 9 °C, respectively, while this study also demonstrates negative effects at lower temperatures (0 ºC and 2.5 ºC). These data suggest that the species has a narrow thermal range for survival and development. Chironomids generally have a developmental rate positively correlated with temperature, as reported in *E. murphyi* eggs by Convey (1992), but this is normally within optimal temperature boundaries following a hyperbolic law (Stratman et al., 2014).

Model predictions: identifying potential distributions under changing conditions

Both the correlative and mechanistic models consistently identified suitable temperatures for *E. murphyi* in the late 20th Century to be geographically centred on South Georgia, where the midge is native. (Figs. 3 and 4b). In the late 20th Century we find favourable conditions at the early stages of establishment on SI.

Recent changes in the trends of regional climates in the Southern Ocean have largely reduced the informative value of historical occurrence records in making accurate predictions of present and future distributions in areas under processes of change. This limitation is particularly relevant for data-poor species (Galante et al., 2017), such as *E. murphyi*. In practice, the problem arises when the bioclimatic centroid inferred from occurrence datasets is largely driven by the uncertainty of the spatial presence data. In the current study occurrence data are from only a few locations in the original native range, and so we suffer from 'static' predictions based on conditions that may have become sub-optimal over time (Booth, 2017). Therefore, present and future risk assessments can benefit strongly from 1) attributing the occurrence data to the climatic period they were specifically reported from (Booth, 2016; Baker et al., 2016), 2) using sources complementary to correlative information, such as mechanistic models based on physiological information (Aragón et al., 2010a; Sánchez-Fernández et al., 2012) and 3) extrapolating outputs of mechanistic models to changing future climatic scenarios (Baker et al., 2016). Projections in response to future climatic scenarios are a common feature of correlative SDMs (Aragón et al., 2010b), but are not frequently generated in mechanistic models (Kearney & Porter, 2009; Aragón and Lobo, 2012). Yet they may offer value to infer climate change impacts under the assumption of niche conservatism (Aragón and Lobo, 2012).

Parts of the Southern Ocean region have experienced a process of recent climate warming. However, despite the widely reported late 20th Century temperature warming in the Antarctic Peninsula region, in recent years this region has shown a cooling trend interpreted as natural variability (Turner et al., 2016; Fig. 2b). It is important to evaluate these dynamics and natural variability in order to optimize predictions. No substantial change of climatic suitability's was identified by the NEX-GDDP dataset between 1950s and 2000s (Fig. 5a), suggesting that this magnitude of change in temperature (0.6 ºC on

average) might not be sufficient to identify impacts on suitability for larval survival. This is consistent with the shallower response in larval survival between 1 and 5 ºC (Fig. 3). Lastly, it also must be noted that the 15 arc-min resolution presumably generates a stronger maritime influence than the WorldClim2 dataset, and so larger thermal changes in ice-free areas could be diluted by the smoothing effect of nearby seas.

When considering predicted changes for 2050 and 2100, climatic change effects on larval survival become more evident. The models predict that survival will decrease in the species' native range, while conditions across most of the Antarctic Peninsula will become more suitable for invasion.

Environmental protection and biosecurity management

Biosecurity provisions must incorporate both climatic suitability's and human movement. Our biosecurity risk mappings reveal that human pressures are a strong component of the introduction risk for *E. murphyi*, as the climatic differences between Antarctic Peninsula sites do not vary as much as the footprint levels do. Pathways and activities that may facilitate the introduction of invertebrates into the Antarctic are diverse (Hughes et al., 2019). Early detection, preferably undertaken outside the Antarctic, is regarded as the most cost-effective mechanism for delivering effective biosecurity "prevention is better than cure" (Hughes and Pertierra, 2016; see also Bergstrom et al., 2017; Houghton et al., 2016). The models and analyses presented here indicate that climatic suitability for future *E. murphyi* invasion in the South Shetland Islands and some limited areas along the Antarctic Peninsula may increase over time (Figs. 2, 6). These areas also contain several locations at high invasion risk due to their levels of human presence and activity (e.g., at Whalers Bay, Deception Island and Fildes Peninsula, Admiralty Bay, King George Island in the South Shetland Islands, and multiple sites around the Gerlache Strait and the east coast of Adelaide Island off the Antarctic Peninsula, Fig. 5). The risk of further expansion of *E. murphyi* distribution within the South Orkney Is. is moderate, due mainly to the low intensity of human activity on islands in the archipelago other than Signy and Laurie (Fig. 5). However, the proximity of Signy Island to ice-free locations on other islands within the archipelago means that the

application of effective biosecurity measures by operators working on Signy Island and within this archipelago is vital.

Antarctic Specially Protected Areas such ASPA 126 Byers Peninsula (Livingston I.) may be subject to a lower risk due to their status, which restricts and controls visitation to the site, but this particular case is also due to a harsher multidimensional thermal regime (Fig. 4a), thus other ASPAs may be more vulnerable. Finally, locations near Rothera Research Station (UK) on Adelaide I. generally face harsher climatic conditions at this more southern location. However, this area does face a moderate risk as at smaller scales as it includes locations with warmer microclimates that might be overlooked at large spatial scales, while it also has a direct connection with Signy I. (South Orkney Is.) and South Georgia, due to the operational footprint of the United Kingdom's National Antarctic Program (the British Antarctic Survey). And it is worth noting that the midge has been found on cargo at Rothera once before (Hughes et al., 2010) The remote South Sandwich Islands will become highly favourable for colonization (Fig. 4), but assuming their continued isolation and minimal human activity they should not be considered strongly threatened. For these reasons' biosecurity procedures should be applied following the precautionary principle, as even lowest risk areas still face a threat. In future, further specific biosecurity measures internationally agreed within the Antarctic Treaty through the Environmental Protocol might be necessary and urgent to minimise the risk of further dispersal of *E. murphyi* and similar cases of fauna and flora with potential to reach and establish in Antarctica (see Duffy et al., 2017).

Climatic scenarios and uncertainties

Future scenarios suggest that the *E. murphyi* population in its native range on South Georgia will face challenges to its climatic niche, being forced to adapt to new conditions or find refugia in montane areas as coastal areas are predicted to warm considerably. This highlights a conservation value of the models employed here, i.e. not just in biosecurity assessments. There is a real risk that *E. murphyi* might collapse at South Georgia as has been reported for other organisms in the sub-Antarctic (Bergstrom et al., 2015). This raises the interesting situation that the accidental transfer of this midge to Signy has

probably delayed the timeframe of its extinction. What our models clearly highlight is that where transfer to new locations does not happen, species must adapt. The capabilities of species to achieve rapid evolutionary changes and shifts in their niches in response to rapid changes in environmental pressures, however, remain to be assessed (Petitpierre et al., 2012).

The future climate change scenarios used here to examine the potential long-term risk of invasion predicted an increase of suitability southwards whereas our empirical assessment suggested a hiatus in the short-term, probably due to natural fluctuations (cf. Turner et al., 2016). Since the seminal paper of Thomas et al. (2004), there has been an exponential increase in the number of studies projecting SMDs as a function of future climate change scenarios in the long term (i.e. 2050, 2100), however with limited if any consideration of short-term natural climatic variability that may deviate from the long-term general trend. Thus, another recommendation of this study is that management actions should integrate both long-term predictions of climate change and short-term empirical observations, since the risk of invasion could increase or decrease depending on the area and the timescale considered.

Final remarks

SDMs can offer a valuable contribution to underpinning stronger biosecurity (and conservation) provisions in general. However, correlative methods alone may be subject to substantial spatio-temporal limitations since recorded occurrence data will become progressively obsolete. Mechanistic approaches offer additional understanding of the underlying processes but are less commonly applied as they rely on the existence of background ecophysiological studies, and obviously reflect only partial information. Such knowledge and integration of techniques can help to develop more informed climate change conservation planning strategies. The identification here of current and future biosecurity risks using the example of an actual threat provides quantitative assessment tools that can be used to strengthen site-specific biosecurity provisions applied by all National Antarctic Programs and tourism operators. A further take-home message is that predictions should take into account not only long-term future scenarios but also short-term more immediate fluctuations in order to identify spatial and temporal variation in future risk.

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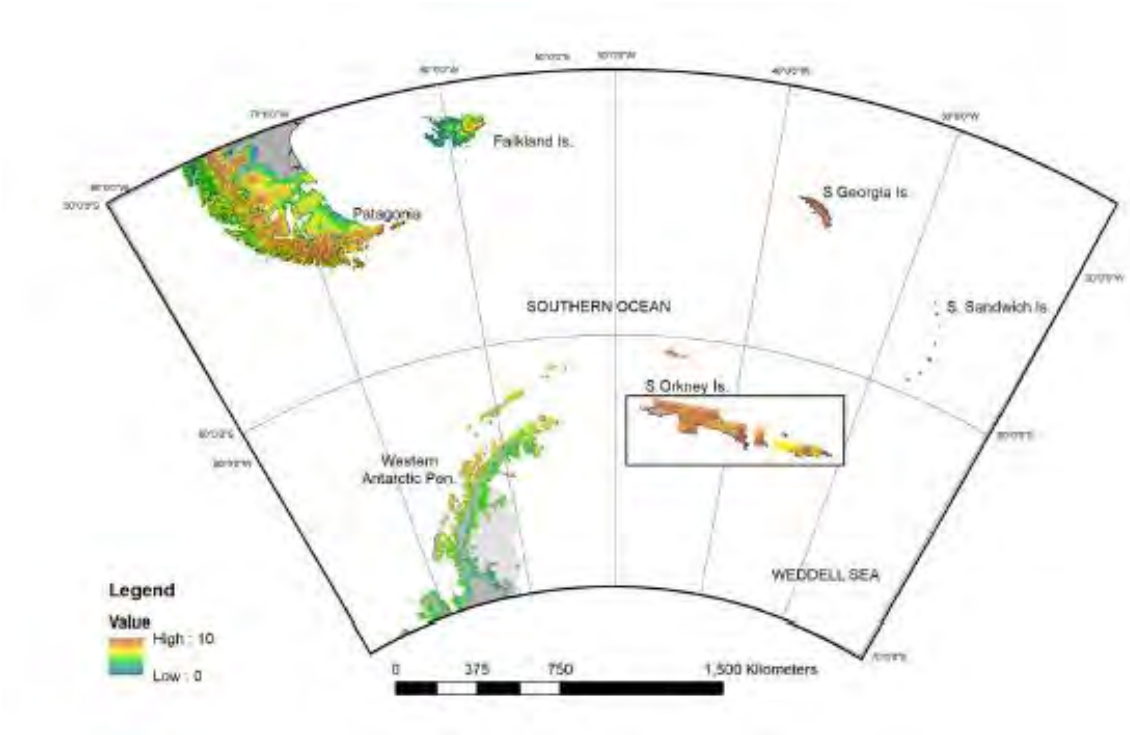
Supporting Information

Supplementary Material 1. *E. murphyi* larval development under different temperature treatments. *N*=10 L4 larvae in saturated soil petri dishes. Survival measured with brush stimulation or observable independent movement. Dead larvae removed from dish. Left in dark in relevant incubators at constant temp.

	Replicate	Survival (*n*=10)	Pupae	Adult	Egg Sacs
0°C	**1**	7	0	0	0
	2	9	0	0	0
	3	9	0	0	0
	4	9	1	0	0
	5	10	0	0	0
	6	10	0	0	0
	7	10	0	0	0
2°C	**1**	10	0	0	0
	2	10	1	0	0
	3	10	1	0	0
4°C	**1**	10	1	1	0
	2	10	0	0	0
	3	10	0	0	0
	4	10	0	1	1
	5	10	1	0	0
	6	10	1	0	0
	7	10	0	0	0
6°C	**1**	9	2	0	0
	2	10	2	0	0
	3	9	1	0	0
8°C	**1**	9	1	0	0
	2	9	1	0	0
	3	10	0	0	0
	4	9	0	0	0
	5	8	0	0	0
	6	9	1	0	0
	7	4	1	0	0



Supplementary Material 2. Averaged consensus between the partition in five quantiles of the correlative and mechanistic SDMs. A value of 10 represents the top quantile obtained in both SDMs, and a value of 1 the lowest quantile for either one that is unsuitable for the other. Image/jpg.

And she lived happily ever after…with tenure.

The End