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Ocean and Earth Science

Ecological plasticity of Southern Ocean bivalves from contrasting environments

by

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For A & A…

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ABSTRACT

FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCES
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ECOLOGICAL PLASTICITY OF SOUTHERN OCEAN BIVALVES FROM CONTRASTING ENVIRONMENTS

By Adam Jerold Reed

The ability of a species to show plasticity throughout its range is suggested to be significant to the survival, maintenance, and expansion of populations. In the Southern Ocean, plastic traits may have enabled resilience since the onset of cooling, and given species the capacity to exploit empty niches after the retreat of ice in interglacial periods. Phenotypic plasticity has rarely been investigated in Southern Ocean invertebrates however, and the cold stenothermal environment, which prevails, has often been considered homogenous in its selection on fauna. Previous ecological studies have often pooled together material collected within predetermined biogeographic regions to overcome the limitations of sampling difficulties. Subtle differences between environments may however, be forcing ecological divergence in species, with possible implications for speciation processes. This thesis investigates the phenotypic plasticity and reproduction among populations of the small shallow-water brooding bivalve *Lissarca miliaris* over its Antarctic range, and of deep-sea protobranch bivalves *Yoldiella ecaudata*, *Y. sabrina*, and *Y. valettei* from contrasting benthic regions.

The reproductive studies of *L. miliaris* revealed a previously unknown hermaphrodite trait, maximising the reproductive efficiency in a short-lived species where the female's capacity to brood its young is limited. Reproduction is also described for the first time in deep-sea Antarctic protobranch bivalves and demonstrates lecithotrophic larval development. Additionally, *Y. valettei* shows evidence of simultaneous hermaphroditism, which may increase the likelihood of successful reproduction in low population densities. Phenotypic plasticity is observed among populations of bivalves, irrespective of geographical proximity, and with no latitudinal trends, but subtle differences in the environment. Significant differences in morphology and growth rates are identified among populations, and reproductive plasticity identified in *L. miliaris* and *Y. sabrina.* Increasing atmospheric temperature is also measured to show an effect on the ecophysiology of intertidal populations of *L. miliaris* at Signy Island over the past 40 years, with increasing growth rates at the cost of smaller offspring and pressure from endolithic algal decay.

Plasticity in cold-stenothermal environments appears underestimated in the Antarctic context, but may be an important driver of ecological divergence and speciation in the Southern Ocean. Responding to subtle environmental changes are the likely mechanisms of resilience to climate oscillations, and this may be important when challenged with future pressures associated with regional warming, ocean acidification, and potential invasive predatory species. This thesis shows that ecological differences between populations are important to consider when comparing species from different Southern Ocean environments, while future genetic studies should consider plastic traits as drivers of ecological divergence and speciation among populations of subtly contrasting conditions.

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DECLARATION OF AUTHORSHIP

I, **Adam Jerold Reed** declare that the thesis entitled;

“Ecological plasticity of Southern Ocean bivalves from contrasting environments”

and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
- where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
- where I have consulted the published work of others, this is always clearly attributed;
- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all main sources of help;
- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
- parts of this work have been published as:

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Chapter 1

Introduction

1.1 Ecological divergence and plasticity

Modern molecular tools are fuelling a revolution in the way we understand evolutionary processes in marine environments (Grant et al. 2011), in some cases replacing the traditional taxonomic descriptions of species (e.g. Webb et al. 2006; Heimeier et al. 2010). No-where is this more true than in the Antarctic, where the processes that have been driving evolution since the onset of cooling ~30 million years ago (Ma) are only just beginning to be explained in the macro-ecological context. The ecological divergence of populations has an important role to play in speciation processes of terrestrial and aquatic ecosystems globally. It is the consequence of natural selection in populations between sufficiently contrasting environments, overcoming gene flow between them and encouraging reproductive isolation (Funk et al. 2006; Schulter 2009; see Hey 2006 for review). For widespread species, ecological divergence can be the primary force behind reproductive isolation, even in overlapping populations (Knowlton 1993), while selection for particular traits is often observed as plasticity in morphology, reproduction, physiology, or behaviour among populations (West-Eberhard 1989). In the Southern Ocean, such plastic traits have only occasionally been investigated, and to overcome the problems associated with small sample sizes, material has often been pooled from relatively wide geographic areas, under the assumption that the widespread cold-stenothermal environment, which prevails, is homogeneous in its selection pressure (see discussion below). This thesis challenges this view however, by examining the plastic traits of marine bivalves from different populations to determine the role of plasticity in maintaining local populations within subtly contrasting conditions.

Plasticity

Phenotypic plasticity is a common adaptive trait and is defined as the capacity of a single genotype to exhibit a range of phenotypes in response to environmental variations (Fordyce 2006; Whitman and Agrawal 2009; Pfennig et al. 2010). Globally, plasticity is most commonly reported in plants (Bradshaw 1965) insects (Atchley 1971), birds (e.g. James 1983), fish (Meyer 1987; Chapman et al. 2000; Stauffer and Gray 2004), reptiles (e.g. Losos et al. 2000), and marine molluscs (see discussion below), and allows an organism to adjust within environments over wide spaces and variable conditions. The ultimate benefit of being plastic is a range of phenotypes to suit a larger range of environments (West-Eberhard 1989; Scheiner 1993), but often at the cost of maintenance of redundant traits, the production of new traits, development instability and genetic costs (DeWitt et al. 1998; Auld et al. 2010, and references therein). Plasticity may also come at the cost of a mismatch in phenotype and environment (expressing the wrong phenotype) or simply the cost of being plastic (Auld et al. 2010). It can occur on different timescales, as an adaptation to a rapidly changing environment, or by long term acclimation and isolation. From an evolutionary perspective, plasticity occurring on generational timescales is of great interest when studying divergence and speciation of populations.

The impact of plasticity on species divergence is highly controversial and has been discussed extensively with ecological modelling (e.g. Price et al. 2003; Ghalambor et al. 2007; Whitman and Agrawal 2009; Pfennig et al. 2010; Fitzpatrick 2012), but in practice few ecological field studies have been conducted which investigate the link with speciation processes. Some of the most studied examples are those of fish such as the diverse rift valley lake cichlids of Africa (Meyer 1987; Wimberger 1991; Turner et al. 2001; Stauffer and Gray 2004; Cooper et al. 2010) and European stickleback (Garduno-Paz et al. 2010, and references therein) relating to diet, temperature, predation, and habitat preference (benthic vs. pelagic), many showing consequent evidence of reproductive isolation or the maintenance of morphologically distinct but continuous populations (e.g. Turner et al. 2001; Kristjánsson et al. 2002; Garduno-Paz et al. 2010).

Plasticity may impede speciation by removing the need for a genetic change and maintaining gene flow between populations by sustaining the reproductive fitness of individuals. Insufficient genetic variation for speciation therefore hinders evolution of traits (DeWitt et al. 1998). Alternative evidence however, suggests that plasticity can over time become genetically controlled and the initial environmental conditions which triggered the plastic response, is no longer needed to maintain the response (see reviews by Price et al. 2003; Ghalambor et al. 2007; Pfennig et al. 2012 for extensive discussion). This can be observed when populations from contrasting environments come together or within overlapping populations that express plastic traits for different habitat preferences. In this instance, sexual or natural selection against contrasting phenotypes can occur either actively or passively through behavioural preferences (habitat selection or favourable morphotypes), and may increase the likelihood of reproductive isolation (Via et al. 1999, 2000; Nosil et al. 2005).

Phenotypic plasticity is a particularly well described and easily measured trait in marine molluscs, usually determined by variations in the shape, size and structure of the calcified shell in response to environmental differences (e.g. Vermeij 1973; Seeley 1986; Nolan 1991; Fuiman et al. 1999; Trussell 2000; Bayne 2004; Sousa et al. 2007; Benaim et al. 2011). These accommodations may have important roles in survival and dispersal success, or promote more efficient energy partitioning. Morphological variations in shell shape can result from the physical effects of wave action or ice (Seed 1968; Beaumont and Wei 1991; de Wolf et al. 1998; Akester and Martel 2000; Steffani and Branch, 2003; Harper et al. 2012), predation (Seeley 1986; Trussell 2000) or food availability (Appeldoorn 1982), and are not necessarily representative of genetic structuring (de Wolf et al. 1998; Hoffman et al. 2010a).

Plasticity in the Antarctic

Phenotypic plasticity and plasticity of ecophysiological characteristics, inferred by growth and reproductive output over a wide distribution, have rarely been investigated in the Antarctic, and yet can give important clues to the species response to changes in temperature (Morley et al. 2009; Reed et al. 2012), food availability (Peck et al. 2005), disturbance (Nolan 1991; Harper et al. 2012), and even water chemistry (Chapter 5). In the Antarctic this is of particular relevance as many species which have wide distributions are known to be sensitive to subtle variations in temperature (Peck et al. 2004; Clarke et al. 2004a; Barnes and Peck 2008; Morley et al. 2010; Reed et al. 2012), which may suggest sensitivities to small variations in other environmental factors. Although subtle in the global context, the differences in temperature, food, and disturbance between different marine ecosystems may be contrasting in the Antarctic given the cold-stenothermal environment which prevails.

Antarctic fish have dominated research into phenotypic plasticity in the Southern Ocean, although plasticity has only recently been recognized in the abundant notothenioid fish (Eastman 2000). Among the first was the observation of differing head and mouth structures of *Trematomum newesi* in the Ross Sea, interpreted as being related to the benthic or semi-pelagic feeding behaviour associated with bathymetry (Eastman and Devries 1997). The same traits have been described in overlapping populations at the South Shetland Islands (Piacentino and Barrera-Oro 2009), although the ecological relationships with this plasticity is unknown. Feeding plasticity has also been described in *T. hansoni* relating to fishery waste products in the Cosmonauts Sea (Pakhomov 1998) and *T. newnesi* feeding in relation to sea ice coverage in the Ross Sea (La Mesa et al. 2000). Having evolved *in situ* in the thermally cold and stable Southern Ocean, one crucial aspect of Antarctic notothenioid fish biology is their apparent lack of plasticity with regard to temperature (Somero and DeVries 1967), in at least one species due to the inability to express heat-shock proteins (Hofmann et al. 2000). A passive selection against genes that are not required in a cold stenothermal environment is likely to have driven this lack of temperature acclimation (Somero 2005). Therefore, the same processes selecting for plastic traits can also passively select against plastic traits.

Calcified organisms often present distinct morphological variations in response to environmental conditions by differing shell formations, and has erroneously resulted in the identification of new species which are later found synonymous with existing species (e.g. *Nacella polaris/concinna*, Powell 1973; *Lissarca miliaris/rubro-fusca/rubricata/bennetti*, Nicol 1966; Dell 1964; *Yoldiella eightsi/woodwardi,* Nicol 1966). At shelf depths in the Southern Ocean, the circum-Antarctic brooding bivalve *Lissarca notorcadensis* demonstrates phenotypic plasticity in shell shape between the Scotia Sea, Ross Sea, and Weddell Sea with a significant difference between the Scotia Sea population with the Weddell and Ross Seas (Cope and Linse 2006). A genetic analysis using 28s rDNA and mitochondrial cytochrome oxidase subunit 1 (CO1), later revealed two distinct lineages, indicating reproductive isolation between the morphotypes. Although brooding, this small bivalve species has mobility by living on sea urchin spines and juveniles may be able to disperse by drifting short distances with mucous threads (Brey et al. 1993). Ecophysiological plasticity (growth, reproduction and somatic production) was also measured in *L. notorcadensis* from 'North' and 'South' Weddell Sea populations and found significant differences between the two, with the northern populations having larger shelled adults, but significantly smaller offspring (Brey and Hain 1992). The shallow water and widespread limpet *Nacella concinna* has also been studied extensively, with clear morphological differences between subtidal and intertidal habitats. Specimens in the intertidal zones are characterised by having a taller and thicker shell, a response to desiccation stress, wave action, and freezing stress, while subtidal specimens have flatter shell shapes (Beaumont and Wei 1991; Nolan 1991; Hoffman et al. 2010a). Additionally, there is a physiological difference between the two morphotypes within the same population in response to freezing (Waller et al. 2006a), desiccation (Weihe and Abele 2008), and the ability to right themselves when disturbed (Morley et al. 2010).

Latitudinal variability in phenotypic plasticity has also been studied in six invertebrate species from six sites 54° - 68°S, at South Georgia and the Antarctic Peninsula, and found significant differences in size, although no correlation with latitude (Linse et al. 2006a). The local factors at each island were expected to have driven differences between sites studied with latitude having no clear effect *per se*. A similar study examined bryozoan growth and mortality between the same distribution from South Georgia and Antarctic Peninsula and found increasing mortality and lowest size-at-age at the lowest latitudes (Barnes and Arnold 2001). Plasticity has also been described in crustaceans; the reproductive plasticity in a serolid isopod over a latitudinal gradient has been described with larger egg size, mass and richer composition at lower latitudes of the Weddell Sea compared to the South Orkney Islands and South Shetland Islands (Wägele 1987; Clarke and Gore 1992). Asynchronous development of gametes in the decapod *Notocrangon antarcticus* from higher and lower latitudes, and higher oocyte numbers with increasing latitude latitudes for *Munida subrugosa* (Lovrich et al. 2005) also demonstrate ecologically driven plasticity affecting reproductive output.

Plasticity in the Antarctic has more recently been used to investigate the adaptation to ice scour damage in the infaunal bivalve *Laternula elliptica* from contrasting environments at the Antarctic Peninsula, McMurdo Sound and Dumont D'Urville

Research Station in the East Antarctic (Harper et al. 2012). The study found bivalves in the heavily ice impacted regions of the Antarctic Peninsula to have significantly thicker shells demonstrating phenotypic plasticity in response to geographic variations in ice impact. Additionally, genetic analysis of the populations using amplified fragment length polymorphisms showed no significant structuring in the populations (Harper et al. 2012), suggesting that the traits observed are plastic in response to the environmental disturbance.

The problem of Southern Ocean plasticity research

With low sample sizes being a regular constraint, some previous studies have pooled together specimens from broad geographic ranges, potentially hiding plasticity on local scales (see Table 1.1). For example, morphological differences in *Lissarca notorcadensis* were examined among 48 stations between 159m and 910m depth, and yet pooled into just 14 sample locations representing the Scotia Arc, Weddell Sea, and Ross Sea (Cope and Linse 2006). The ecophysiological studies on *L. notorcadensis* in the north and south Weddell Sea were collected over a greater range, with samples representing 65 stations covering a depth range of 80 to 1108m (Brey and Hain 1992), including areas of the East Antarctic Shelf, South Shetland Islands, and South Orkney Islands in the Scotia Sea. While growth rates were only taken from specimens at subtly contrasting depths of 200-350m in the northern population, and 300-500m in the southern population, the variations within the pooled populations were not compared, possibly a consequence of low sample sizes to establish significant differences. Environmental variability between this depth range and latitude would be expected to select for different traits associated with subtly contrasting temperature ranges, food availability, and physical disturbance, and pooling such a wide collection may hide variations in ecophysiological and phenotypic traits on narrower scales.

An example of how this can be a problem is highlighted by the extensive studies conducted on the intertidal and subtidal limpet *Nacella concinna*. Molecular analysis initially identified distinct morphotypes within a populations to be the same (Beaumont and Wei 1991) but a later study found significant genetic differentiation between the two morphotypes at Potter Cove, King George Island, South Shetland Islands, using simple sequence repeat markers (Aranzamendi et al. 2008). However, when this study was repeated at Rothera on Adelaide Island, Antarctic Peninsula, using specimens collected from the intertidal to 25m, no genetic differentiation was found (Hoffmann et al. 2010a). One explanation for such a difference could have been the geographic isolation of the two populations; a recent study using amplified fragment length polymorphism bands identified strong genetic structuring in the Scotia Sea and Antarctic Peninsula (Hoffman et al. 2011). Although currently unstudied, phenotypic plasticity between the two populations at King George Island and Adelaide Island, driven by habitat variations, may be responsible for further unexplored ecological divergences.

Environmental variability on even smaller scales has also been ignored in past studies, and specimens collected from various sites on one island have been previously pooled to increase sample sizes. This can be demonstrated by the study into latitudinal gradients in body size and growth which pool together samples collected at up to 10 sites at each island under the assumption of homogenous environment with respect to temperature and food availability (Linse et al. 2006a), while the study on bryozoan growth and mortality does not specifically detail where the specimens were collected within each island used (Barnes and Arnold 2001).

Although important to increase samples sizes, and appropriate in the correct context, such data pooling and omission of sample collection data, may lead to greater error in results, and hide information on the variability at scales which to date have not been examined in the Southern Ocean. Plasticity on fine scales is an important but largely ignored area of investigation, and yet can give information on the selection pressures on fauna. Understanding the ability of a species to adapt and acclimate to subtle variations within its range, together with modern molecular tools, creates a greater macro-ecological approach to studying Southern Ocean past and future ecologies, extinctions and radiations. It also can give us a variable with which to measure change against in the future which may be crucial in understanding the future ecological pressures associated with global climate change.

Taxa	Plastic Trait	Pooled samples?	Reference
Lissarca notorcadensis	Shell morphology	yes	Cope and Linse 2006
Lissarca notorcadensis	Growth & reproduction	yes	Brey and Hain 1992
Laternula elliptica	Shell morphology	no	Harper et al. 2012
brachiopods	Feeding	no	Peck et al. 2005
Nacella concina	Shell morphology	no	Beaumont and Wei 1991 Nolan 1991 Hoffman et al. 2010a
Nacella concina	Feeding, physiology	unclear	Waller et al. 2006a
Nacella concina	Desiccation	no, but collected over 24 months	Weibe and Abele 2008
Nacella concina	Righting ability	no	Morley et al. 2010
various	Phenotypic plasticity	yes	Linse et al. 2006
bryozoan	growth	yes	Barnes and Arnold 2001
serolid isopods	Reproduction	yes	Wägele 1987; Clarke and Gore 1992
decapods	Reproduction	yes, within island groups	Lovrich et al. 2005
Trematomus newnesi	Morphology	no, but collected over four years	Eastman and Devries 1997
Trematomus newnesi	Morphology	no, but samples between 1968 and 2006	Piacentino and Barrera-Oro 2009
Trematomus hansoni	Feeding	no	Pakhomov 1998

Table 1.1 Studies into plastic traits and if pooled specimens were used. 'Unclear' denotes unspecific collection locations or methodology.

1.2 The Southern Ocean

Southern Ocean fauna has evolved and radiated throughout 200 million years (Ma) of history in its formation (Table 1.2), climatic variations, isolation and environmental differences forcing ecological divergence and speciation. The marine environment is often referred to as one of the most isolated places on earth; isolated physically by the strong Antarctic circum-Polar Current (ACC) and physiologically by the Polar Front (Figure 1.1), which acts as a limiting thermal barrier to many taxa (Clarke and Johnston 2003). Ice formation has formed much of the physical environment which characterises the benthic Southern Ocean today. This includes the unusually deep and long continental shelf with average depths of 450m extending to below 1000m (Clarke and Johnston 2003), formed by pressure of previous ice shelves acting on it and past ice scouring events (Huybrechts 2002; Clarke and Johnston 2003).

Time	Event	Consequence
200 Ma	Gondwana Breakup	Isolation of Eastern Gondwana
100 Ma	Rifting between Antarctic and Australian continent	Isolating seaway between continents
65 Ma	Deep submergence of South Tasman Seaway	Separation and start of cooling of Antarctic
50-40 Ma	Proto-circum-Antarctic current through Australia and Antarctic	Formation of southern ice cap
30-23Ma	Deep-water seaway at Drake Passage	Major cooling and ice sheet expansion
20-17 Ma	Global warming	Possible trans-Antarctic seaway
16 Ma	Cooling step	East Antarctic Ice sheet formation, cooling of Antarctic and sub-Antarctic water
6-5 Ma	Cooling Step	West Antarctic ice sheet formation, global ocean circulation complete, expansion of ice at both poles
5 Ma - present	Series of glacial-interglacial periods on Milankovitch timescales	Advance and retreat of ice on the continental shelf

Table 1.2 Evolution and formation of the Antarctic continent (summarised from Kennet 1977; Barker and Burrell 1987; Veevers et al. 1991; Lawver et al. 1992; Lazarus and Caulet 1993; Flower and Kennett 1995; Zachos et al. 1996; Crame 1999; Zachos et al. 2001; Lawver and Gahagan 2003; Thatje et al. 2005).

The role of ice on Antarctic evolution in the marine ecosystem

Over the past 5 Ma, glacial and inter-glacial periods have continued to shape the continental shelf, with the ice sheets reaching their maximum extent by 15 thousand years ago (Huybrechts 2002). Of considerable interest and importance are the effects of this past glacial event on the composition and diversity of benthic life seen today (see review by Convey et al. 2009). The past glacial maximum is modelled to have advanced and grounded to the edge of the continental shelf seen today (Huybrechts 2002), potentially forcing benthic fauna onto the slopes or deep-sea, a theory supported by the high levels of extended eurybathy seen in many benthic taxa today

(Brey et al. 1996). However, there are strong suggestions and evidence that ice advance (and retreat) did not geographically occur simultaneously in the Antarctic, and provided temporary benthic shelters on the continental shelf for species to survive, if they had capacity to disperse from one to another (Thatje et al. 2005; Convey et al. 2009). An ecological problem for displaced or persistent populations was the extremely low food supply that would be encountered (Thatje et al. 2008). Much of the Southern Ocean benthos that was not grounded by ice sheets would have been under multiannual sea ice with no light available for primary production, and benthic fauna will have been greatly affected by starvation (Thatje et al. 2008). This poor food supply may also have selected for brooding and non-feeding larval traits, with insufficient primary productivity to support pelagic larvae (Poulin et al. 2002; Thatje et al. 2008; Pearse et al. 2009; Thatje 2012). Evidence of this is seen today with an unusually high incidence of brooding and non-pelagic reproductive traits (see below). Some benthic regions may have resembled current benthic faunal compositions under sea ice, made up of filter feeding organisms nourished by the advection of very fine food particles (Dayton and Oliver 1977). Similar environments today exist under extensive ice sheets and benthic communities have been found under the Amery Ice shelf, 480m under ice and 100km from open water (Riddle et al. 2007). Although living away from a primary food source, a diverse population dominated by filter feeders exists, relying on drifting primary productivity from over 100km away (Riddle et al. 2007).

Polynyas (areas of open water surrounded by ice), may have existed as hotspots for primary production during glacial periods and could have potentially maintained benthic populations under the sea ice around them by advection of particles (Thatje et al. 2008). Today, coastal polynyas are widespread on the continental shelves of Antarctica, formed when the katabatic winds push the sea ice from the ice shelf leaving an open area, which is often replaced by bottom waters and refreezes to form more sea ice before being broken down again (Martin 2001). Alternatively, in open ocean polynyas, seamounts or other undersea elevations cause upwelling of deeper nutrient rich warm waters, encouraging rich primary productivity (Comiso and Gordon 1987; Martin 2001; Thatje et al. 2008). The ice over winter in these areas is much thinner and the earlier melting of this ice fertilises and extends the length of primary productivity of the region. Open Ocean polynyas are very rare in the Southern Ocean, with Maud Rise polynya being the most prominent one, but may have been the only sites of large-scale primary productivity during the Last Glacial Maximum (Thatje et al. 2008).

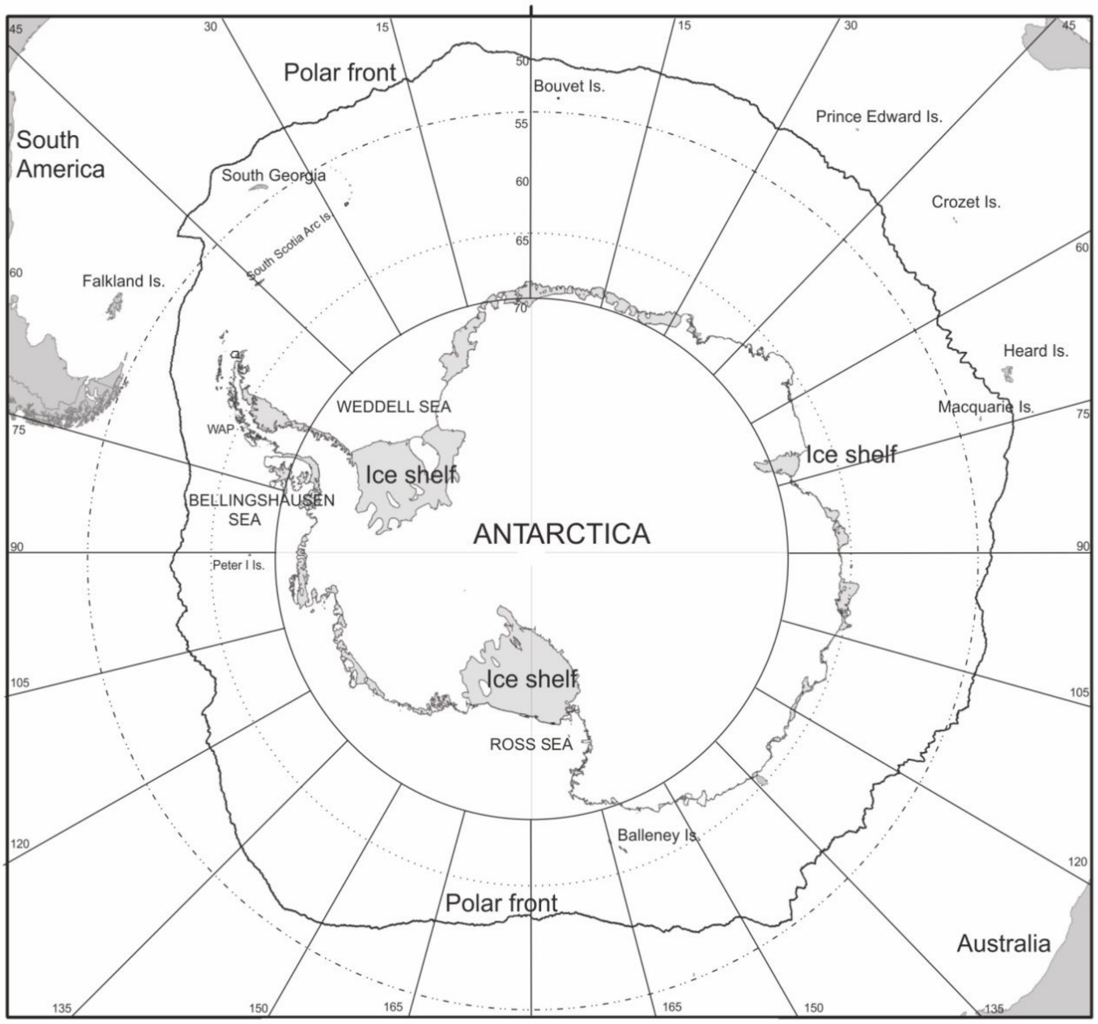


Figure 1.1 Overview map of the Antarctic, Southern Ocean and surrounding islands with approximate path of the Polar Front.

Extinction events in the Southern Ocean, triggered by the decline in temperature after isolation and ice sheet advance over the shelf, created many niches encouraging radiation and colonisation of surviving and invading species (Brandt 2000; Lörz and Held 2004; Raupach et al. 2010). Additionally, the deep continental slope to depths of 1000m (Clarke and Johnstone 2003) with little thermal gradient, will have been important in the migration and radiation of taxa into and from the deep-sea, and many species with deep-sea origins are seen today in the Southern Ocean (Rogers 2007; Strugnell et al. 2008; Barco et al. 2012).

Modern Southern Ocean environment constraints

Presently in an interglacial period, ice is plays a major role in the structuring of the benthos on short time scales (Barnes 1995; Barnes and Conlan 2007). In the winter, intertidal areas can freeze rapidly, forming an ice foot on the sea floor and pushing away any settled fauna (Waller et al. 2006b). In water up to 30m, anchor ice can be a major disturbance for benthic fauna and in waters up to 550m in the high Antarctic, icebergs can scour paths 15km long and 15m deep into the sediment (Hotzel and Miller 1983; Gutt et al. 1996; Gutt 2000). In these regions the faunal composition can be rapidly altered from a steady population of slow recruiting filter feeding organisms to opportunistic scavengers in a short space of time (Barnes and Conlan 2007). Meanwhile, large expanses of the deep-sea are covered for most of the year if not all year, by multiannual sea ice supporting limited or no primary productivity.

Of all the characteristics which control the Antarctic fauna, the most notable is the extreme thermal stability south of the polar front. Shallow water environments are stenothermal with annual temperature variations of only up to 5˚C at the lowest latitudes and just 1.5˚C at continental locations (Barnes et al. 2006a). Deep-water benthic fauna are affected by changes in oxygen and temperatures as water masses mix over the long and deep continental shelf (Klinck 1998; Smith et al. 2000). The differences with depth are possibly most significant in the Southern Ocean as there are defined water masses interacting and seasonally influencing water temperature and salinity at different depths. Antarctic surface water (AASW) reaching up to 150m have been shown to vary in temperature between -1.8˚C and 1˚C (Smith et al. 2000). Antarctic winter water (WW) lies underneath this as cooled water around 0˚C. The significant water masses affecting fauna however, are the Upper circum-polar deep water (UCDW), lower circum-polar deep water (LCDW) and Weddell Sea deep water (WSDW). The UCDW is found at depths of 200-800m and can be identified by an oxygen minima (4ml l^{-1}) and temperatures up to 1.6˚C. These temperatures may drop to 1.0˚C over the continental shelf. Below this, the LCDW can be identified with a salinity maximum and temperatures between 1.3-1.6˚C (Klinck 1998; Smith et al. 2000). The WSDW is a deep and stable water mass that originates in the Weddell Sea and moves west into the Scotia Sea and Drake Passage until it meets the deep water influence from the South Pacific travelling east. It can be found below 5000m in the Weddell Sea and fills the deep trench in the South Scotia Sea (Sievers and Nowlin 1984; Smith et al. 2000). It is this cold bottom water formation in the Weddell Sea and Ross Sea that is responsible for much of the ventilation throughout the Atlantic, and maintains global ocean circulation.

The Antarctic Peninsula and surrounding Southern Ocean is currently under pressure from rapid regional atmospheric warming, at a rate of around 0.56°C decade^{-1} since 1950 (Vaughan et al. 2001, 2003; Turner et al. 2005). This atmospheric warming is also being detected by changes to the surface temperatures of local seas, most notable being a 1°C increase at the surface of the Bellingshausen Sea (Meredith and King 2005), and up to 2.3°C in the upper 100m near South Georgia (Whitehouse et al. 2008). Attributed to this increase in temperature have been glacial retreats (Cook et al. 2005) and reduced sea ice formation (Stammerjohn et al. 2008), with ecosystem responses including changing plankton biomass (Schofield et al. 2010), krill abundance, and shifting penguin distributions (Fraser and Hofmann 2003). With further warming predicted (Bracegirdle et al. 2008), the fate of thermally sensitive marine fauna is of concern to ecologists (Pörtner 2001; Peck et al. 2002; Peck et al. 2002, 2004, 2010; Clarke et al. 2009; Schofield et al. 2010; Aronson et al. 2011).

1.3 Benthic fauna today

The evolution of benthic fauna in the Southern Ocean has been driven by the physical isolations of the ACC, physiological isolation by historic cooling events and forced migration and extinctions from the shelf during the last glacial maximum. It is made up of fauna that has evolved *in situ*, colonised from the deep-sea and radiated from South America through the Scotia Arc islands (Clarke and Crame 1992; Arntz et al. 2005; Aronson et al. 2007). The current Antarctic fauna is dominated by sessile filter feeders associated with sediment type (Gili et al. 2006; see review by Gutt 2007, and a top trophic level dominated by echinoderms and large nemerteans (Dayton et al. 1974; McClintock 1994; Arntz et al. 2005; Aronson et al. 2007)

A key characteristic which defines much of the diversity of the benthos is the lack of durophagous predators, an extinction associated with Antarctic cooling (Dayton et al. 1994) and poor physiological tolerance to cold conditions preventing recolonisation (Peck 2002; Thatje et al. 2005; Aronson et al. 2007). While there is a reduced diversity in bivalves and gastropods, and striking absence of durophagous decapods, elasmobranchs and teleost fish (Crame 1999; Clarke 1993; Clarke et al. 2004b), some taxa appear to thrive. The peracarid crustaceans, sponges, pycnogonids, echinoderms and opportunistic filter feeders show high diversity (Brandt et al. 1999, 2007), linked to the reduced predation pressure (Aronson and Harms 1985; Clarke and Johnston 2003; Aronson et al. 2007). Endemism is also high, predicted to be between 50 and 90% of species (Arntz et al. 1997; Griffiths et al. 2009), and may be underestimated with the discovery of more cryptic species, suggesting a long period of evolution in isolation. Under ice, communities of sponges and associated epifauna are specially adapted to living on fine food particles (Dayton and Oliver 1977), while intertidal faunal diversity is often very low from the constant formation and melting ice (Barnes and Brockington 2003) although some areas can be surprisingly diverse (Waller et al. 2006b).

Life cycles and dispersal play an important role in the maintenance of population diversity in the Antarctic (see review by Thatje 2012) and often discussed in relation to the controversially described high incidence of direct development at the poles (Thorson 1936, 1950; Mileikovsky 1971). Wide dispersal is possible through pelagic larval drifting, demersal larval drifting, rafting, and an increasingly modern problem of anthropogenic dispersal, facilitated by the influential Antarctic Circumpolar Current (ACC) (see review by Thatje 2012).

The prevalence of direct development

Brooding and direct development is a common reproductive trait in the Antarctic, especially in peracarid crustaceans, small bivalves and echinoderms, representing an extreme of parental trade-offs between quantity and quality (Strathmann 1978, 1985; Poulin et al. 2002; Ripley and Caswell 2008). The evolution and prevalence of brooding traits in some taxa may be directly related to isolation by the Polar Front although lecithotrophy appears the dominant larval type of the benthic fauna. It has been suggested that any brooding reproductive trait observed currently, is not necessarily a consequence of the current conditions in the Antarctic, but may be an adaptation to a previous environmental regime (see review by Pearse et al. 2008), or from migration from another habitat (Strathmann 1978).

Planktotrophy has often been assumed to be a primitive larval form from which non-feeding larval traits have evolved. Strathmann (1978) reviewed the reproductive traits of ten phyla to examine the evolutionary history of larval feeding and concluded that once planktotrophic larval traits are lost, they are unlikely to ever be reacquired, and this is considered to be the case for all pelagic developmental types (Strathmann 1978, Pearse et al. 2008). The adaptation to non-feeding larvae also reduces the larval features associated with feeding (Strathmann 1974, 1978) and in some species of echinoid, intermediate forms between planktotrophic and lecithotrophic development have been described (Emlet 1986; Raff 1987; Olson et al. 1993).

Maintaining populations over large distributions in the Antarctic would challenge brooding species during previous glacial periods, when the extent of ice over the shelf may have maintained only small isolated ice refuges and would have required constant migrations to and from to persist (Thatje et al. 2005). Non-feeding larval traits may have been selected in favour of planktotrophic traits given the lack of primary productivity under thick sea ice (Poulin and Féral 1996; Poulin et al. 2002; Thatje et al. 2005, 2008; Pearse et al. 2008), while brooding taxa would need to rely on dispersal by rafting on floating material. This conveys doubt over the true origins of brooding in the Antarctic and whether current conditions, biotic or abiotic, instigated evolution in favour of direct development. Instead, direct development and brooding may be present from earlier physical and physiological constraints which favoured this form of development, and evolution *in situ* has left entirely brooding clades of taxa (Strathman 1978; Gallardo and Penchaszadeh 2001; Poulin et al. 2002; Pearse and Lockhart 2004; Pearse et al. 2008).

In comparison to the wide diversity of the Antarctic benthos, there is a distinct lack of planktotrophic larvae, a likely consequence of the shorter period of food availability against the longer time of development in the cold, referred to as 'Thorson's Rule' (Thorson 1950; Mileikovsky 1971; see Thatje et al. 2005). Developmental times in many asteroid species with lecithotrophic larvae can take up to three times as long in the Antarctic (Bosch and Pearse 1990), pelagic planktotrophic asteroids taking up to 6 times as long (Pearse and Bosch 1986), and some small bivalves brood their young for 18 - 24 months before releasing the juveniles (Richardson 1979; Brey and Hain 1992; Higgs et al. 2009; Reed et al. 2013). The Antarctic infaunal clam *Laternula elliptica* and scallop *Adamussium colbecki* reared in the laboratory developed 4 and 18 times slower, respectively, than non-Antarctic relatives (Peck et al. 2007). Of an estimated 15,000 species of benthic invertebrate, less than 250 larval types have been described (Stanwell-Smith et al. 1997, 1999; Thatje 2012). As more are discovered however, and identified using molecular techniques (Heimeier et al. 2010), there will certainly be more discussion on Thorson's concepts of latitudinal declines in pelagic larvae in the foreseeable future (Thatje 2012). There are many effects that may be associated with a shift towards direct development, such as slow recruitment and a trade-off between mortality and fecundity. Conversely, these traits have an evolutionary effect from the ability of a species to re-colonise areas of disturbance after ice scouring of the sea floor, especially during past and present glacial-interglacial periods (Thatje et al. 2005).

Rafting the Southern Ocean

Rafting as a dispersal mechanism for brooding invertebrates is likely to maintain reproductive continuity between populations, enables colonisation of new habitats, and highlights the importance of the ACC as a dispersing system. A recent study examined three kelp holdfast associated invertebrate species among the sub-Antarctic islands near New Zealand and found evidence of significant gene flow among island populations hundreds of kilometres apart (Nikula et al. 2012). The protection and food supply afforded to rafting invertebrates may facilitate dispersal over extended periods of time (Smith 2002), exceeding the dispersal time of pelagic larvae and therefore acting as a highly effective mechanism for dispersal (Highsmith 1985; Nikula et al. 2010). An example of this was found in the Ross Sea when populations of over 1000 reproductively active brooding bivalves, *Adacnarca nitens,* were found rafting on a hydrozoan colony at 100m (Higgs et al. 2009, Figure 1.2). Rafting is also known to disperse the brooding bivalve *Gaimardia trapesina* (Helmuth et al. 1994) and demonstrates the dispersal potential for small invertebrate species which continue to reproduce while drifting. It is estimated that over 70 million macroalgal rafts are actively drifting at any one time, 20 million of which may support a holdfast capable of supporting a high diversity of invertebrate species (Smith 2002).

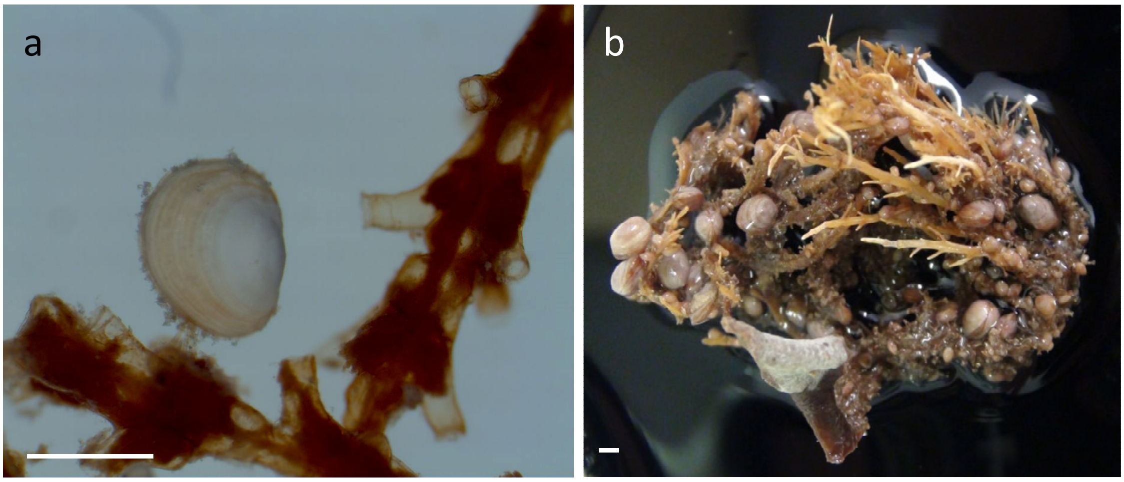


Figure 1.2 Brooding bivalves attached to hydrozoan and macroalgal rafts. a) *Adacnarca nitens* from the Ross Sea on floating hydrozoan; b) *Lissarca miliaris* and *Kidderia bicolor* from King George Island on floating macroalgae. Scale bars = 2mm.

Alternative drifting flotsams have also been found with rafting organisms in the Southern Ocean. Pumice from a submarine volcanic eruption at the South Sandwich Islands in 1962 was found two years later, washing up on the shores of South Island, New Zealand, with live barnacles attached (Coombs and Landis 1966). More recently, plastic debris was found washed up at Adelaide Island, Antarctic Peninsula, and had at least 10 species of invertebrate present (Barnes and Fraser 2003), demonstrating the potential anthropogenic facilitation of rafting in the Southern Ocean.

Speciation

Speciation is the process of species subdivision, often resulting from ecological divergence or reproductive isolation (Cook 1906; see West-Eberhard 1989 for discussion), and has been regularly discussed in the Antarctic context in relation to the isolated nature of the Southern Ocean. A phenomenon in Antarctic species distribution, challenged by evidence of speciation, is the apparent circum-Antarctic distribution of many species, or wide distributions spanning the island groups of the Scotia Sea into Magellan regions (Dell 1972; Clarke and Johnston 2003). Speciation has different definitions according to the processes involved (Table 1.3) but in the Antarctic context, the most common speciation processes are expected to be allopatric and parapatric speciation, where populations become geographically split and reproductively isolated.

Speciation Process	Definition
Allopatric	A population splits into two or more geographically isolated populations which become genetically distinct.
Peripatric	Peripheral populations become reproductively isolated from main populations and become distinct.
Parapatric	Partial separation of geographically distinct populations with limited gene flow between them.
Sympatric	Two or more distinct species from a common ancestor within the same geographic location.

Table 1.3 Definitions of the different forms of speciation.

Cryptic speciation

Recent studies have identified genetic structuring in many brooding and broadcasting species of circum-Antarctic distributions, and some may represent multiple cryptic species, indistinguishable from each other by morphology alone and yet with significant genetic differences. Many of these studies have been designed to test wide distributions reported for many Antarctic species, especially in species where dispersal capabilities may not be obvious (e.g. brooding species). Panmictic populations have been described for planktotrophic decapod shrimp species (Raupach et al. 2010) and in crinoids (Hemery et al. 2012), despite limited dispersal in some regions (Wilson et al. 2007), supporting the hypothesis of circum-Antarctic distributions. There are also surprisingly wide distributions in the brooding ophiuroid *Astrotoma agassizii* (Hunter and Halanynch 2008), the brooding amphipod *Durvillaea antarctica* (Nikula et al. 2010) which rafts on macroalgae, and some connectivity in the brooding gastropod

Margarella antarctica although a degree of population fragmentation has also been found within the latter (Hofmann et al. 2012).

Cryptic speciation or strong population structuring have been described in many other species with direct development, lecithotrophic and planktotrophic larval forms, including the brooding Antarctic molluscs (Linse et al. 2007; Page and Linse 2002; Wilson et al. 2009), echinoderms (Hunter and Halanynch 2008; Heimeier et al. 2010), pericarid crustaceans (Held 2003; Held and Wägele 2005; Raupach and Wägele 2006; Raupach et al. 2007; Leese and Held 2008; Baird et al. 2011), pycnogonids (Mahon et al. 2008; Krabbe et al. 2009; Arrango et al. 2010), and the planktotrophic nermertean *Parbolasia corrugatus* (Thornhill et al. 2008). It may be suggested that Antarctic species richness is significantly higher than stated as a consequence of such speciation, as previously discussed fordeep-sea environments (Wilson and Hessler 1987; Vrijenhoek 2009). Furthermore, genetic structuring with depth found in the deep-sea (Etter et al. 2005; Held and Wägele 2005; Brandão et al. 2010), may continue to challenge initial observations of eurybathy in shelf fauna (Brey et al. 2006; Rogers 2007). It is likely that the factors that may result in panmixia are a complex relationship of dispersal method (by pelagic larvae or adult drift), geographic distance, depth, and currents (Hunter and Halanych 2008), and with further improvements in molecular techniques and wider areas of the Antarctic sampled, new evidence of genetic divergence and connectivity will be described (Thatje 2012).

1.4 Bivalves

Deep-sea and Antarctic bivalves were among the first organisms from their respective environment to be studied and continue to be of interest with their extreme longevity, variations in feeding modes, reproductive traits, small sizes, and the ease with which growth (usually incremental with age) and reproduction can be studied (e.g. Ralph and Everson 1972; Brey and Hain 1992; Higgs et al. 2009; Brey et al. 2011). Bivalves also contribute 8-10% of deep-sea macrobenthic fauna, third in abundance after polychaetes and peracarid crustaceans (Gage and Tyler 1991). Both deep-sea and Antarctic bivalves tend to be small in size (<5mm) although there are several larger examples in shallow Antarctic environments (Nicol 1966). They often present with no coloration and commonly have a pelagic lecithotrophic or brooding reproductive traits

(Dell 1972). As a broad rule, lamellibranch bivalves are dominant at shelf depths, while the protobranch bivalves dominate bivalve diversity on the abyssal plains (Allen 2008).

Antarctic bivalves

Bivalves in the Antarctic are less speciose than elsewhere in the world's oceans (Clarke 1993; Crame 1999; Clarke et al. 2004b) and there are currently about 158 known species in Antarctic waters, south of the Polar Front (Clarke and Johnston 2003; Linse 2004; Linse et al. 2006b). Sampling however has been restricted to certain regions such as the Ross and Weddell Seas, while the deep-sea and Eastern Antarctic benthos are particularly under sampled (Clarke at al. 2007a). Early studies of Antarctic bivalves have suggested an unusually high number of brooding species and small sizes (generally <10mm, many <5mm) (Dell 1972; Picken 1980; Hain and Arnaud 1992) although pelagic development is also observed in some larger species such as *Adamussium colbecki* and *Limatula* spp. (Berkman et al. 1991; Linse and Page 2003; Peck et al. 2007). Recent expeditions have uncovered more information of the Antarctic deep-seas (ANDEEP I&II, summarised by Brandt et al. 2007) and in regions not previously explored such as the Amundsen Sea (BIOPEARL II, Enderlein and Larter 2008) which will give us a better idea on the population diversity of all benthic fauna and their relationship with the shallow waters. Many species of bivalve are considered to be eurybathic (Brey et al. 1996) to at least depths found on the continental slope (~500m) with only a few being considered shallow water (e.g. *Lissarca miliaris, Mysella* sp., *Yoldia eightsii*) (Dell 1964; Nicol 1966).

Most studies on bivalves in the Southern Ocean have focussed on the shallow water species and those down to 100m, probably because of the ease of sampling in comparison to deep-sea bivalves. The ecology, growth, and reproduction of few shallow water species have been described, especially for some of the shallower lamellibranchs from the family of brooding bivalves Philobryidae (Richardson 1979; Brey and Hain 1992; Janssen 1997; Cope and Linse 2006; Linse et al. 2007; Higgs et al. 2009, Reed et al. 2012). These bivalves tend to be small in size, live in high densities, are regularly found in trawls and epibenthic sledge samples attached to other flora and fauna and reproduce by brooding their young within the mantle cavity (Dell 1964, Richardson 1979, Brey and Hain 1992).

Deep-sea protobranchs

The deep-sea Antarctic protobranch bivalves are known to occur to depths below 5000m (Linse 2004), although the ecologies of these species are largely unknown, and species identification can be notoriously questionable (Allen et al. 1978). Protobranchs are of interest to deep-sea and Antarctic biologists as they are highly successful at living in deep and cold oligotrophic abyssal plains, and have evolved unique life histories, including extreme longevity and a unique pericalymma larval stage (Zardus 2002). There are now over 600 species of protobranch described globally, with origins from the late Cambrian (approx. 500Ma) (Zardus 2002). Despite a poor deep-sea fossil record, there are fossil records of several families of protobranch bivalve from the Paleozoic (10 families), Mesozoic (20 families), and Cenozoic (12 families) and these have helped to understand the evolutionary history of this ubiquitous group of bivalves (Allen 1978). It has also been suggested that the protobranchs may have evolved as a separate lineage to other bivalves, which gave rise to the unique reproductive traits observed (Gustafson and Lutz 1992).

Morphologically, protobranch bivalves share similar features; they all have simple unfolded gill structure, are usually small (<5mm), and coiled guts. They are separated into two Orders, Nuculoida and Solemyoida, by thickness of the shell and the presence of hinge teeth. Size of ctendia is also different as they are solely for respiration in Nuculida, but for feeding and respiration in Solemyida (Allen 1978). Solemyoids are also known to inhabit reducing environments (Zardus 2002). The thickness of shell has been associated with sediment type, lighter shells found on fine sediments and heavier shells found on courser sediments (Zardus 2002).

Growth in protobranchs is variable and life expectancy can be from 7 - >100 years. Turekian et al. (1975) describes the protobranch *Tindaria callistiformis* as living for over 100 years to a maximum size of 8mm, while gonad development does not begin until the animal is 4mm or approximately 50 years old (Turekian et al. 1975). In contrast, species of the genus *Microgloma* grow to a maximum size of 1.2mm with unknown lifespans (Allen 1978). It is likely that growth is dependent on food availability; most protobranch bivalves are deposit feeders and possess very long coiled guts to maximise the digestion time. Allen (2008) suggested that it was the impoverished food supply available to the abyssal protobranchs that have driven their evolution and is likely to be the primary factor affecting their reproductive patterns (Gage and Tyler 1991; Tyler et al. 1992).

Reproduction in deep-sea protobranchs has become the focus of some iconic publications, including the study of larval types (Thorson 1950; Milekovsky 1971) and early observations of seasonality in the deep-sea (Lightfoot et al. 1979; Tyler et al. 1992). Periodicities in deep-sea bivalves are driven by the presence or absence of seasonal fluxes of phytodetritus from surface waters (Tyler 1988). Most protobranchs have pelagic lecithotrophic development and non-planktonic direct development. With large energy reserves in the egg, larvae are able to delay metamorphosis until a suitable settlement site is found (Scheltema and Williams 2009). No planktotrophic traits are known although for most species, reproduction is only inferred by the relationship between egg diameter, prodissoconch size, and mode of development (Ockelman 1965). The unique pericalymma larvae are a short dispersing lecithotrophic larvae spending as little as hours to eight days in the water (Zardus and Morse 1998). Although highly variable in form, the pericalymma characteristically have a test made from thin external layer of cells and have various bands of locomotive cilia. The larval shell (prodissoconch I) is secreted underneath the layer of cells and is characteristically smooth while the prodissoconch II is absent. The test is shed during metamorphosis, the organs re-arrange and the muscles develop before settlement (Zardus and Morse 1998).

The abundance of protobranch species globally increases with depth and they have been found at depths below 5000m in all deep-sea basins (Allen 1978; Allen and Sanders 1996; Allen 2008). High levels of endemism are also reported with around 80% of all species in each basin being endemic (Zardus and Morse 1998; Zardus 2002). Subtle differences in morphology have been identified in some species, which may represent morphological plasticity to different conditions. For example, *Ledella pustulosa* have four subtle morphotypes at the Rockall Trough, North Atlantic, associated with bathymetry (Fuiman et al. 1999). Conversely, there is evidence cryptic speciation in *Ledella ultima* from the Atlantic at bathyal depths (Etter et al. 2005) although little genetic structuring between deep basins throughout the Atlantic (Etter et al. 2011).

1.5 Shallow water study species*: Lissarca miliaris* (Philippi 1845)

Lissarca miliaris is a small philobryid bivalve (Figure 1.3) often found living on macroalgae in shallow bays (<30m) but also to 270m (Dell 1990; Richardson 1979). It does not have a circum-Antarctic distribution unlike its congeners although is found along the Antarctic Peninsula, Scotia Sea, Magellan Straits, and Falkland Islands. Morphological variations in *L. miliaris* have historically caused many problems with their accurate identification (Dell 1964). It has been identified four times as different species; *Pentunculus miliaris* Philippi 1845, *Lissarca rubro-fusca* Smith 1879, *Lissarca media* Thiele 1912, *Lissarca bennetti* Preston 1916. These descriptions represent different populations ranging from the Magellan Strait, Scotia Sea Islands, and Kerguelen Islands, and were described as separate species from subtle differences in morphology and coloration until being placed together as one species after examination of all previous material together (Soot-Ryen 1951; Dell 1964; Nicol 1966). Much of the confusion of taxonomy arose over the morphological variations with ontogeny (Dell 1972). A population from Signy Island was studied by Richardson (1979) to reveal an 18 month reproductive cycle linked to seasonal primary productivity and two cohorts developing at the same time. At Signy Island, *L. miliaris* is the dominant epifaunal species on macroalgae in the shallow sub-tidal and intertidal.

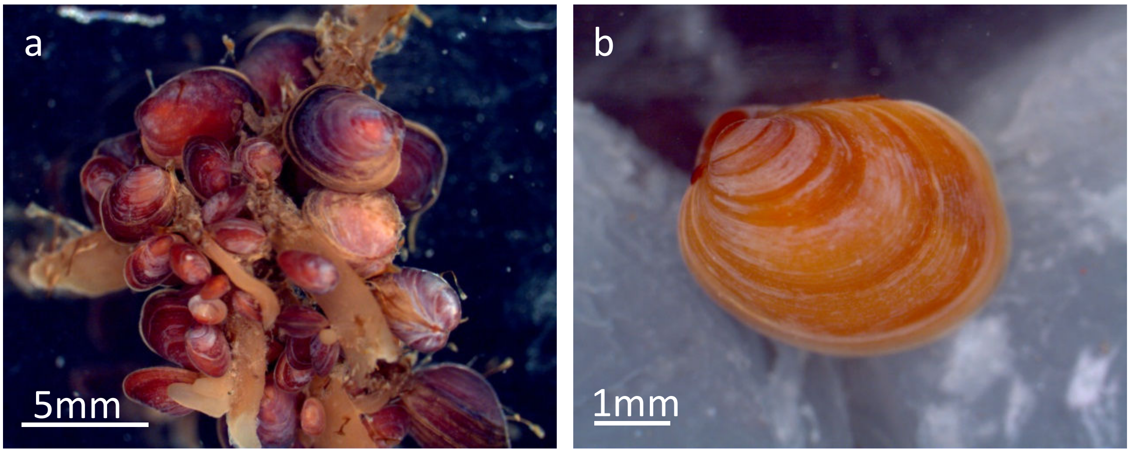


Figure 1.3 *Lissarca miliaris* from the Scotia Sea. a) adults and juveniles attached to macroalgae found intertidally at Livingston Island, South Shetland Islands; b) adult *Lissarca miliaris* from the intertidal zone at Signy Island, South Orkney Islands.

1.6 Deep-sea study species: *Yoldiella ecaudata*, *Yoldiella sabrina*, and *Yoldiella valettei*

The genus *Yoldiella* are a deep-sea group of bivalves and are commonly found in the Antarctic on the Antarctic shelf to depths of >5000m (Linse 2004). *Yoldiella ecaudata*, *Y. sabrina*, and *Y. valettei* are likely to have circum-Antarctic distributions, and *Y. sabrina* is known to exist in the South Atlantic. They are generally small (*Y. ecaudata* and *Y. valettei* <5mm, *Y. sabrina* <14mm), and yellow/white in appearance with little evidence of growth increments or shell sculpture (Figure 1.4).

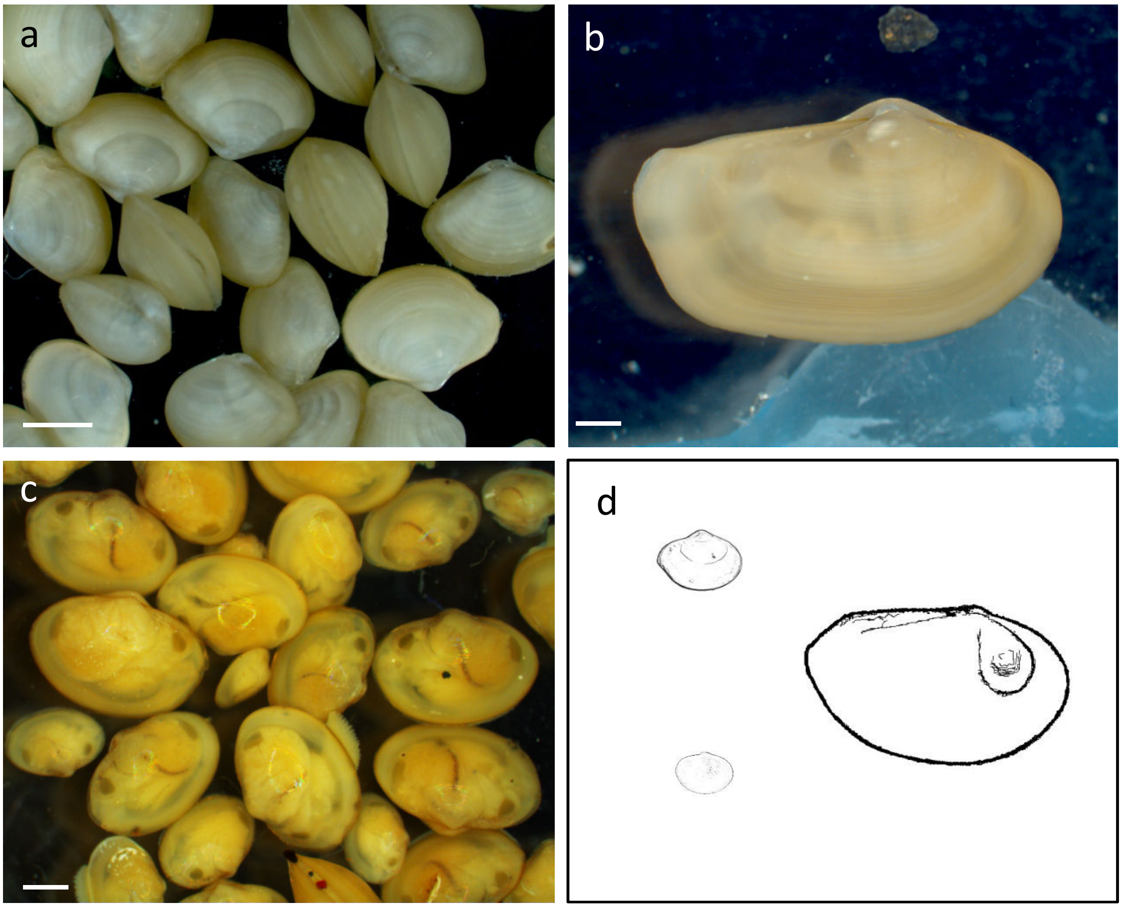


Figure 1.4 *Yoldiella ecaudata*, *Yoldiella sabrina,* and *Yoldiella valettei* from the Southern Ocean. a) *Y. ecaudata* from the Scotia Sea; b) *Y. sabrina* from the Scotia Sea; c) *Y. valettei* from the Amundsen Sea; d) Relative scaled drawings of all three protobranchs at adult size from *Y. ecaudata* (top left), *Y. sabrina* (middle right) and *Y. valettei* (bottom left). Scale bars = 1mm.

Very little is known regarding the ecology of deep-sea protobranch bivalves, and in the Antarctic, no more than distribution and frequency analyses have been conducted (e.g. Linse 2004). Wide geographic distributions and bathymetric ranges make this group of bivalve intriguing regarding their adaptability between different deep-sea and shelf environments. It is likely that this group of bivalves are represented by many different genera based on shell morphology and anatomical geography, but within the Antarctic, *Y. ecaudata*, *Y. sabrina*, and *Y. valettei* appear as distinct morphologies and are prolific in ancient literature (e.g. Lamy 1906; Thiele 1912). Although described as *Yoldiella*, this genus of bivalve requires revision (La Perna 2004), and likely represents three different genera. For the purposes of this study however, all species will be regarded as *Yoldiella*.

1.7 Aims, Hypothesis and Objectives

The Southern Ocean provides a unique setting to study the effects of subtle environmental differences on populations of invertebrate taxa. Adaptations to the cold stenothermal environment have driven the evolution of traits, which appear to demonstrate poor tolerance to change (Peck et al. 2004, 2010), and very few studies have examined phenotypic plasticity over both wide and local species distributions. This is important however, as the ability to be plastic to contrasting habitats could result in wider distributions, radiation into new environments, and persistence in changing environments. In the Antarctic context, plasticity to changing environments could have sustained populations of shallow water species in past glacial and interglacial periods. With increasing atmospheric warming trends at the Antarctic Peninsula (Meredith and King 2005; Turner et al. 2005), plasticity to a warming climate and associated pressures of invasive species, and ocean acidification, may also be vital to future survival. This thesis aims to investigate evidence of phenotypic and ecophysiological plasticity between populations within a cold stenothermal environment, which has previously been assumed as having low ecological divergence over wide geographic ranges.

Hypotheses

Hypothesis 1: *Lissarca miliaris* demonstrates morphological plasticity with ecophysiological trade-offs indicative of ecological divergence between subtly contrasting environments over their Antarctic geographic range of the Scotia Sea and Antarctic Peninsula.

Hypothesis 2: *Lissarca miliaris* shows evidence of an ecophysiological response with associated trade-offs to subtle changes in environmental conditions since the onset of recent warming at Signy Island.

Hypothesis 3: Deep-sea protobranch bivalves demonstrate morphological and growth plasticity over wide geographic ranges in the Southern Ocean with associated environmental differences.

Hypothesis 4: Reproductive traits in *Lissarca miliaris* and the deep-sea protobranchs maximise reproductive output and show evidence for possible wide dispersal.

Objectives

Objective 1: Investigate the reproductive traits of *Lissarca miliaris* and the deep-sea protobranchs *Yoldiella ecaudata*, *Yoldiella sabrina*, and *Yoldiella valettei* from the Southern Ocean (test of hypothesis 4)

Objective 2: Measure and analyse the morphological differences in adult and larval shell shapes between populations of *Lissarca miliaris* across their Antarctic range in the Scotia Sea and Antarctic Peninsula to determine phenotypic and reproductive plasticity (test of hypothesis 1).

Objective 3: Using museum collections, measure and analyse changes in morphological plasticity and ecophysiology (growth and reproduction) within a population of *Lissarca miliaris* at Signy Island between 1972 and 2012 to determine a plastic response to increasing atmospheric temperature (test of hypothesis 2).

Objective 4: Measure and analyse the phenotypic plasticity between populations of the deep-sea protobranchs *Yoldiella ecaudata*, *Yoldiella sabrina*, and *Yoldiella valettei* from the Weddell Sea, Scotia Sea and Amundsen Sea over wide bathymetric ranges to investigate ecological divergence with depth and geographic region (test of hypothesis 3).

Expected Outcome

It is expected that different populations of Southern Ocean bivalves from both the deep-sea and shallow water environments will express plasticity, selected by the environmental constraints within each habitat, regardless to geographic proximity or latitude. Reproductive traits will be consistent in demonstrating high energy investment by adults, and give clues to their dispersal potential in the Southern Ocean.

Chapter 2

Methodology

This chapter describes the methodology used in science chapters 3 to 7, including sample collection. Details of numbers specimens used and statistics used for each study are described in the respective chapters. This study focussed on four species of bivalve, the shallow water/intertidal lamellibranch *Lissarca miliaris* (Philippi 1845), and the deep-water deposit feeding protobranch bivalves *Yoldiella ecaudata* (Pelseneer 1903)*, Yoldiella sabrina* (Hedley 1916), and *Yoldiella valettei* (Lamy 1906).

2.1 Sample collection

Bivalve specimens were collected during a number of research expeditions to the Southern Ocean from research ships or by hand near research stations. Numbers of specimens used are detailed in each chapter.

***Lissarca miliaris* (Philobryidae)**

Lissarca miliaris were collected from Islands in the Scotia Sea and Antarctic Peninsula (Figure 2.1). Available for this study were specimens from South Georgia (Cumberland Bay, 54°17’S, 36°30’W, intertidal, 1972, Figure 2.2); Signy Island (South Orkney Islands, Shallow Bay, 60°42’S, 45°36’W, intertidal, 1976, 2002, 2011, 2012, Figure 2.3); King George Island (South Shetland Islands, Admiralty Bay, 62°10’S, 58°25’W, 10m, 1997; Potter Cove, 62°14’S, 58°42’W, 10m, 1994, Figure 2.4a); Livingston Island (South Shetland Islands, Byers Peninsula, 62°38’S, 61°05’W, 2009, Figure 2.4b) and Stepping Stones (Anvers Island, 64°47’S, 64°00W, 10m, 2012, Figure 2.6). The specimens from Signy Island 2002 were collected as part of the R/V *Polarstern* LAMPOS (ANT XIX/5) expedition (Arntz and Brey 2003). Specimens from South Georgia and Signy 1976 were made available through a loan from the National Museum Wales. All samples were fixed in 4% buffered formalin and subsequently stored in 70% ethanol. Ten specimens from Signy Island 2012 were dried at 30°C shortly after collection.

***Yoldiella* spp.**

Yoldiella valettei, Y. ecaudata and, *Y. sabrina* were collected during a number of research expeditions to the Weddell Sea, Scotia Arc, Antarctic Peninsula and Amundsen Sea (Figure 2.2) between 2002 and 2012 (ANDEEP I&II (Fütterer et al. 2003); LAMPOS 2002 (Arntz and Brey 2003); BENDEX 2003 (Arntz and Brey 2005); ANDEEP III 2005 (Fahrbach 2005); BIOPEARL 2006 (Linse 2006); BIOPEARL II 2008 (Enderlein and Larter 2008); JR275 (Griffiths et al. 2012)). Benthic samples were collected primarily using an epibenthic sledge (EBS) (Brandt & Barthel 1995; Brenke 2005) or Agassiz trawl (AGT), and the deployment methods are described in the relating reports. Samples containing the target protobranch species were taken in depths ranging from 192m to 4900m (Figure 2.6). Specimens collected by EBS were fixed in 96% ethanol immediately after coming on deck and later sorted, while specimens collected by AGT were sorted alive and then fixed in 96% ethanol or 4% buffered formalin. Differences in fixing/sorting procedure were the consequence of different science priorities.

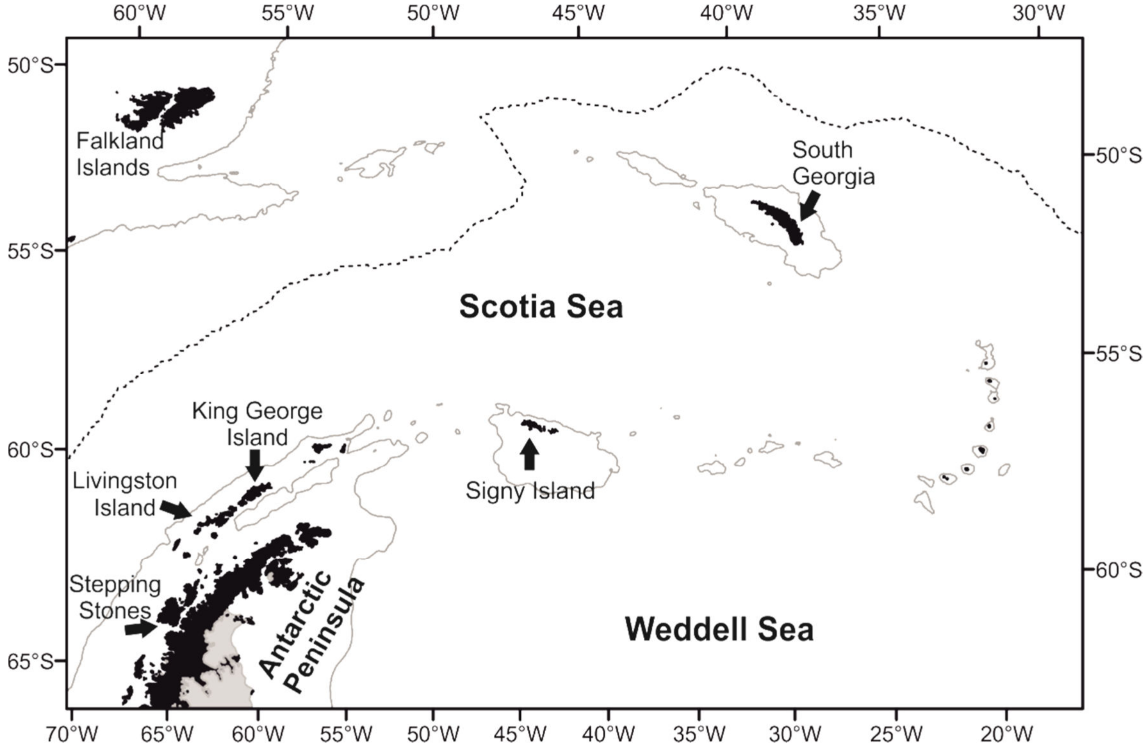


Figure 2.1 Map showing relative location of islands studied in relation to the Antarctic Peninsula where *Lissarca miliaris* were collected.

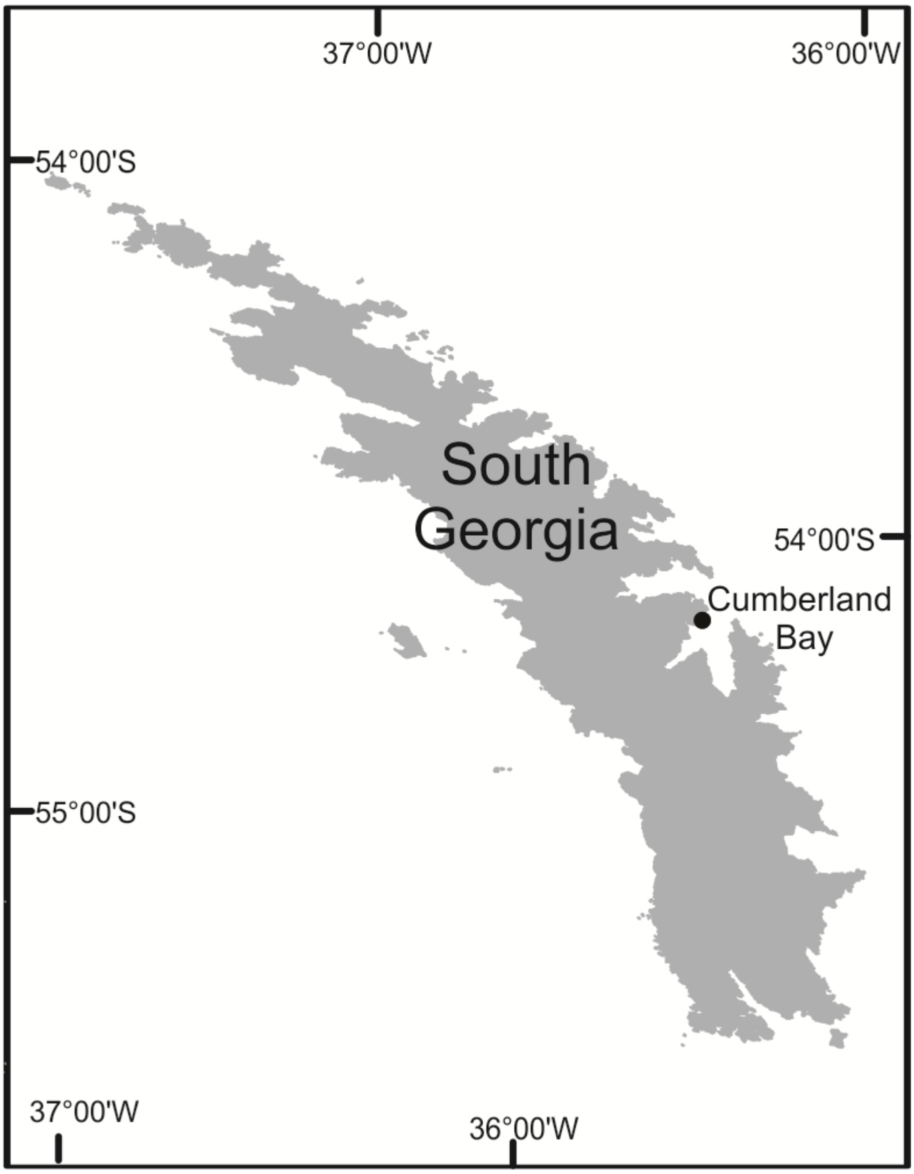


Figure 2.2 Map of South Georgia locating Cumberland Bay where *Lissarca miliaris* were collected in 1972.

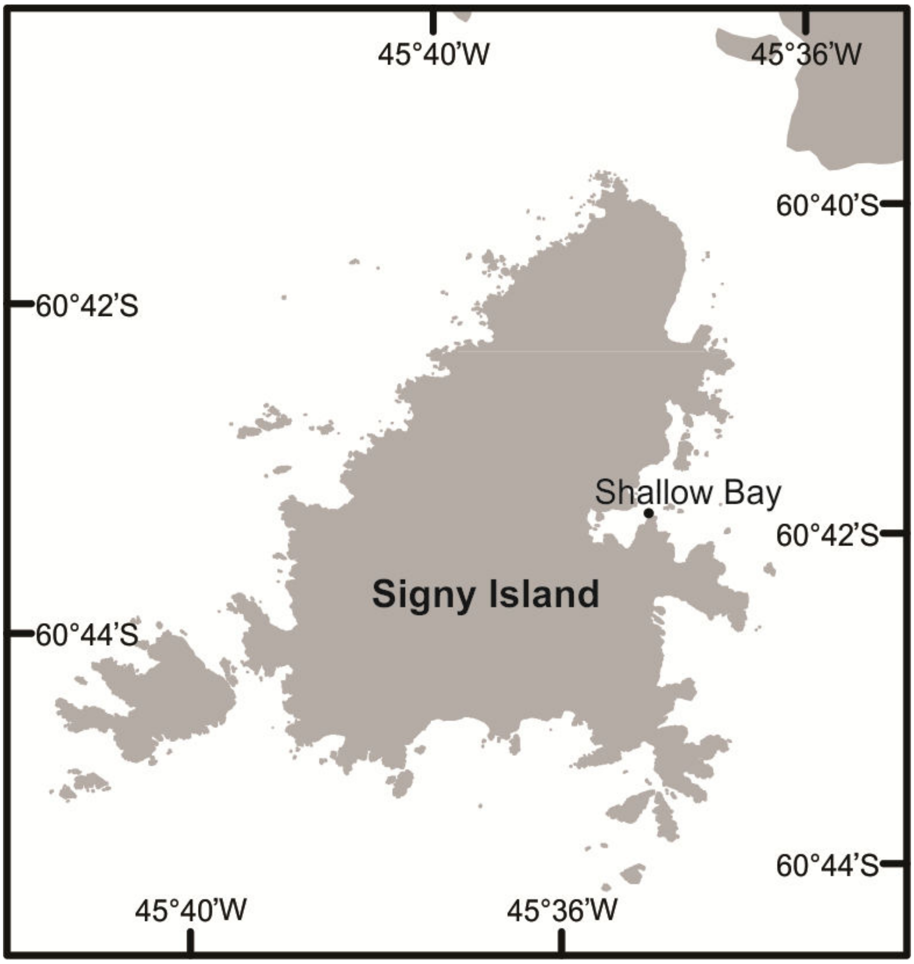


Figure 2.3 Map of Signy Island in the South Orkney Islands showing Shallow Bay near the Signy Research station where *Lissarca miliaris* were collected between 1972 and 2012.

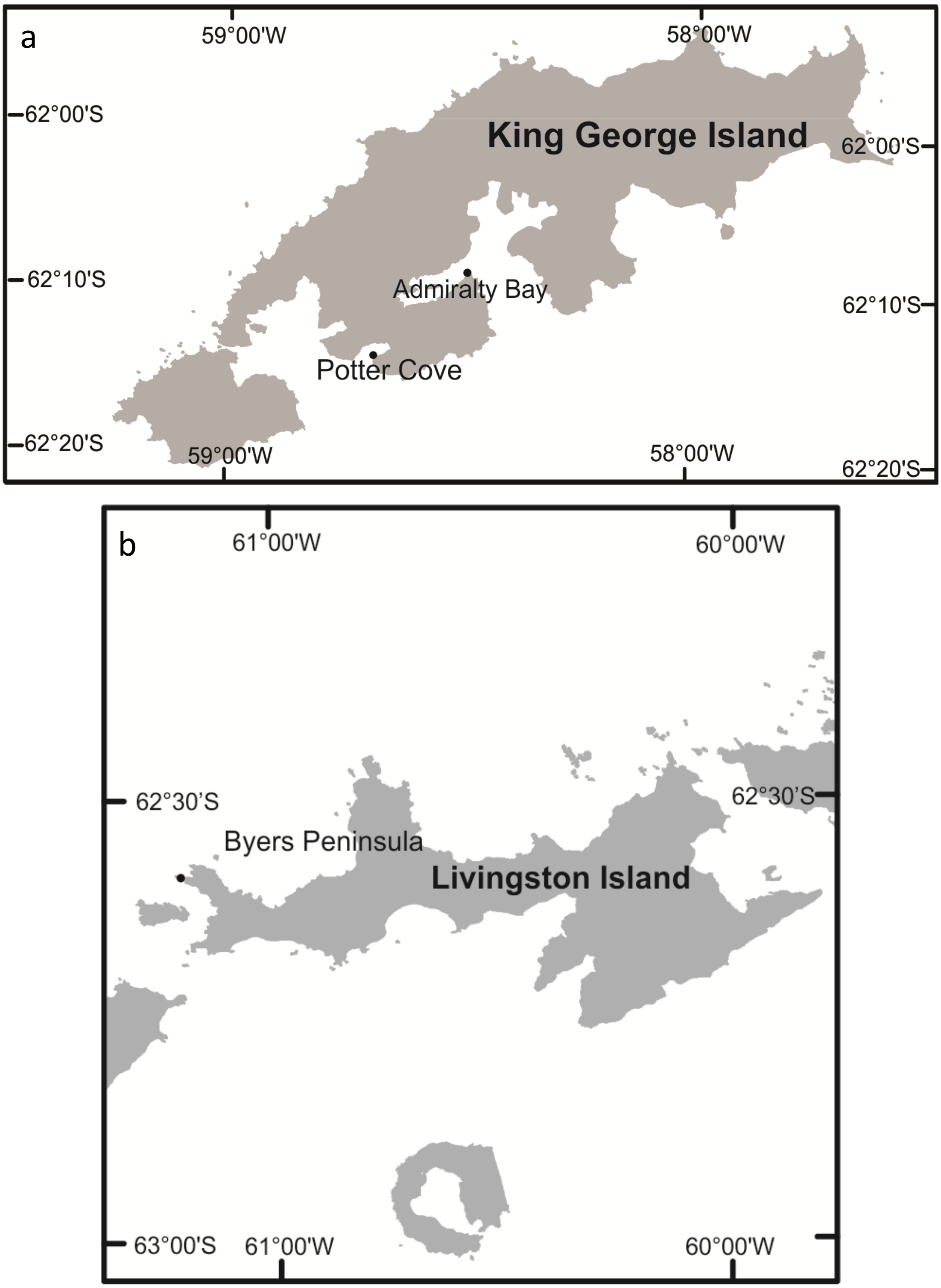


Figure 2.4 Map of South Shetland Islands King George Island and Livingston Island showing locations of *Lissarca miliaris* collections. a) King George Island displaying location of Potter Cove and Admiralty Bay; b) Livingston Island displaying location of Byers Peninsula.

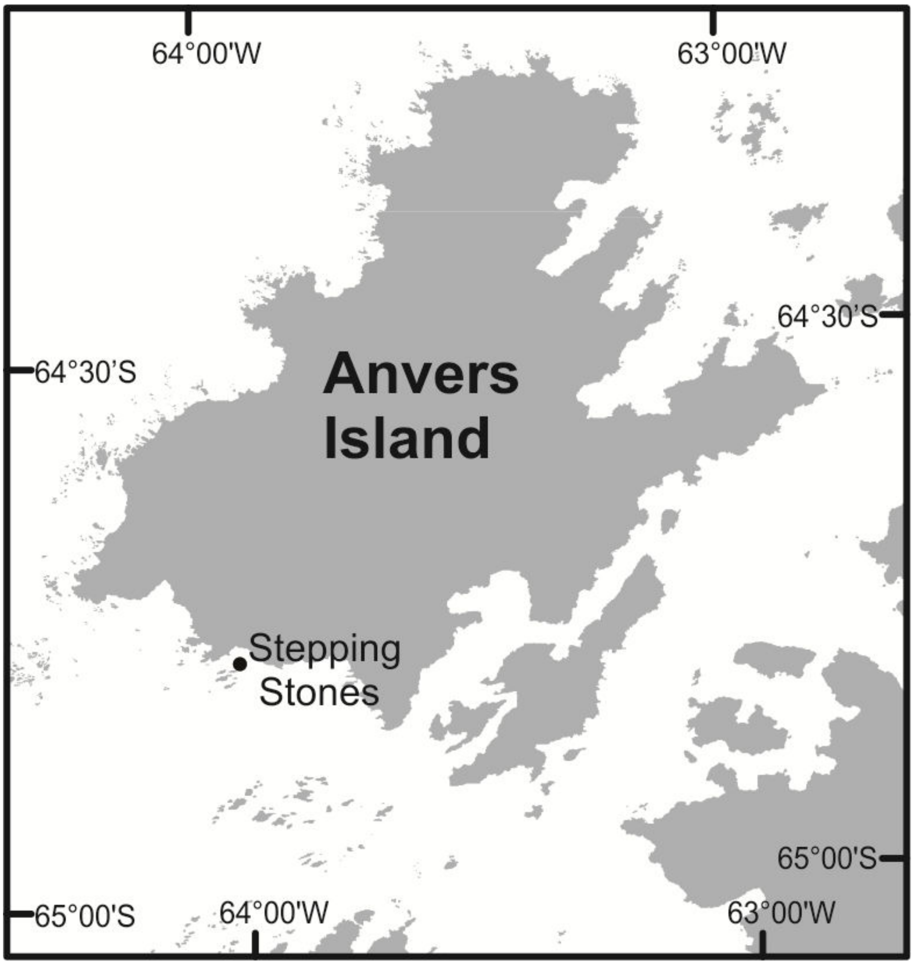


Figure 2.5 Map of Anvers Island showing location of *Lissarca miliaris* collections from Stepping Stones 2011.

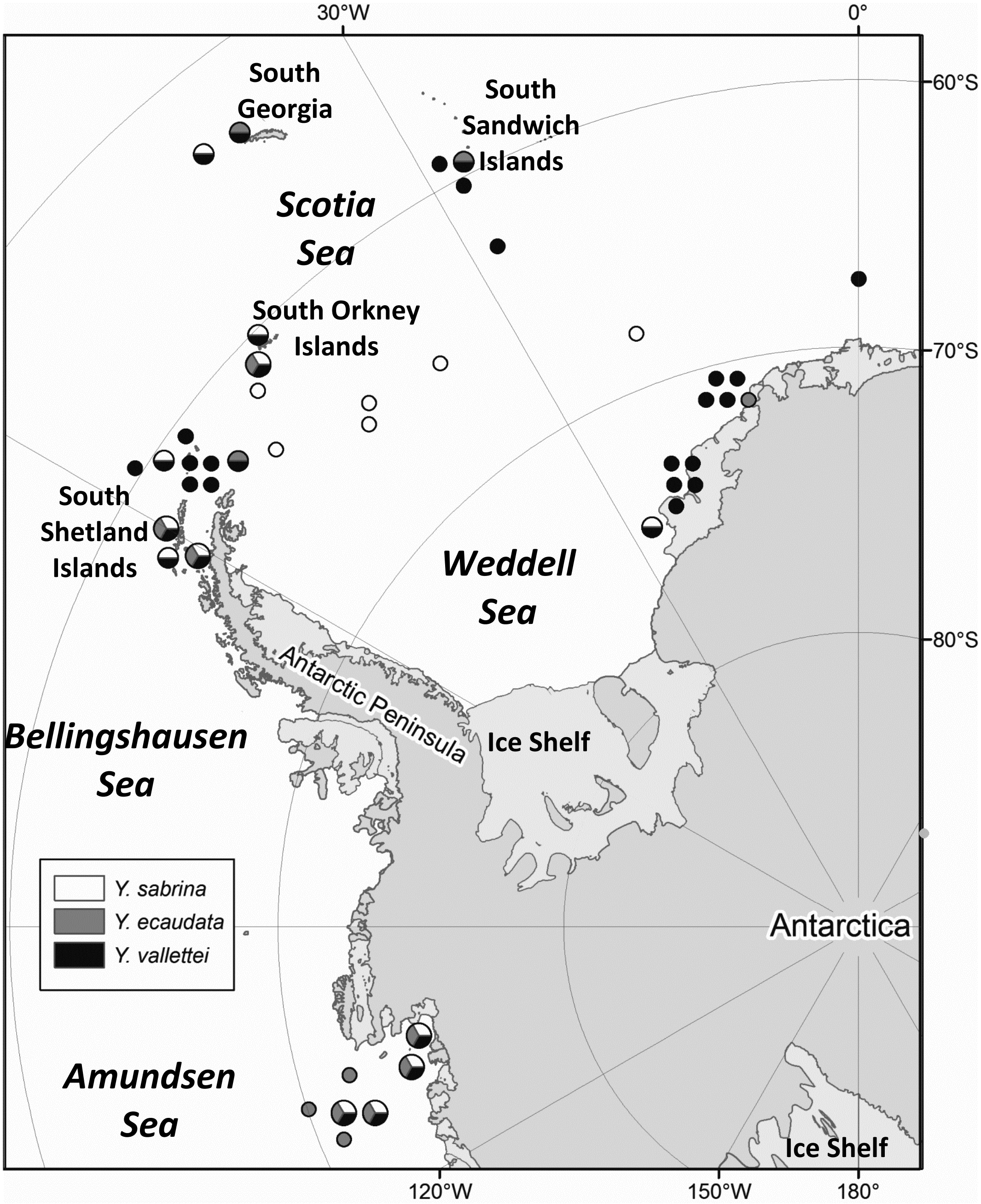


Figure 2.6 Map showing study areas and presence/absence of *Yoldiella valettei, Yoldiella ecaudata*, and *Yoldiella sabrina* at the Antarctic stations of the research cruises ANDEEP I&II, LAMPOS, BENDEX, ANDEEP III, BIOPEARL I&II between 2002 and 2008.

2.2 Bivalve Morphometrics

Shell measurements

Bivalves were measured using traditional terminology and techniques. Shell length (l) was taken as the maximum distance anterior to posterior along a horizontal axis, shell height (h) the dorsal to ventral distance along a vertical axis and shell width (w, also referred to as tumidity, obesity or depth), maximum inflation of the two valves (Figure 2.7a-b). Specimens under 10mm shell length were measured under a Leica light microscope with a calibrated eyepiece micrometre to an accuracy of ±0.05mm. Specimens over 10mm were measured using digital vernier callipers to an accuracy of ±0.01mm. Broken or damaged specimens were only measured where complete valves were available. Those with an obliquely ovate shell outline (e.g. *Lissarca* spp.) were measured as demonstrated in Figure 2.7c-d.

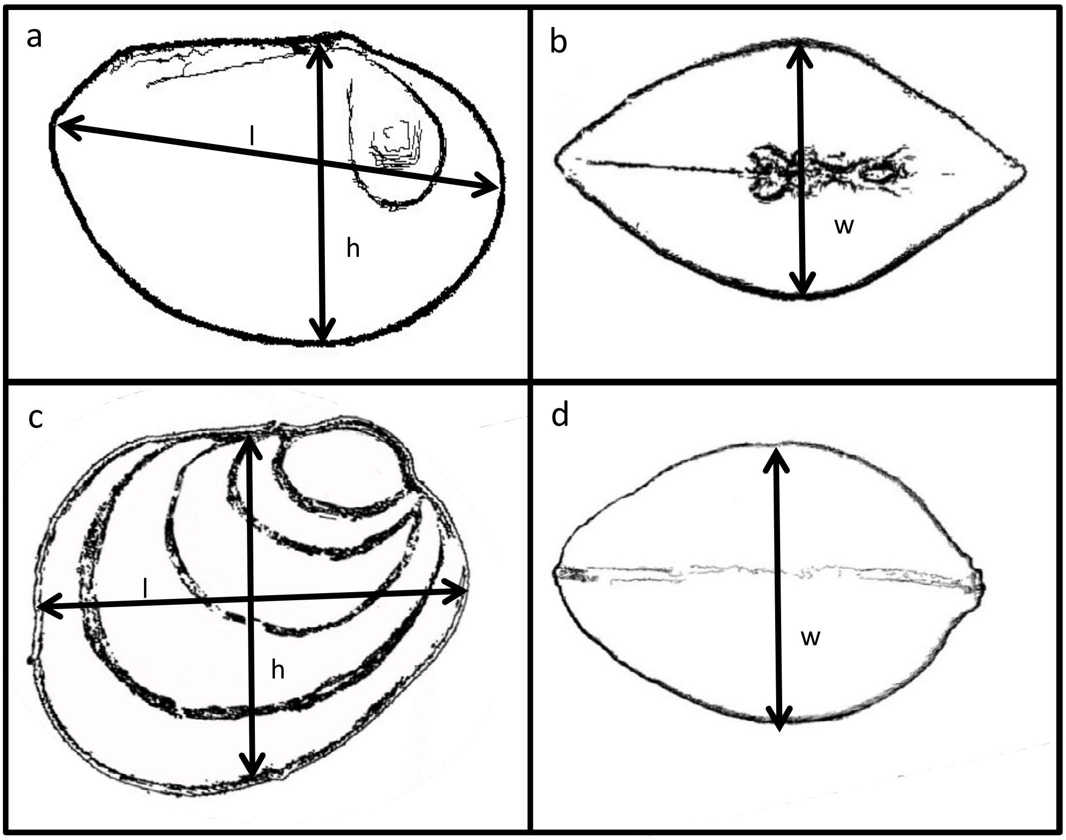


Figure 2.7 Schematic of standard bivalve shapes. a-b) protobranch bivalve; c-d) philobryid bivalve. Dimensions used to measure bivalves are shown as l (length), h (height) and w (width).

The relationship of length/height and length/width can be used to visualise the shape of the shells, and differences among different shell shapes within/between populations. The higher the value of the length/height ratio the more elongate the shell shape is, while the closer the value is to 1, the rounder the shell shape. Similarly, the closer the length/width ratio is to 1 the more obese the shell shape while as the value increases, the flatter the shell shape becomes (Figure 2.8).

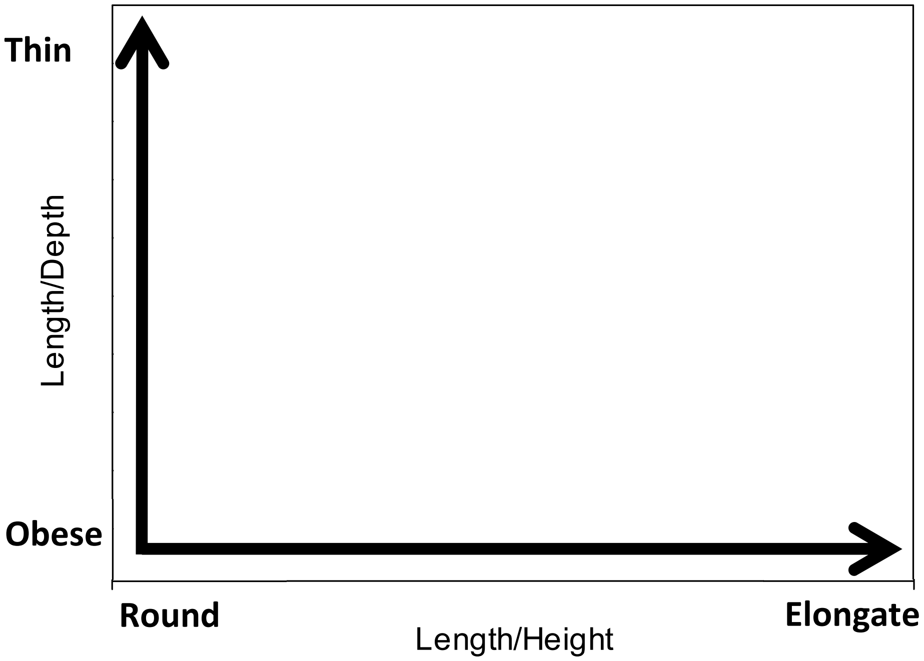


Figure 2.8 Relationship of shell length, height and width to the overall shape and morphology of the bivalve.

Prodissoconch I measurements

Prodissoconch sizes were measured by Scanning Electron Microscopy (Leo 1450 VP SEM) of the umbo region of the shells, or measured from micrographs taken on a camera mounted Leica stereomicroscope. Specimens for SEM were prepared and viewed as described in section 2.3. The maximum length and height of the prodissoconch I were measured using sub-adult shells (protobranchs) in good condition or year 0-1 cohort shells (*Lissarca miliaris*). Larger shells were positioned so that prodissoconch was lying flat to the camera or SEM beam. At least three measurements were taken of each length until the maximum length was obtained.

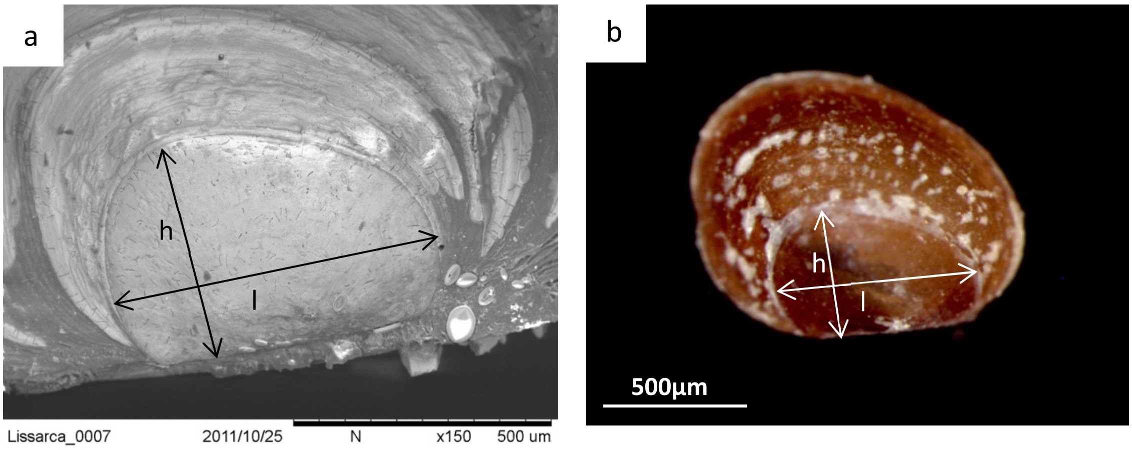


Figure 2.9 Prodissoconch measurements from *Lissarca miliaris*. a) Scanning Electron microscope image of the prodissoconch; b) micrograph of prodissoconch taken under a microscope. Dimensions used to measure the larval shell are shown as l (length) and h (height).

To retain consistency when measuring the maximum length, the line of measurement was always kept level with the hinge line. Maximum height was always perpendicular to the length as demonstrated in Figure 2.9. Length/height ratio was used to determine differences in morphology between specimens while the length of the PI is directly linked to size of offspring, representing the size of shell secreted by the larval stage, and so linked to egg size and nutritional content (Ockelmann 1965; Goodsell and Eversole 1992).

2.3 Scanning Electron Microscopy

SEM analyses were used under both high and low vacuum and are described separately.

High Vacuum

Bivalve specimens or bits of broken shell were mounted onto stubs using carbon cement on a conductive carbon adhesive pad (Figure 2.10a). For prodissoconch analysis, separated valves were positioned with the umbo facing upwards to ensure a squared surface for accurate measurement. For growth line and shell sculpture analysis, specimens were placed flat onto their left or right valve. Samples were pulse sputter coated with a 40nm gold coating to prevent charging of the material under high voltage conditions. A Leo 1450 VP SEM was used at the University of

Southampton, National Oceanography Centre, Southampton, to image the samples and, where appropriate, measurements were made and high-resolution images taken.

Low vacuum

Using the SEM under low vacuum requires a lower voltage and so reduces the likelihood of charging the sample. Although getting a lower magnification and resolution, the material used does not require coating with a conductive material and can therefore be retained for other studies. This is particularly useful when specimens available for destructive methods are limited. For low vacuum use specimens were mounted onto stubs by use of conductive carbon adhesive pads. Where material was to remain as untouched as possible, photographic film was attached to the adhesive pad on the stub, and slightly dampened before the sample was mounted (Figure 2.10b). The water melts the surface of the photographic film creating a liquid cellulose layer that dries hard to hold the sample in place. To remove the sample after SEM work, the stub can be re-dampened with water to wash away the cellulose and clean the specimen.

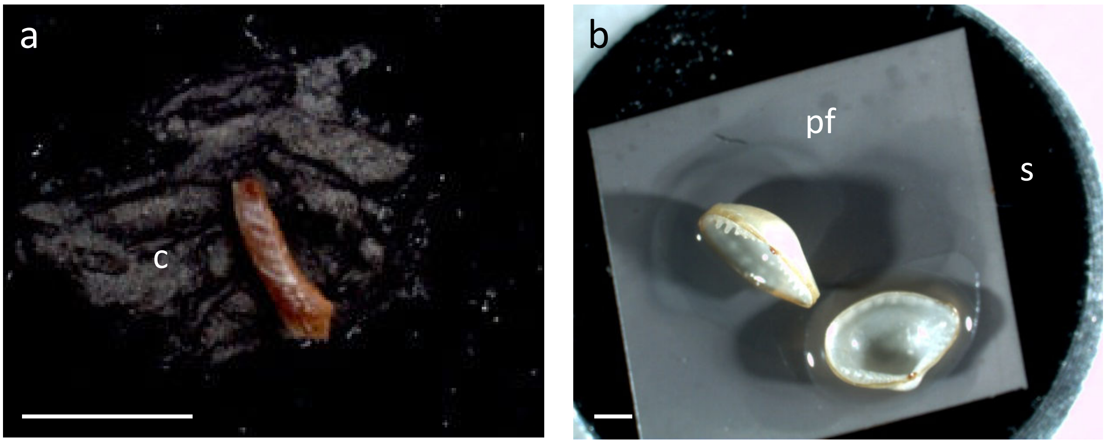


Figure 2.10 Scanning electron microscope stubs a) broken shell fragment attached to stub with carbon cement prior to gold coating; b) shells attached by photographic film by water prior to low vacuum SEM analysis. **c** = carbon cement; **pf** = photographic film; **s** = SEM stub. Scale bars = 1mm.

2.4 Growth

Many benthic invertebrates from seasonal environments show distinct growth marks representing an interval of time. This may be driven by environmental cues (e.g. food, temperature), reproduction or disturbance and can be identified in echinoids (Gage and Tyler 1985), polychaetes (e.g. Valderhaug 1985; Britayev and Belov 1994), gastropods (e.g. Ekaratne and Crisp 1984) and bivalves (e.g. Thompson et al. 1980;

Brey and Hain 1992; Higgs et al. 2009; Brey et al. 2011). The importance of seasonal growth marks observed on fish otoliths to assess fish stocks, have driven new techniques in accurately determining growth and analysing size-at-age data.

To measure growth in bivalves, each specimen was measured along the maximum distance across the shell using a Leica light microscope with a calibrated eyepiece micrometre to an accuracy of ±0.05mm. Growth data were collected by counting growth increments on the shells and were assumed to be annual or at regular interval (Richardson 1979; Higgs et al. 2009; Brey et al. 2011). Where shell sculpture was difficult to define, SEM analysis was used on specimens to observe finer growth rings. Size-at-age data were analysed using the von-Bertalanffy growth function (vBGF);

$$S_t = S_\infty 1 - e^{-K(t - t0)}$$

where S_t is length, S_∞ is asymptotic length, K is growth coefficient, t is age and t_0 is age when size equals zero. This method, commonly used for fish growth analysis, has more recently been used for bivalve growth (Brey and Hain 1992; Brey 2001; Heilmayer et al. 2004; Brey et al. 2011). As individual growth is non-linear, it is difficult to compare the overall growth between individuals. The derived parameters from vBGF can however, be used further to compare the overall growth performance (OGP) of species of similar size;

$$\mathrm{P} = \log(K * S_\infty)$$

where P is the overall growth performance, K is the growth coefficient and S_∞ is asymptotic length derived from vBGF (Pauly and Munro 1984; Brey 1999; Brey 2001). The plotted values of OGP can act as a graphical representation of growth differences between populations or act as values for statistical analysis between species.

2.5 Histology

Histology was used to study the reproduction of different bivalve species as in previous studies (Tyler et al. 1992; Higgs et al. 2009; Lucas and Reed 2009; Lucas and Reed 2010). Specimens were dissected from their shell or decalcified in rapid decalcifying solution (CellPath UK). Where possible, specimens were photographed before and after shell removal to help with identification of parts post processing. This also was used to identify mature male and female gonads, or brooding in some specimens.

Given the small size of bivalves studied (generally <5mm), whole animals were used for histology to avoid damaging gonads in dissection (see Higgs et al. 2009). Whole animals were dehydrated in graded isopropanol (starting at 50% for formalin stored samples, 70% for ethanol stored samples) increasing 10% per hour with two washes at 100%. Samples were then cleared in Histoclear (CellPath UK) or Xylene overnight before being dabbed dry and placed into molten wax and into an oven at 70°C to embed. The wax was replaced after 12 hours to remove excess clearing agent that may affect infiltration of wax into the tissue.

Procedure	Time (minutes)
Histoclear	5
Isopropanol 100%	1
Isopropanol 70%	1
Haemotoxylin 'z'	4
Water Rinse (running water)	15
Eosin 'y'	2
Water Rinse	2
Isopropanol 100% x2	2
Histoclear	2

Table 1.1 Chemicals and times for histology staining procedure. As used by Tyler et al. 1992; Higgs et al. 2009; Lucas and Reed 2009; Lucas and Reed 2010.

After a total of 24-36 hours in the oven, samples were removed and placed into appropriately sized moulds (10 x 10mm or 15 x 15mm) and left overnight to harden. Wax blocks were placed on ice to chill before cutting. Each sample was serial sectioned at seven micron to ensure every chance of cutting through the gonad. Slides were dried and stained by soaking slides as described in Table 1.1. After staining, slides were mounted with a cover slip using DPX (CellPath UK) and allowed to harden before microscope viewing.

2.6 Image analysis

Image analysis was used for measuring oocyte sizes, prodissoconch sizes (from SEM or photomicrographs) and generating size-at-age data. Feret diameter of oocytes were measured using the Sigma Scan Pro 4 image analysis software from calibrated micrographs taken on a Nikon CoolPix or Nikon D5000 camera mounted on a microscope. Eggs removed from dissected bivalves were photographed on a camera mounted Leica stereomicroscope and Feret diameter measured in the same way. Adobe Photoshop CS5 was used to measure prodissoconch sizes and shell lengths between growth increments from micrographs taken on a camera mounted Leica stereomicroscope. Colour and contrast enhancement using Photoshop CS5 was also used to identify subtle shell growth increments and to increase contrast in stained histology slides for accurate measurement of oocytes.

2.7 Energy Dispersive Spectrometry

Energy dispersive spectrometry (EDS) was used to make qualitative and quantitative measurements of the dominant trace elements in shell structure. The right valves of bivalve specimens of the same age (by growth increment) were used for analysis. Shells were embedded into Epoxy resin (Struers), placed in a vacuum to remove trapped air and left to harden at 30°C. Hardened blocks were cut with a 100 x 0.37 diamond low speed saw along the longest growing margin from the umbo and polished with graded diamond-coated sanding cloths to 1µm. The exposed shell surface was cleaned with ethanol and carbon coated before SEM analysis (Figure 2.11).

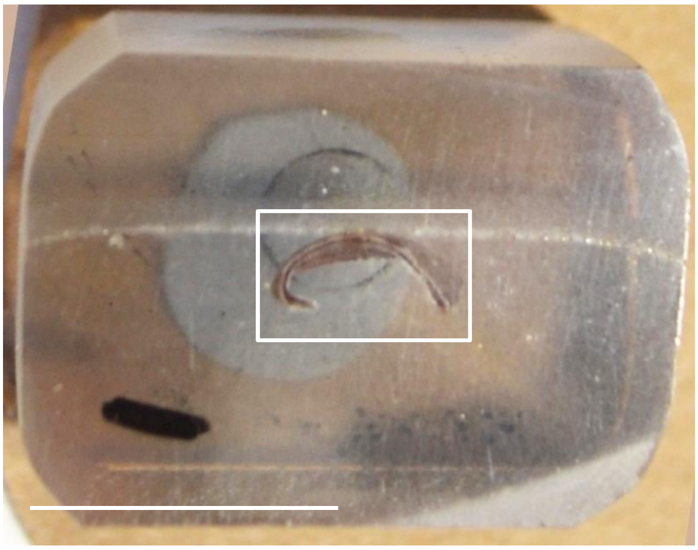


Figure 2.11 Epoxy resin block with cut and polished shells, carbon coated ready for scanning electron microscopy and energy dispersive spectrometry. White box marks cut shell. Scale bar = 1cm.

The SEM EDS analyser was calibrated with standard blocks for elements to be quantitatively measured. EDS analysis was taken through the shell at transects 600µm apart, from the umbo, representing average growth per year to ensure all years of growth could be accounted for (6-8 transects per shell, three spots per transect). The middle layer showed least variability in shell chemistry and was used generate quantitative measurements, although measurements in the upper and lower shell layers were also recorded (~20µm from the inner and outer edge of shell, depending on porosity). Contamination of resin in the measurements was more likely to occur in the surface layers of shell from porosity and could be detected by a chlorine peak in the analysis (present in the epoxy resin). Sections were analysed with a Leo 1450 VP scanning electron microscope with a PGT microanalysis energy dispersive system. The backscatter detector was used to ensure analysis did not occur on an area of changing crystal structure, and to measure the shell thickness (Figure 2.12). An area of around 5µm was required for EDS measurements. Elements Ca, Sr, S, O and P were analysed quantitatively with EDS as the most abundant and reliably measured chemical constituents of the shells and all were within the detection limits of EDS. The element composition by ratio with calcium was used to examine changes in composition between specimens.

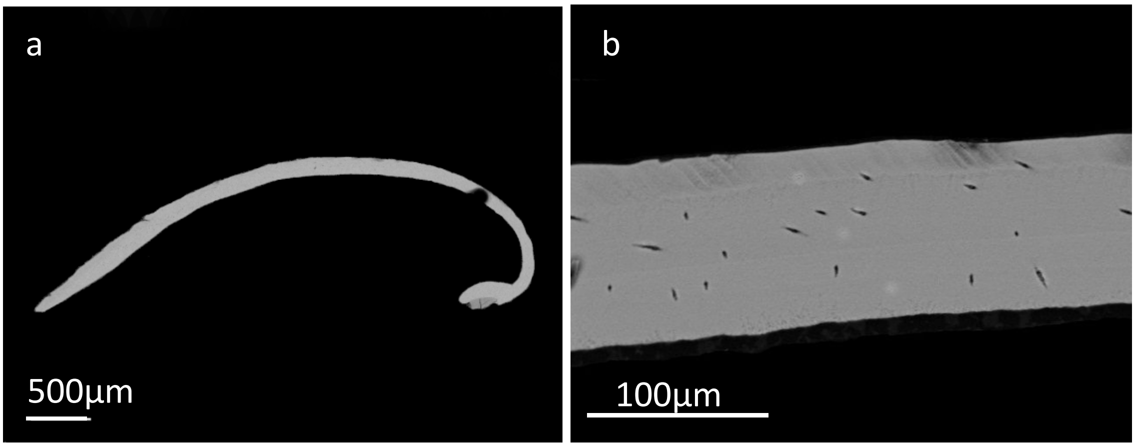


Figure 2.12 Scanning electron microscope backscatter images of sectioned adult *Lissarca miliaris* shells. a) overview of shell section, Signy Island 1976; b) close up of shell section showing some internal structure.

2.8 Statistical Analysis

Data were organised in Microsoft Excel 2003/10 and statistical analysis using Sigmaplot 12.3, MiniTab 16 and the free Palaeontology analysis software PAST. Statistics used are described for each chapter and include linear regression, fully factorial analysis of covariance (ANCOVA), analysis of variance (ANOVA), Kruskal-Wallis ANOVA (non-parametric) and post hoc analysis (Tukey and Dunn's methods). Graphs and auximetric plots were produced in SigmaPlot 12.3. All data analysed were checked for normality and if transformation was required details are given in the individual chapter methodology.

Chapter 3

Reproduction of the Antarctic brooding bivalve *Lissarca miliaris* (Philobryidae) from the Scotia Sea, Southern Ocean

Study published as: Reed AJ, Thatje S, Linse K (2013) An unusual hermaphrodite reproductive trait in the Antarctic brooding bivalve *Lissarca miliaris* (Philobryidae) from the Scotia Sea, Southern Ocean. Polar Biology. 36, 1-11.

Abstract

The Antarctic marine environment is extreme in its low temperatures and short periods of primary productivity. Invertebrates must therefore adapt to maximise reproductive output where low temperature and limited food slows larval development. Brooding is a common reproductive trait in Antarctic marine bivalves; larval development occurs within the mantle cavity, and larvae are released as fully developed young. *Lissarca miliaris* is a small, short lived, shallow-water brooding bivalve of circum-Antarctic distribution and found most abundant in the sub-Antarctic Magellan Region and islands of the Scotia Arc. Here, an unusual hermaphrodite reproductive trait is described for *L. miliaris* from King George Island (62˚14’S, 58˚38’W) and Signy Island (60˚42’S, 45˚36’W), Antarctica, using histological and dissection techniques. Specimens demonstrate simultaneous and sequential hermaphrodite traits; male and female gonads develop simultaneously but the production of oocytes is reduced while testes are ripe. Functional females are more abundant in specimens above 3mm shell length, although male reproductive tissue persists and functional males are found in all size classes. The number of previtellogenic oocytes produced by far exceeds the number of oocytes extruded and brooded, which may indicate an ancestral link to a planktotrophic past. Hermaphroditism in *L. miliaris* maximises reproductive efficiency in a short-lived species in which the female’s capacity to brood its young is limited, and demonstrates a specialised adaptation to a cold stenothermal and food limited environment prevailing in the Southern Ocean.

3.1 Introduction

Brooding is a common and successful reproductive trait in many Antarctic and deep-sea invertebrates representing an extreme of parental trade-offs between quantity and quality, with lowest mortality and lowest fecundity (Vance 1973; Ripley and Caswell 2008). In some invertebrate groups from the Antarctic, such as echinoids and bivalves, the predominance of brooding traits is striking (for review see Poulin et al. 2002; Thatje et al. 2005) but the suggestion that this has been driven by the current cold stenothermal and stable environmental constraints is often questioned (Strathmann 1978; Poulin et al. 2002; Pearse and Lockhart 2004; Thatje et al. 2005; Pearse et al. 2008). It has been discussed that the phyletic constraints on the reproduction and extinction of many planktotrophic traits during previous glacial periods have driven the evolution of entirely brooding clades of taxa (Strathmann 1978; Emlet et al. 1985; Gallardo and Penchaszadeh 2001; Thatje et al. 2003; Pearse and Lockhart 2004; Pearse et al. 2008). Southern Ocean brooding species may have become isolated in ice free shelf refuges on multiple occasions during glacial periods, forming fragmented and isolated populations, preventing gene flow and facilitating speciation of brooding clades over time (Pearse et al. 2008; Thatje et al. 2005, 2008; Thatje 2012).

Reproduction in polar and deep-sea benthic invertebrates has always been a subject of contrasting opinions and controversy but there are still few detailed descriptions on the reproductive ecology of many benthic species, especially in the Antarctic. There is a well-documented mismatch between the long developmental times of indirect planktotrophic larvae and the short period of primary production in Polar Regions, potentially selecting for non-feeding larval forms (Thorson 1950; Mileikovsky 1971). Vance (1973) stated that selection must favour the reproductive trait with the greatest efficiency. Lecithotrophy and direct development often have higher investment of energy per egg and lower fecundity compared to planktotrophy. This is balanced by reduced dependence on external food sources, settlement sites and a lower mortality (Strathmann 1974), limiting factors for deep-sea and Antarctic invertebrates. Recent studies have challenged the early views on the dominance of direct development in the Antarctic after the description of species with successful planktotrophic larval development in the Southern Ocean (Arntz and Gili 2001; Poulin et al. 2002; Raupach et al. 2010; but see Thatje 2012) and the belief that the isolation of the Southern Ocean has driven evolution and endemism in many taxa (Arntz et al. 1997; Clarke and Johnston 2003; Pearse and Lockhart 2004; Thatje et al. 2005).

Philobryidae are a notably speciose and increasingly studied family of bivalves, typical of the Southern Ocean and often occurring in high abundances in shallow bays and the continental shelf (Tevesz 1977; Arntz et al. 1997; Linse 2004). A characteristic important to the success of this group is the ability to brood young to juvenile stages, facilitated by the reduced or absent anterior adductor muscle and strongly constructed modified filibranch ctenidia (Tevesz 1977; Morton 1978; Prezant et al. 1992; Brey and Hain 1992; Hain and Arnaud 1992; Higgs et al. 2009).

The philobryid *Lissarca miliaris* (Philippi, 1845) is a small ubiquitous bivalve (<6mm shell length) often found attached to macroalgae by byssal threads between the intertidal and 270m (Dell 1964). With a life span of up to seven years, it is relatively short-lived for a cold-water invertebrate and broods up to 70 young in clusters within the mantle cavity for 17-18 months (Richardson 1979). Populations are known to occur along the West Peninsula Islands, Scotia Sea islands, and in the Ross Sea, as well as reaching into the sub-Antarctic Magellan Region, Falkland and Kerguelen Islands (Huber 2010, www.scarmarbin.be). They are most commonly found in large numbers and dense aggregations in shallow bays (<30 m). The brooding characteristics of *L. miliaris* were described by Richardson (1979) from a population at Signy Island but no study has described reproduction from gametogenesis. Only few studies have comprehensively described reproduction in any Antarctic philobryid species (Morton 1978; Brey and Hain 1992; Higgs et al. 2009). Philobryids are normally considered to be dioecious (Morton 1978; Richardson 1979; Brey and Hain 1992; Higgs et al. 2009) although a small percentage of *Lissarca notorcadensis* within certain size classes possess both testes and ovaries, indicating protandric hermaphroditism in a small proportion of a population (Prezant et al. 1992). Studies on the reproductive ecology of benthic invertebrates from the Southern Ocean are important for our knowledge of reproductive trade-offs in Polar Regions.

Aims

This study aims to describe the reproductive biology in *L. miliaris* using histology and dissection techniques, and discusses the reproductive trade-offs associated with hermaphroditic traits within the species.

Hypothesis

H_1 *Lissarca miliaris* demonstrates large vitellogenic oocytes consistent with brooding traits with two cohorts developing simultaneously.

Objectives

O_1 To investigate the reproductive biology and brooding traits of *Lissarca miliaris* from Signy Island and King George Island using histology and dissection techniques.

3.2 Materials and Methods

Sample collection

Specimens of *L. miliaris* used in this study were from 10m depth in Potter Cove, King George Island (Chapter 2.1) in December 1993 and January 1994 and fixed in 4% buffered formalin, and from Shallow Bay, Signy Island (Chapter 2.1) in April 2002 fixed in 96% ethanol.

Histology

Fifty-three individuals from King George Island (Potter Cove) and nineteen specimens from Signy Island (Shallow Bay) between 2.0 and 5.0 mm shell length were used for histological analysis. Maximum shell length, height, and width were measured using a stereomicroscope. Tissue processing and image analysis is described in Chapter 2.5.

Dissection

A total of 402 specimens from King George Island were selected for dissection. Bivalves were opened and the gonads examined. Separate sexes were easily identified in specimens above 2.5 mm shell length. The vitellogenic eggs of 150 females from 2.5 - 5.8 mm shell length were manually removed from the gonads and counted; 849 eggs from 76 adults were photographed with a Leica Microsystem camera and Feret diameter calculated as described in Chapter 2.6.

Sex Ratios

Sex ratios were determined from specimens collected at King George Island. Histology was used to determine functional sex of specimens below 3 mm; males determined by mature ripe testis and females from the presence of previtellogenic oocytes and no testis. Above 3mm shell length, dissection of specimens was done to observe functional sex. Hermaphrodite percentage in the population was determined by using histology data of all size classes and is observed as functional males with previtellogenic oocytes developing in a small portion of gonad.

3.3 Results

Lissarca miliaris demonstrate traits of both simultaneous and sequential hermaphroditism; sequential by a change in sex that can occur once within their lifetime and simultaneous by the occurrence of both male and female tissue present throughout the life of some specimens. Gametogenesis is observed from 2.00 mm shell length with oogenesis occurring in two small portions of gonad beside the posterior adductor muscle from 2.15 mm (Figure 3.1a, b). Developing testis is observed from 2 mm as gonad extending from the posterior adductor muscle to the antero-dorsal half on both the left and right sides (Figure 3.1c). Up to and over 200 previtellogenic oocytes develop in both males and females and vary in size between 19-50 µm (Figure 3.2a, 3.4a, 3.5). They appear densely packed or loosely arranged and often exceed the maximum number of eggs that become vitellogenic and brooded (Figure 3.5).

Vitellogenesis occurs from 2.9 mm length; no more than 35 oocytes from the site of oogenesis develop on each side in dorsal parts of gonad extending from the posterior adductor muscle to the anterior-dorsal half (Figure 3.2b, 3.4b, 3.6a,b). Oocytes are lined up in tightly packed rows in the dorsal parts of the gonad and measure between 200 and 500 µm Feret diameter at King George Island (mean 362.5 µm, SD=34, n=849) depending on degree of vitellogenic accumulation (Figure 3.4b). The observed number of eggs per female increased with shell length from 2.9 mm (r^2=0.378, $F_{1,148}$=89.908, p<0.001, King George Island) but also showed large variation within size groups (Figure 3.3).

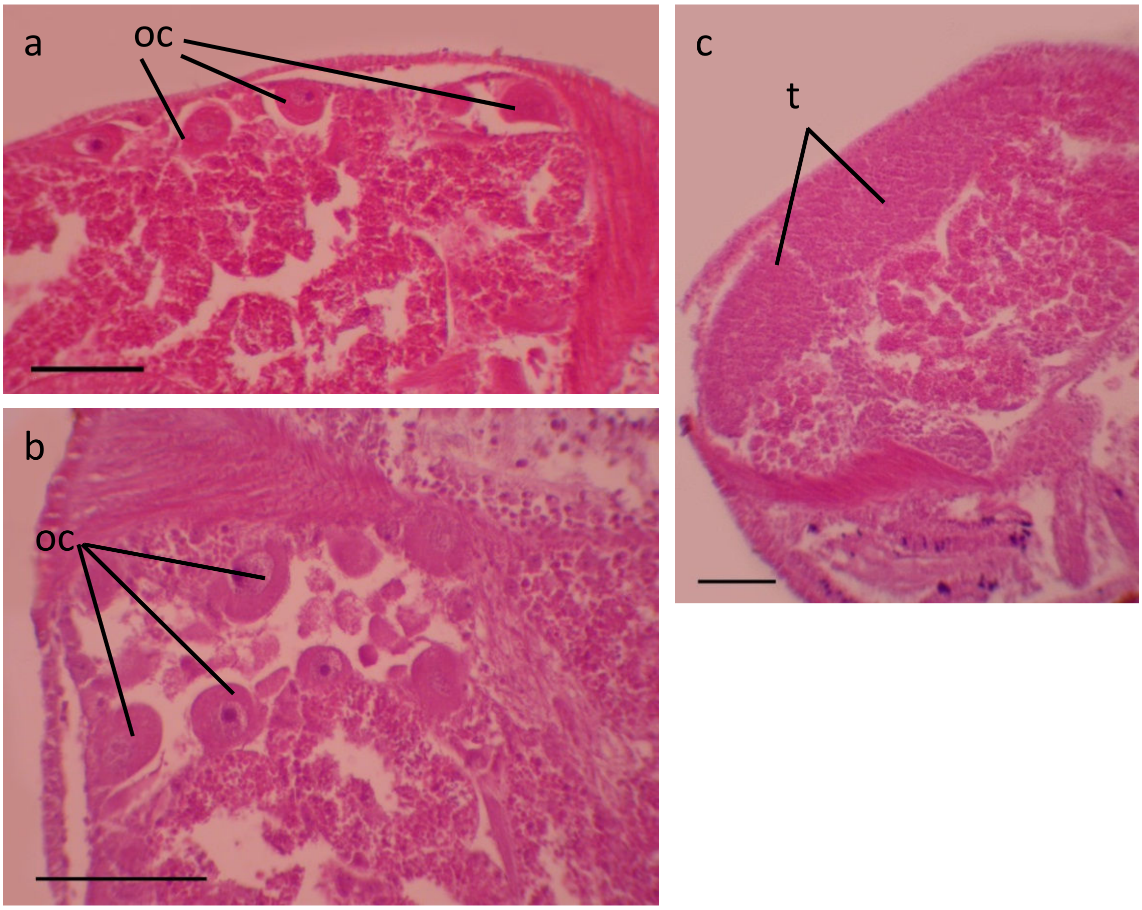


Figure 3.1 Histology sections through posterior part of *Lissarca miliaris* from King George Island showing early gametogenesis. a) oocytes in sub-adult female (2.15mm shell length); b) oocytes in sub-adult female by anterior adductor muscle (2.15mm shell length); c) testis in dorsal portion of gonad from sub-adult male (2.00mm shell length). **oc** – oocytes, **t** – testis. Scale bars = 100µm.

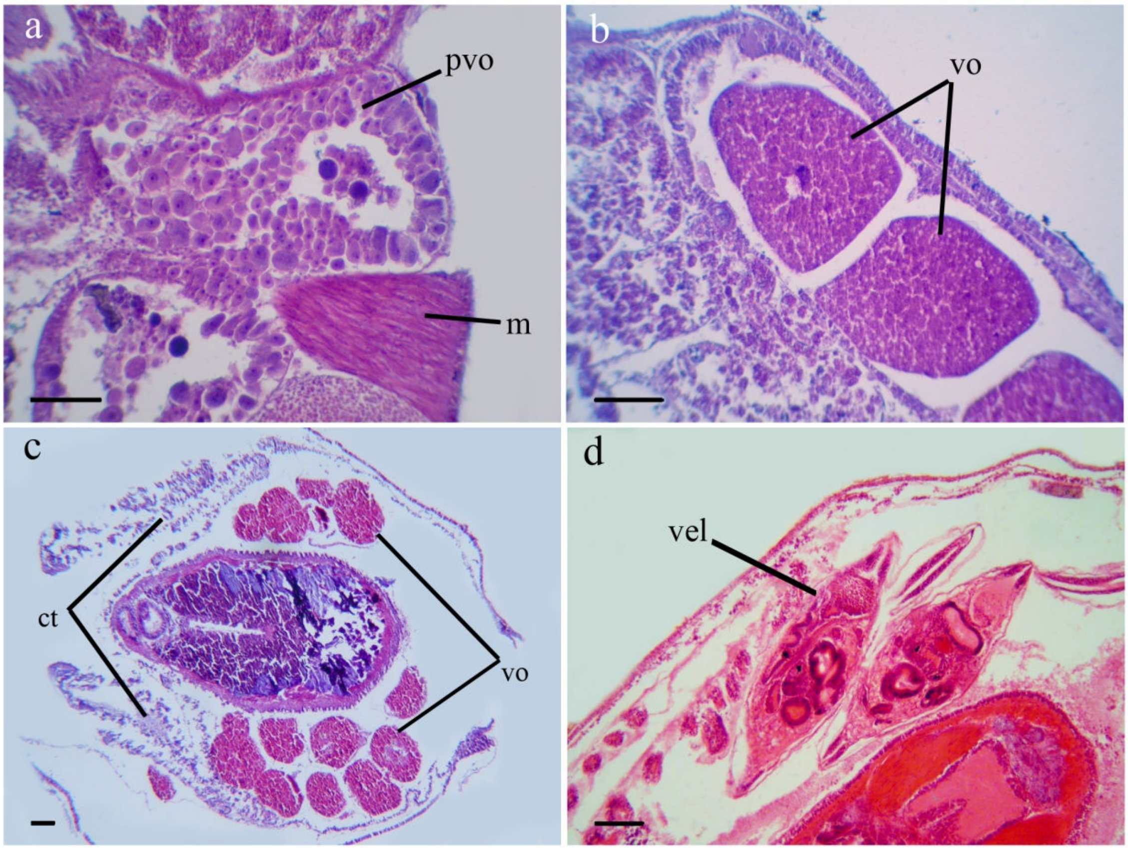


Figure 3.2 *Lissarca miliaris* a,b) King George Island January 1994, c,d) Signy Island April 2002. a) section through the posterior part of the body of functional male (3.6mm shell length) showing previtellogenic oocytes in a small portion of gonad next to posterior adductor muscle; b) transverse section through dorsal portion of gonad in a functional female (4.2mm shell length) showing vitellogenic oocytes; c) transverse section of entire brooding female (4.2mm shell length) showing vitellogenic oocytes within mantle cavity; d) transverse section of mantle cavity of brooding female (3.1mm shell length) showing advanced brooded veliger. **pvo** – previtellogenic oocytes; **m** – muscle; **vo** – vitellogenic oocytes; **ct** – ctenidia; **vel** – veliger. Scale bar = 100µm.

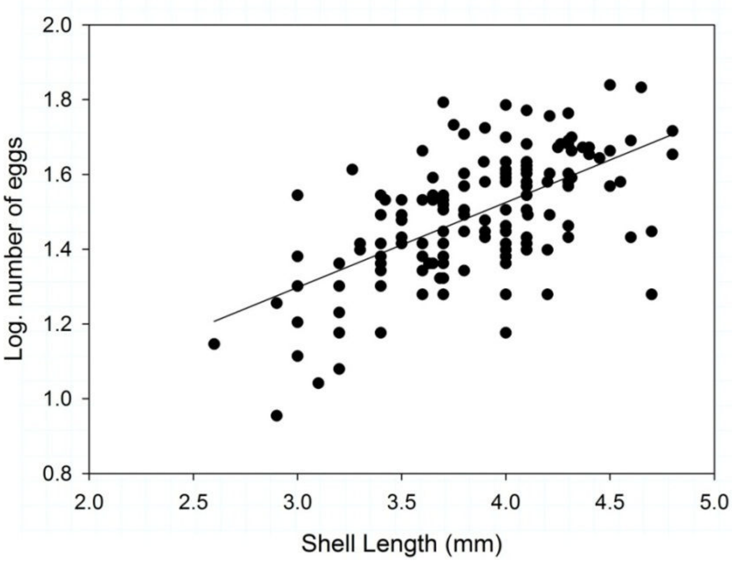


Figure 3.3 *Lissarca miliaris* from Potter Cove, King George Island. December and January. Log. number of vitellogenic oocytes plotted against maximum shell length. $r^2=0.378$, $n=150$, $p<0.001$.

Between 15 and 69 eggs were found in females of 2.9 to 5.8 mm shell length. Fully developed but unfertilised eggs are extruded into the mantle cavity, and are initially held together in two clusters on each side (Figure 3.2c, 3.7a), by an unidentified maternal tissue (Figure 3.4c, d, 3.6c, d). This tissue breaks down, probably after fertilisation, but the clusters remain loosely held together by thin chords resembling thin strands of byssus (Figure 3.4c, 3.7b). Embryos develop larval shells and the large prodissoconch (Signy Island mean length 699.2 µm, SD=34, n=47) often displays a growth disturbance mark (Figure 3.8). Fully developed but pre-release shelled veligers were only found in specimens from Signy Island and were loose in the mantle cavity (Figure 3.2d) with a mean shell length of 0.715 mm (SD 0.04, n=13). The smallest released shelled young from King George Island had a shell length of 0.775 mm.

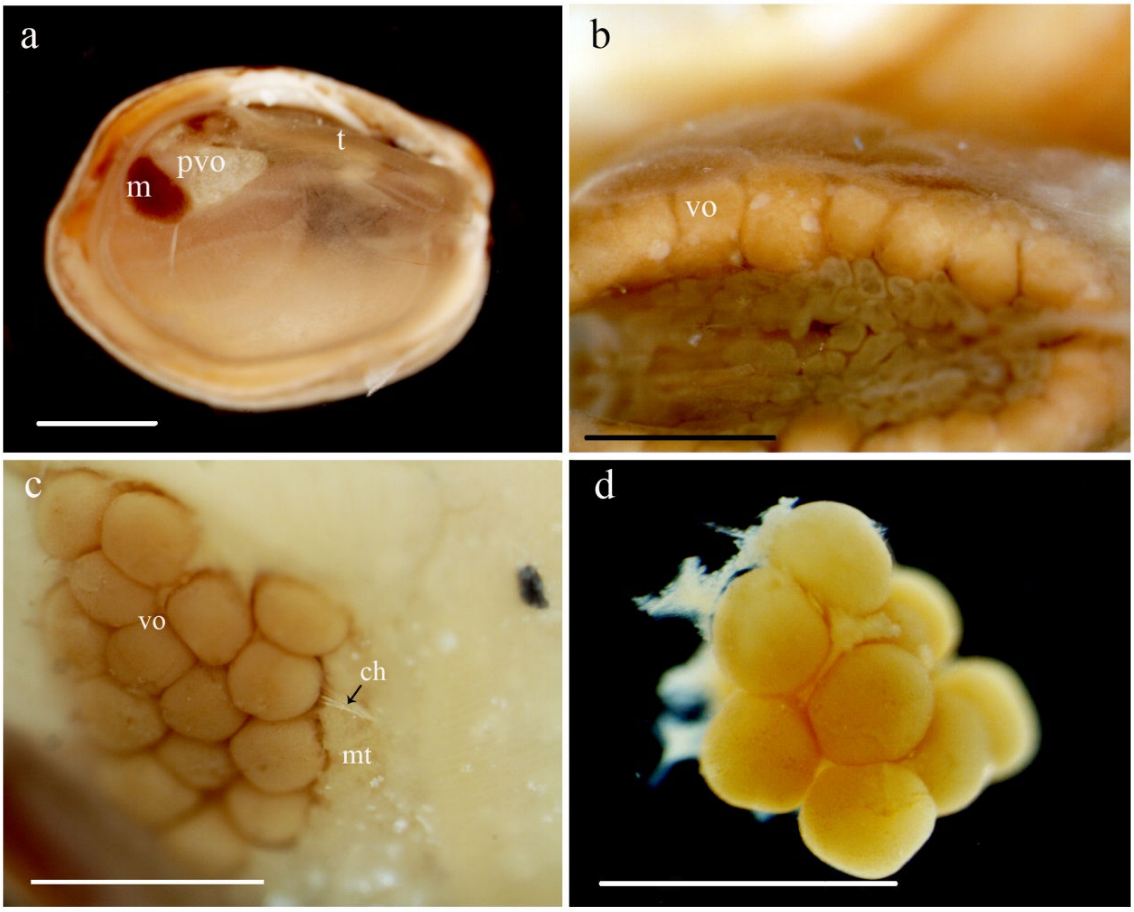


Figure 3.4 *Lissarca miliaris*, King George Island January 1994. a) micrograph showing previtellogenic oocytes developing next to posterior adductor muscle, and testis in a functional male (4.2mm shell length); b) micrograph showing dorsal view of vitellogenic oocytes in dorsal portion of gonad (4.9mm shell length); c) micrograph showing brooded vitellogenic oocytes in the mantle cavity held in place by maternal tissue and thin chords (4.7mm shell length); d) micrograph of cluster of vitellogenic oocytes brooded in the mantle cavity (4.7mm shell length). **m** – muscle; **pvo** – previtellogenic oocytes; **ch** – thin chords; **mt** – maternal tissue. Scale bars = 1mm.

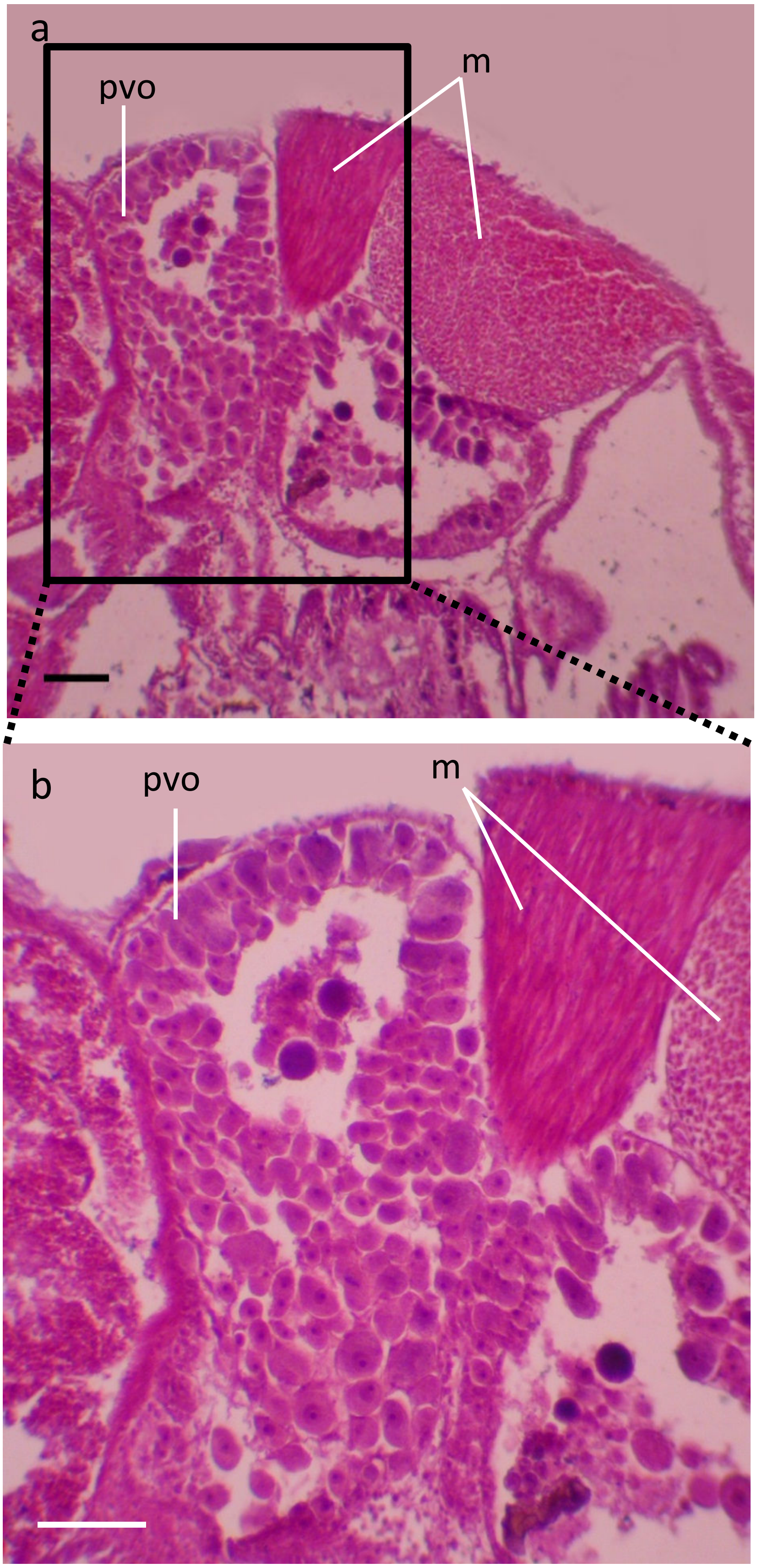


Figure 3.5

Histology of 3.49 – 3.99mm *Lissarca miliaris* from Potter Cove, King George Island with zoomed inlay. a) previtellogenic oocytes tightly packed under and around the posterior adductor muscle; b) higher magnification of previtellogenic oocytes.

m – muscle; **pvo** – previtellogenic oocytes.

Scale bars = 100µm.

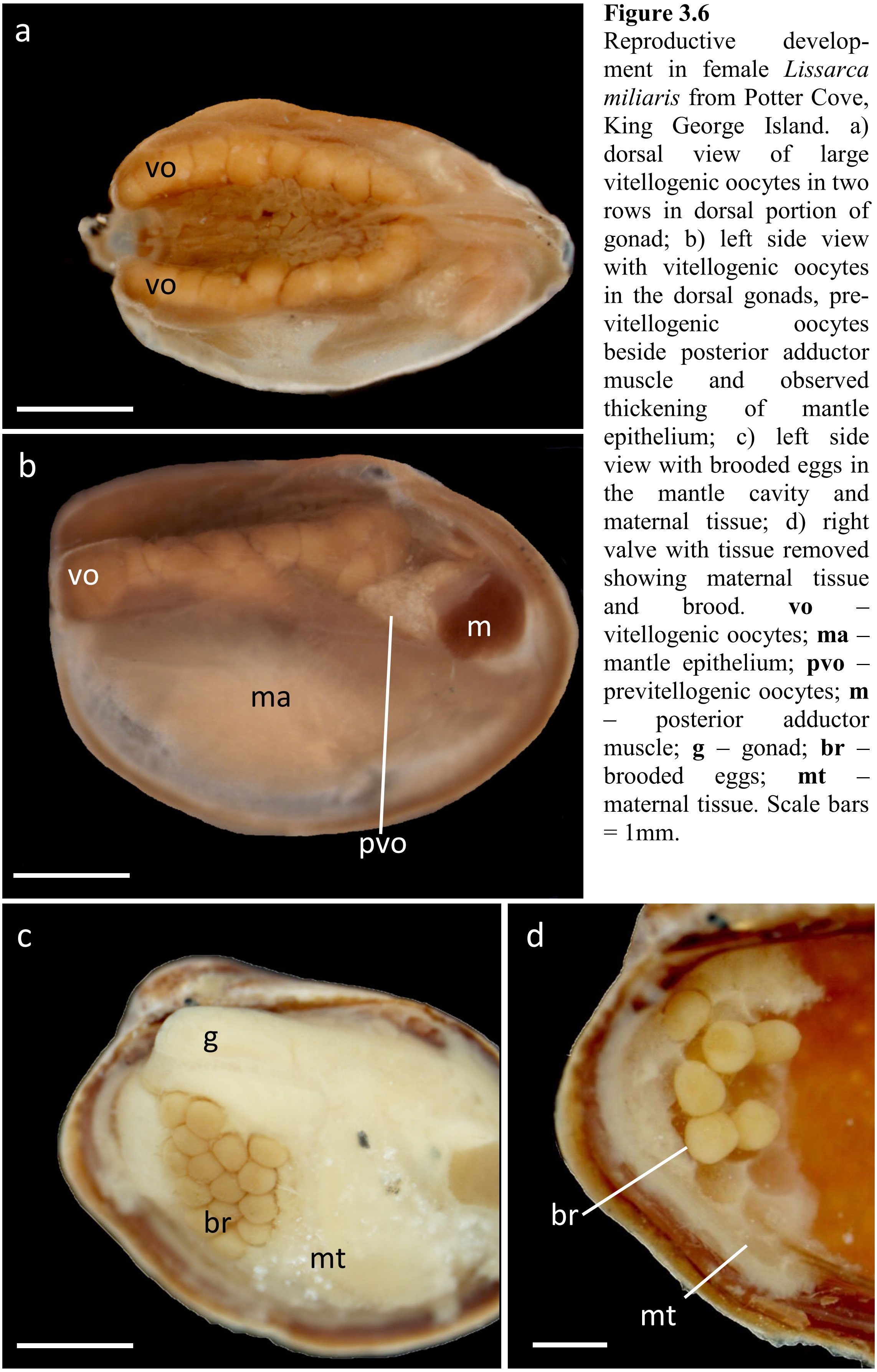


Figure 3.6
Reproductive development in female *Lissarca miliaris* from Potter Cove, King George Island. a) dorsal view of large vitellogenic oocytes in two rows in dorsal portion of gonad; b) left side view with vitellogenic oocytes in the dorsal gonads, pre-vitellogenic oocytes beside posterior adductor muscle and observed thickening of mantle epithelium; c) left side view with brooded eggs in the mantle cavity and maternal tissue; d) right valve with tissue removed showing maternal tissue and brood. **vo** – vitellogenic oocytes; **ma** – mantle epithelium; **pvo** – previtellogenic oocytes; **m** – posterior adductor muscle; **g** – gonad; **br** – brooded eggs; **mt** – maternal tissue. Scale bars = 1mm.

Figure 3.7

Transverse histology section of 4.1mm brooding *Lissarca miliaris* from Signy Island. a) brooded clusters of eggs held in the mantle cavity; b) cluster of eggs in the mantle cavity held together by byssus like threads. Arrows indicate chord like structure. **br** – brooded eggs.

Hermaphroditism is most commonly observed as functional males with ripe testis and previtellogenic oocytes developing in the small portions of gonad beside the posterior adductor muscle (Figure 3.4a, 3.10a, b, c, d, 3.11, 3.12c). In functional females, hermaphroditism is observed above 2.9 mm shell length with broods or large vitellogenic oocytes and reduced testis at the edges of the dorsal portion of the gonad (Figure 3.10e, 3.13). Sex Ratio data from specimens at King George Island demonstrate an increase in functional females from 2.5 mm and a corresponding decrease in male abundance (Figure 3.9). The proportion of functional males with previtellogenic oocytes increases in stages from 15-17% between 2 and 2.99 mm, 33-37% between 3 and 3.99 mm and 83-100% between 4 and 4.99 mm. The changing ratios, supported by evidence of hermaphrodite stages, suggest that the dominant sex of the species changes with age but both male and female portions of gonad persist. Testis appears to be the dominant functional gonad up to 3mm shell length but previtellogenic oocytes are also developing simultaneously.

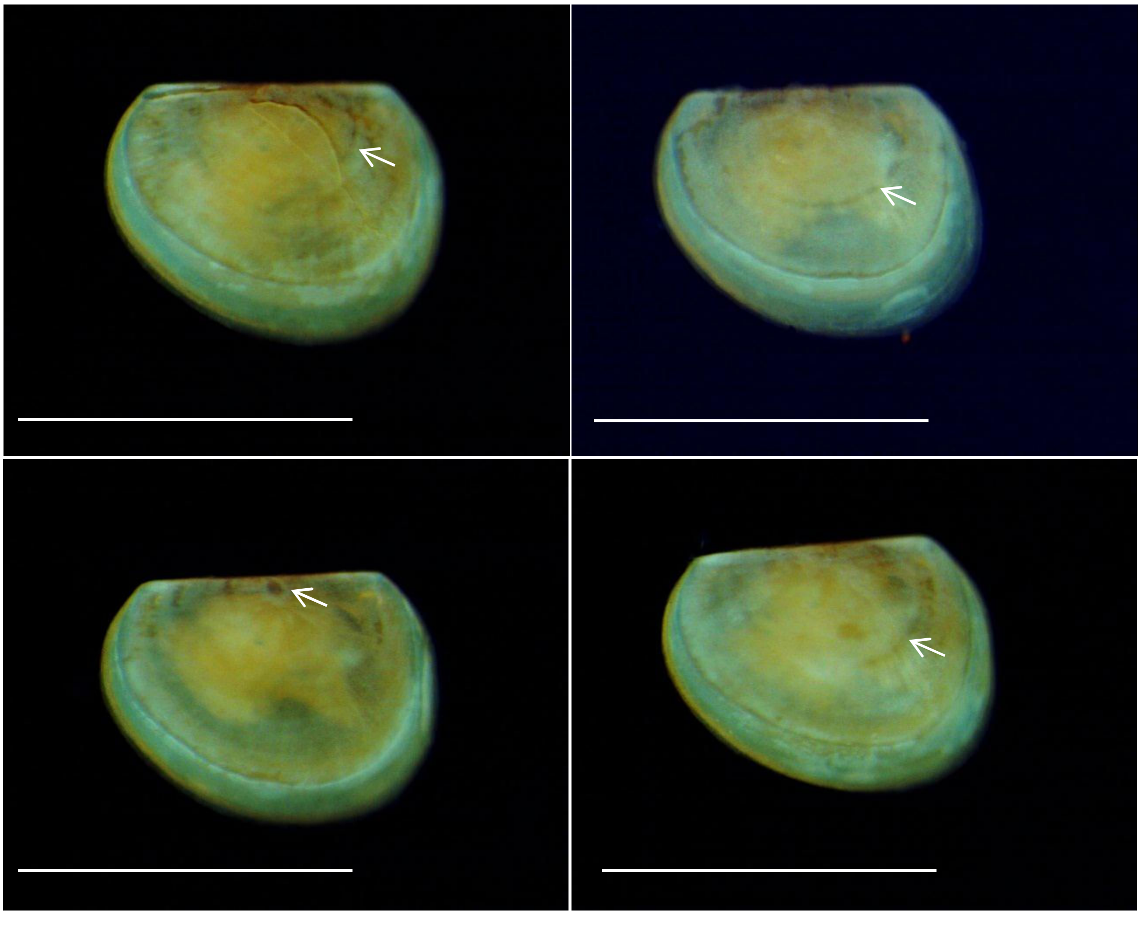


Figure 3.8 Prodissoconch sculpture of *Lissarca miliaris* from Signy Island. Arrows indicate disturbance marks. Scale bars = 1mm.

During vitellogenesis, the testis becomes inactive and reduced while the oocytes develop in ovaries that form dorsally, in the position that testis had previously occupied (Figure 3.9c, d). Brooding females with part shelled veligers were found to have large (250-500 µm) vitellogenic oocytes developing in the dorsal parts of the gonad (Figure 3.12) and there is no evidence that suggests the male portions of gonad become active at larger sizes once reduced. No differences in reproductive trait were observed between the two populations studied and despite the temporal difference between collections; no differences in the hermaphrodite stage were observed between sites.

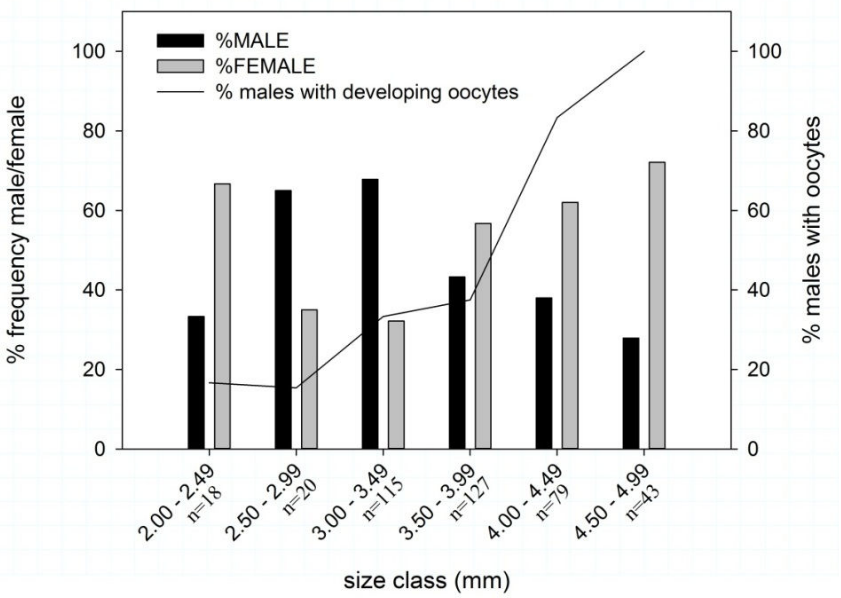
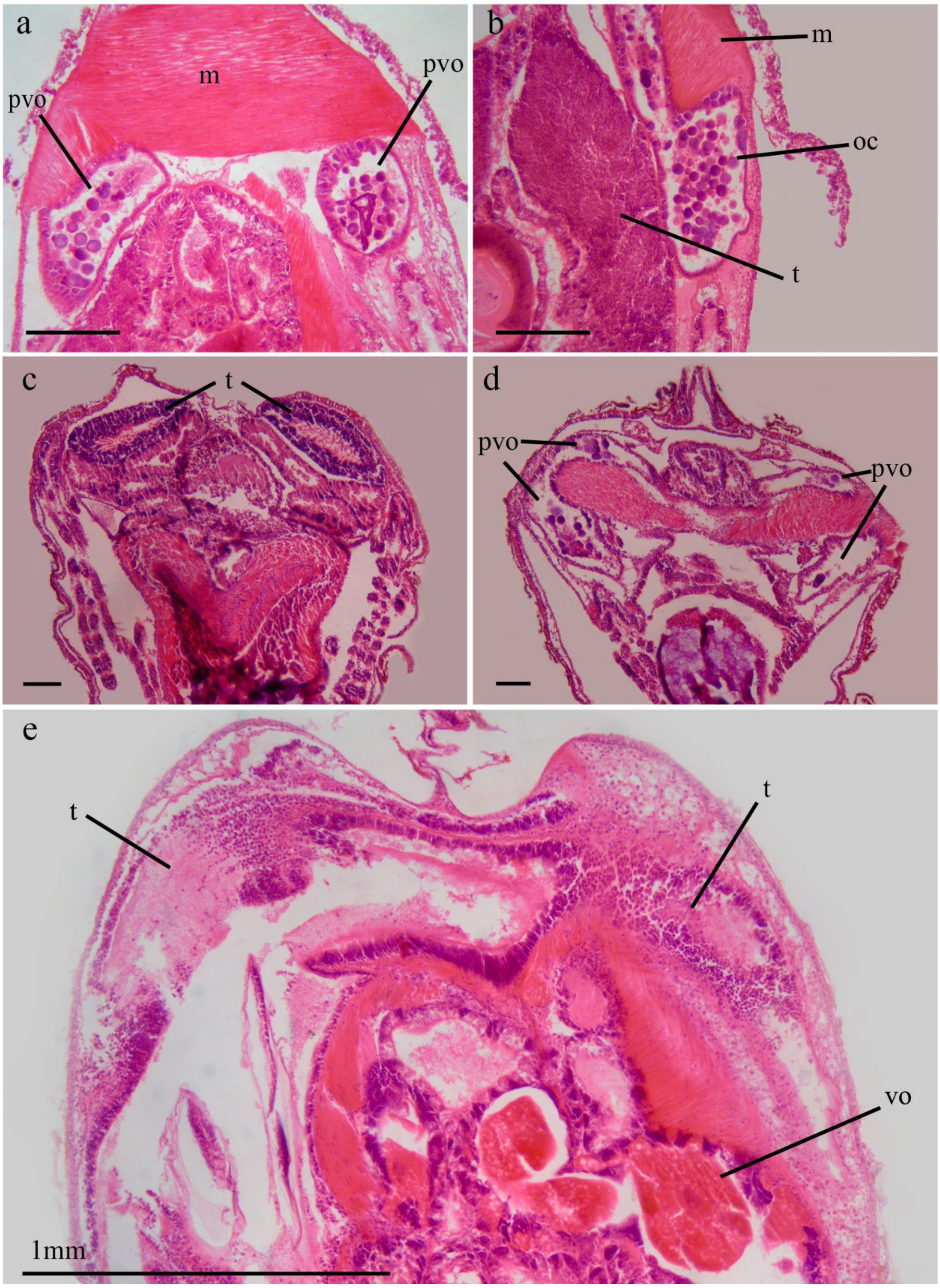


Figure 3.9 *Lissarca miliaris* from Potter Cove, King George Island, January 1994. Percentage frequency male:female sex ratios with increasing size classes using histology <3mm and dissection >3mm shell length. Percentage frequency of males with developing oocytes derived from histology on functional males.

Figure 3.10 → *Lissarca miliaris*, Signy Island April 2002. a, b) transverse section through posterior part of body in functional male (3.5mm shell length) showing a) previtellogenic oocytes developing in two areas of gonad beside the posterior adductor muscle; b) previtellogenic oocytes developing beside the posterior adductor muscle next to mature testis; c, d) two dorsal-ventral sections from the same specimen (3.1mm shell length) demonstrating c) testis reduced in size and low in density; d) oocytes from portion of gonad by posterior adductor muscle spreading into portion of gonad the testis occupies; e) dorsal-ventral section showing functional female (4.1mm shell length), which contained shelled brood (not seen) with vitellogenic oocytes and reduced testis. **m** –muscle; **pvo** – previtellogenic oocytes; **t** – testis; **vo** – vitellogenic oocytes. Scale bar = 100µm unless stated.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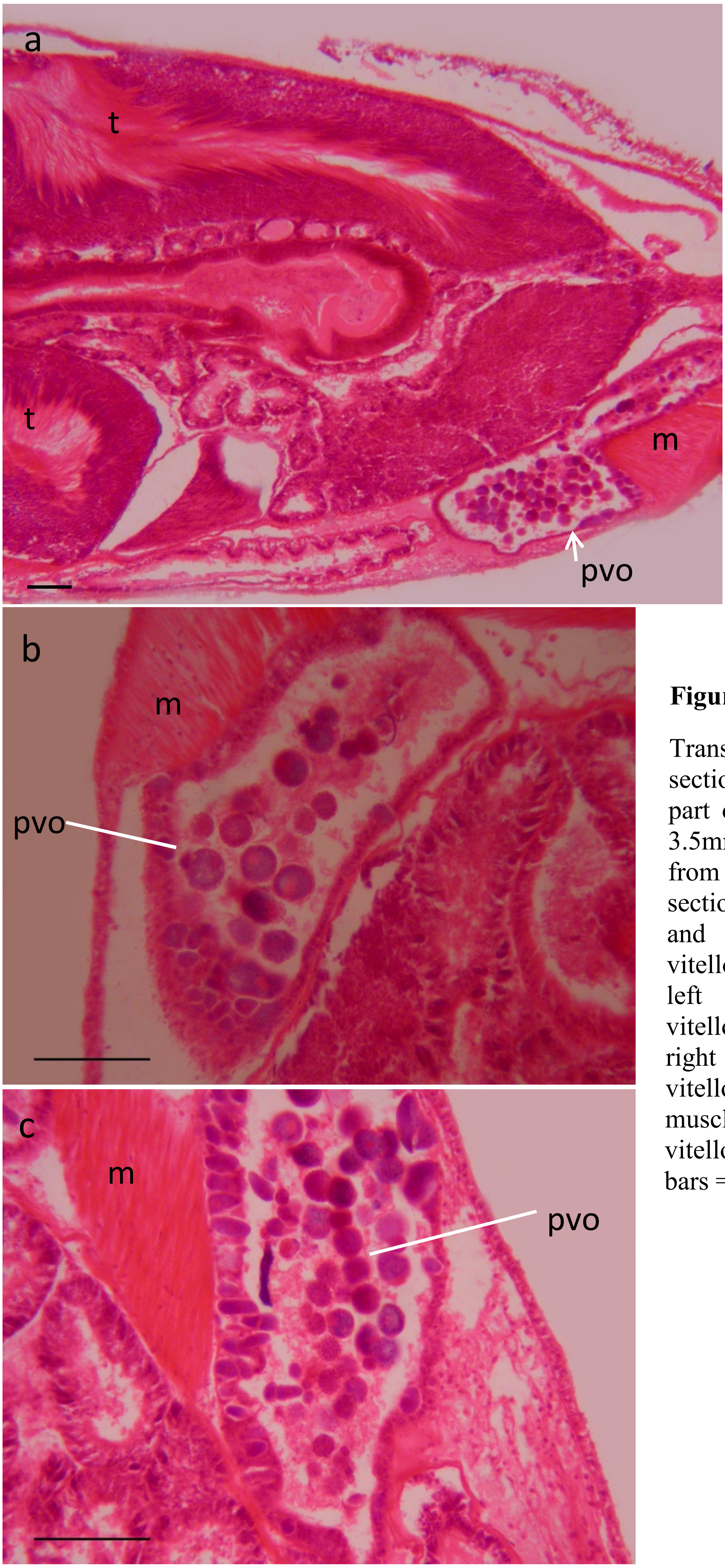


Figure 3.11

Transverse histology sections through posterior part of a functional male 3.5mm *Lissarca miliaris* from Signy Island. a) section overview of testis and position of pre-vitellogenic oocytes; b) left gonad with pre-vitellogenic oocytes; c) right gonad with pre-vitellogenic oocytes. **m** – muscle; **pvo** – pre-vitellogenic oocytes. Scale bars = 100µm.

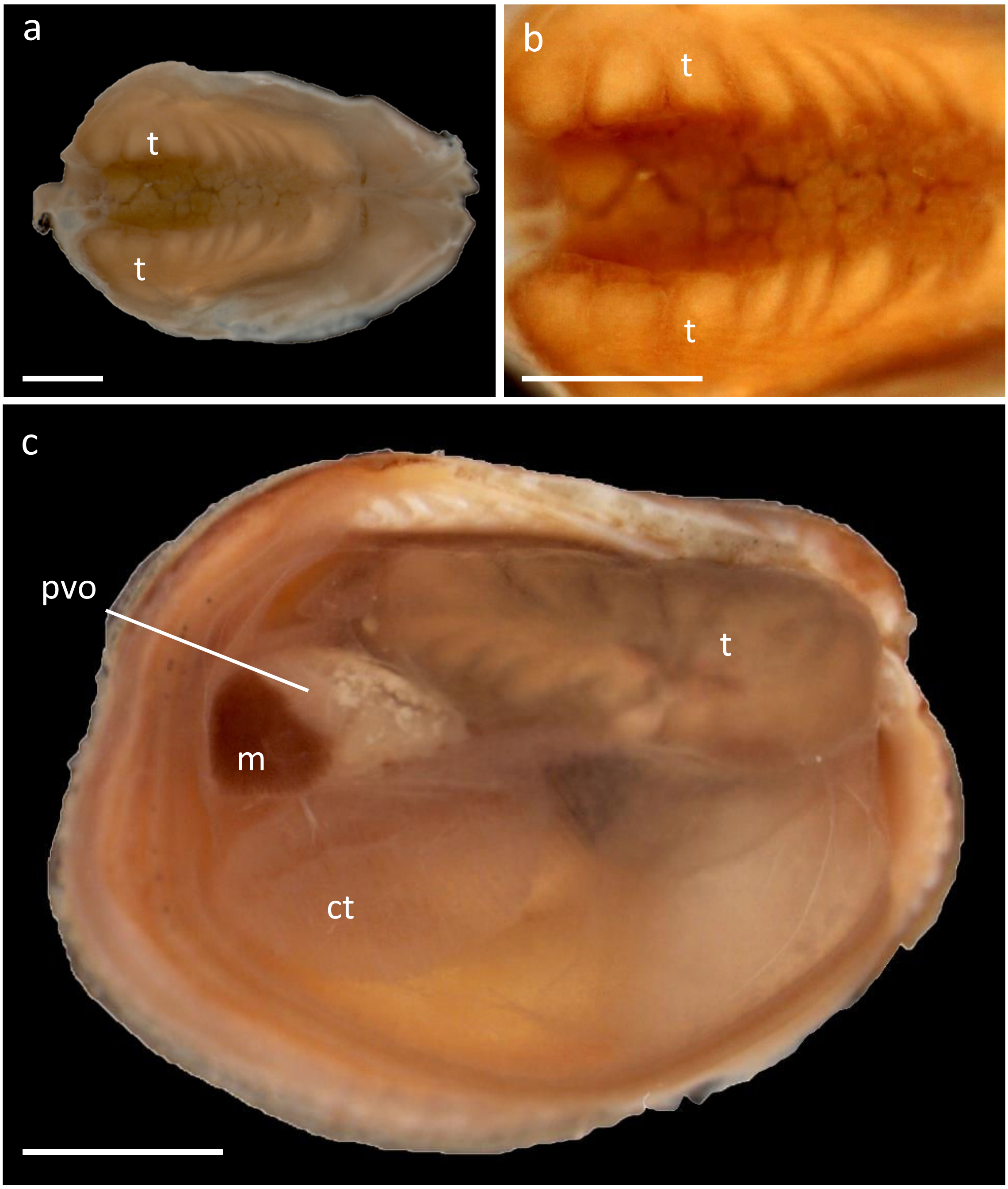
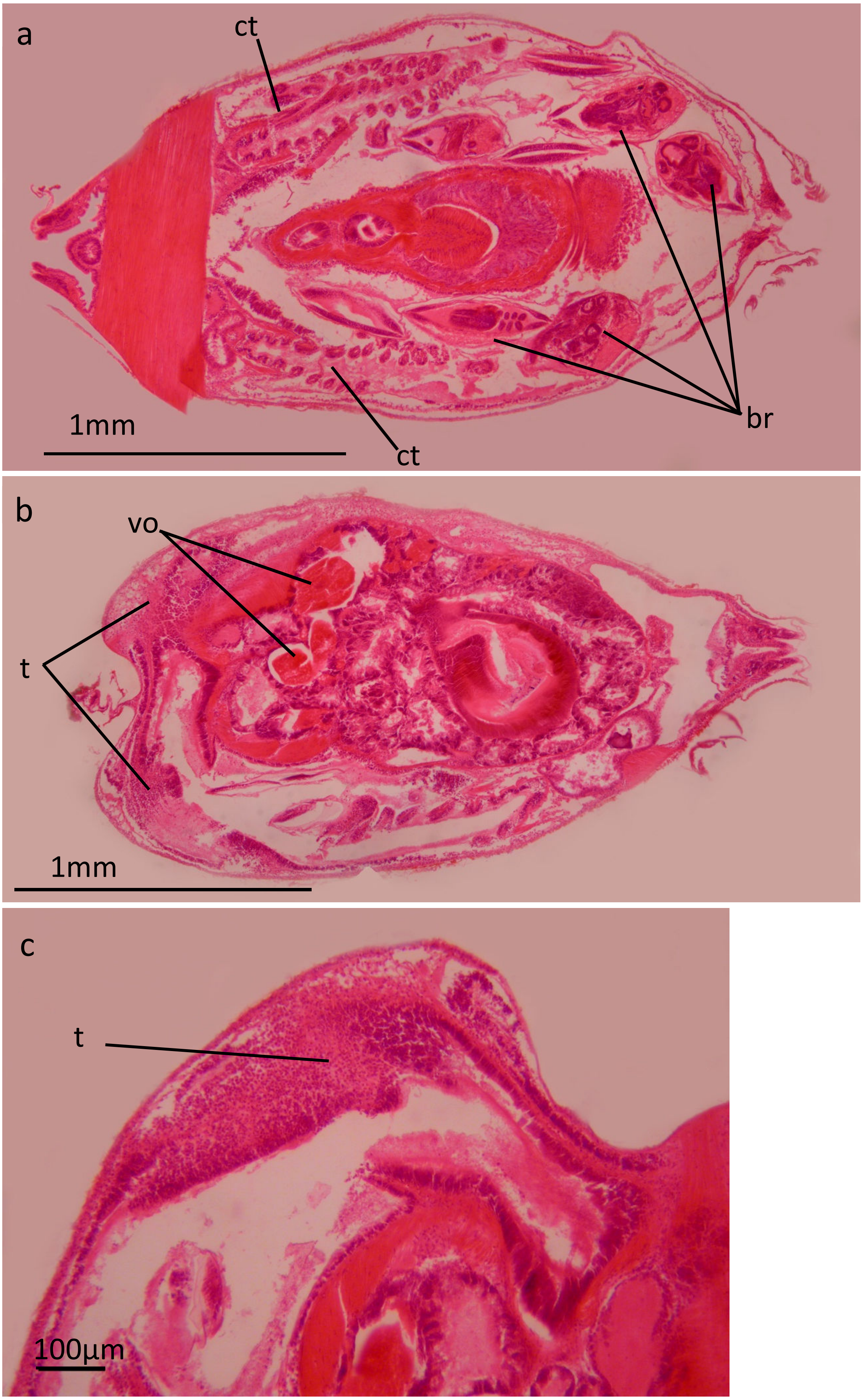


Plate 3.12 Photomicrograph of the stages in reproductive development in male *Lissarca miliaris* from King George Island. a) dorsal view of two rows of testes in the dorsal portion of gonad; b) dorsal view showing the structure of the testes in the dorsal portion of gonad; c) right side view showing testis in dorsal portion of gonad and previtellogenic oocytes developing next to posterior adductor muscle. Scale bars = 1mm.

Figure 3.13 → Transverse histology sections of 3.1mm shell length brooding female *Lissarca miliaris* from Signy Island. a) whole animal showing fully developed shelled larvae within the mantle cavity; b) whole animal section showing large vitellogenic oocytes in lower dorsal gonad; c) degenerating testis along dorsal ventral margin. **ct** – ctenidia, **br** – brood, **t** – testis, **vo** – vitellogenic oocytes.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1mm
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t
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3.4 Discussion

Brooding and Gametogenesis

The Antarctic shallow water environments are dynamic and diverse habitats, disturbed by ice scouring, cold temperatures and high concentrations of short-lived primary production (Gutt 2001; Thatje et al. 2005; Barnes and Conlan 2007). In such limiting conditions, a diverse range of benthic fauna have evolved traits which not only increase the survival of young but also maximise reproductive output. The brooding traits of *L. miliaris* are not unusual for philobryids although there are distinct differences to the described traits of *L. notorcadensis* (Brey and Hain 1992) and *Adacnarca nitens* (Higgs et al. 2009). *Lissarca miliaris* are typically abundant in shallow water, commonly in high abundances in the intertidal and sub-tidal shallow bays in the sub-Antarctic (Dell 1964; Richardson 1979) whereas the previously studied *L. notorcadensis* were found between 80-1108m in the Weddell Sea and *A. nitens* at 84m in the Ross Sea (Brey and Hain 1992; Higgs et al. 2009). Different environmental constraints associated with these depths and latitudes may influence reproductive cycles, investment of energy into offspring, and brooding characteristics.

Despite a smaller shell size, *L. miliaris* have larger broods than *L. notorcadensis* but this is balanced by smaller young; *L. miliaris* producing young with shell lengths of 0.77 mm compared with 1.25 mm in *L. notorcadensis* (Table 3.1). Both species have seasonal reproduction but *L. miliaris* have a shorter 18 month reproductive cycle compared to the 24 month cycle of *L. notorcadensis* (Richardson 1979; Brey and Hain 1992). In contrast, *A. nitens* are asynchronous producing few eggs at a time in small numbers continuously through the year (Higgs et al. 2009). Availability of food is the single most likely factor explaining these differences with *L. miliaris* in this study being exposed to the high concentrations of phytoplankton during the summer months at Signy Island (Clarke et al. 1988) and Potter Cove, King George Island (Klöser et al. 1994; Schloss et al. 2002; Tatian et al. 2002) while their deeper relatives may only be exposed to sinking phytodetritus or low concentrations of phytoplankton during blooms.

Species	Depth range (m)	Brood Size (*n*)	Size at Release (mm)	Reference
Lissarca miliaris	0-275	15 - 69	0.77	Richardson (1979) this study
Lissarca notorcadensis	100-1000	15 - 30	1.25	Brey and Hain (1992)
Adacnarca nitens	8-2350	16 - 55	0.84	Hain and Arnaud (1992) Aldea et al. (1992) Higgs et al. (2009)

Table 3.1 Brooding traits of Antarctic philobryid bivalves *Lissarca miliaris*, *L. notorcadensis* and *Adacnarca nitens.*

Egg sizes of *L. miliaris* are similar to *L. notorcadensis* and *A. nitens*, developing up to 500 µm and as described in *A. nitens* appear to have a high yolk content, demonstrating a high investment of energy into each egg (Higgs et al. 2009). It is not clear how fertilisation occurs although it is likely that the unidentified maternal tissue (Figure 3.4c, d, 3.6c, d) may play a role in holding the ova in position or in fertilisation.

A previously unknown trait observed in *L. miliaris* is the large number of previtellogenic oocytes that develop in a small portion of gonad beside the posterior adductor muscle, and far exceed the number of young an individual can brood, perhaps in the lifetime of the animal. Brooding traits are considered to have evolved from more primitive planktotrophic traits as a consequence to environmental conditions and once lost, these traits are not likely to be reacquired (Strathmann 1985). These previtellogenic eggs are akin in size (19–50 µm), number (200+), and arrangement to planktotrophic species and may be indicative of a planktotrophic past of an ancestor species.

Hermaphroditism

Hermaphroditism is not a rare reproductive trait in molluscs and in Southern Ocean has been described in the bivalves *Laternula elliptica* (Bosch and Pearse 1988), *Thracia meridonalis* (Sartori and Domaneschi 2005) and the brooding bivalves *Mysella charcoti* and *M. narchii* (Passos and Domaneschi 2009), all demonstrating simultaneous hermaphroditism. Sequential protandric hermaphroditim is also common in molluscs where an organism functions initially as a male and then female at a later stage (Heller 1993) and is potentially advantageous for small brooding species which are limited by space available to brood their young (Ghiselin 1969; Heath 1977; Wright 1988; Heller 1993). The occurrence of different types of hermaphrodite traits within populations is known to persist in species of *Crassostrea* (Heller 1993). This group of oysters is mostly single sex but also demonstrates protandric and simultaneous hermaphrodite traits, the cues of which are genetically or environmentally driven (Coe 1943; Guo 1998; Ruiz-Verdugo et al. 2000).

Sequential protandric hermaphroditism was described in *L. notorcadensis* when 5% of a population from the Scotia Sea was found to possess both testes and ovaries while the proportion of males was highest in the smallest size classes (Prezant et al. 1992). Hermaphroditism in *L. miliaris* is different however, as it demonstrates both simultaneous and sequential traits. The long retention of male gonads in mature females and development of primary oocytes in mature males are evidence of simultaneous gonad development, but the reduction of one of the gonads is indicative of sequential hermaphroditism. Sex ratio data in this study support the change of sex from male to female and the proportion of hermaphrodites in the entire population is 30%, higher than the 5% observed in *L. notorcadensis* (Prezant et al. 1992). The higher percentage of females in the 2.00-2.49 mm size class may relate to the difficulty of determining immature testis from other tissue types and the development of previtellogenic oocytes may occur before the formation of determinable testis.

The occurrence of reduced testes in brooding *L. miliaris* could also be evidence of the rarer alternating sex hermaphrodite trait where specimens change sex more than once in their lifetime. This is commonly found in temperate and tropical *Ostrea* spp., which change sex seasonally (Asif 1979; Heller 1993) and is supported in this study by the observation of reduced testis in late brooding females, indicating a possible male reproductive stage whilst brooding young. Reduced testis at this stage of reproduction is curious as its position is in the portion of gonad where vitellogenesis of oocytes occurs. Evidence of undeveloped testis in the dorsal portion of gonad and vitellogenic oocytes (Figure 3.9e) may be in a transitional stage where the oocytes are being extruded into the mantle cavity and the testes are re-developing however, only regular sampling of *L. miliaris* would confirm this reproductive stage. Alternating sexuality would enable females to allocate energy to male gonad production and remain sexually active during long periods of brooding. In a short lived species with a long period between gametogenesis and larval release, alternating sexuality would maximise the reproductive output of *L. miliaris* that may otherwise only reproduce up to 3 times within their life span. Ripe testis have not however been observed in a brooding *L. miliaris* and larger brooding females have been observed with small vitellogenic oocytes. It is not possible to determine the percentage of functional females with testis in *L. miliaris* accurately, as reduced testes are not easily distinguished from surrounding tissue.

L. miliaris may have evolved hermaphrodite brooding traits in response to a number of different constraints including small adult size and isolation. Small size is commonly associated with brooding as larger animals can often produce more young than they can brood (Strathmann and Strathmann 1982; Strathmann et al. 1984). In the Antarctic context brooding is likely to be so high due to speciation within brooding clades and the possible extinction of non-brooding species during glacial periods (for discussion see Poulin et al. 2002; Thatje et al. 2005). Hermaphroditism can also be linked to small sizes however, as the female’s limited capacity to brood may select for sequential protandric hermaphroditism (Ghiselin 1969; Heath 1977; Strathmann and Strathmann 1982; Wright 1988) and the retention of unused gonad may represent an intermediate stage of development. Isolation and small population size may also select for hermaphrodite traits as this may increase the chances of successful reproduction (Ghiselin 1969; Heller 1993).

Evolutionary consequences

The limited capability for dispersal in brooding species can be overcome by rafting and the unique oceanographic conditions offered by the Antarctic circumpolar current allow for connectivity between separated island groups (Highsmith 1985; Martel and Chia 1991; Smith 2002; Nikula et al. 2010). It has to be recognised however, that genetic diversity in brooders appears high, frequently resulting in cryptic speciation and species clusters of circum-Antarctic distribution (Held 2003; Held and Wägele

2005; Cope and Linse 2006; Linse et al. 2008; Hunter and Halanych 2008; Leese et al. 2008; for discussion see Thatje 2012). The distribution of *L. miliaris* in the Scotia Sea is likely to be the consequence of dispersal via macroalgal rafting (Highsmith et al. 1985, Higgs et al. 2009) or juvenile byssus drifting (Sidgurdsson et al. 1976, Beaumont and Barnes 1992). Hermaphroditism may have facilitated founding populations and population expansion following habitat contraction during glacial periods (Thatje et al. 2008, Thatje 2012) as small populations in ice free refugia would be likely to have both sexes for reproduction. Future molecular studies into the origins of *L. miliaris* may provide evidence of the Scotia Sea Islands acting as stepping-stones into the Antarctic from the Magellan region during re-colonisation events (Arntz 1999).

Conclusions

L. miliaris demonstrates a specialised reproductive trait in the Southern Ocean and is consistant with the hypothesis that brooding in small bivalves, may provide an evolutionary advantage over other Antarctic fauna (Dell 1972; Poulin et al. 2002; Thatje et al. 2005, 2008; Higgs et al. 2009). The occurrence of hermaphroditism in this species is a further adaptation that has enabled the species to maximise their reproductive output and perhaps to colonise islands on both sides of the polar front. Philobryid bivalves represent many species of small benthic Antarctic fauna by demonstrating common traits (small size, brooding, and variable Antarctic/sub-Antarctic distributions) and this study highlights the need for further investigation into brooding traits and the processes that may have driven unique characteristics. *Lissarca* spp. also make a good model species for future studies of population genetics, potentially revealing in detail the origins and radiation processes of the philobryid bivalves and increasing our understanding on the processes that have driven the evolution of Antarctic benthic diversity.

3.5 Chapter Summary

Lissarca miliaris show an unusual reproductive trait which may function to maximise reproduction in a cold stenothermal and food limited environment and are distinct from other Antarctic bivalves by;

- A transitional hermaphrodite phase in *L. miliaris* from male to female occurring throughout every size class and functional males are found in the largest size class.
- Male reproductive tissue persisting long after changing sex and observed in late brooding females, 18-24 months after the change in sex.
- The production of a large number of previtellogenic oocytes in functional males, where the number of oocytes observed exceeds the number of young an individual is likely to brood in a lifetime.

Chapter 4

Spatial plasticity in morphology, growth and reproduction in *Lissarca miliaris* (Philobryidae) from the Scotia Sea islands and Antarctic Peninsula

Abstract

The cold stenothermal nature of Antarctic marine ecosystems, and highly adapted fauna living within, raises the question of how much intra-specific plasticity there is among populations, and how any plastic traits have a role in speciation processes through ecological divergence, natural selection and reproductive isolation. Despite decades of collecting specimens around the Antarctic, this question remains largely unanswered with most studies only comparing ‘populations’ of pooled material from a wide geographic range. Many Antarctic invertebrates have wide distribution ranges including areas of the sub-Antarctic, with some having true circum-Antarctic distributions. However, subtle differences in temperature, food availability, and ice conditions among populations could be contrasting in the Antarctic context. In this study, plasticity in ecophysiological traits is explored by measuring growth, reproduction, and shell morphology of six populations of the small bivalve *Lissarca miliaris* from five islands in the Scotia Sea and Antarctic Peninsula, which experience different stenothermal temperature ranges, disturbance, and chlorophyll peak and bloom duration. There are significant differences in morphology and growth among the different populations but no trends with latitude, relationships with population proximity or between those collected intertidally and subtidally. Prodissoconch sizes showed an inverse ‘U’ shaped relationship with latitude and correlated with egg sizes at South Georgia and King George Island Potter Cove. Higher brood sizes at South Georgia represent a trade-off with lower egg size and may be associated to shell morphology offering lower internal capacity to brood young. Lower investment into offspring, phenotypic, and ecophysiological plasticity in *Lissarca miliaris* may be evidence of ecological divergence among populations, and highlight the importance of local scale environmental variations on plastic traits in the Southern Ocean.

4.1 Introduction

Biogeographic mapping of Antarctic invertebrates has found species distributions to cover wide geographic ranges, representing different habitats, bathymetry, and latitudes (Linse et al. 2006a; Barnes and Griffiths 2008; Brandt et al. 2007; Griffiths et al. 2009). Some species have ranges which include the sub-Antarctic Magellan regions to Antarctic Peninsula (e.g. Dell 1964; Nicol 1966) while others have true circum-Antarctic distributions (Raupach et al. 2010), and a high proportion still appear to be endemic to particular regions (Griffiths et al. 2009). The extent to which these populations mix is of much recent discussion, especially with the widening use of molecular tools to identify cryptic speciation and separate distinct species (Held 2003; Held and Wägele 2005; Linse et al. 2007; Grant et al. 2011; see Thatje 2012). However, a response to different local ecosystem conditions, sometimes overlooked, is the phenotypic plasticity in morphology and ecology of widely distributed invertebrate populations (Seed 1968; Beaumont and Wei 1991; de Wolf et al. 1998; Akester and Martel 2000; Steffani and Branch 2003; Harper et al. 2012). While molecular tools are important in understanding population connectivity, plasticity in the ecology of a species, observed among different populations, demonstrates ecological divergence to potentially contrasting environments. Plasticity can also maintain gene flow between populations by sustaining reproductive fitness, hindering the evolution of traits (DeWitt et al. 1998), although reproductive isolation may drive speciation independently of plasticity (Pfennig et al. 2012). Plastic traits may therefore have an important role in understanding the past radiation and resilience of species in the Southern Ocean in response to environmental changes over Milankovitch cycles (Thatje et al. 2005; 2008). This may have consequences on the response to new challenges including regional warming (Vaughan et al. 2003), ocean acidification (McClintock et al. 2009), and invasions from migrant species (Aronson et al. 2007; 2011).

Phenotypic plasticity is a commonly described and easily measured trait in marine molluscs, usually determined by variations in the shape, size, and structure of the calcified shell in response to environmental differences (e.g. Vermeij 1973; Seeley 1986; Nolan 1991; Fuiman et al. 1999; Trussell 2000; Bayne 2004; Sousa et al. 2007;

Benaim et al. 2011). These changes may have important roles in survival and dispersal success, or promote more efficient energy partitioning. Morphological variations in shell shape can result from the physical effects of wave action or ice (Seed 1968; Beaumont and Wei 1991; de Wolf et al. 1998; Akester and Martel 2000; Steffani and Branch 2003; Harper et al. 2012), predation (Seeley 1986; Trussell 2000) or food availability (Appeldoorn 1982), and are not necessarily representative of genetic structuring (de Wolf et al. 1998; Hoffman et al. 2010a). Physiological plasticity, inferred by growth and reproductive output throughout a wide distribution, have rarely been investigated in the Antarctic, and yet can give important clues to the species response to changes in temperature (Morley et al. 2009; Reed et al. 2012), food availability (Peck et al. 2005) and even water chemistry. In the Antarctic this is of particular interest as many species which have wide distributions are known to be sensitive to subtle variations in temperature (Peck et al. 2004; Clarke et al. 2004a; Barnes and Peck 2008; Peck et al. 2008; Morley et al. 2010; Reed et al. 2012).

Although subtle in the global context, the amplitude of variations in conditions between different shallow marine ecosystems is contrasting in the Antarctic context with respect to temperature, ice cover and food availability. With low sample sizes, previous studies on plasticity have often pooled together specimens from broad geographic ranges which may hide plasticity on local scales. For example, morphological differences in *Lissarca notorcadensis* were examined among 48 stations between 159 and 910m, and yet pooled into just 14 sample locations representing the Scotia Arc, Weddell Sea, and Ross Sea (Cope and Linse 2006). Ecophysiological plasticity (growth, reproduction and somatic production) was also measured in *L. notorcadensis* from 'Northern' and 'Southern' Weddell Sea despite samples representing 65 stations covering a depth range of 80-1108m (Brey and Hain 1992) (See Chapter 1 for more examples).

Water temperature ranges vary with latitude in the Southern Ocean with an increase of approximately 0.2°C/100Km between 54°S (South Georgia) and 67°S (Antarctic Peninsula) (Barnes et al. 2006a), and this mostly translates to an increase in the upper temperatures recorded. For example, South Georgia has annual shallow water temperature fluctuations of in excess of 5°C (Whitehouse et al. 1996) compared to the

South Shetland Islands of only ~2°C (Clarke and Leakey 1996). Few studies have measured intraspecific plasticity in Antarctic invertebrates against latitude with the most notable studies on bryozoans (Barnes and Arnold 2001) and gastropods (Linse et al. 2006a) giving contradicting results.

Lissarca miliaris make a good model bivalve species in which to study plasticity given its geographic range from the Antarctic Peninsula, Scotia Sea, sub-Antarctic, and Magellan regions (Huber 2010). They are relatively short lived (up to 7 years) with distinct growth increments and brood their young 18 months in the mantle cavity (Richardson 1979; Reed et al. 2013; Chapter 3). The brooding traits potentially limit the dispersal capability of this species, although rafting in the circum-polar current and large scale eddies are likely dispersal mechanisms for many brooding Antarctic invertebrates (Highsmith 1985; Martel and Chia 1991; Helmuth et al. 1994; Higgs et al. 2009; Leese et al. 2010; Nikula et al. 2010; Nikula et al. 2012). The long periods of brooding in *L. miliaris* may also be a critical time for early shell development and disturbances marked by shell characteristics (Foighil et al. 1986). Protection from physical disturbances from within the maternal brood chamber makes temperature a significant variable affecting prodissoconch size and morphology.

Aims

This study aims to investigate the phenotypic and reproductive plasticity of *Lissarca miliaris* from different island populations spanning the Scotia Sea to the Antarctic Peninsula. Although all populations are characteristically stenothermal, all experience different environmental conditions, especially temperature range, and the intensity and duration of food availability. The potential for ecological divergence through plasticity to subtle environmental variations, and its impacts on speciation, is discussed in an evolutionary context.

Hypotheses

H_1 *Lissarca miliaris* demonstrates morphological plasticity among different populations in the Scotia Sea and Antarctic Peninsula.

H_2 *Lissarca miliaris* demonstrates plasticity in growth rate, maximum size, and longevity among populations.

H_3 *Lissarca miliaris* demonstrates reproductive plasticity identified by differing egg sizes and prodissoconch sizes among populations.

Objectives

O_1 Measure and compare the morphometric variation in shell shape among different populations of *Lissarca miliaris* to test environmental constraints on morphology (test of hypothesis 1).

O_2 Compare the rates of growth, maximum size, longevity, and growth performance among different populations of *Lissarca miliaris* to test effect of environmental variations on energy allocation to growth (test of hypothesis 2).

O_3 Investigate the reproductive plasticity in *Lissarca miliaris* among populations by measuring larval shell morphology, egg size and brood counts bringing together morphology and growth and associated trade-offs with reproductive output (test of hypotheses 1, 2 and 3)

4.2 Methods

Bivalve Collection

Lissarca miliaris were collected during a number of research expeditions to the Southern Ocean. Available for this study were specimens from South Georgia (Cumberland Bay, intertidal, 1972, n=626); Signy Island (Shallow Bay, intertidal, 2002, n=460); King George Island (Admiralty Bay, 10m, 1997, n=117); King George Island (Potter Cove, 10m, 1994, n=2271); Livingston Island (Byers Peninsula, 2009, n=58) and Stepping Stones (10m, 2012, n=133) (Figure 4.1). The specimens from Signy Island 2002 were collected as part of the R/V *Polarstern* LAMPOS (ANT XIX/5) expedition (Arntz and Brey 2003). All samples were separated on board and fixed in 4% buffered formalin and subsequently stored in 70% ethanol.

Shell Morphology

Shells of *Lissarca miliaris* from South Georgia (n=626), Signy Island (n=460), King George Island Admiralty bay (n=117), King George Island Potter Cove (n=2271), Livingston Island (n=58) and Stepping Stones (n=133) were measured as described in Chapter 2.2. Corrected mean length/height and length/depth ratios, calculated from the analysis of covariance (adjusted least squares means) on normally distributed data were used to plot and investigate statistical differences between populations. Shell length was used as the covariate to standardise the effect of ontogenetic morphological differences within populations. A post-hoc Tukey test was used to identify groups of significantly different populations using log transformed data. A plot of the corrected length/height and length/depth ratio was also used to identify the variations in shape between populations as described in Chapter 2.2.

Growth

Growth was measured using the von Bertalanffy growth function (vBGF) and calculated overall growth performance (OGP) as described in Chapter 2.4. As many bivalves available for each population were measured and growth increments counted cumulating in South Georgia (n=558), Signy Island 2002 (n=458), King George Island Admiralty Bay (n=117), King George Island Potter Cove (n=1444), Livingston Island (n=58) and Stepping Stones (n=133). For comparison of OGP, a general vBGF was used for each population and parameters ‘K’ and ‘S_∞’ used to derive an OGP value. An analysis of covariance and post-hoc Tukey test was used to identify significant differences in growth among populations.

Size-at-age was also measured and data analysed for each growth increment with corresponding Kruskal-Wallace One-Way Analysis of Variance and multiple comparison test (Dunn’s Method). The mean size and standard error for each growth increment was plotted and statistically distinct groups identified. Where growth increments were low in number and resulted in high standard error, populations were removed from statistical analysis.

Prodissoconch Sizes

Prodissconch I (PI) sizes were measured by microphotographs and image analysis as described in Chapter 2.2. Populations at South Georgia, Signy Island 2002, King George Island Admiralty Bay and Stepping Stones had appropriate shell condition and size classes for this analysis. A general linear model Analysis of Variance and Tukey test was used to show significant differences among populations. Other populations were not used due to poor condition of shells making measurements impossible or inaccurate.

Egg Sizes

Egg sizes were measured by dissection from the brood chamber and image analysis from micrographs as described in Chapter 2.6. Eggs were removed and counted from the brood chamber of adult females or from the mature dorsal gonad from specimens at South Georgia and King George Island Potter Cove. All eggs were at the same stage of development (before observed blastula stage) and same time of year (January). Feret diameter was used to measure egg size, 125 eggs from 11 adults were measured from South Georgia while 845 eggs from 75 adults were measured rom King George Island Potter Cove. Egg counts per brood were taken from 16 adults from South Georgia and 150 adults from King George Island Potter Cove. An analysis of covariance was performed on the data and the adjusted means used to xompare populations.

4.3 Results

Species Identification

Lissarca miliaris was originally described as *Pentuculus miliaris* from the Straits of Magellan by Phillippi (1845) and was suggested to be congeneric with *Lissarca rubro-fusca* by Smith (1879). Nicol (1966) synonymised the species after examining the morphology of both *miliaris* and *rubro-fusca* morphotypes but suggested that the two might be distinguished by a slightly more elongate shell form of *L. rubro-fusca*. Dell (1964) also included *Lissarca bennetti* (Preston 1916) from the South Orkneys as a synonym. In this study, the different populations were identified as *Lissarca miliaris* according to the description by Smith (1879) and Nicol (1966) (Plate 4.1). Key features include;

Shell size small, usually less that 5mm, robust, tumid, equivalve, subquadrate, anterior side rounded, outline oval, umbones project from dorsal line. Shell reddish brown to purple/pink, thick brown periostracum, thicker at margins. Valves with concentric striae, sometimes with radial striae. Hinge with four or five strongly developed posterior chevron shaped teeth, three to four anterior. Ligament central to teeth, small, alivuncular indistinct, hinge line straight. Pallial line continuous without sinus, posterior adductor muscle scar large, slightly pear shape, anterior muscle absent.

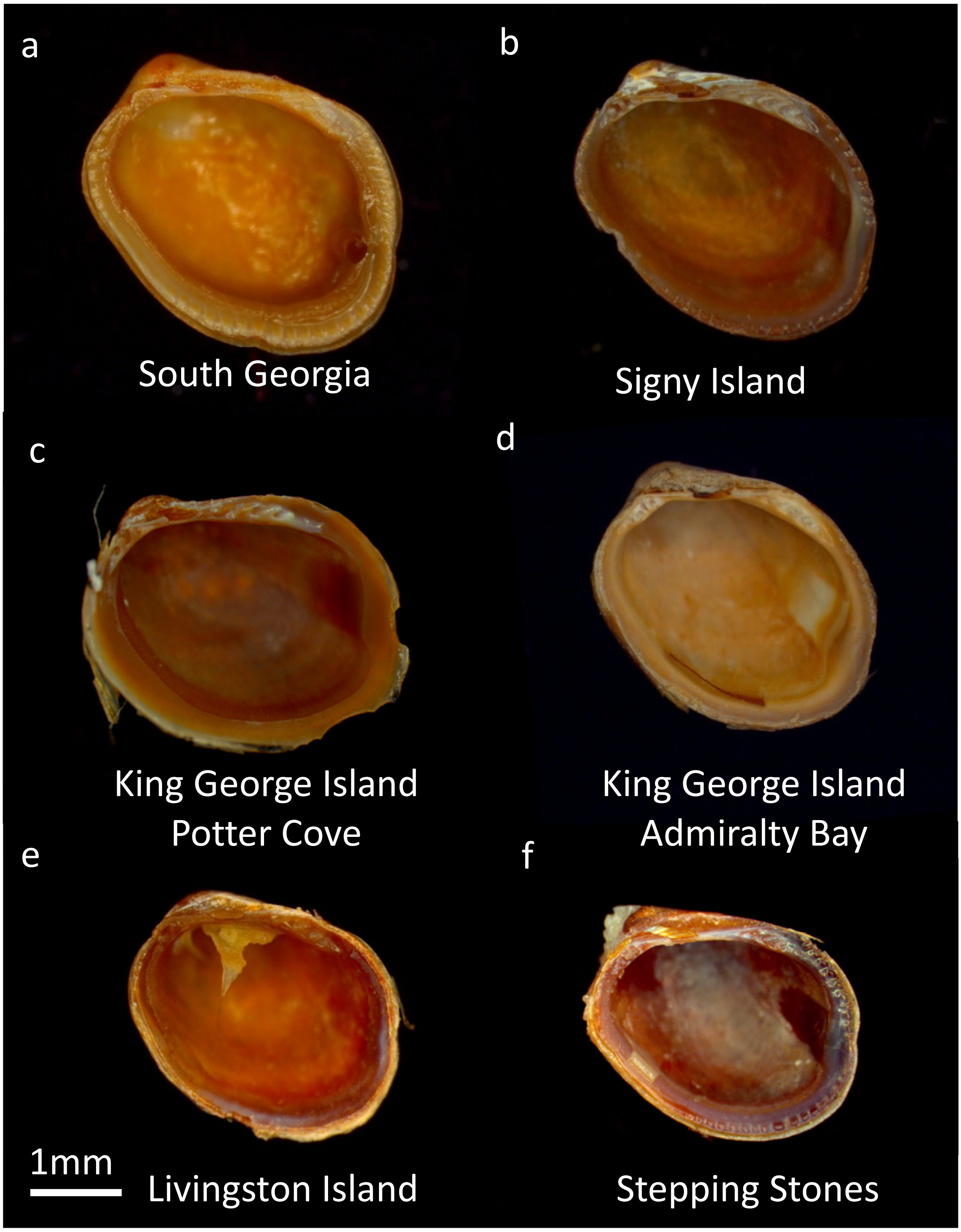

Figure 4.1 *Lissarca miliaris* shell types from six Antarctic populations used in this study at the same age (four growth increments). a) South Georgia, Cumberland Bay, 1972; b) Signy Island, Shallow Bay, 2002; c) King George Island, Potter Cove, 1994; d) King George Island, Admiralty Bay, 1997; e) Livingston Island, Byers Peninsula, 2009; f) Stepping Stones, 2011.

Shell Morphology

The six populations of *L. miliaris* from South Georgia, Signy Island, King George Island, Livingston Island, and Stepping Stones all show strong linear relationships between shell length and height (Figure 4.2a) and shell length and width (Figure 4.2b) (length/height r^2 values all above 0 .98, length/width r^2 values all above 0.93) (Table 4.1). An analysis of covariance (ANCOVA) of shell height from the measured values identified three distinct morphologies of log transformed length against height ($F_{5, 3654}$=8.87, p<0.001). A post hoc Tukey test (95% similarity) found King George Island (Admiralty Bay) and Stepping Stones to make up a distinct group (Figure 4.2c) by having more elongate shells than other populations (Figure 4.3a).

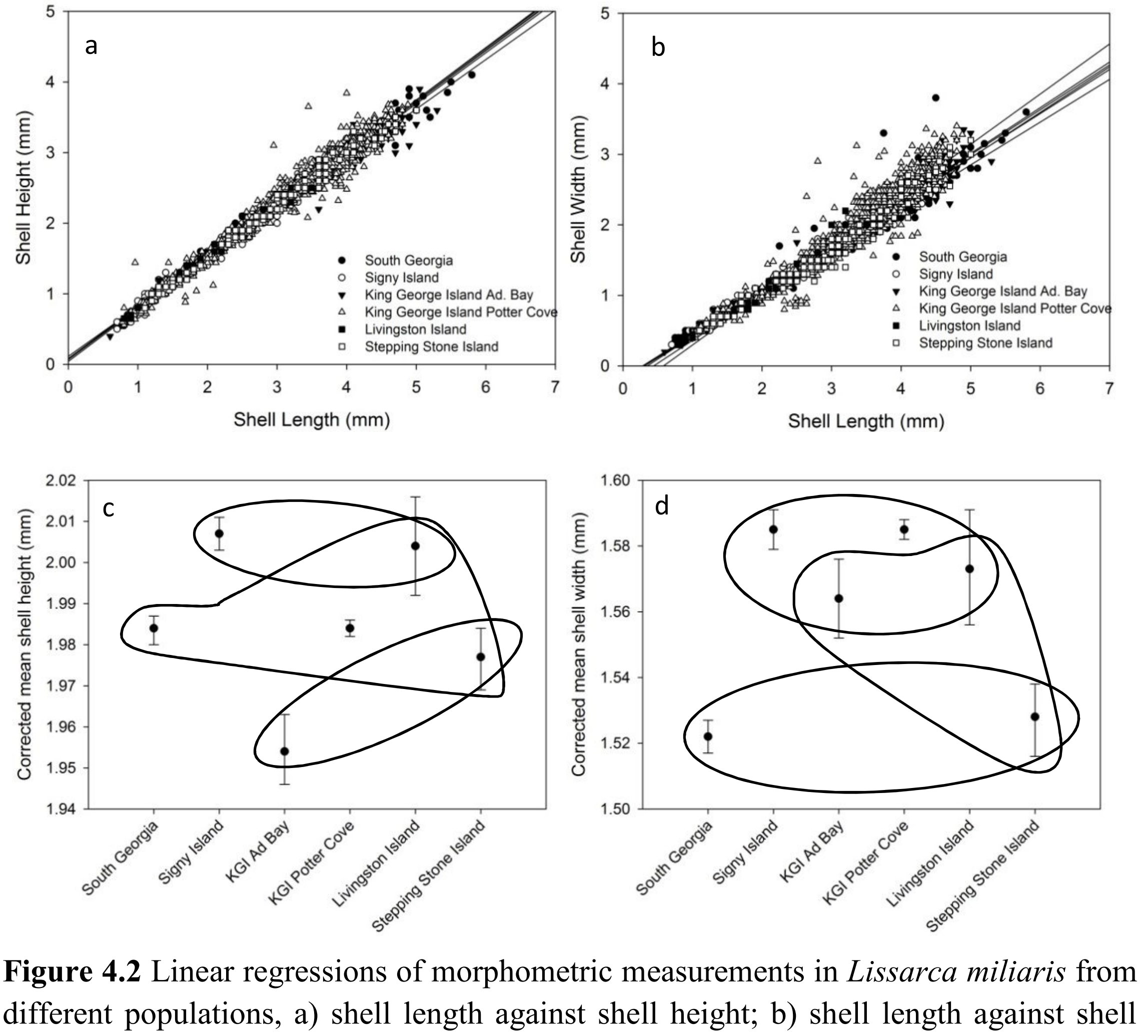

Figure 4.2 Linear regressions of morphometric measurements in *Lissarca miliaris* from different populations, a) shell length against shell height; b) shell length against shell width. a,b) Linear regressions significant p<0.001; c,d) Circled groups represent statistically indistinct populations, Tukey test, p<0.05. KGI = King George Island.

South Georgia, King George Island Potter Cove, Stepping Stones and Livingston Island made up a second statistically distinct group (Figure 4.2c) being between the extremes of shell roundness and slenderness (Figure 4.3a), while Signy Island and Livingston Island made the third distinct group (Figure 4.2c), having the most rounded shell shape (Figure 4.3a). Shells became more obese in shape with age but there was no trend in the length/height relationship with growth increment (Figure 4.3b). There was also a significant difference between log transformed length and width among sites (ANCOVA, $F_{5,\ 2971}$=25.75, p<0.001), and a post hoc Tukey test (95%) identified two distinct groups with only Livingston Island overlapping in both. South Georgia, King George Island Admiralty Bay and Stepping Stones made up a distinct group (Figure 4.2d) with a thinner shell shape (Figure 4.3a) while Signy Island and King George Island Potter Cove made up a second group (Figure 4.2b) being more obese in shell shape (Figure 4.3a).

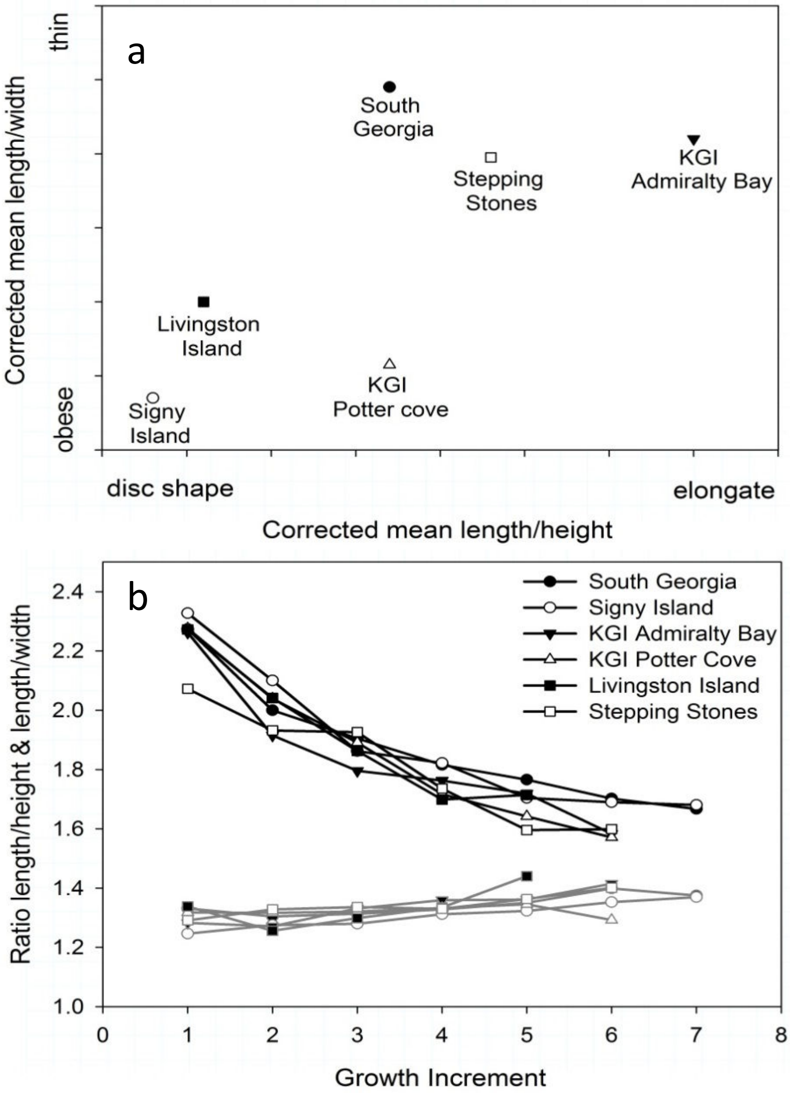

Figure 4.3 ← Morphology of *Lissarca miliaris* by populations and with age. a) corrected length/height v corrected length/width among populations identifting differing shell shapes; b) shell morphology with age among popualtions. Black line shell length/shell width, grey line shell length/shell height. Decreasing ratio value indicates increaseing obesity or roundness, respectively.

Growth

A von Bertalanffy growth curve for each population showed variations in the rate of growth among *L. miliaris* (Figure 4.4a, Table 4.1). South Georgia showed the fastest growth, and had the largest observed adult at 6mm shell length after 7 growth increments. In contrast, Stepping Stones and Livingston Island have the slowest growth and smallest sizes at each age cohort; both populations observed only having up to 5 and 6 growth increments, respectively. An ANCOVA identified a significant difference in size-at-age among populations ($F_{5,\ 2763}$=51.74, p<0.001). A post-hoc multiple comparison test (Tukey at 95%) of size-at-age measurements found the growth at South Georgia and both King George Island populations (Potter cove and Admiralty Bay) to be statistically indistinct, while Signy Island, Livingston Island and Stepping Stones all had significantly different and distinct growth. The overall growth performance (OGP), calculated from the parameters of the vBGF (taking into consideration the calculated growth constant (*k*) and size after infinite growth (S_∞), show higher OGP for South Georgia (0.443) and the King George Island populations (Admiralty Bay 0.437, Potter Cove 0.404) compared to Stepping Stones, Livingston Island and Signy Island (Figure 4.4b, Table 4.1). Calculated OGP is lowest at Signy Island at 0.318, followed by Livingston Island (0.346) and Stepping Stones (0.373).

Size-at-age data for each population show how the growth rate of *Lissarca miliaris* changes with growth increment (Figure 4.5). A Kruskal Wallis one way ANOVA at each growth increment found significant differences between populations (all p<0.001) and a multiple comparison test (Dunn's method, 95% similarity) identified where the differences were found (Figure 4.5). Specimens from South Georgia are initially small in size (Figure 4.5a-c) but growth is consistently high between growth increments (Figure 4.5g) and has the largest shell size from the 4th increment (Figure 4.5d).

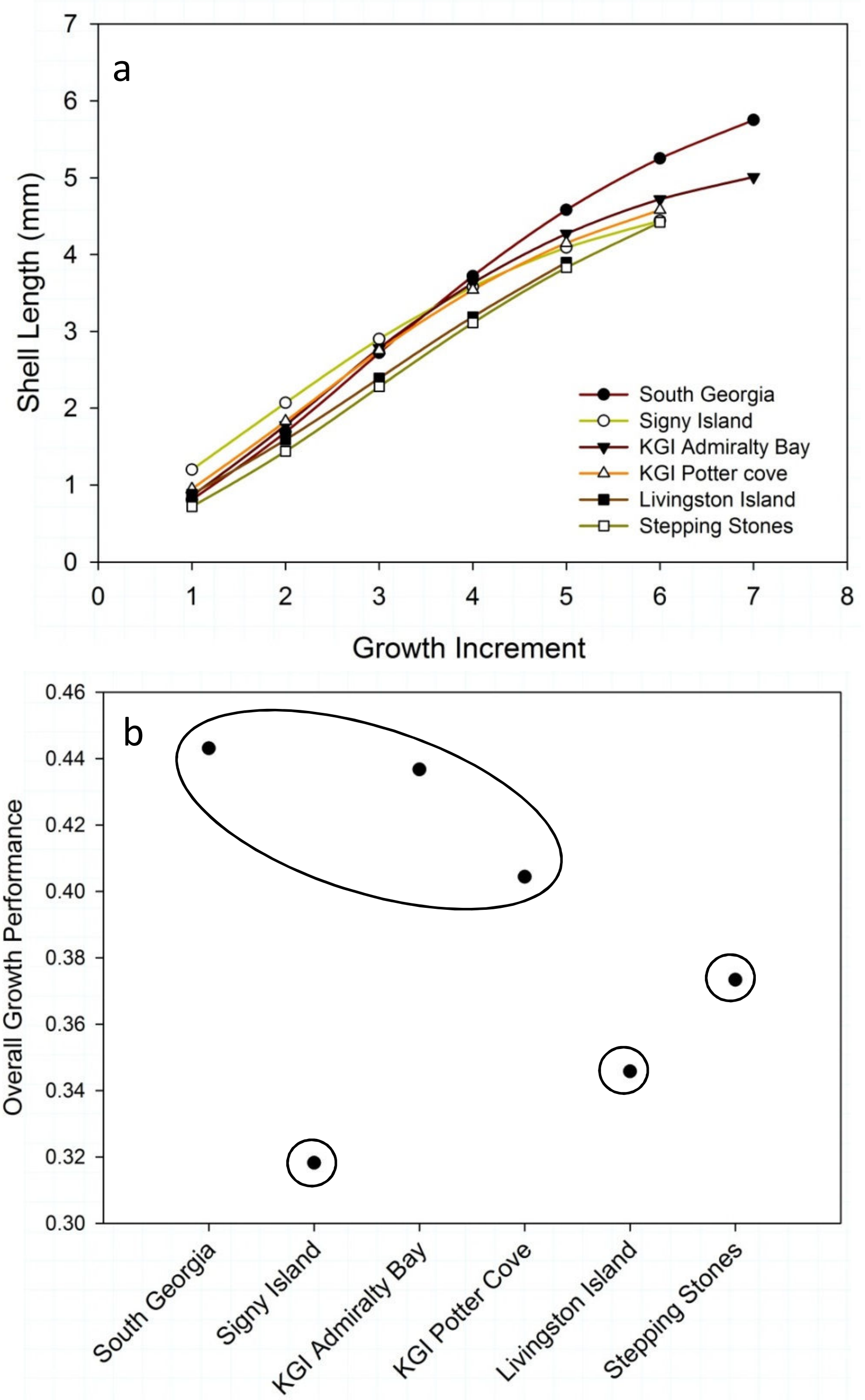

Figure 4.4 Growth parameters of *Lissarca miliaris* among populations. a) von Bertalanffy growth function from age-at-size data of *Lissarca miliaris* from different populations; b) overall growth performance of *Lissarca miliaris* from different populations derived from residuals of von Bertalanffy growth function. Separate circles represent significantly different growth, $p<0.001$. KGI = King George Island.

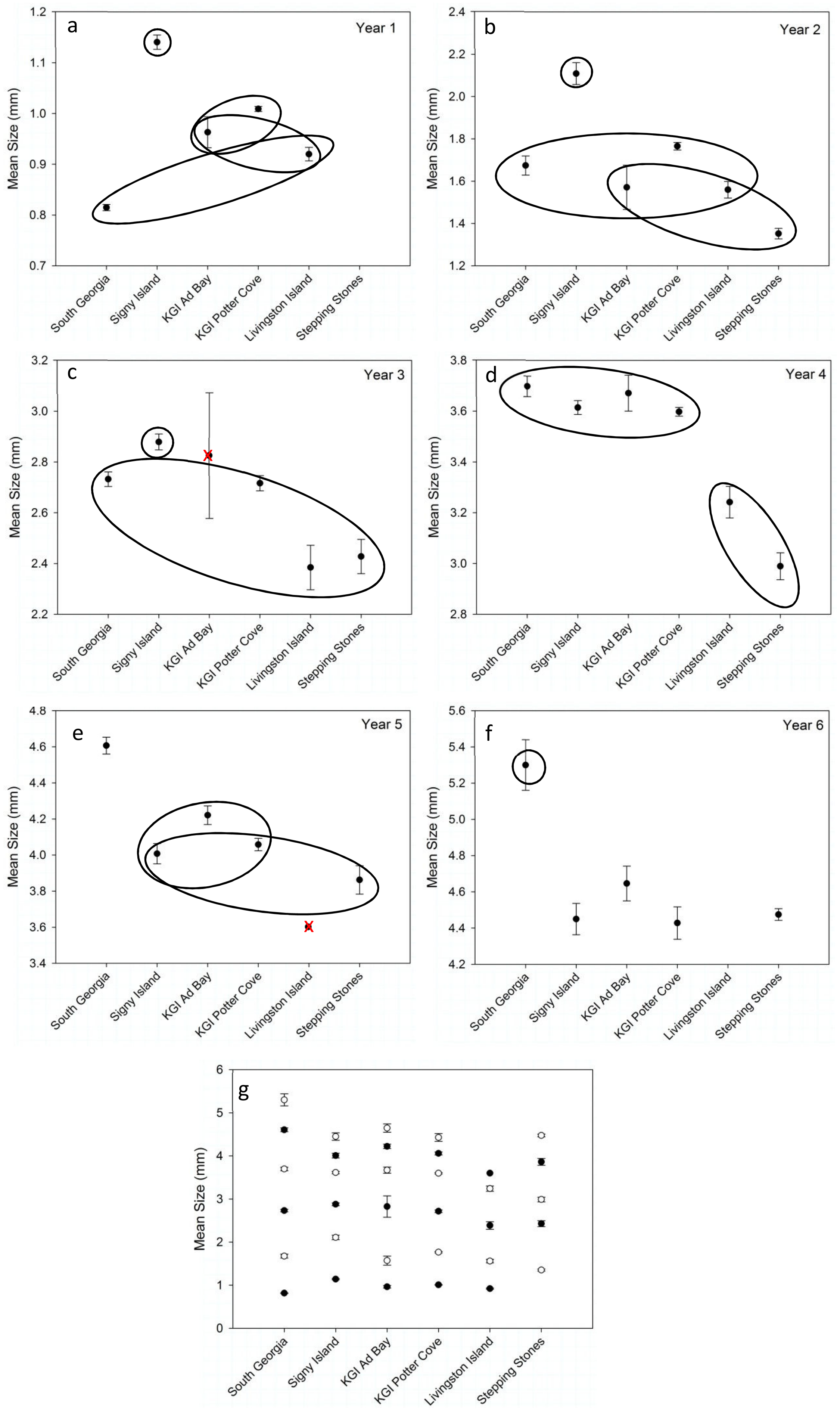
a
Year 1
1.2
1.1
1.0
0.9
0.8
0.7
b
Year 2
2.4
2.2
2.0
1.8
1.6
1.4
1.2
c
Year 3
3.2
3.0
2.8
2.6
2.4
2.2
d
Year 4
3.8
3.6
3.4
3.2
3.0
2.8
e
Year 5
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
f
Year 6
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
g
6
5
4
3
2
1
0
Mean Size (mm)
South Georgia
Signy Island
KGI Ad Bay
KGI Potter Cove
Livingston Island
Stepping Stones

Figure 4.5 ← Mean size-at-age for each growth increment a) year 1; b) year 2; c) year 3; d) year 4; e) year 5; f) year 6; g) all growth years 1-6 plotted together. Circles represent significant differences (Multiple comparison test, Dunn's method, $p<0.05$). Red cross marks populations not tested due to low sample 'n' value. No statistics done on year 6 due to low 'n' value. KGI = King George Island; Ad bay = Admiralty Bay. n.b. no year one value at Stepping Stones and no year six value at Livingston Island.

Signy Island has the largest shell size at the 1st, 2nd and 3rd growth increments (Figure 4.5a-c), but growth rapidly slows after the 4th increment (Figure 4.5d) and the shell sizes in the smallest statistical group by the 5th increment (Figure 4.5e). Both King George Island populations have a similar pattern of growth (Figure 4.5g) and are statistically indistinct at all growth increments tested (Figure 4.5a-f). Livingston Island specimens only have 5 observed growth increments and are in the smallest growth increment statistical group in all year classes with the Stepping Stones population which are only larger at the 3rd (Figure 4.5c) and 5th increment (Figure 4.5e). Statistics were not done on the 6th (Figure 4.5f) and 7th growth increments due to low 'n' values.

Prodissoconch Size

There is a significant difference among mean prodissoconch length (Pl) at South Georgia, Signy Island, King George Island's Admiralty Bay, and Stepping Stones (ANCOVA $F_{3,\,180}$=5.6, $p<0.001$). A multiple comparison test (Tukey test) identifys South Georgia and Stepping Stones to be statistically grouped with smaller Pl lengths of 687.72µm ±1.57SE and 680.52 µm ±6.71SE respectively (Figure 4.6a, Table 4.1). Signy Island and King George Island were also statistically grouped together with larger Pl lengths of 699.21µm ±4.96SE and 701.13µm ± 5.41SE respectively.

Measurements of prodissoconch height identified a significant difference (ANCOVA $F_{3,\,180}$=57.84, $p<0.001$) and three statistically distinct groups (Tukey test at 95% similarity) (Figure 4.6b). South Georgia and King George Island Admiralty Bay had the largest Pl heights of 478.73µm ±1.53SE and 472.46µm ± 4.45SE respectively. Signy Island had a significantly smaller Pl height of 458.04µm ± 3.79SE and Stepping Stones had the smallest significantly distinct Pl height of 402.26µm ±7.86SE.

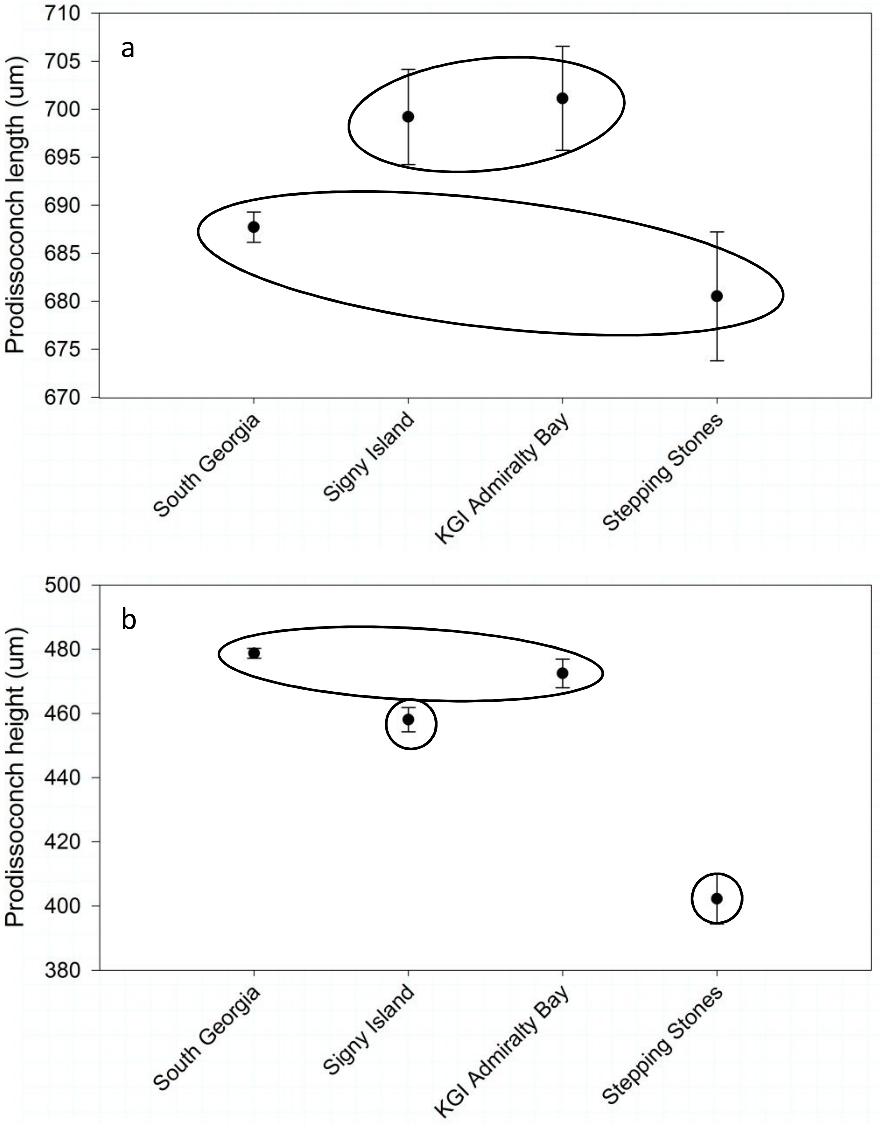

Figure 4.6 Mean prodissoconch sizes for *Lissarca miliaris* at South Georgia, Signy Island, King George Island Admiralty Bay and Stepping Stones. a) mean prodissoconch length (µm); b) mean prodissoconch height (µm). Circles represent significant differences ($p<0.05$). KGI = King George Island.

The ratio of prodissoconch length and height were significantly different (ANCOVA $F_{3, 180}$=64.99, $p<0.001$) and a Tukey test 95% similarity) identified South Georgia and Stepping Stones to be distinct with ratios of 1.44 ± 0.0046 SE and 1.70 ± 0.0240 SE respectively. Signy Island and King George Island Admiralty Bay, were grouped together with ratios of 1.53 ± 0.013 SE and 1.49 ± 0.016 SE (Figure 4.7)

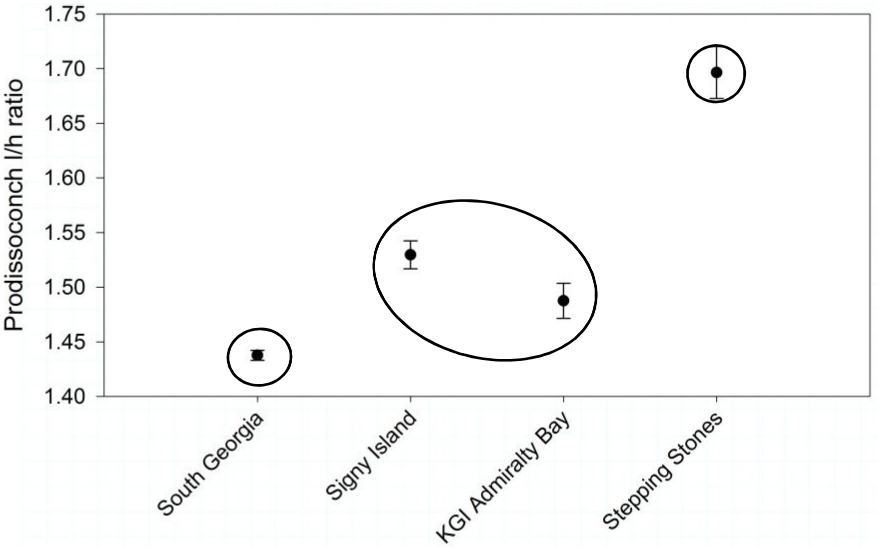

Figure 4.7 Ratio of prodissoconch length and height at South Georgia, Signy Island, King George Island Admiralty Bay and Stepping Stones. Significant differences are shown by circles ($p<0.05$).

Egg Sizes

Size of brooded oocytes of the same stage measured at King George Island Potter Cove and South Georgia were significantly different in size and number. An ANCOVA found a significant difference between King George Island and South Georgia ($F_{1,\ 82}=75.64$, $p<0.001$) with corrected mean Feret diameters of 355.3µm ± 3.64 SE and 263.0µm ± 9.98 SE respectively (Figure 4.7a). The number of eggs brooded were correlated with shell size (King George Island $r^2=0.390$, $p<0.001$, $n=150$; South Georgia $r^2=0.215$, $p<0.05$, $n=16$) (Figure 4.7b) and were significantly different between populations. King George Island had significantly lower numbers per cohort than South Georgia (ANCOVA $F_{1,\ 163}=74.85$, $p<0.001$) with corrected mean brood sizes of 32.95 ± 0.97 SE and 60.00 ± 2.97 SE, respectively (Figure 4.7c).

Figure 4.7 → Mean egg size and number of eggs per brood in *Lissarca miliaris* from King George Island Potter Cove and South Georgia. a) Mean egg size (Feret Diameter µm) and Standard error for Potter Cove (filled circle) and South Georgia (open circle). Solid line represents corrected mean egg size for Potter Cove, Dashed line corrected mean egg size for South Georgia; b) Log egg number against length for South Georgia (open circle) and Potter Cove (filled circle); c) Number of eggs per brood against shell length at Potter cove (filled circle) and South Georgia (open circle). Solid line represents corrected mean egg number for Potter Cove. Dashed line represents corrected mean egg number for South Georgia. Egg Counts - Potter Cove, King George Island sample n=150; South Georgia sample n=16. Egg Sizes – 845 eggs from 75 adults from Potter Cove, King George Island; 125 eggs from 11 adults South Georgia.

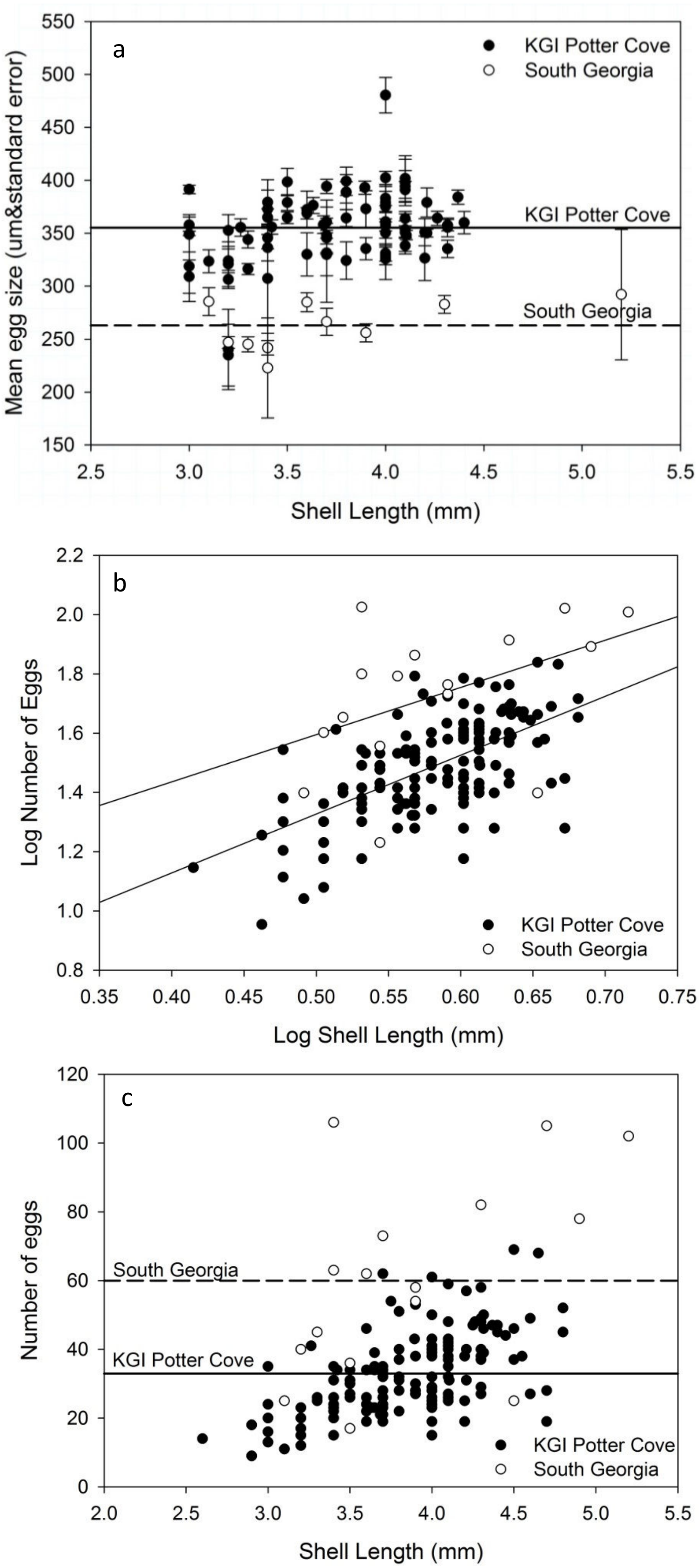
a
KGI Potter Cove
South Georgia
KGI Potter Cove
South Georgia
Mean egg size (um&standard error)
550
500
450
400
350
300
250
200
150
2.5
3.0
3.5
4.0
4.5
5.0
5.5
Shell Length (mm)
b
Log Number of Eggs
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.35
0.40
0.45
0.50
0.55
0.60
0.65
0.70
0.75
Log Shell Length (mm)
KGI Potter Cove
South Georgia
c
Number of eggs
120
100
80
60
40
20
0
South Georgia
KGI Potter Cove
KGI Potter Cove
South Georgia
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
Shell Length (mm)

Table 1	Morphology				Growth				Prodissoconch Size		
	Length/Height		Length/Width		vBGF parameters				(µm)		
Site	r^2	n	r^2	n	S_∞	K	OGP	n	L	H	L/H
South Georgia	0.99*	626	0.95*	626	6.90	0.402	0.443	558	687.7	478.7	1.438
Signy Island	0.99*	460	0.97*	458	5.10	0.408	0.318	458	699.2	458.0	1.530
KGI Admiralty Bay	0.98*	117	0.97*	111	5.50	0.497	0.437	117	701.1	472.5	1.488
KGI Potter Cove	0.98*	2271	0.93*	1592	5.41	0.469	0.404	1444	-	-	-
Livingston Island	0.99*	58	0.98*	58	6.21	0.357	0.346	58	-	-	-
Stepping Stones	0.99*	133	0.96*	133	6.03	0.375	0.373	133	680.5	402.3	1.697

Table 4.1 Morphology, growth and prodissoconch size parameters from linear regression, von Bertalanffy Growth Function and prodissoconch corrected means. ‘*’ denotes statistical significance of linear regression $p<0.001$. KGI = King George Island.

4.4 Discussion

Despite the cold stenothermal conditions of the Southern Ocean, *Lissarca miliaris* demonstrate phenotypic and reproductive plasticity between populations of close geographic proximity. This result finds plasticity on local scales which may have been hidden in previous studies which pooled local populations together, often when comparing plastic traits across broader regions. In reality, subtle environmental differences become important at the local scale by selecting for traits with species specific ecological benefits. Evidence for reproductive trade-offs associated with plastic traits in the Southern Ocean may also be a driver for ecological divergence in *L. miliaris* with the potential for speciation through ecologically driven reproductive isolation.

Although plasticity may constrain evolution by buffering the need for genetic change, plasticity itself is subject to natural selection processes (West-Eberhart 1989). Phenotypes may select for particular habitats, or mate selection, with the potential reduction in fitness of migrants (Via et al. 1999, 2000; Nosil et al. 2005). Over time such differences in phenotype may become genetically controlled and speciation processes occur, even in the face of moderate levels of gene flow (Knowlton 1993; Schluter 2009). The ability to be plastic, both physiologically and phenotypically, could be significant in understanding the radiation of *L. miliaris* in the Antarctic. The genus *Lissarca* dates back in the fossil records to the Miocene (Tevesz 1977), and the last significant cooling step ~17 Ma; (Lawver et al. 1992). Unlike many Antarctic species, its range extends to the South American continent and it is prevalent in the Magellenic Region. Their possible persistence in the Antarctic through this cooling step, and subsequent glacial-interglacial periods, will have required resilience to changing conditions, especially in the absence of extended eurybathy observed in other benthic invertebrates (Brey et al. 1996). An alternative radiation in the Antarctic since the onset of cooling from South American populations could also have occurred, with ecophysiological and phenotypic responses to the available niches allowing radiation within and between shallow bays.

Morphological variants

Morphological variations in molluscs are often attributed to the physical environment in which they are exposed, and sheltered and exposed shores have classically been used to explain differences in shell shapes, sizes and characteristics (Seed 1968; De Wolf et al. 1998; Akester and Martel 2000; Steffani and Branch 2003). Each population of *L. miliaris* studied lives in a different environment and was collected at contrasting heights of the shore. Most notably, specimens collected from South Georgia, Signy Island, and Livingston Island were all collected intertidally, while those collected at both sites in King George Island and Stepping Stones were collected sub-tidally at around 10m water depth. However, this difference alone cannot explain all of the morphological differences among populations. The two King George Island populations, geographically closest together and collected by SCUBA divers at the same water depth, are significantly different by all morphological measures and similar only by growth rate. Meanwhile, significant differences exist in the morphology of adult shell, larval shell and growth between intertidal populations at Signy Island, South Georgia.

Exposure to wave action also appears to have little effect on shell morphology in *L. miliaris*; specimens from Livingston Island were collected at the exposed rocky Byers Peninsula and yet show no significant differences in morphology to the Signy Island population collected on the sheltered intertidal mud flats at Shallow Bay. Both of these populations show an obese ball shaped morphology, often associated with wave exposed areas (Seed 1968; Akester and Martel 2000), in comparison to the elongate populations of King George Island, South Georgia and Stepping Stones. This is in contrast to the intertidal limpet *Nacella concinna* which has genetically identical but morphological distinct morphotypes in the inter- and sub-tidal populations at Rothera associated with predation pressures, desiccation, and disturbance (Powell 1973; Nolan 1991; Beaumont and Wei 1991). Ice scour can also have a physical effect on the morphology of bivalve shells. Harper et al. (2012) found phenotypic plasticity in the infaunal *Laternula elliptica* related to geographic variations in ice scour disturbance between Hangar Cove at Rothera, and McMurdo Sound, with no genetic differences between populations identified. Although ice cover and disturbance varies between all populations of *L. miliaris* studied, their small size and epifaunal settlement reduce the impacts of ice scour, and populations are more likely to be dislodged rather than crushed or damaged by ice. Differences in population density can also force shell morphology variations by overcrowding (Seed 1968). *Lissarca miliaris* usually live in dense clumps (Dell 1964; Richardson 1979; personal observation); population density is known to have been high at Signy Island, King George Island, Livingston Island and Stepping Stones and given the high recruitment of Antarctic brooding bivalves to the immediate environment (Richardson 1979; Brey and Hain 1992; Brey et al. 1993; Higgs et al. 2009), this factor is assumed to be constant among all populations studied.

Although predation of bivalves in the Antarctic is documented, to date the effects of predation are poorly known. Predatory shell boring gastropods such as the *Trophon longstaffi* and *Neobuccinum eatoni* and the asteroids *Odantaster validus* and *Cryptasterias turqueti* (Dayton et al. 1974; Stockton 1984; Zamorano et al. 1986; Harper and Peck 2003) are known consumers of larger infaunal bivalve species *Laternula elliptica, Yoldia eightsii* and *Adamussium colbecki*. Of the *L. miliaris* collected in this study, few empty shells were observed and no evidence of shell boring occurred at any site.

Growth

The growth rate and adult sizes of *L. miliaris* demonstrate a decreasing trend with latitude, contrasting with previous growth work on bryozoan and limpet growth in the Antarctic (Barnes and Arnold 2001; Clarke et al. 2004a) and the early concepts of larger body sizes at low temperatures in the Antarctic (Chapelle and Peck 2004). Brey and Hain (1992) found no significant differences in growth rates of *L. notorcadensis* (from residuals of vBGF) but shells were distinctly larger from lower latitudes. Growth and shell development is commonly dependent on food availability and temperature (Appeldoorn 1982; Brey et al. 2011; Harper et al. 2012; Reed et al. 2012) and the ability to extract calcium from sea water at low temperature (Harper 2000), but species sensitivity to these factors is complex.

Food availability in the Antarctic is often limiting with respect to short durations of highly seasonal primary productivity (Clarke et al. 1988; Clarke and Leaky 1996) and has a close relationship to sea ice and its associated inter-annual variability (Whitaker 1982; Lizotte 2001; see Peck et al. 2007). The length and concentrations of blooms among the studied populations are variable (Table 4.2), and related to the heterogeneity of hydrographic conditions which prevail in the Southern Ocean. The exposed shores of Livingston Island are in contrast to the sheltered Potter Cove at King George Island which although deep is protected by a 30m sill at the entrance to the inner cove (Klöser et al. 1994; Schloss et al. 2002). Admiralty Bay meanwhile is a large embayment with a maximum depth of 500m and a high freshwater input from glacial melt (Rajusa-Suszczewski 1980).

Peak chlorophyll-*a* ranges from 50.9mg m^{-3} at Signy Island (Clarke et al. 1988) to peaks of just 1.7 mg m^{-3} at King George Island Admiralty Bay (Lange et al. 2007) and 7 mg m^{-3} at Cumberland Bay, South Georgia (Whitehouse et al. 1996). No data are available for Byers Peninsula at Livingston Island but given the exposed open water to the west it is likely that surface chlorophyll-*a* concentrations would not exceed the local Scotia Sea standing stock, which is usually between 0.5-1.0 mg m^{-3}, with possible peaks up to 14.6 found at the Antarctic Peninsula and central Scotia Sea (Holm-Hansen et al. 2004). The populations studied at King George Island are contrasting in primary production with peaks of up to 25mg m^{-3} at Potter Cove (Tatian et al. 2002; Schloss et al. 2002) but only up to 1.7 mg m^{-3} within Admiralty Bay (Brandini 1993; Lange et al. 2007), while the population at Stepping Stones near Palmer station may receive peaks between 20.9 – 35 mg m^{-3} from nearby waters (Holm-Hansen et al. 1989; Baker et al. 1996) although this has high inter-annual variability (Baker et al. 1997).

Temperature ranges vary also among populations (Table 4.2); the most northern population of South Georgia being the warmest with water temperatures reaching 4.4°C (Whitehouse et al. 1996). Signy Island is comparatively cold with water temperatures from freezing (-1.8°C) to only 0.3°C (Clarke et al. 1988) while King George Island populations have water temperatures from freezing (-1.8°C) to 1.76 and 1°C in Admiralty Bay and Potter Cove, respectively (Rakusa-Suszczewski 1980; Klöser et al. 1994). At the highest latitude studied, surrounding waters of Stepping Stones have surface water temperatures between -1.8°C and 1.5°C, but in the upper range for fewer months (Baker et al. 1997; see Barnes et al. 1996).

The high growth rates, overall growth performance, and larger adult sizes of *L. miliaris* at South Georgia are likely to be in response to the higher water temperatures and longer period of phytoplankton availability within Cumberland Bay. Both King George Island populations have similar growth and overall growth performance despite contrasting peaks chlorophyll levels. This suggests that low temperature may be limiting the growth of adult *L. miliaris* at King George Island over food availability. Both of these ecosystems are particularly impacted by high levels of seasonal melt water from retreating glaciers, creating high sedimentation rates and fluctuating salinities (Klöser et al. 1994; Lange et al. 2007).

Population	**Temperature Range (°C)**	**Chlorophyll Peak (mg m^{-3})**	**Chlorophyll Peak duration**	**Reference**
South Georgia	-0.95 to 4.4	4 to 7	5 months	Whitehouse et al. 1996
Signy Island	-1.8 to 0.3	50.9	3 months	Clarke et al. 1988
King George Island Admiralty Bay	-1.8 to 1.76	0.1 to 1.7	4 months	Rakusa-Suszczewski 1980 Lange et al. 2007
King George Island Potter Cove	-1.0 to 1.0 (Summer)	25	4 months	Tatian et al. 2002; Schloss et al. 2002 Klöser et al. 1994
Livingston Island	?	?	?	
Stepping Stones	-1.8 to 1.5	15 to 35	?	Holm-Hansen et al. 1989 Baker et al. 1996

Table 4.2 Environmental parameters for South Georgia, Signy Island, King George Island, Livingston Island, and Stepping Stones derived from published literature (references within).

Signy Island has smaller adult sizes despite larger immature sizes and the lowest overall growth performance. The low water temperatures of Signy Island and extreme variance in air temperature when exposed at low tide (Walker 1972; Reed et al. 2012) could be retarding growth. Populations at Livingston Island and Stepping Stones with the smallest sizes are perhaps more restricted by food availability, lower average temperatures and greater impacts of ice cover. Given the intertidal ecology of *L. miliaris*, short term acute thermal tolerance might be of importance to survival, but care should be taken in comparing inter- and sub tidal populations as physiological responses to emersion may differ (Weihe and Abele 2008).

Prodissoconch development in *L. miliaris* occurs within the maternal brood chamber and is largely protected from the physical disturbances of predators, ice and wave action. Temperature may be the driver of morphological differences in larval shells with the most contrasting morphologies South Georgia and Stepping Stones being most contrasting in maximum temperature range.

Reproduction

Few studies have investigated reproductive plasticity throughout a species range, although inter-annual variability within single populations have been reported in Antarctic brittlestars (Grange et al. 2004) and bivalves (Ahn et al. 2003; Tyler et al. 2003). Reproductive output and plasticity in *L. miliaris* can be discussed in relation to growth rate and the energetic trade-off between large adult size and large eggs/juveniles. Linse et al. (2006a) demonstrated differences in body size in two gastropod and one echinoid species in the Southern Ocean across different populations, and a significant effect of body size on reproduction for the gastropod *Margarella antarctica*. Morphology can have a significant impact on the reproductive output in brooding as typically brood size is limited by the females' capacity to carry young.

The difference in egg size observed between South Georgia and King George Island Potter Cove may also be the effect of local temperature and food availability, but also shows a strong relationship with adult shell obesity. The significantly larger number of eggs per brood may be associated with the faster growth and larger sizes of *L. miliaris* from South Georgia but the trade-off is a reduction in the mean egg size. Kraeuter et al. (1982) found that small differences in the egg size of the bivalve *Mercenaria mercenaria* and *Argopectin irradians* are important for survival and smaller size may reflect a lower energy store. Several studies have identified differences in egg size and quality between populations of invertebrates attributed to food availability in echinoderms (George 1994, 1996; Bertram and Strathmann 1998) and bivalves (Barber and Blake 1983; Phillips 2007). The small egg sizes at South Georgia relate to the small prodissoconch sizes measured and the smaller size-at-age during the first three growth increments when compared to King George Island's Potter Cove. It is the morphological differences in adult shell that are striking however, as both populations show the same length/height relationship yet are significantly different in shell obesity with the South Georgia populations being thinner than the rounded Potter Cove specimens (Figure 4.3b).

Measurements of prodissoconch maximum length at four of the populations could be measured and reveal an inverse 'U' shape trend with increasing latitude. Secreted by the larval form, prodissoconch I (PI) sizes are usually directly linked to egg size and differences in egg size as small as 10µm can be reflected in significant differences in PI length (Goodsell and Eversole 1992). The largest prodissoconch sizes, statistically grouped together, were measured at Signy Island and King George Island Admiralty Bay, suggesting largest egg and juvenile size. Parental shell morphology is however most morphologically dissimilar with Signy Island having a rounded shape to Admiralty Bays more elongate shape (Figure 4.3a). The two populations are in the same size at age group from the minimum age of observed brooding (3 growth increments, Figure 6c-e), which may suggest an effect of shell length on egg size. Prodissoconch lengths at South Georgia and Stepping Stones are the shortest with Stepping Stones having the smallest overall morphology (Figure 4.7). Both populations have shells of similar morphology, being more flattened and oval in shape and potentially less capacity to brood (Figure 4.3a).

Increased volumes from differing morphologies in *L. miliaris* are cannot be conclusive from prodissoconch measurements alone; however the smallest egg sizes of South Georgia and small prodissoconch sizes of Stepping Stones do correlate restricted internal capacity. The larger prodissoconch sizes at King George Island Admiralty Bay, despite limiting morphology, may be related to an unmeasured trade-off with brood size. Evolutionary implications of plasticity in reproductive output are potentially significant but would require further analysis of energetic contents of eggs to interpret. Investment of energy into eggs can affect larval fitness of marine invertebrates with consequences for future adult fitness (Vance 1973; Emlet and Hoegh-Guldberg 1997; Oliphant et al. *under review*), although small egg size may not correlate with energy composition (Byrne et al. 2008; McAlister and Moran 2012). Smaller offspring sizes inferred from prodissoconch sizes at South Georgia and Stepping Stones would suggest a lower investment of energy per offspring with reduction in larval fitness, ability to disperse long distances, and over winter mortality where food and temperature are particularly limited. Such ecological divergence and selection may act against these individuals even with significant dispersal by rafting, and lead to reproductive isolation eventually influencing speciation processes.

Conclusions

Lissarca miliaris demonstrate a wide range of plastic traits over its distribution south of the Polar Front including statistically significant differences in growth, morphology, and reproduction but there are trade-offs between morphology (potential capacity to brood), growth, egg size, and fecundity. These plastic traits are occurring despite only subtle environmental differences, and highlight the importance of local scale variations in ecology when comparing populations. Temperature and food availability are the most likely drivers of plasticity in *L. miliaris,* and the wide geographic range and similarities in morphology among certain populations reveal the adaptive capabilities within a cold stenothermal environment. Despite the suggestion of latitudinal trends in Antarctic mollusc morphology and ecophysiology (Brey and Hain 1992; Linse et al. 2006a; Barnes and Arnold 2001), no trend other than an inverse 'U' shaped relationship with prodissoconch size exist with increasing latitude in *L. miliaris.* The intertidal ecology of *L. miliaris* may indicate a wider tolerance to extremes in temperature and food supply which have helped this species to survive a radiation north and south of the Polar Front. Distinct morphologies and ecological responses to subtle environmental variations also suggest a possibility for resilience to current challenges, particularly the rapid warming of the Antarctic Peninsula which may rival climatic events since the origin of *Lissarca* in the Southern Ocean (Vaughan et al. 2003; Turner et al. 2005; Clarke et al. 2009).

4.5 Chapter Summary

Lissarca miliaris demonstrate phenotypic and ecophysiological plasticity in relation to subtle variations in the environmental conditions. Such traits are likely to have been important in the resilience of this species in the Southern Ocean and suggest adaptability to new environments. The study also shows the importance in differentiating between populations in other ecological studies regardless to how geographically close each population may be. Key findings demonstrate;

- Subtle differences in morphology among populations irrespective of geographical proximity or latitude.
- Differences in growth rate among populations attributed to subtle differences in food availability and temperature among populations.
- A reproductive trade-off at the extreme ranges of latitude associated with morphology and growth rates.

Chapter 5

Temporal plasticity in morphology, growth and reproduction in *Lissarca miliaris* (Philobryidae) from Signy Island, Antarctica

Study published as: Reed AJ, Thatje S, Linse, K (2012) Shifting baselines in Antarctic ecosystems; ecophysiological response to warming in *Lissarca miliaris* at Signy Island, Antarctica. PLoS ONE. 7, e53477

Abstract

The Antarctic Peninsula has experienced a rapid increase in atmospheric temperature over the last 50 years. Whether or not marine organisms thriving in this cold stenothermal environment are able to cope with warming is of concern. Here, changes to the growth and shell characteristics of the ecologically important, small and short lived brooding bivalve *Lissarca miliaris* from Signy Island, Antarctica are presented. Using material collected from the 1970s to the present day, this study shows an increase in growth rate and adult shell deterioration accompanied by a decrease in offspring size, associated with an increase in annual average temperatures. A change in shell morphology is also identified, potentially driven by increased growth rates and further affecting reproduction by reducing the females' capacity to brood. Critical changes to the bivalve's ecology seen today evidence the problem of a shift in baseline since the onset of warming recorded in Antarctica. *Lissarca miliaris* are demonstrating ecophysiological responses to subtle warming that, provided warming continues, could soon surpass a physiological tipping point, adding to warming associated threats such as increased predatory pressure and ocean acidification. This study highlights the importance of historic data in polar areas, the striking response of shelled invertebrates to subtle changes in temperature, and the risk of shifting baselines affecting our perception of the 'pristine' Antarctic ecosystem.

5.1 Introduction

The rate of atmospheric warming at the Antarctic Peninsula has been around 0.56˚C decade^{-1} since 1950 (Turner et al. 2005), higher than the global average and most extreme in winter with an increase of 5-6˚C over the past 50 years (Vaughan et al. 2003). Observed glacial retreat (Cook et al. 2005), reduced sea ice formation (Stammerjohn et al. 2008) and a regional increase of 1˚C in the upper ocean layer in summer (Meredith and King 2005), have all been attributed to this temperature increase. Ecosystem response can be difficult to identify but to date include changes in plankton biomass (Schofield et al. 2010), penguin distribution, and krill abundances (Fraser and Hoffman 2003). Investigating physiological responses to thermal stress may also be important in understanding ecosystem changes occurring at population levels (Pörtner 2001; Pörtner 2010).

A problem facing ecologists when studying ecosystem change is finding a baseline in which to measure change against. The term 'shifting baselines' was first used to describe fishery scientists who failed to use historic data to evaluate the status of the ecosystem, instead using the ecosystem status at the start of their career as the baseline for change (Pauly 1995; Pinneger and Engelhard 2008). This concept has since been discussed to include a wide range of ecosystems that are only studied whilst in decline; in rare cases no historic data being available to develop a suitable baseline (Jackson et al. 2001). This is certainly the situation for many coral reefs (Knowlton and Jackson 2008), benthic environments (Villnäs and Norkko 2011) and in rocky shore ecology (Roy et al. 2003), where often no adequate baselines are currently attainable.

Long-lived marine bivalves are often used to study faunal response to environmental change as different variables may be recorded by growth increments and shell chemistry. A commonly studied species is the sub-Arctic bivalve *Arctica islandica* that can live in excess of 350 years (Schöne et al. 2005), while in the Antarctic, 40 year old *Laternula elliptica* shells have been modelled to infer details into past growth rate and production (Brey et al. 2011). While providing an invaluable insight into responses to climatic variability, the slow growth rates of long-lived species may hide inter-decadal variation. Interpretation of results can also be difficult in a macro-ecological context when communities are dominated by comparatively small, short lived and faster growing species. Southern Ocean bivalves are typically thin shelled (Nicol 1967) with calcium difficult to extract from seawater at low temperatures (Harper 2000), making them potentially vulnerable to changes in temperature, decreasing $CaCO_3$ saturation (Fabry et al. 2009; McClintock et al. 2009) and predation from invasive durophagous predators in the course of warming (Aronson et al. 2007; 2011).

The philobryid bivalve *Lissarca miliaris* (Philippi, 1845) is a small species (typically <5mm) commonly found in the inter- and sub-tidal regions attached to macro-algae by byssal threads around Signy Island, Antarctica. They are relatively short lived, living up to 7 years, and brood a maximum of 70 young for 18 months (Richardson 1979; Reed et al. 2013). Populations occur along the Antarctic Peninsula, Scotia Sea, and sub-Antarctic, often in dense aggregations. Within Borge Bay, Signy Island, *L. miliaris* are the most dominant species of mollusc both by weight and number living on the abundant macroalgae *Desmarestia anceps* (Richardson 1979). As an ecologically important species with a wide distribution and comparatively short life-span, *L. miliaris* make a good model species for identifying changes in the Antarctic environment.

Aims

Using published data from 1972 (Richardson 1979), specimens collected in 1976, 2002, 2011, and 2012, this study aims to investigate the effects of regional atmospheric temperature increases on the growth rates and shell characteristics of *L. miliaris* from Signy Island, South Orkney Island, Antarctica.

Hypothesis

H_1 *Lissarca miliaris* shows evidence of an ecophysiological response to subtle changes in environmental conditions at Signy Island, from 1972-2012.

Objectives

O_1 Analyse and compare shell morphology for each year *Lissarca miliaris* were collected at Signy Island.

O_2 Analyse and compare shell growth rates for each year *Lissarca miliaris* were collected at Signy Island.

O_2 Analyse and compare larval shell size for each year *Lissarca miliaris* were collected at Signy Island.

5.2 Materials and Methods

Collection

A total of 808 hand-collected intertidal *Lissarca miliaris*, collected near the British Signy Base at Shallow Bay (see Chapter 2.1) were used in this study. This was made up of 226 specimens fixed in formalin but stored in ethanol from 1976, 462 specimens from April 2002 in 96% ethanol, 68 specimens in February and March 2011 fixed in 96% ethanol and 52 specimens in March 2012, 10 dried at 30˚C, 42 fixed in 96% ethanol.

Growth and Morphology

Growth rates were calculated using a general von Bertalanffy growth function from size-at-age data as described in Chapter 2.4 with overall growth performance calculated from the given parameters. Morphology was analysed as described in Chapter 2.2.

Prodissoconch sizes

Prodissoconch sizes were measured by image analysis of micrographs taken with camera mounted stereo-microscope as described in Chapter 2.4. Only ‘0 year’ and ‘1 year’ specimens with an undamaged prodissoconch were used for this analysis. 2011 and 2012 data were pooled as sampling of these later specimens included low numbers of ‘0 year’ animals. A total of 84 measurements were made (1976 n = 26, 2002 n = 47, 2011/2012 n = 11).

Shell analysis

The right valve from three specimens in each collection with 5 growth rings was used for shell analysis. Shells were embedded into Epoxy resin and were cut with a 100 x 0.37mm diamond low speed saw along the longest growing margin from the umbo. Cut blocks were polished with graded diamond-coated sanding cloths to 1µm and carbon coated before Scanning Electron Microscopy (SEM) analysis. Full methods described in Chapter 2.7.

5.3 Results

Using monthly temperature date from the Argentinean Orcadas research station on Laurie Island in South Orkney Islands, there is an increase in mean annual air temperature since temperature records began and since the collections of *L. miliaris* at Signy Island began in 1972 (r^2=0.256, p<0.001, Figure 5.1a). This is particularly notable in the summer months (December, January, February), where despite high inter-annual variability, there was a significant increase in air temperature (r^2=0.402, p<0.001, Figure 5.1b). The increase in air temperature for our collections can be described by the number of months a year the average temperature was above 0˚C, over the seven-year life span of the specimens collected (Figure 5.2, 5.3).

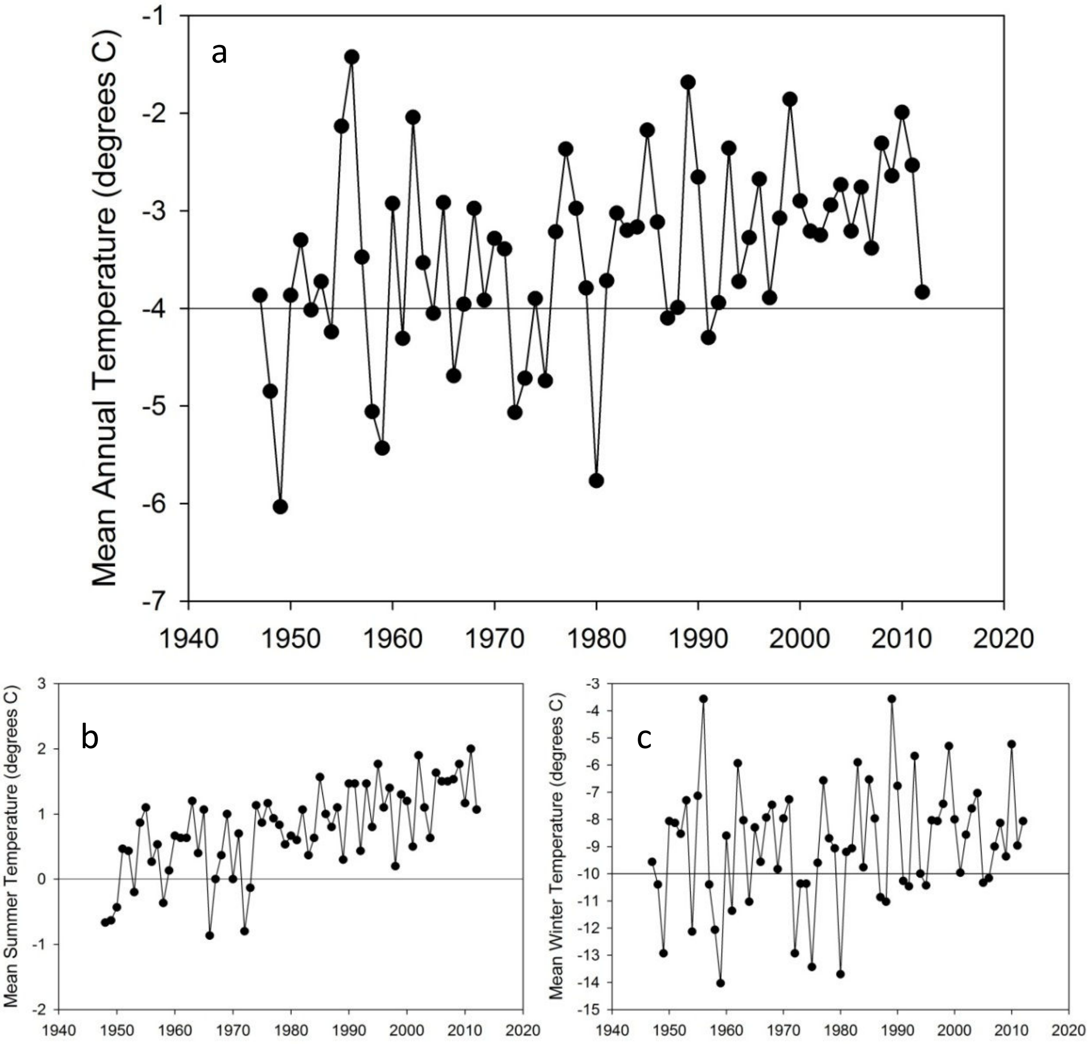

Figure 5.1 Mean air temperatures between 1947 and 2012 at South Orkney Islands. a) mean annual temperature; b) mean summer temperature (December, January and February); c) mean winter temperature (June, July, August). Lines added as point of reference at -4°C, 0°C and -10°C respectively.

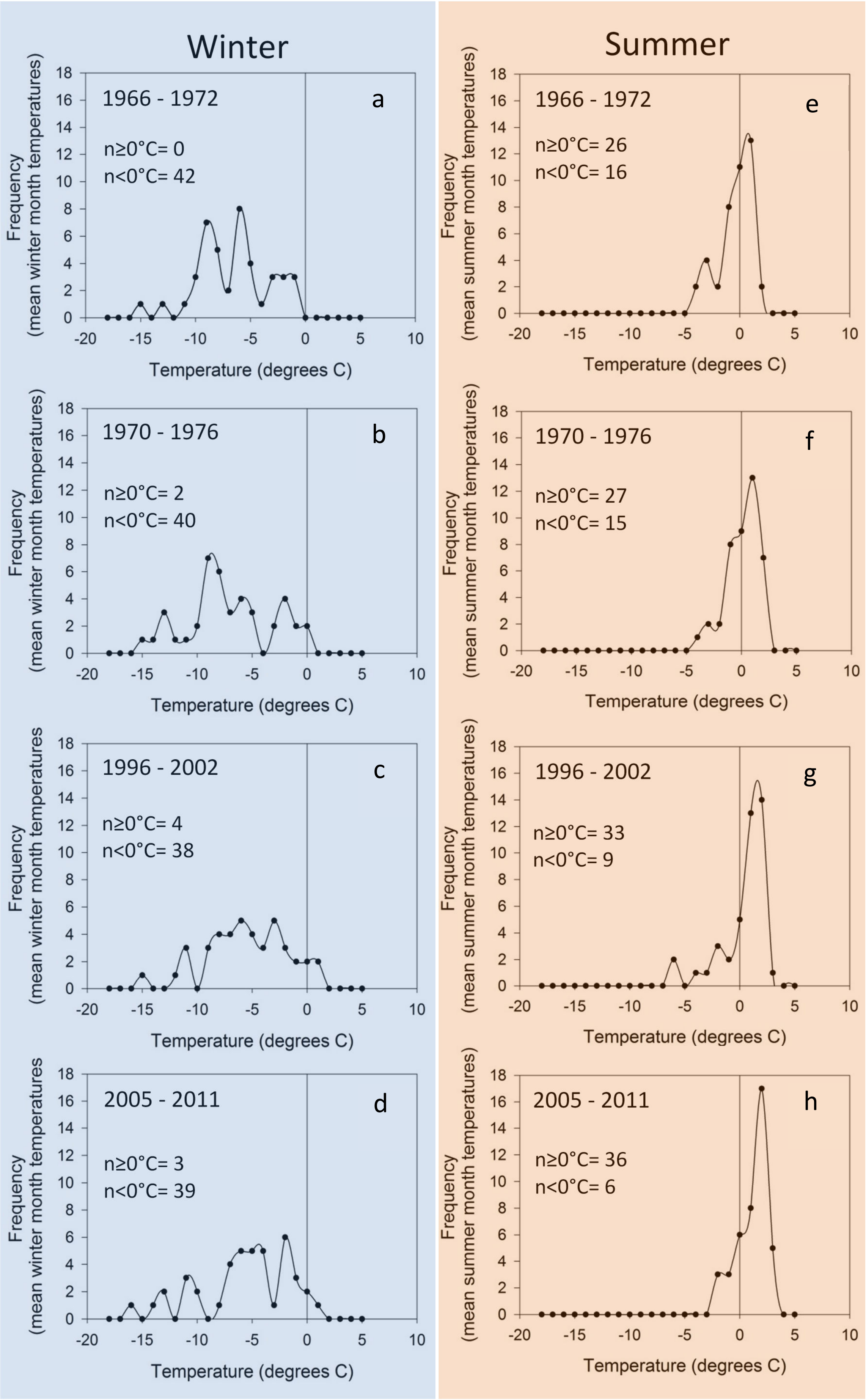
Winter
Summer
Frequency (mean winter month temperatures)
Frequency (mean summer month temperatures)
Temperature (degrees C)
1966 - 1972
a
n≥0°C= 0
n<0°C= 42
1970 - 1976
b
n≥0°C= 2
n<0°C= 40
1996 - 2002
c
n≥0°C= 4
n<0°C= 38
2005 - 2011
d
n≥0°C= 3
n<0°C= 39
1966 - 1972
e
n≥0°C= 26
n<0°C= 16
1970 - 1976
f
n≥0°C= 27
n<0°C= 15
1996 - 2002
g
n≥0°C= 33
n<0°C= 9
2005 - 2011
h
n≥0°C= 36
n<0°C= 6
0
2
4
6
8
10
12
14
16
18
-20
-15
-10
-5
0
5
10

Figure 5.2 ← Frequency of mean monthly temperatures at Laurie Island, South Orkney Islands for the 7 years up to the specimen collection dates in Winter (December, January, February) and Summer (June, July, August), demonstrating the number of months averaging below and above 0°C over the life of an adult *Lissarca miliaris*. Vertical line represents 0°C.

The number of winter months above 0°C between 1966-1972 and 2005-2011 have gone up from 0 to 3 months (Figure 5.2a-d) while in the summer have increased from 26 to 36 months (Figure 5.2e-h). There is a corresponding shift in the peak number of months at a mean winter temperature of -6°C in 1966-1972 to a peak of -2°C in 2005-2011. In the summer months, this shift in peak is from just under 1°C in 1966-1972 to a peak of 2°C in 2005-2011. Mean monthly temperature over the entire year show an increase in months above 0˚C, from 26 months between 1966 and 1972 to 39 months between 2005 and 2011 (Figure 5.3).

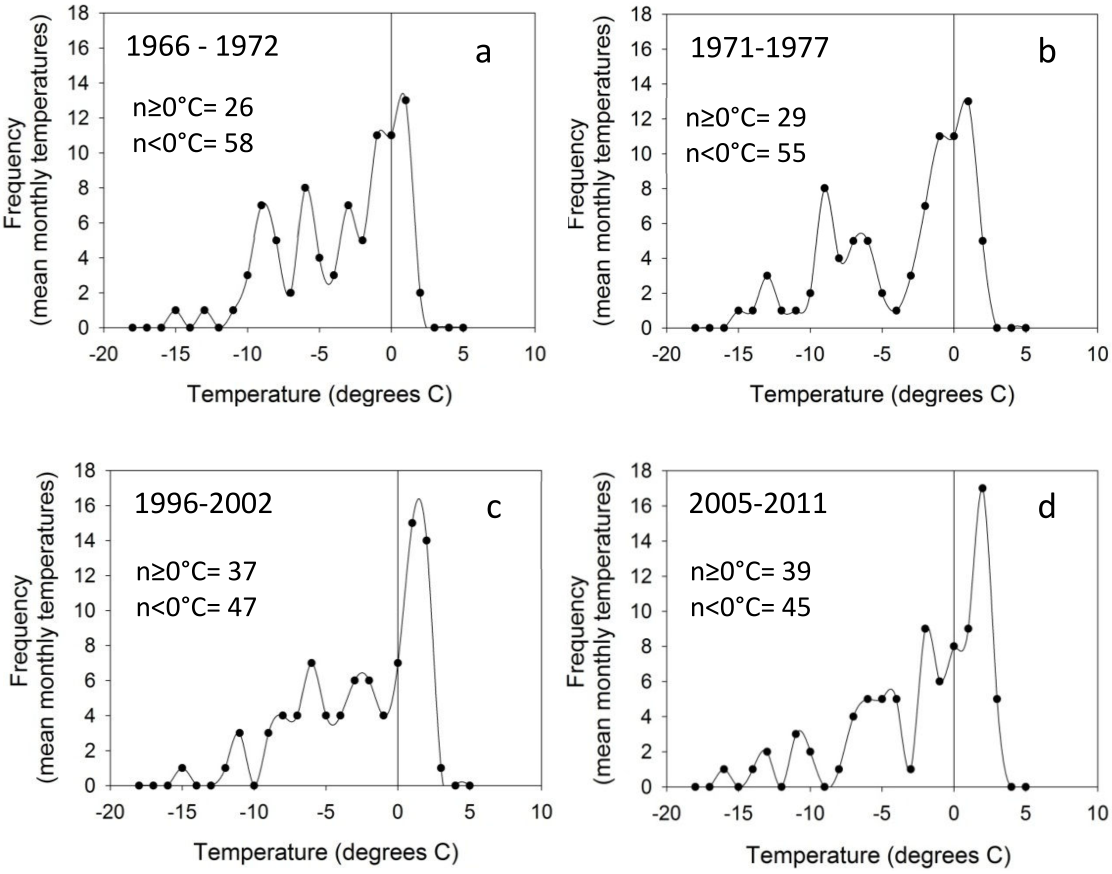

Figure 5.3 Frequency of mean monthly air temperatures at Laurie Island, South Orkney Islands, for the 7 years up the specimen collection dates. Vertical line represents 0°C.

The calculated growth constant *K*, representing 'rate of growth', increases from 0.130 in 1972 to 0.208 in 2002 and 0.290 in 2011 while the asymptotic maximum size (S_∞) decreases from 8.61mm in 1972 to 5.88mm in 2011 (Figure 5.4a, Table 5.1). When plotted on a graph the calculated and observed size-at-age shows a similar pattern between years (Figure 5.4). The overall growth performance (OGP) for each sample shows a five-fold increase from the 1970s samples to 2011 (Figure 5.5, Table 5.1).

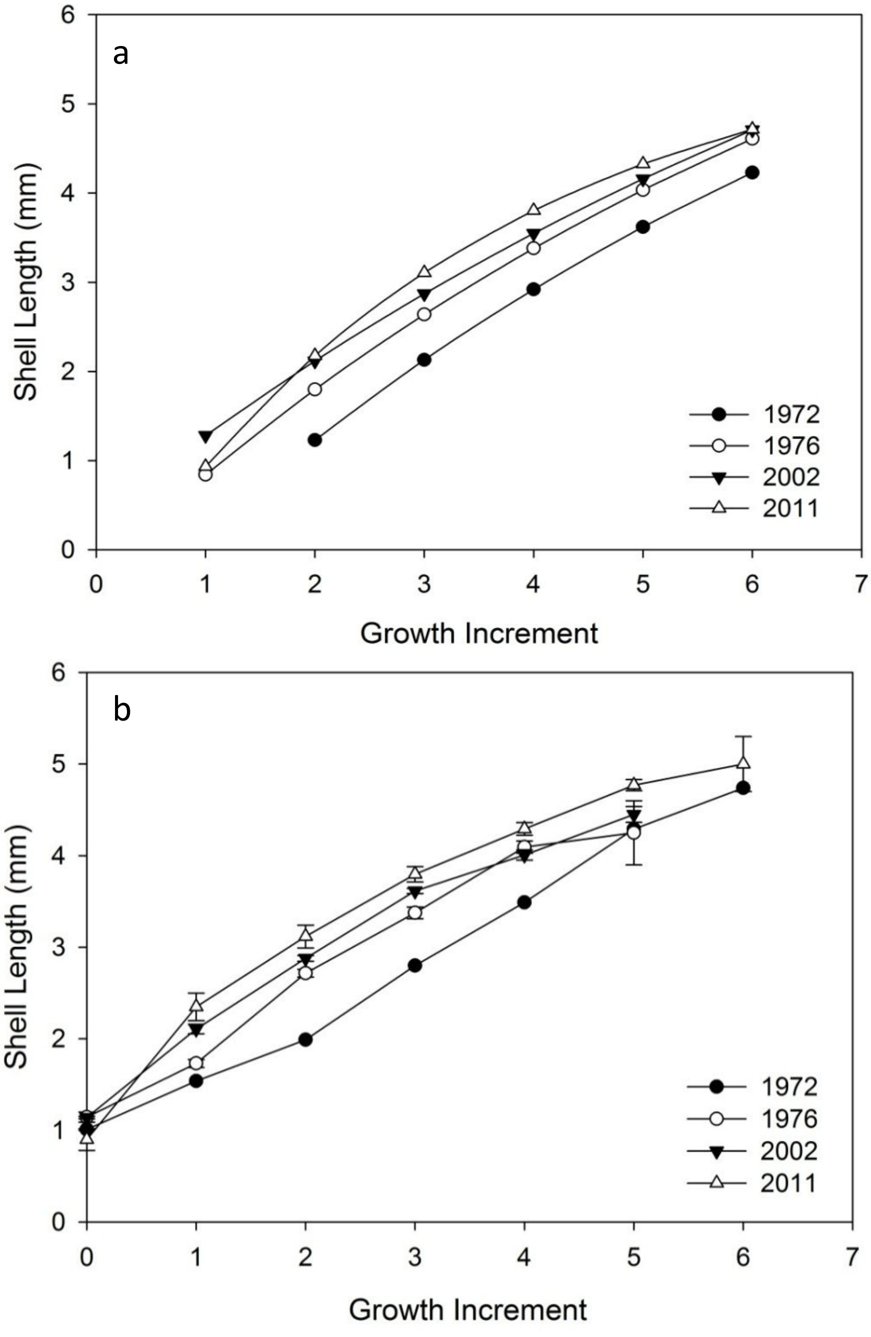

Figure 5.4 Growth rates of *Lissarca miliaris* at Signy Island collected in 1972, 1976, 2002 and 2012. a) special von Bertalanffy growth equation dervived curves; b) observed size-at-age data and standard error. 1972 data taken from Richardson (1979).

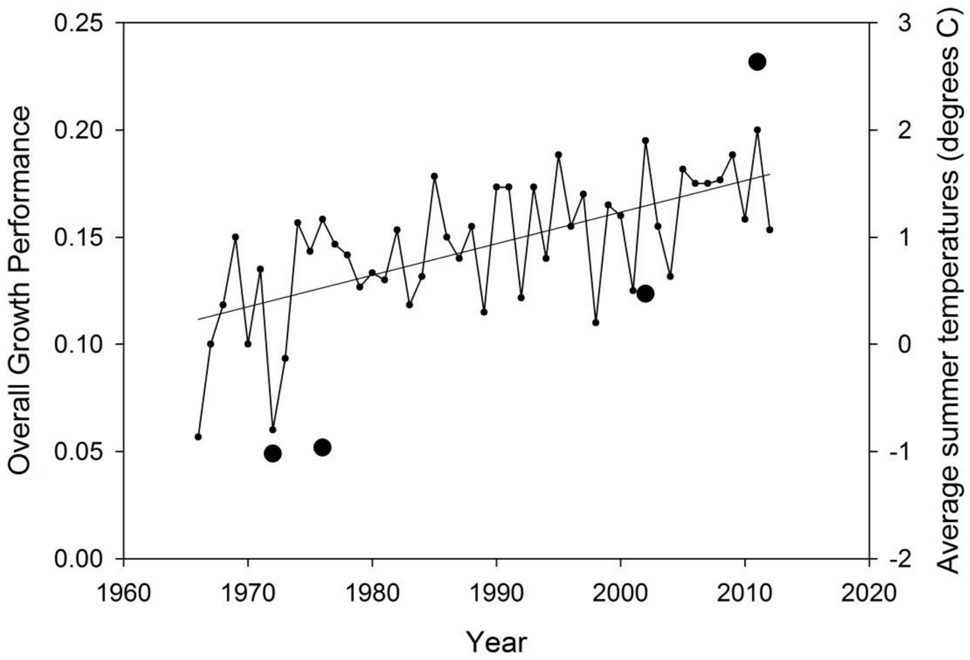

Figure 5.5 Overall growth performance of *Lissarca miliaris* from 1972 to 2011 (large circles) displaying average summer temperature from 1966 to 2012 (small circles).

Year	S_∞	*K*	t_0	OGP	n	ref
1972	8.61	0.130	0.821	0.049	?	Richardson 1979
1976	8.87	0.127	0.212	0.052	236	this study
2002	6.39	0.208	0.065	0.124	439	this study
2011	5.88	0.290	0.403	0.232	66	this study

Table 5.1 Parameters of the von Bertalanffy growth function and calculated overall growth performance (OGP) for *Lissarca miliaris* at Shallow Bay, Signy Island.

Prodissoconch sizes have also changed over time (Figure 5.6, Table 5.2). Maximum prodissoconch lengths (PI) of *L. miliaris* have decreased from 714.5µm ± 5.17 in 1976 to 694.3µm ± 6.46 in 2011 and 2012 (Figure 5.6a). The maximum height however, shows a slight increase from 449.0µm ± 6.31 to 458.9µm ± 3.63 (Figure 5.6b). No P1 data were available for the collection in 1972. The resulting decrease in length/height ratio from 1.598 ± 0.017 to 1.516 ± 0.029, was significant (Kruskal-Wallis H=10.10, df=2, p<0.01) (Figure 5.6c).

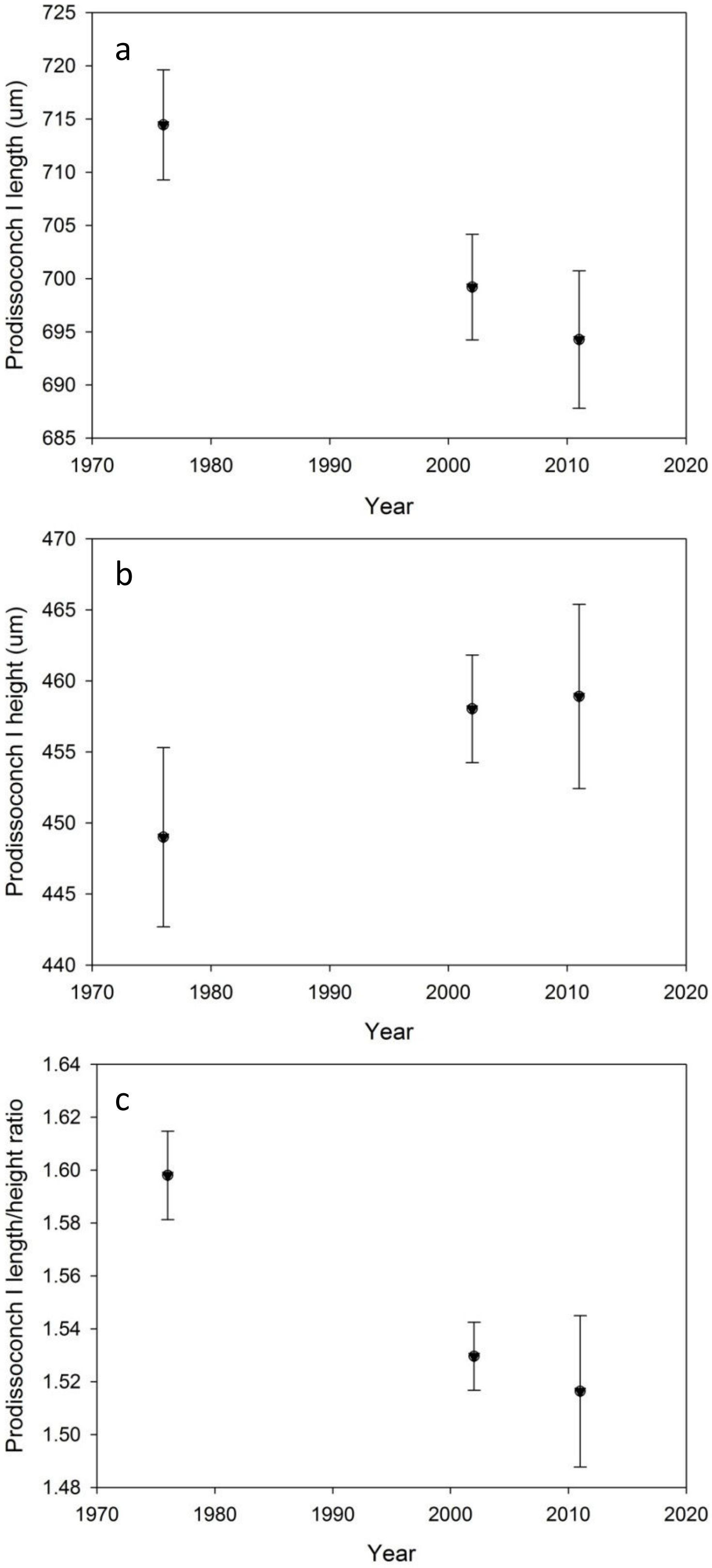

Figure 5.6 Mean prodissoconch measurements for *Lissarca miliaris* at Signy Island in 1976, 2002 and 2011 & 2012. a) mean prodissoconch length and standard error; b) mean prodissoconch height and standard error; c) mean prodissoconch length:height ratio and standard error.

Year	Length (µm)	Std. Error	Height (µm)	Std. Error	Length/Height ratio	Std. Error	n
1976	714.5	5.17	449.0	6.31	1.60	0.017	26
2002	699.2	4.96	548.0	3.79	1.53	0.013	46
2011&2012	694.3	7.14	458.9	3.63	1.52	0.029	11

Table 5.2 Maximum prodissoconch length, height and ratio including standard error for *Lissarca miliaris* collected at Shallow Bay, Signy Island in 1976, 2002, and 2011/2012.

The Strontium:Calcium ratio increased from 0.0012 ± 0.0007 in 1976 to 0.0029 ± 0.0011 in 2011 (Figure 5.7). Strontium in 2002 was similar to 1976 (0.0017 ± 0.0007) while the dried material from 2012 was highest (0.0063 ± 0.0013). The Phosphorus:Calcium ratio was very low but decreased from 0.0013 ± 0.0003 to 0.0006 ± 0.0002 between 1976 and 2012. Algae cells were observed living on the external shell surface of fresh dried material from 2012 (Figure 5.8) and despite occasional encrusting bryozoans, no other epifauna was observed on shells from other years. Maximum shell thickness increased from 166µm in 1976 to 276µm in 2011 and 206µm in 2012 and is associated with deteriorating shell quality and increased shell repair (Figure 5.9-12).

The integrity of the shells from 2011 and 2012 is compromised by endolithic decay causing erosion of the upper layer of shell and the subsequent secondary shell deposition results in a thicker shell (Figure 5.11, 5.12). Decay is not observed in the 1976 collection (Figure 5.9), and cannot be confidently identified in the 2002 collection (Figure 5.10). To confirm dissolution was not an artefact of preservation, the 2012 specimens were dried without contact with formalin/ethanol, and only the 1976 material had been fixed in formalin (subsequently ethanol stored). Diatoms, dried to the surface of the shells from 2011 (Figure 5.11d, e) support the erosion of shells prior to fixation.

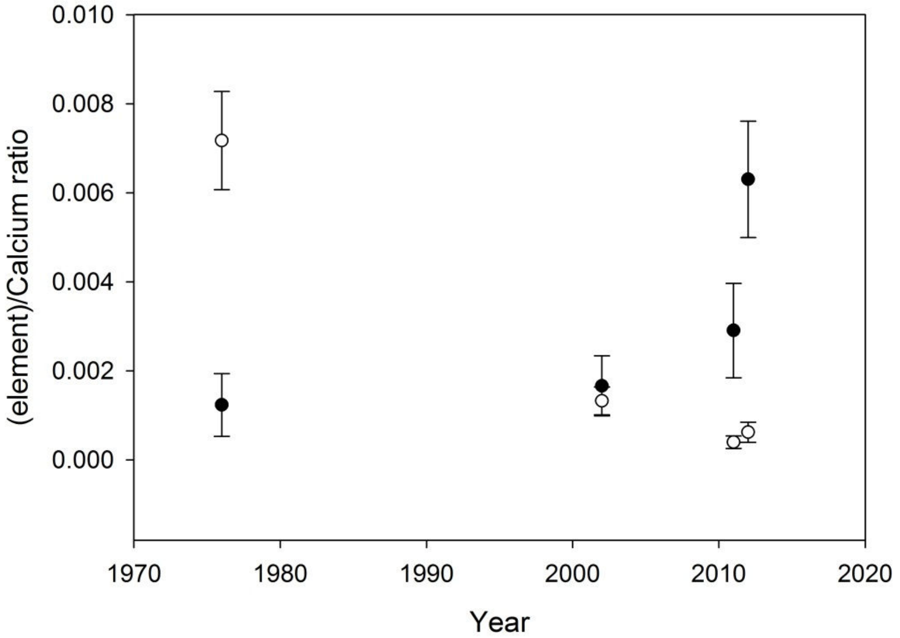

Figure 5.7 Element/calcium rations (mean ± standard error) of *Lissarca miliaris* shells from Signy Island in 1976, 2002, 2011 and 2012. Strontium/calcium ratios (filled circles) and phosphorus/calcium ratios (open circles).

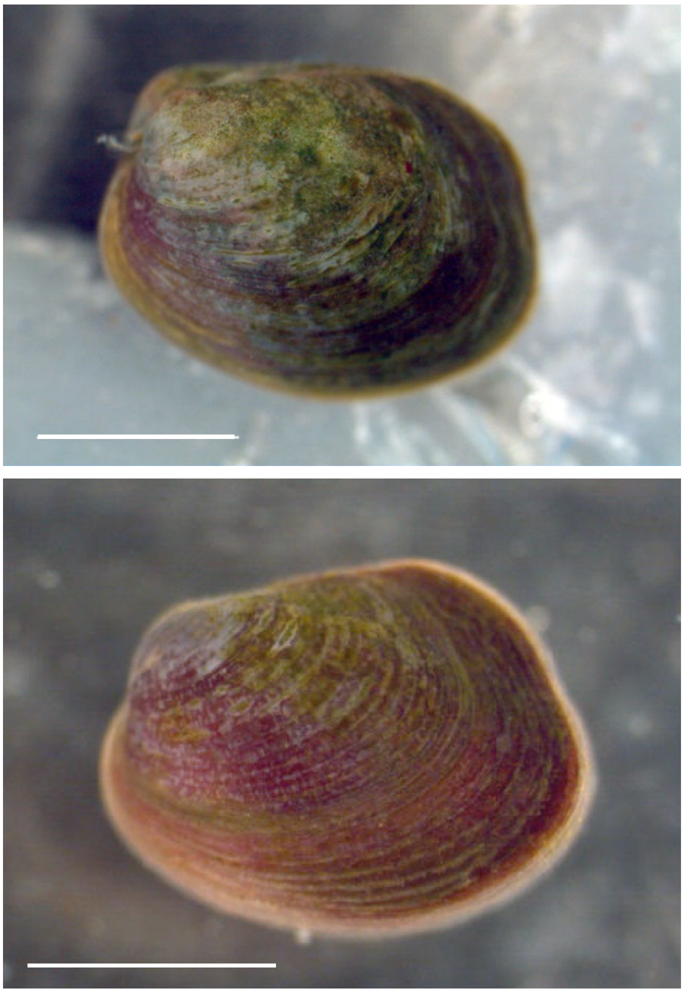

Figure 5.8

Adult *Lissarca miliaris* from Shallow Bay, Signy Island collected in March 2012. Endolithic algae can see be seen in green, covering the shells shortly after fixation. Scale bars = 2mm.

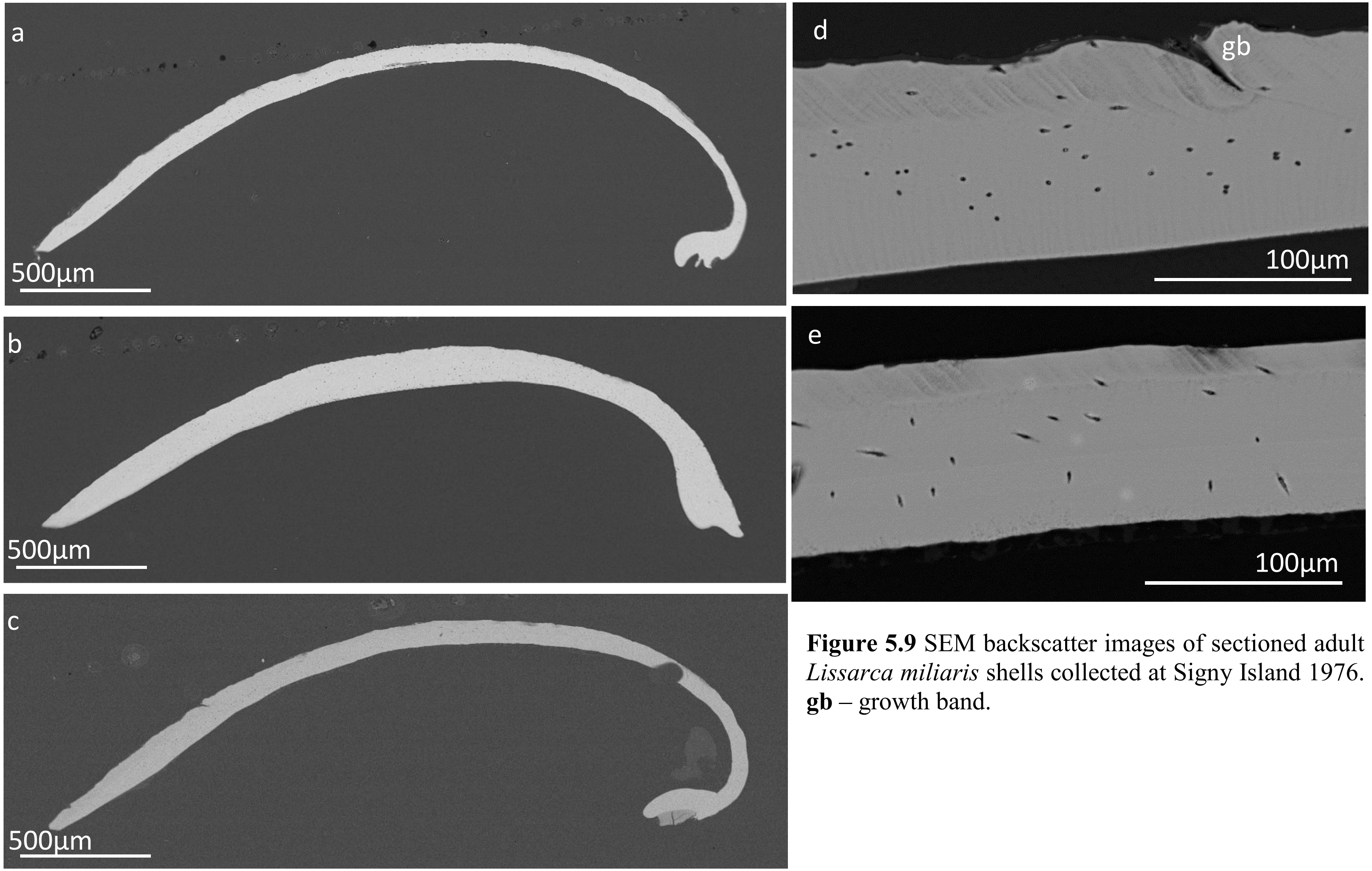

Figure 5.9 SEM backscatter images of sectioned adult *Lissarca miliaris* shells collected at Signy Island 1976. **gb** – growth band.

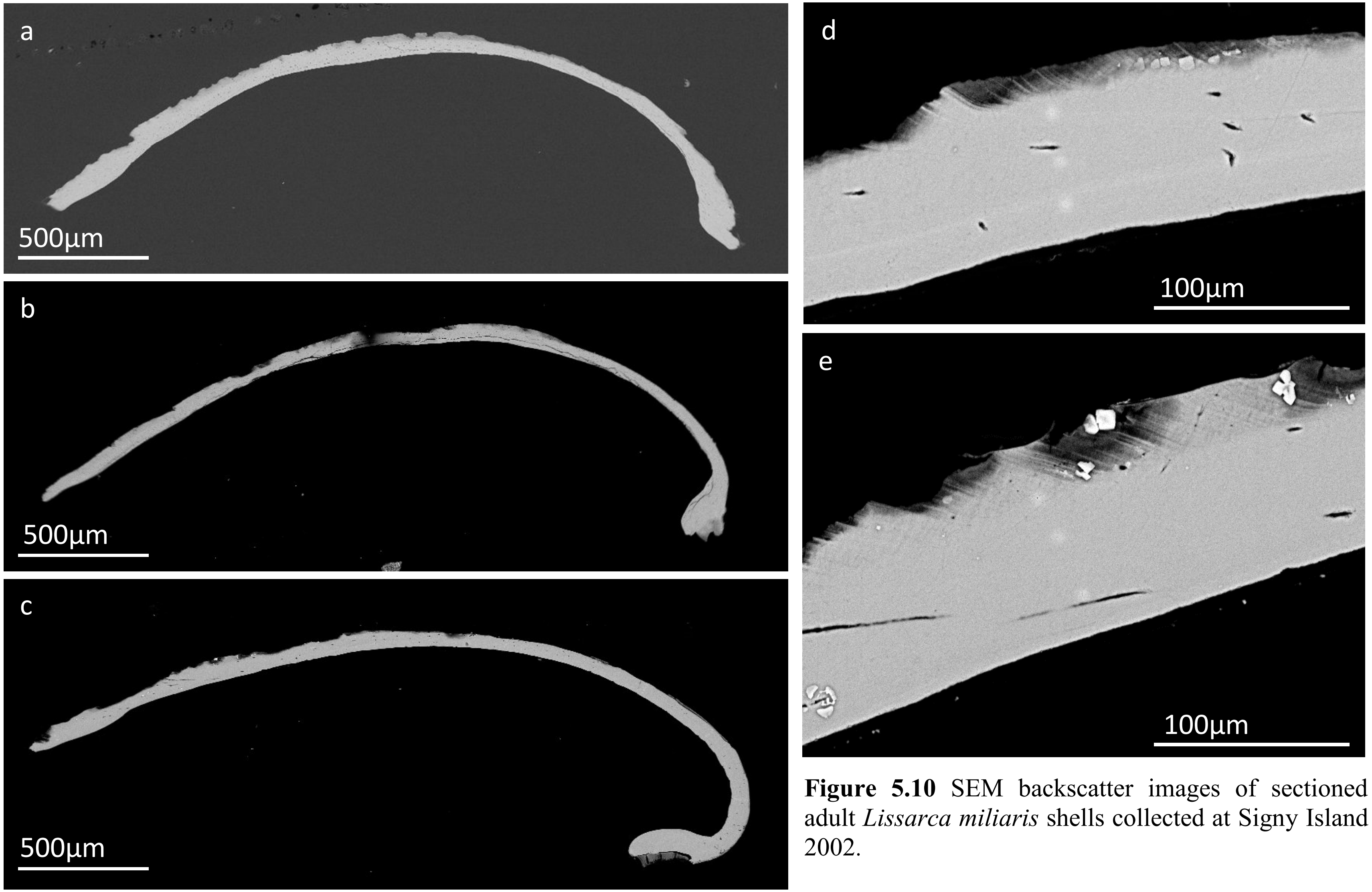

Figure 5.10 SEM backscatter images of sectioned adult *Lissarca miliaris* shells collected at Signy Island 2002.

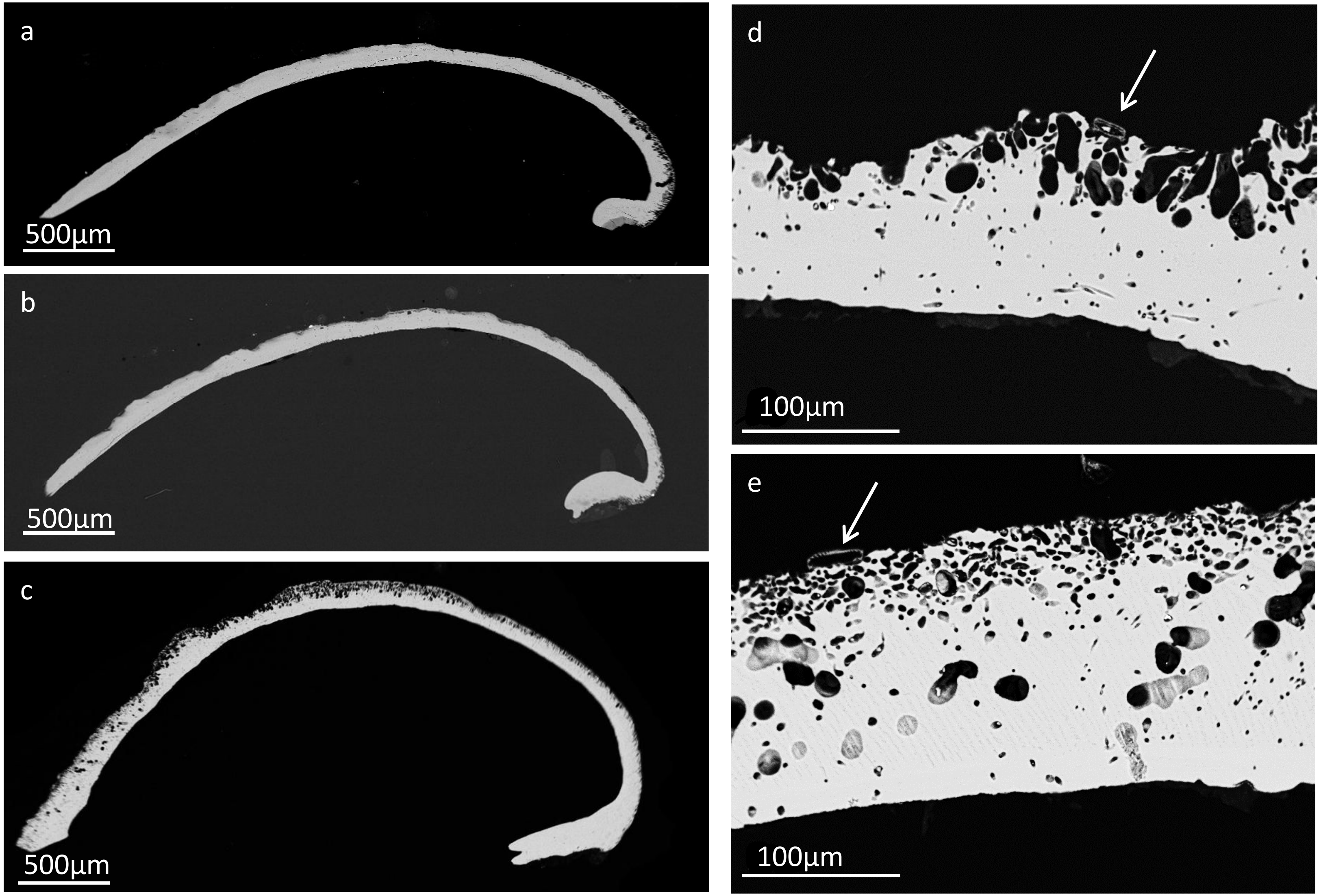

Figure 5.11

SEM backscatter images of sectioned adult *Lissarca miliaris* shells collected at Signy Island 2011. Arrows point to diatoms on shell surface.

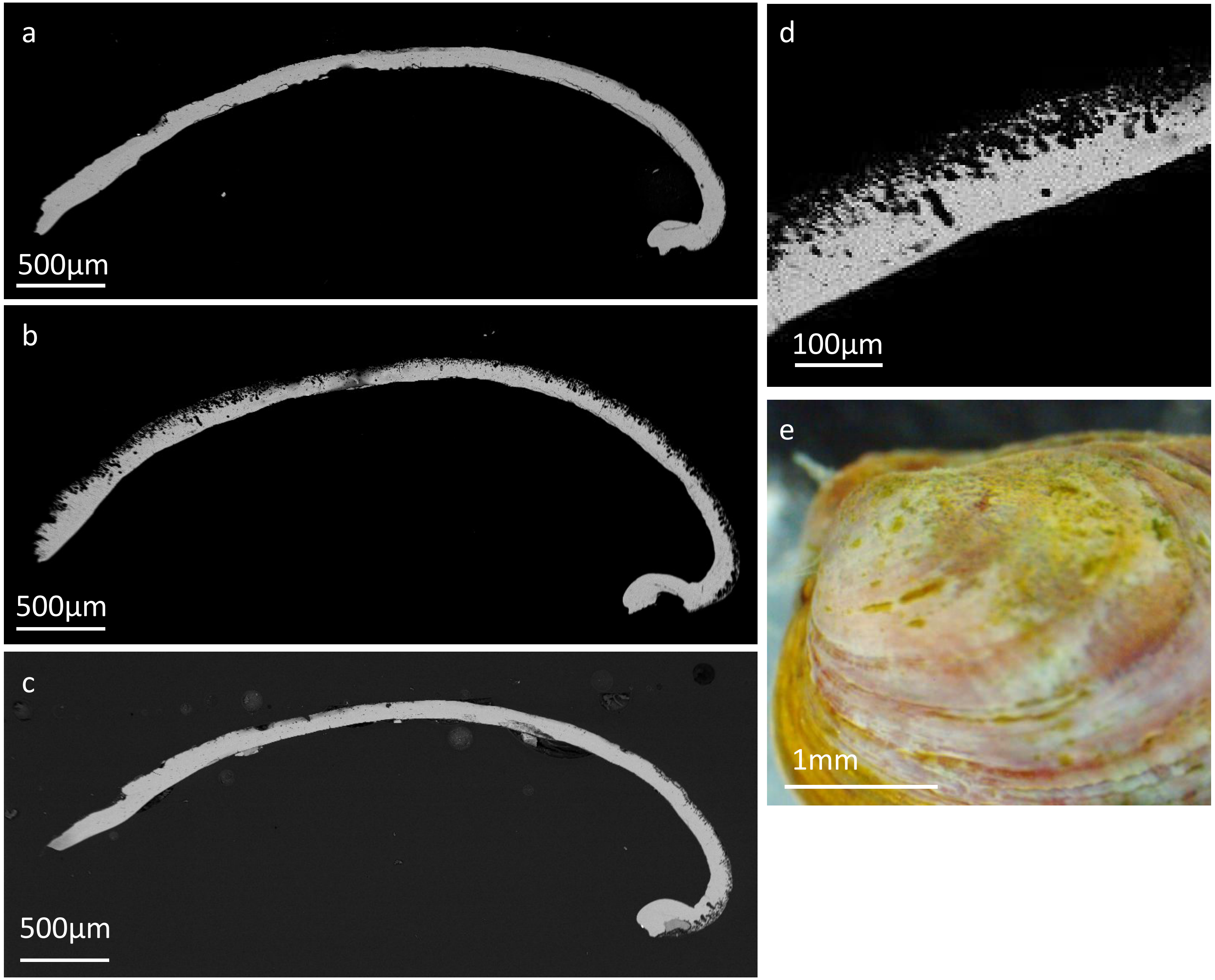

Figure 5.12

Shells of *Lissarca miliaris* from Signy Island 2012. a-d) SEM backscatter images of sectioned adult *Lissarca miliaris*; e) photomicrograph showing shell deterioration and green algae on freshly sampled *Lissarca miliaris*.

Shell morphology changed with time, the overall shape changing most significantly over the past decade (2002-2011). An analysis of covariance, corrected for differences in length, identified a change in length/height from 1.321 and 1.305 in 1976 and 2002 respectively to 1.362 in 2011 ($F_{2,\ 756}$=27.63, p<0.001), and an increase in length/width from 1.865 and 1.845 in 1972 and 2002, respectively, to 1.937 in 2011 ($F_{2,\ 756}$=15.75, p<0.001) (Figure 5.13c, d). This overall change in morphology suggests the bivalve shape has changed from a round ball shaped shell in 1976 and 2002, to a thinner and elongate shell shape (Figure 5.13a). Data from Richardson (1979) suggest the population from 1972 to have length/height of 1.301 ±0.160 and length/width of 1.913 ± 0.0339 (Figure 5.13) but the low sample 'n' (n=13) and high standard error make this unsuitable for statistical analysis.

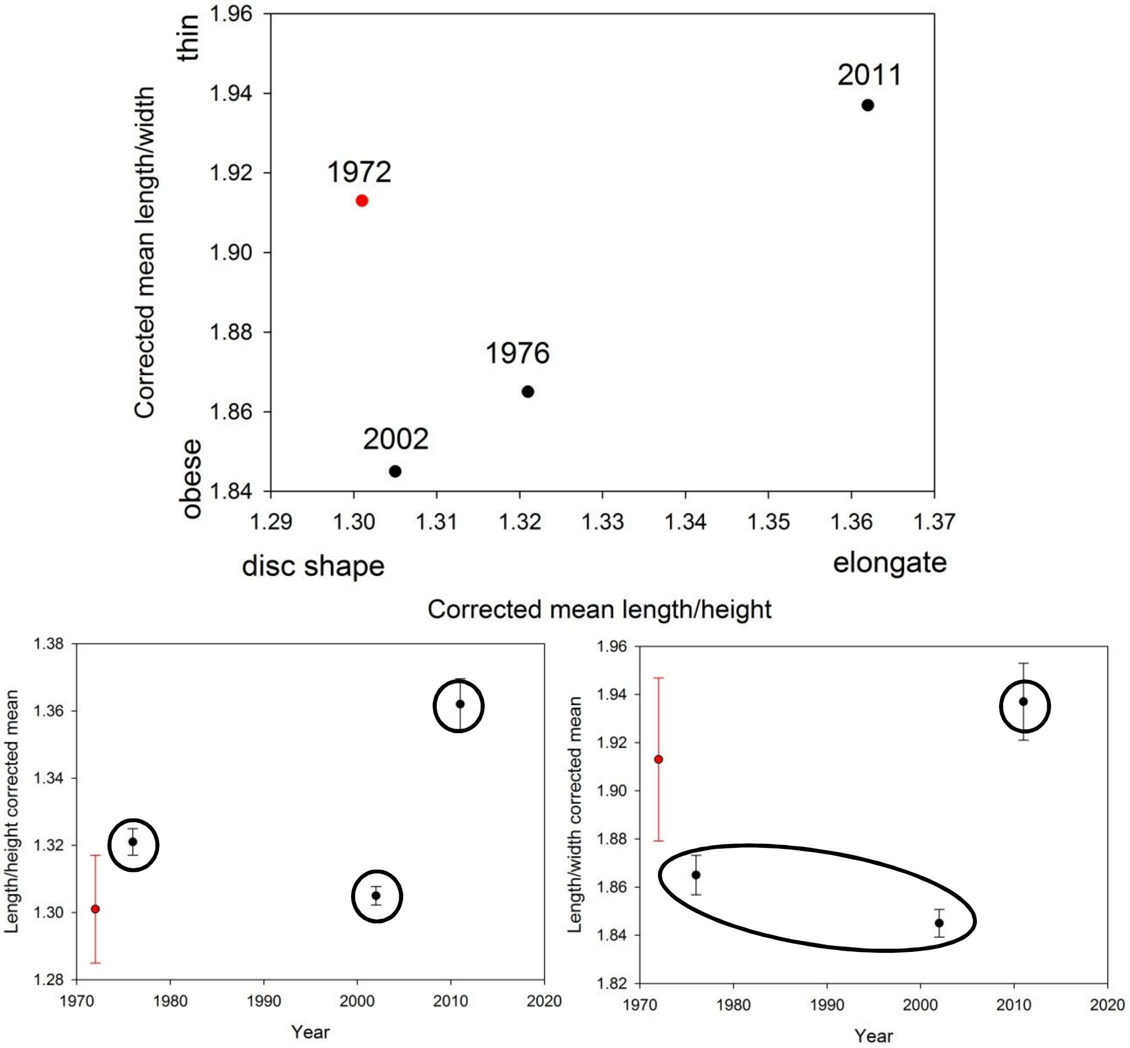

Figure 5.13 ←Morphology of *Lissarca miliaris* from Signy Island between 1976 and 2011. a) corrected length/height v corrected length/width among populations identifying differing shell shapes; b) corrected mean length/height ratio with year; c) corrected mean length/width ratio with year. Significant differences among populations marked by circled groupings, Tukey test, $p<0.05$. Red marks (1972) not included in statistical analysis.

5.4 Discussion

The South Orkney Islands have one of the longest time series of recorded air temperature, dating back to 1903 at the Argentinian research base Orcadas. Temperatures recorded fit closely to the monthly air temperature data collected at the British Signy base from 1945 until 1995 when data collection halted (Figure 5.14) and have shown an average increase of 0.20˚C decade^{-1} over 100 years (Turner et al. 2005), although much of this change appears to be in the last 50 years.

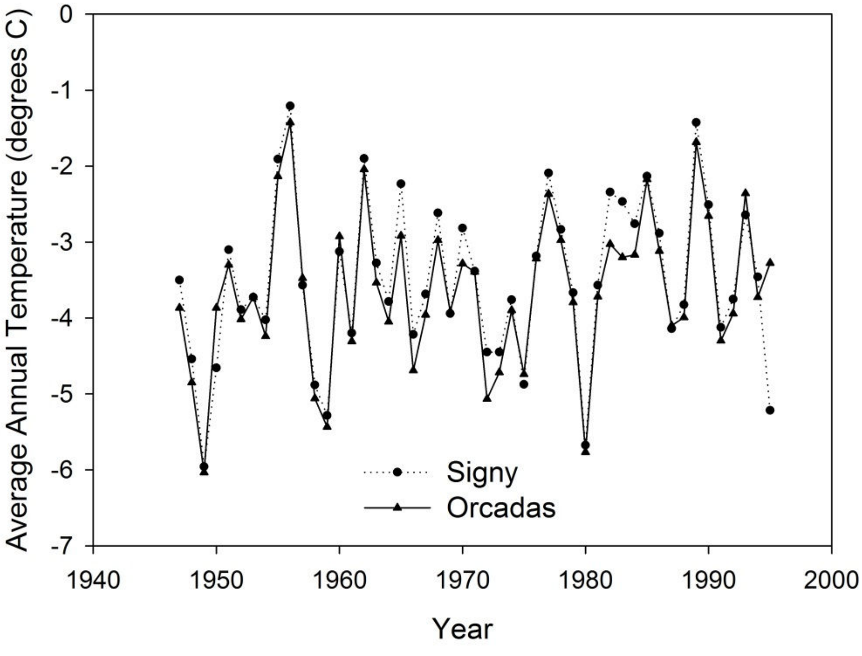

Figure 5.14 Mean annual temperature from 1947-1995 from the British Antarctic Survey base (dotted line) and Argentine Orcadas, Laurie Island base (solid line). Signy Island temperature data collection stopped in 1995.

The increased growth rate of *L. miliaris* over the past 40 years is likely to be a response to this changing temperature in the region. Although no record of sea-water temperature is available for this period at Shallow Bay, the intertidal distribution of *L. miliaris* would make an increase in air temperature a significant factor affecting its physiology. Growth rate is closely linked with two factors, food availability and temperature (Brey et al. 2011; Appeldoorn 1982). Chlorophyll *a* values in Borge Bay show high levels of interannual variability and are considered to be high in the summer while extremely low in the winter (Clarke et al. 1988; Clarke and Leaky 1996). The duration of these blooms might be expected to increase with air temperature as nutrients are rarely limiting, although no evidence of this is observed in the water sampling programmes of 1972-1994 (Clarke et al. 1988; Clarke and Leaky 1996).

L. miliaris has a wide distribution and is commonly found as far north as South Georgia (Dell 1964, Huber 2010). Air temperatures experienced at Signy Island still remain cooler than the temperatures experienced by other populations in the sub-Antarctic and assuming connectivity between populations, the increase in temperature at Signy Island is unlikely to have reached the species thermal limit. OGP provides a method to compare 'how well' an organism grows (Brey 1999; Heilmayer et al. 2004) and a graphical representation of growth, a higher OGP suggesting a less stressful environment in which to grow. The five-fold increase in OGP over 40 years is in contrast to the larger, infaunal bivalve *Laternula elliptica* from King George Island (Brey et al. 2011) which showed a decrease in OGP over 40 years, inferred from shell growth per year in old specimens. However, the two species differ in their distribution and ecology, with *L. miliaris* perhaps favouring the conditions that regional environmental changes have brought.

The most striking change between collections was the condition and composition of the shells. Strontium in aragonite structures is very well studied and its relationship with temperature is used as a climatic proxy (de Villiers et al. 1995; Rosenheim et al. 2004). During faster crystal growth rates, near-surface migration of ions, which expel impurities, are less efficient and an increase in strontium replacement of calcium ions might be expected (Watson 2004). Strontium has also been found to be correlated with temperature indirectly, by increased growth rates in marine gastropods (Sosdian et al. 2006). The increased ratios of strontium in the shells of *L. miliaris* are therefore likely to be linked to the increased growth rates observed, although other abiotic influences may also affect this relationship. Phosphorus is incorporated from the surrounding seawater, suggesting a change in dissolved phosphorus levels. Although detected in each shell, there was no significant difference in sulphur levels between among shells with values only just above the detection limits of EDS.

The shell damage observed in 2011 and 2012 shells resembles the decay caused by photosynthetic bacteria (Webb and Korrûbel 1994; Kaehler 1999). Shell dissolution in the Antarctic is very poorly understood (McClintock et al. 2009) and only one study has described endolithic algae on the shells of the bivalve *Adamussium colbecki* (Cerrano et al. 2001), and at Signy Island a single example of macro algae causing shell erosion in the limpet *Nacella concinna* (Nolan 1991). The dissolution observed appears to be greater on older specimens where the protective periostracum has become eroded; algal cells are observed on the freshly preserved adult 2012 specimens (Figure 5.8, 5.12e). All *L. miliaris* shells in this study were of the same age, but the degree of shell dissolution was much greater in the 2011/2012 specimens compared to the almost perfect shell surface in 1976 specimens. This is also reflected in the thickening of shells as the bivalves attempt to repair their damaged shells with secondary calcification (Figure 5.11-12). The temperature increase in the region may be encouraging faster growth of such endolithic bacteria and the warmer winters experienced over the past decade may be increasing the survival of such organisms during the light and temperature limiting months.

Shell repair is energetically costly and requires an energy reallocation, potentially negatively affecting reproductive output (Gellar 1990; Kaehler and McQuaid 1999) and the decrease in prodissoconch 1 sizes is indicative of this decrease in reproductive effort per egg. Secreted by the bivalve in early development, the prodissoconch size can be directly linked to egg size and differences in egg size as small as 10µm can be reflected in significant differences in prodissoconch 1 length (Goodsell and Eversole 1992). Smaller egg sizes in *L. miliaris* might suggest less energy available for development and a higher risk of larval mortality. The observation of thickened shells and reduced reproductive output demonstrates the energetic trade-off involved in maintaining shell integrity. Some brooding bivalves are known to decrease immune responses leaving a greater susceptibility to parasite infection (Taskinen and Saarinen

1999). Many Antarctic invertebrates brood for over a year; if *L. miliaris* have suppressed immunity for 18 months of brooding, their reduced resistance to endolithic parasites and cost of shell repair may impact their survival.

Phenotypic plasticity driven by environmental conditions has been seen by shell thickening the Antarctic molluscs *Nacella concinna* (Nolan 1991) and *Laternula elliptica* (Harper et al. 2012) in response to wave action and ice damage respectively; in neither case has any genetic evidence of divergence been identified as a result of change. This study however may represent the first case of climate warming induced phenotypic plasticity in molluscs from the Antarctic. Changing conditions at Signy Island have potentially driven a change in the morphology seen in this study. Such a shift in morphology to a more thin and elongate shape is likely to have a negative effect on the reproduction of brooding species as this further reduces the females capacity to brood young. The thinner shape may be a response to the faster growth observed in the 2011 specimens. A similar response to higher temperature range was found in *L. miliaris* from the warmer South Georgia (See Chapter 4) where smaller offspring were also found. In this case, it may be assumed that the most recent population of *L. miliaris* at Signy Island is morphological and perhaps ecophysiologically comparable to sub-Antarctic populations, despite the temperature range still being lower than those at lower latitudes. The results of this study provide sufficient evidence to support the hypothesis of ecophysiological changes to *L. miliaris* at Signy Island in response to environmental changes over time.

Conclusions

L. miliaris at Signy Island show the vulnerability of Antarctic fauna by rapidly responding to only a subtle change in temperature over four decades, demonstrating the importance of having a realistic baseline for measuring change. The growth rate response already occurring is startling but before a physiological tipping point is reached other factors, including the shell dissolution described in this study, may affect future growth and survival. This highlights the need to investigate other small, shelled organisms that may already be in a highly altered ecological state in polar ecosystems, although only by using historic and archived material can the true extent of environmental change be measured. It is likely too late for realistic baselines to be established for measuring such change and any management approach must therefore be assessed against an already altered ecosystem state. Antarctic ecosystems are often perceived to be pristine (Halpern et al. 2008) but this study demonstrates changes that have been occurring unnoticed for at least 40 years, and other critical changes could be occurring in ecosystems that are assumed to be stable. The immediate concern for *L. miliaris* and the polar shelled organisms it represents is the energetic cost of shell repair affecting reproduction, but also the reduced resilience to the inevitable pressures associated with invasive species, ocean acidification and predictions of further warming of the Southern Ocean (Aronson et al. 2011) that by far exceed the comparatively subtle warming seen to date.

5.5 Chapter Summary

Over the past forty years, rapid climate warming at the South Orkney Islands have resulted in changes in the population of *Lissarca miliaris* in the inter-tidal areas at Signy Island. Atmospheric temperature has increased regionally by about 1.5°C, and changes to *L. miliaris* include;

- An increase in observed and calculated growth rate between 1972 and 2011 including a five-fold increase in overall growth performance.
- A decrease in prodissoconch length and morphology suggesting a decrease in offspring size.
- Changes in inner shell chemistry; measured increase in strontium and decrease in phosphorus suggesting changes in the chemistry and possibly temperature of sea water over time.
- Decrease in shell condition with more shell decay caused by possible endolithic decay from photosynthetic algae.
- Change in shell morphology to a thinner and elongated shell in 2011 compared to 2002 and 1976.

Chapter 6

Reproduction in deep-sea protobranch bivalves *Yoldiella ecaudata, Yoldiella sabrina*, and *Yoldiella valettei* from the Southern Ocean

Abstract

Deep-sea protobranch bivalves are globally an important macrofaunal group within soft sediment communities, achieving calcification at great depths and are responsible for high sediment turnover. Despite regular sampling, studies on the ecology and taxonomy are challenging with regard to the protobranchs, and larger taxa have generally been favoured for past investigations. Protobranch bivalves are potentially an interesting group to study however, with an evolutionary history dating back to the early Ordovician, and radiations in all deep-sea basins around the world. In the Southern Ocean, deep-sea protobranchs occur at much shallower depths than their congeners in the Atlantic and have widespread circum-Antarctic distributions. Despite their likely origin from the deep-sea, their method of dispersal and reproduction are unknown, and rarely studied in any deep-sea ecosystem. This chapter investigates the reproductive biology of three deep-water protobranchs; *Yoldiella ecaudata* from 500m in the Amundsen Sea; *Y. sabrina* from between 200-4730m in the Amundsen Sea, Scotia Sea, and South Atlantic; and *Y. valettei* from 1000m in the Scotia Sea. All species demonstrate evidence of lecithotrophic larval development with maximum egg size of 130.4µm, 187.9µm, and 120.6µm respectively, further supported with prodissoconch I measurements. There is evidence for simultaneous hermaphroditism in *Y. valettei* with well-developed testis identified next to vitellogenic oocytes. Further evidence demonstrated asynchronous oocyte development within specimens of *Y. ecaudata* and *Y. valettei* and between populations of *Y. sabrina* separated by depth. The reproductive characteristics are comparable to previous studies on North Atlantic deep-sea protobranch species and are discussed in the context of the cold stenothermal and potentially food rich deep-sea conditions prevailing on the deep Antarctic continental shelf and the deep sea. The requirement for reclassification of this complex subclass is also discussed in relation to soft anatomy and shell characteristics described.

6.1 Introduction

The protobranchs are a subclass of small deposit feeding bivalves containing over 600 species globally and represent one of the oldest clades of bivalve with fossil records dating back to the early Ordovician ~500 Ma (Allen 1978). They share in common highly specialised characteristics for their preferred fine sediment environment including a strong gill structure solely used for respiration, long hindguts for feeding in food impoverished environments, and adapted feeding palps to consume large quantities of sediment (see review by Zardus 2002). Most commonly found in the deep-sea, protobranchs are also well represented in shallow water environments and are of interest because of their long evolutionary history (Allen 1978; Zardus 2002), potentially long life spans (Turekian et al. 1975; Nolan and Clarke 1993; Gage 1994; Peck and Bullough 1993; Peck et al. 2000) and diverse ecology.

Despite representing between 50 and 90% of bivalve species on the slope and abyssal plain, respectively, deep-sea protobranch bivalves are ecologically poorly understood. Their high abundances make them an important and dominant macrofaunal taxon in the deep-sea (Sanders et al. 1965; Allen 2008) and yet have a confusing taxonomic history with many synonyms after previous mis-identifications of new species. This problem was highlighted early by Verrill and Bush (1898) and subsequently Knudson (1970) before being reclassified and organised by Allen and Hannah (1986) who stated that names used within the group are ‘a confusing array of regional uses that are not collated’. Subsequent studies outlined the defining characteristics of different genera and are valuable tools for the identification of species using shell morphology and soft anatomy (Allen and Sanders 1973, 1982, 1996; Allen and Hannah 1989; Allen et al. 1995). More recently, genetic tools have been used to analyse widespread distributions of species revealing contrasting levels of genetic differentiation and homogeneity among different species relating to bathymetry and geography (Chase et al. 1998; Etter et al. 2005, 2011; Zardus et al. 2006).

Reproduction of deep-sea protobranchs has been studied for only a few species, in most detail from the Rockall Trough, where a research station at ~2900m was sampled extensively between 1973 and 1983 (Lightfoot et al. 1979; Tyler et al. 1992), but also the Gay Head-Bermuda transect (Scheltema and Williams 2009). These studies identified seasonality in *Ledella pustulosa, Yodliella jeffreysi, Nucula delphinodonta*, and *N. annulata* while asynchronous reproduction in *Malletia cuneata, Ennucula similis, E. granulosa, Deminucula atacellana*, and *Brevinucula verrilli,* and both lecithotrophy and direct development. To date, this represents the most detailed reproductive biology known within deep-sea protobranch bivalves from non-chemosynthetic environments.

Protobranch bivalves have a unique, short dispersing pericalymma larva, in which the larval shell develops beneath an external layer of cells and cilia (the test) to help movement (Zardus and Morse 1998; Zardus and Martel 2001). This distinct shell development results in a smoother prodissoconch I (first larval shell) and absent prodissoconch II (second larval shell) (Ockelmann 1965). To date, known larval development in protobranchs can be pelagic lecithotropthic or direct development with parental care, inferred by the size of larval shell and oocyte size (Ockelmann 1965; Scheltema and Williams 2009), with prodissoconch and oocyte size above 135 and 90µm, respectively, suggesting lecithotrophic development over planktotrophic development (Ockelmann 1965).

In the Antarctic, the protobranch bivalves are well represented between 1 and 5000m but research has predominantly focussed on the ecology of the comparatively large and shallow water protobranch *Yoldia eightsii* (Davenport 1988; Nolan and Clarke 1993; Peck and Bullough 1993; Peck et al. 2004). Despite more regular sampling, the reproduction of *Yoldia eightsii* is still poorly understood and is a demonstration of the difficulties associated with time constraints for sampling in the Antarctic. The Southern Ocean protobranchs *Yoldiella sabrina* (Hedley 1916), *Yoldiella ecaudata* (Pelseneer 1903) and *Yoldiella valettei* (Lamy 1906) are commonly found from the Antarctic shelf to the deep-sea basins (200-4000m+) and wide distributions (Huber 2010) that are likely to be circum-Antarctic. Additionally, *Yoldiella sabrina* has a known distribution into the South Atlantic (Linse et al. 2007). Although currently unknown, the reproductive traits of these protobranchs may have enabled survival or colonisation of the Antarctic continental shelf following previous glacial periods, and may remain important today in maintaining widespread populations across a bathymetric range.

Aims

This study aims to describe for the first time the reproduction of deep-water Southern Ocean bivalves *Yoldiella ecaudata, Y. sabrina*, and *Y. valettei* collected from the South Atlantic, Amundsen Sea and Scotia Sea. This will enhance the understanding the benthic ecology in the cold stenothermal environments of the deep Southern Ocean.

Hypothesis

H_1 *Yoldiella ecaudata, Y. sabrina*, and *Y. valettei* will demonstrate evidence of lecithotrophic larval development.

Objective

O_1 To describe the reproductive biology of the protobranch bivalves *Yoldiella ecaudata, Y. sabrina*, and *Y. valettei* from the Southern Ocean using histology and dissection techniques.

6.2 Materials and Methods

Sample collection

Specimens of *Yoldiella ecaudata, Y. sabrina*, and *Y. valettei* were collected during a number of research expeditions to the Weddell Sea, Scotia Arc, Antarctic Peninsula and Amundsen Sea between 2002 and 2008, as described in Chapter 2.1.

Histology

A total of 38 *Yoldiella sabrina* from the 500m in the Amundsen Sea, 4730m in the South Atlantic and from 200-3500m in the Scotia Sea; 34 *Y. valletei* from the 1000m in the Scotia Sea; and 15 *Y. ecaudata* from 500m in the Amundsen Sea, were used for histology (Table 1), where possible representing the size range of each species. Each specimen was photographed before and after decalcification as described in Chapter 2.5 and 2.6. Soft anatomy and internal shell characteristics was used to confirm identification of species. Tissue processing and image analysis is described in Chapter 2.5. Serial sectioning of tissue was done where possible to ensure the gonads were cut.

Prodissoconch size

Prodissoconch length of fifteen *Y. ecaudata* and *Y. valettei* were measured by scanning electron microscopy at low vacuum as described in Chapter 2.3.

6.3 Results

Reproductive ecology of *Yoldiella sabrina*

In total 25 animals were identified with active gonads and 12 with no identifiable gonads. The gonad occupies an area dorsal laterally on both the left and right external to the digestive gland and internally around the digestive tissue and gut loop (Figure 6.2a,b, 6.3a, 6.4a). Oocytes and testis were observed beside the intestinal loop wall and there was no evidence of hermaphroditism.

The smallest female was found at 3.3mm shell length from the Scotia Sea with previtellogenic oocytes observed internally around the digestive gland (Figure 6.1, 6.3a). Small vitellogenic oocytes were observed from 5.5mm shell length with up to 22 oocytes in the Scotia Sea (Figure 6.3, 6.4b,c), the largest measuring 55.7µm in diameter. No more than ten large vitellogenic oocytes were measured in specimens of 9.1 shell length from the Scotia Sea (Figure 6.4a) and the largest female at 10.3mm from the South Atlantic (Figure 6.3b). The largest eggs measured 187.9µm and 174.0µm respectively (Figure 6.1). Only one female with small vitellogenic oocytes were observed in the Amundsen Sea with 5.8mm shell length.

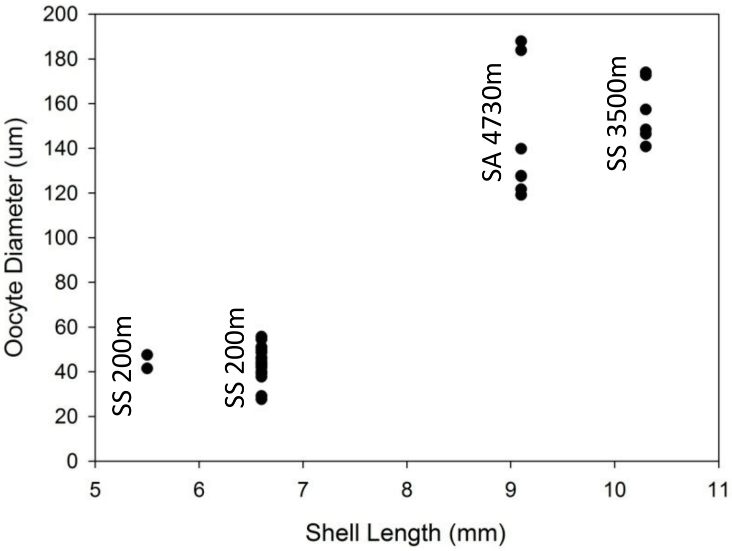

Figure 6.1 Oocyte diameters measured from histology sections against shell length in *Yoldiella sabrina* collected in February from the South Atlantic (SA) and Scotia Sea (SS).

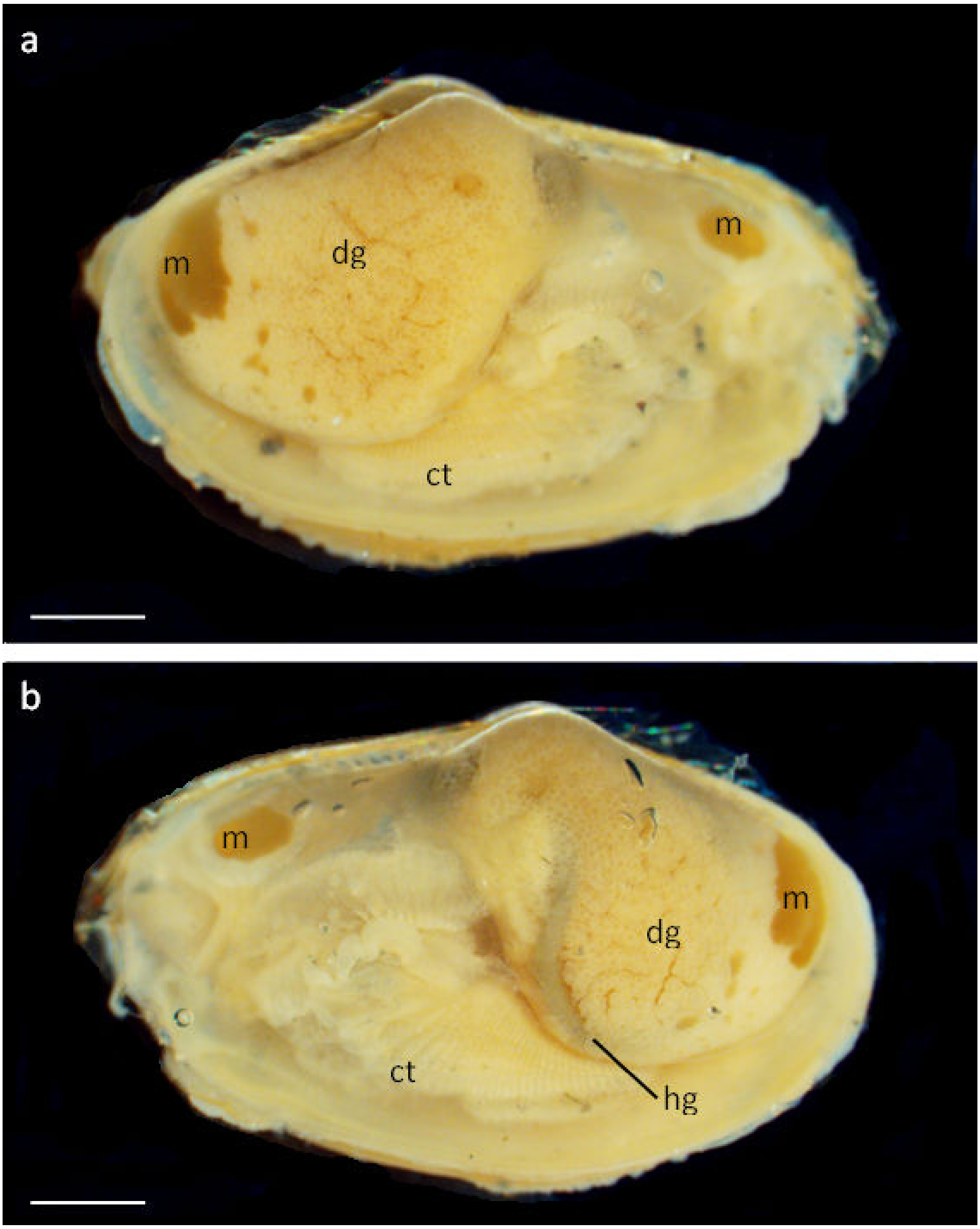

Figure 6.2 Photomicrographs of decalcified female *Yoldiella sabrina* before histology sections (shell length 7.75mm) from 200m in the Scotia Sea. a) view of the left showing large digestive gland; b) view of the right showing digestive gland around hind gut. Digestive appears gland enveloped by thin layer of gonad. **m** – muscle; **ct** – ctenidia; **dg** – digestive gland; **hg** – hind gut. Scale bar = 1mm.

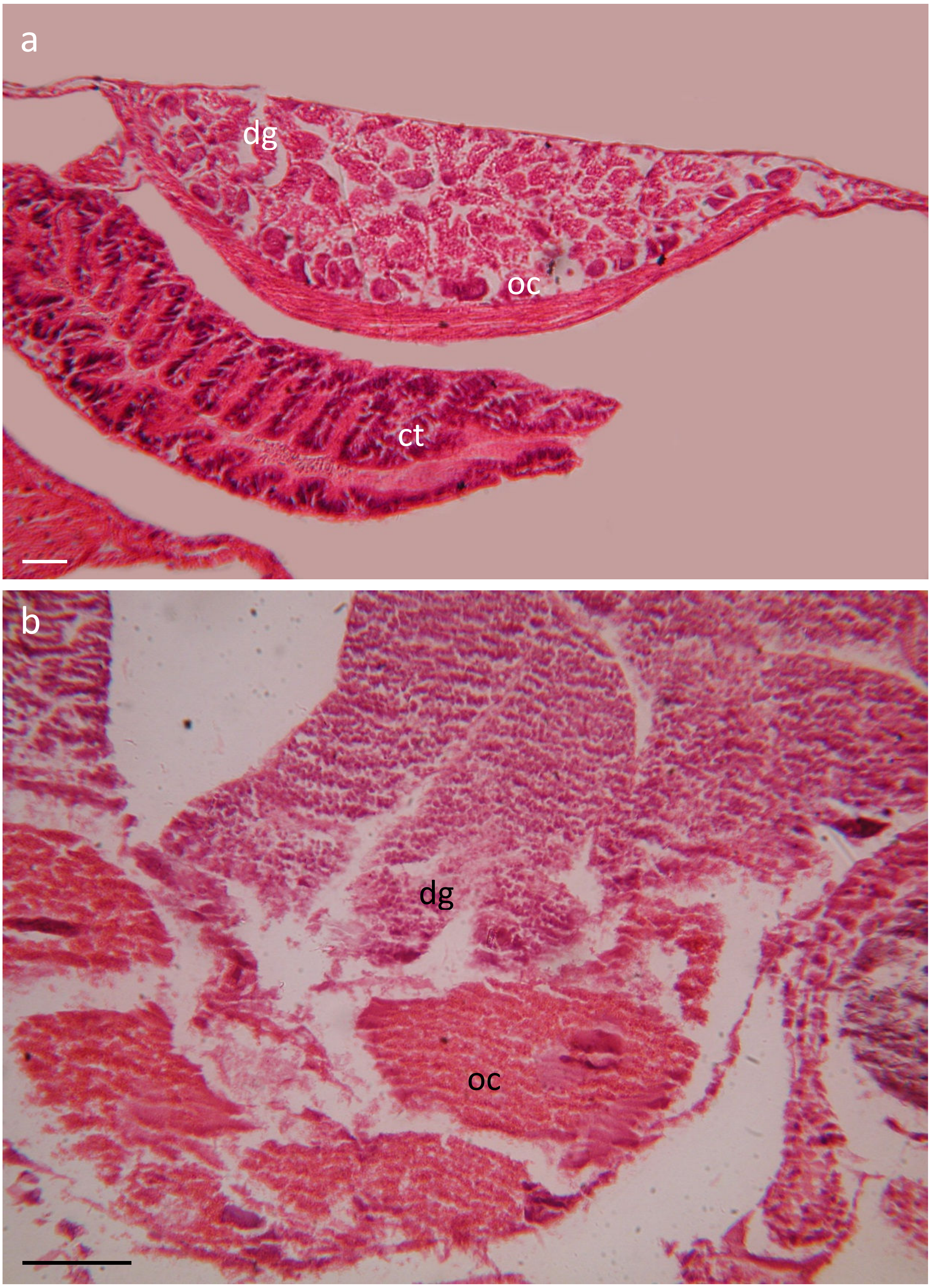

Figure 6.3 Transverse histology section of digestive glands and surrounding female reproductive tissue of *Yoldiella sabrina*. a) small previtellogenic oocytes developing interior to the digestive gland from 3.3mm shell length specimen from 200m in the Scotia Sea; b) large vitellogenic oocyte and digestive gland from 10.3mm shell length specimen from 4700m in the South Atlantic. **ct** – ctenidia; **oc** – oocyte; **dg** – digestive gland. Scale bars = 100µm.

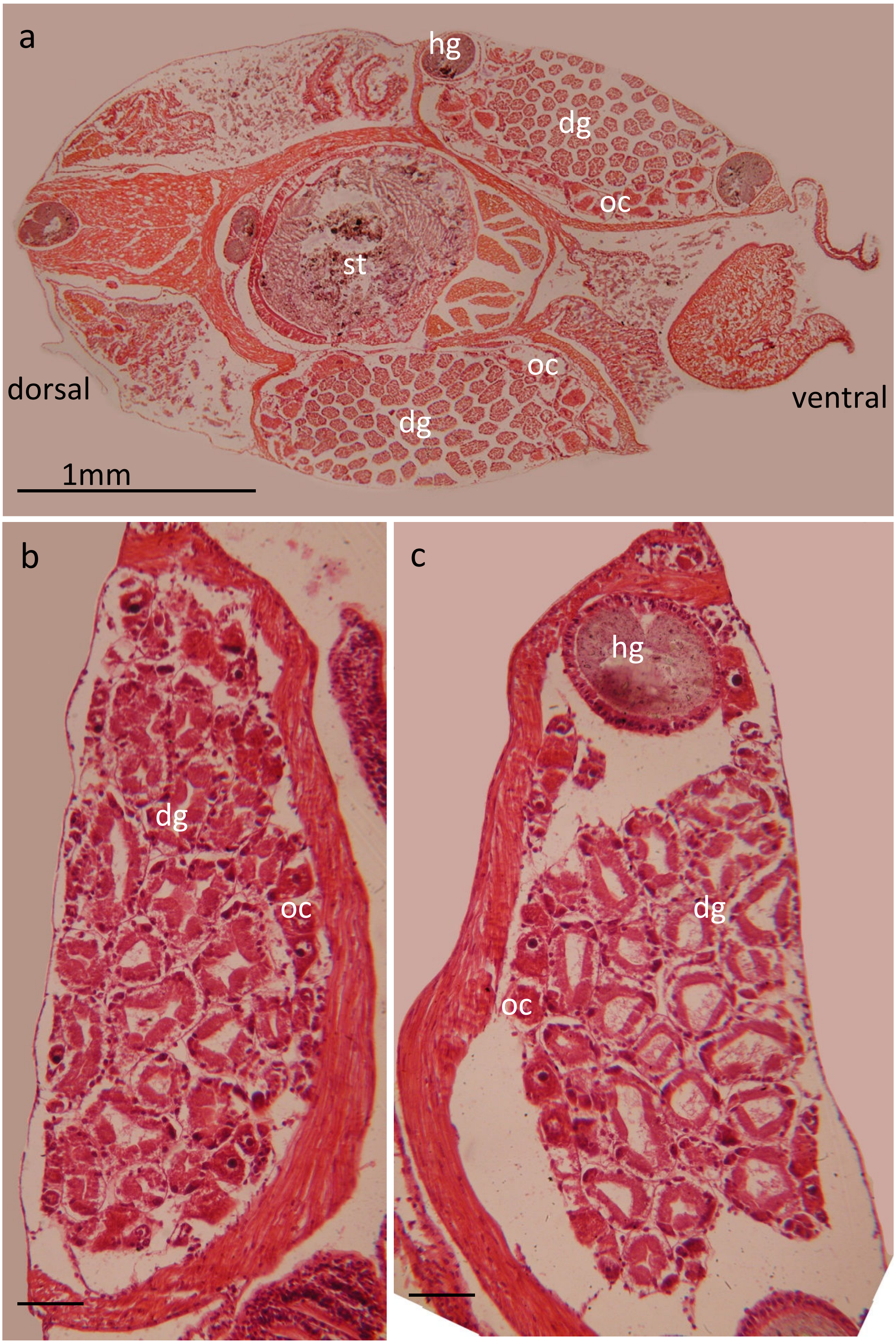

Figure 6.4 Transverse histology sections *Yoldiella sabrina.* a) 9.1mm shell length from 3500m Scotia Sea showing anatomy; b,c) digestive gland from 5.5mm shell length from 200m Scotia Sea; b) left digestive gland; c) right digestive gland. **ct** – ctenidia; **oc** – oocyte; **dg** – digestive gland; **hg** – hind gut; **st** - stomach. Scale bars = 100µm.

Males were observed from 3 – 13mm shell length, identified by extensive testis surrounding the digestive glands, and hind gut (Figure 6.5, 6.6). The right dorsal-ventral gonad surrounding the hind gut does not appear as extensive as the left dorsal ventral gonad (Figure 6.5, 6.7). Spermatogonia and spermatocytes were observed but no accumulations of spermatozoans were visible in the specimens from any location. Males were well represented in specimens studied from the Scotia Sea, Amundsen Sea and South Atlantic, with 16 males to 8 females in all areas. This relationship of 2:1 or higher was also observed within each area studied.

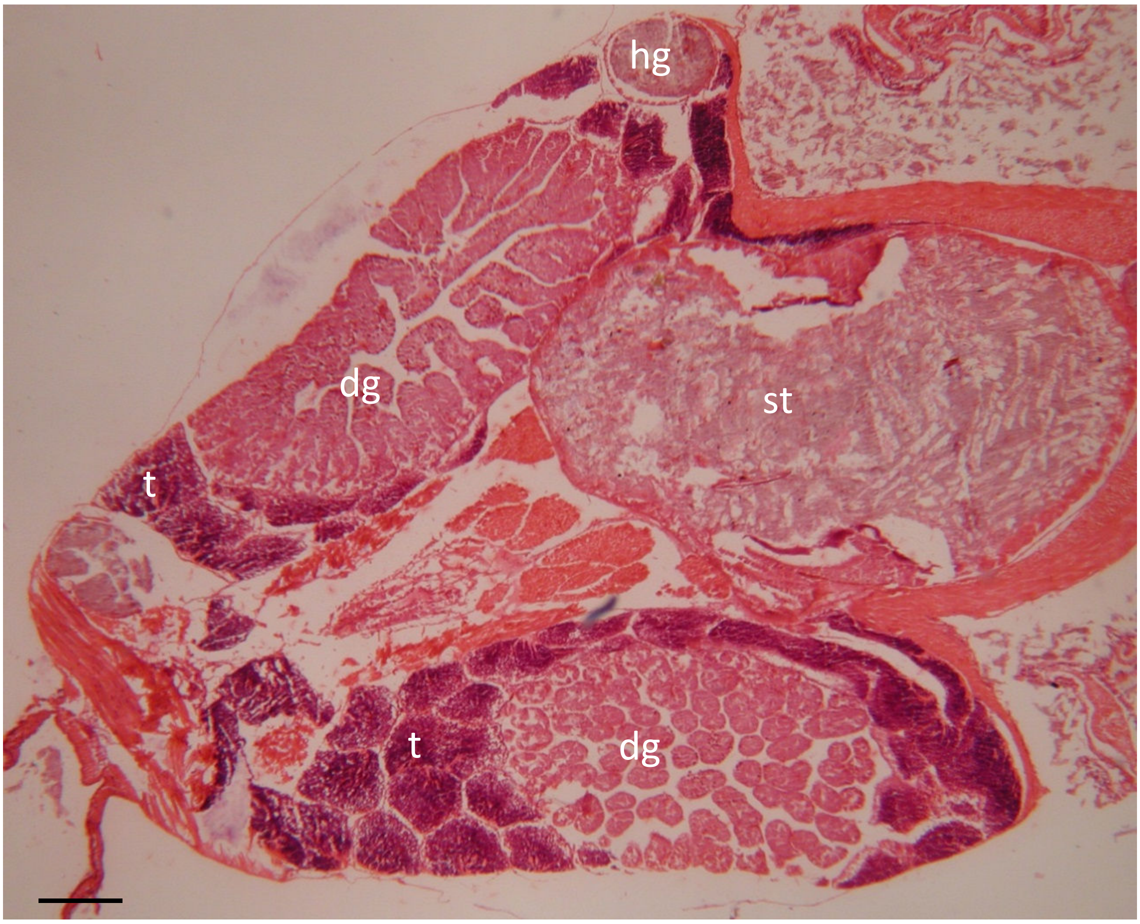

Figure 6.5 Transverse histology section of a male *Yoldiella sabrina* of 9.7mm shell length from 3500m in the Scotia Sea showing testis surrounding digestive gland. **t** – testis; **dg** – digestive gland; **hg** – hind gut; **st** – stomach. Scale bar = 100µm.

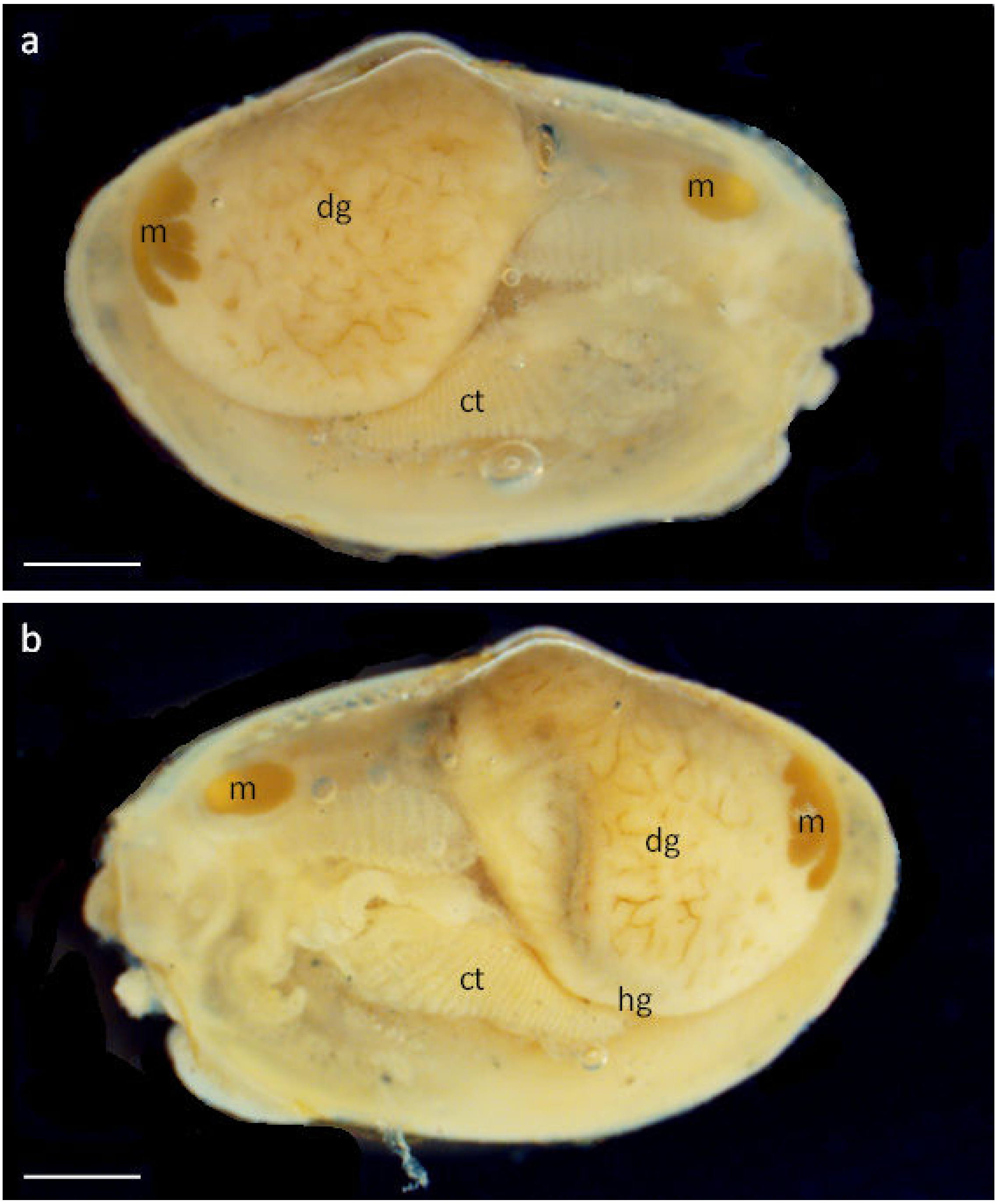

Figure 6.6 Photomicrographs of decalcified male *Yoldiella sabrina* before histology to confirm sex (shell length 7.8mm) from 200m in the Scotia Sea. N.B. structure of the digestive gland identifies specimen as a male when comared to female digestive gland a) view of the left showing large digestive gland/testis; b) view of the right showing digestive gland/testis around hind gut. **m** – muscle; **ct** – ctenidia; **dg** – digestive gland; **hg** – hind gut. Scale bar = 1mm.

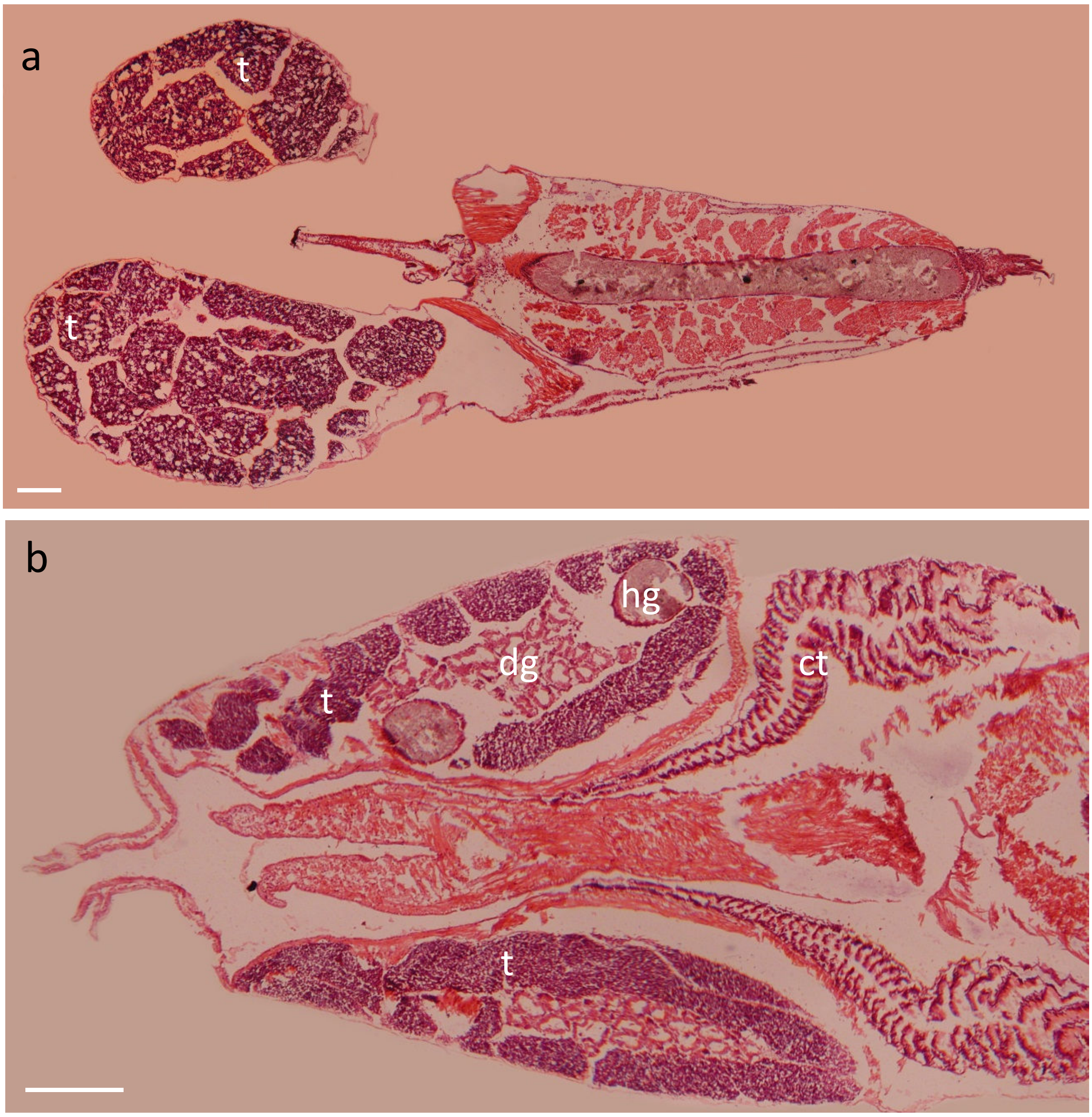

Figure 6.7 Transverse histology sections of male *Yoldiella sabrina* from 200m in the Scotia Sea showing testis in enveloping the digestive gland. a) 9.4mm shell length posterior digestive gland section; b) 7.8mm shell length cut through anterior section of digestive gland. **t** - testis; **dg** – digestive gland; **hg** – hind gut; **ct** – ctenidia. Scale bars = 100µm.

Reproductive ecology of *Yoldiella ecaudata*

Nine specimens of *Y. ecaudata* were identified has having active gonads and six where no reproductive tissue was found. The gonad is situated dorsal-laterally, external to the digestive gland and around the hind gut (left and right), and internally around the digestive gland (Figure 6.8 digestive gland; 6.9, 6.10 for gonad). Testis or oocytes were observed in areas otherwise occupied by the digestive gland which appears reduced in specimens with well-developed gonad; there is no evidence of hermaphroditism.

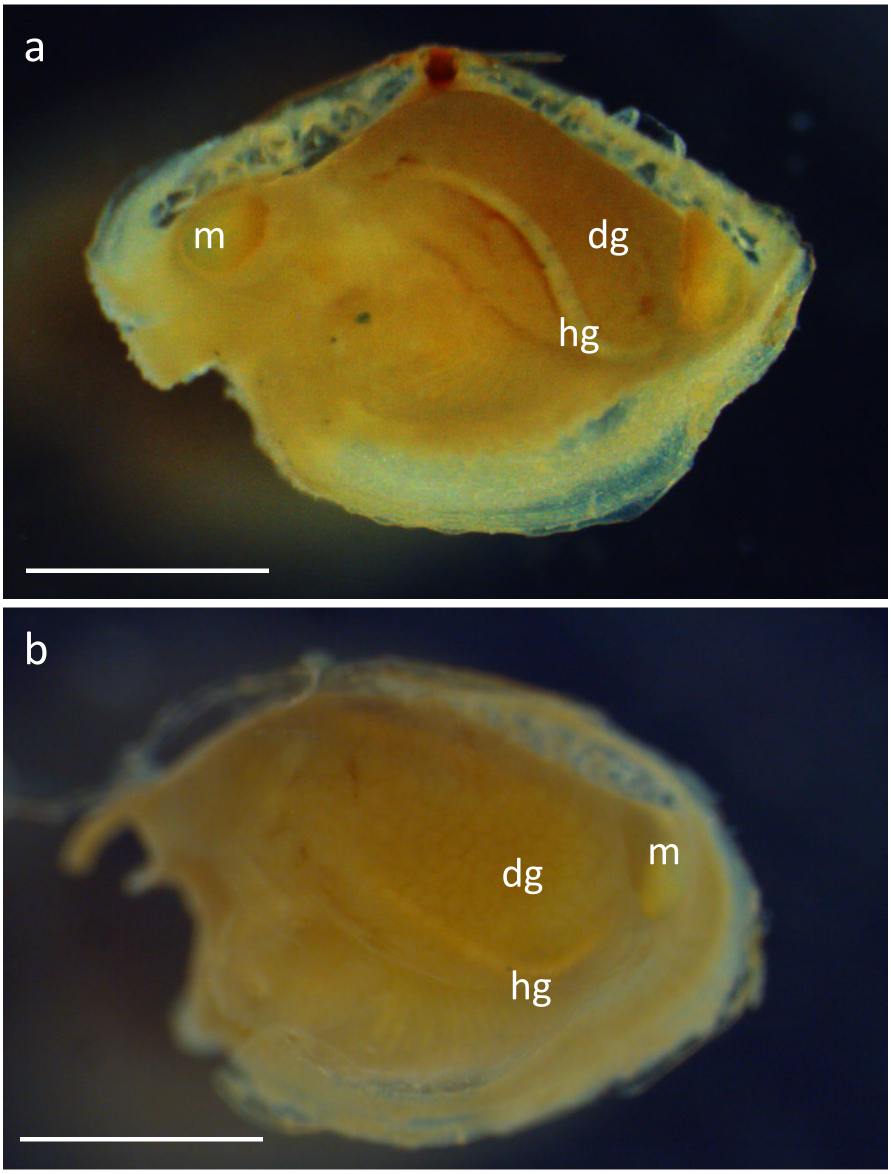

Figure 6.8 Photomicrographs of decalcified *Yoldiella ecaudata* from 500m in the Amundsen Sea a) 2.8mm shell length showing digestive gland and hind gut loop; b) 3.1mm shell length showing extensive digestive gland and hind gut loop. **m** – muscle; **dg** – digestive gland; **hg** – hind gut. Scale bars = 1mm.

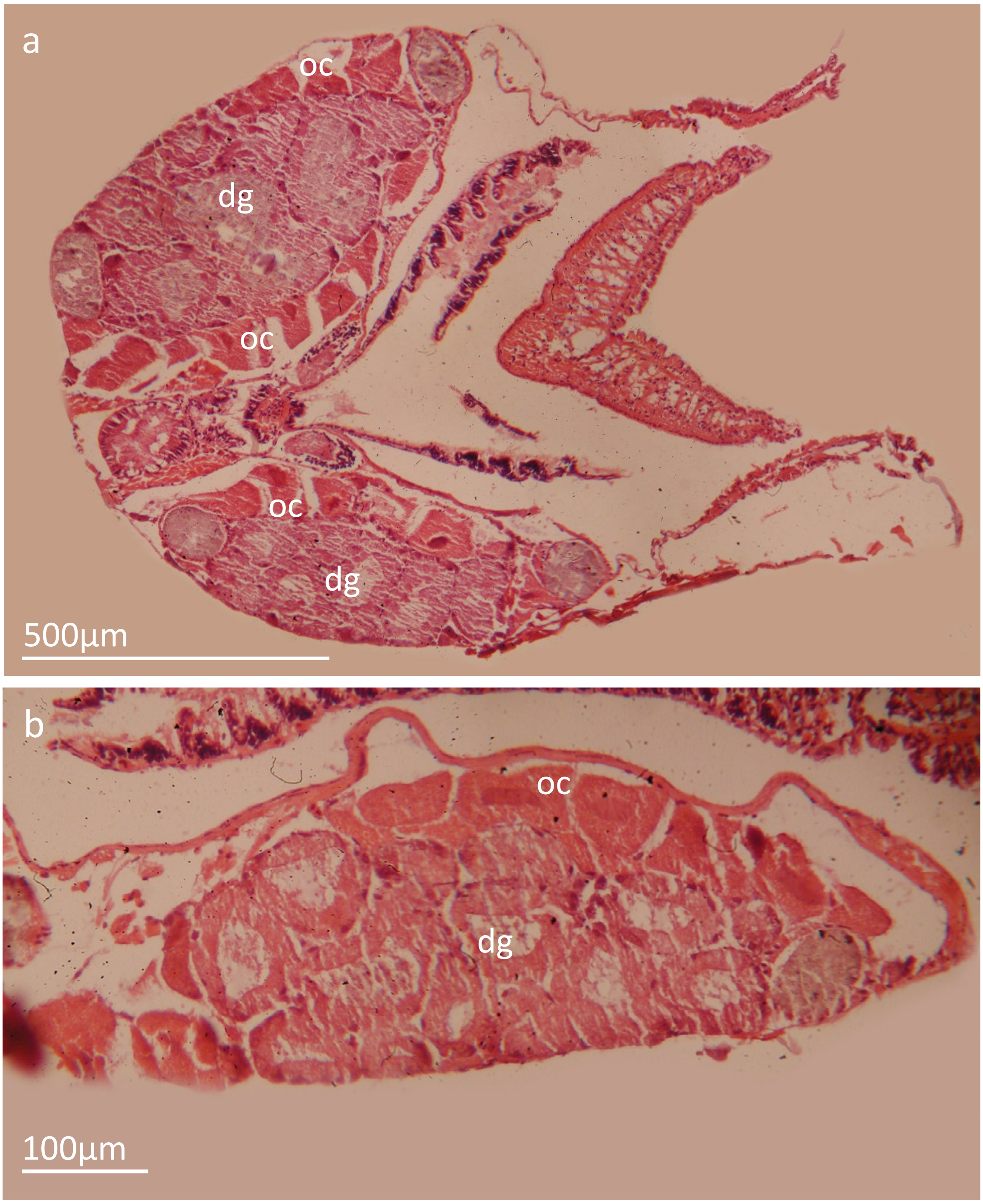

Figure 6.9 Transverse histology sections of a 2.8mm shell length *Yoldiella ecaudata* from 500m in the Amundsen Sea. a) section through whole animal showing oocytes internal to digestive gland; b) oocytes in relation to digestive gland and hind gut loop. **dg** – digestive gland; **oc** – oocytes.

Female *Y. ecaudata* were found between 2.2 and 2.8mm shell length with the largest oocyte measured 130.4µm in a female of 2.8mm shell length (Figure 6.11). Up to 13 eggs were counted in a single female of 2.6mm shell length. Only large vitellogenic oocytes were observed in females although the size range measured varied within and between individuals (Figure 6.11). Only three male *Y. ecaudata* were observed between 2.4 and 2.7mm shell length but no evidence of spermatazoan accumulation.

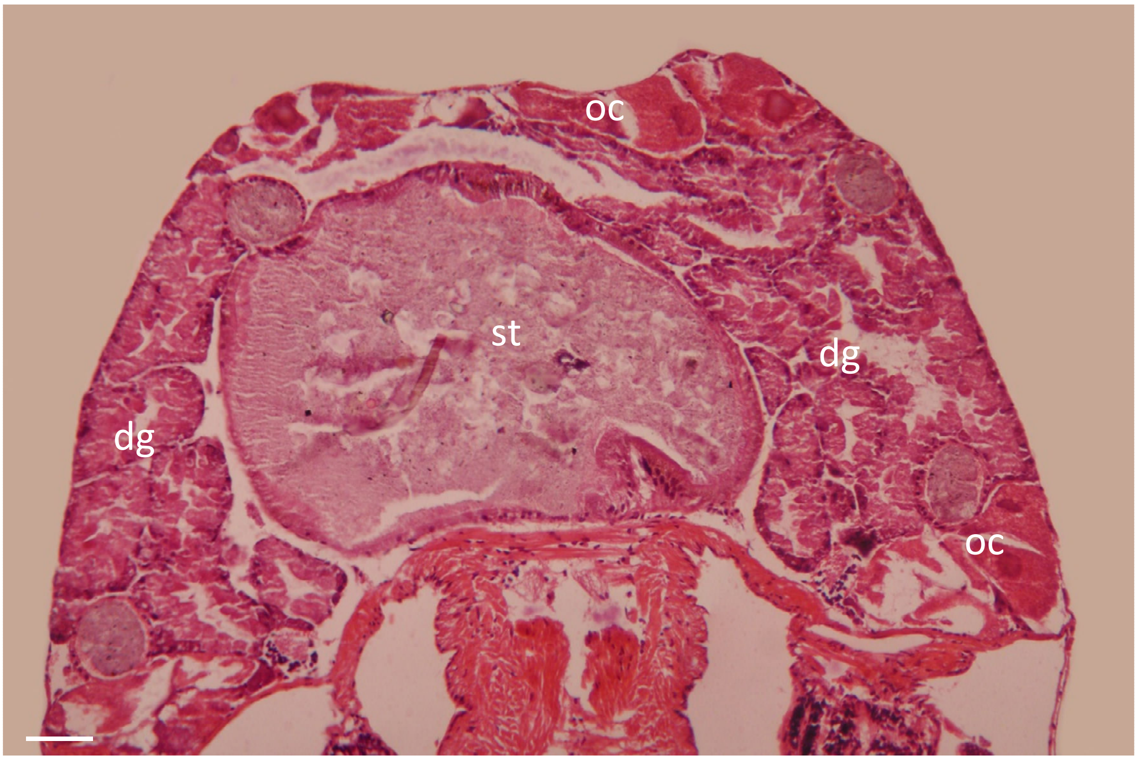

Figure 6.10 Transverse histology section of a 2.6mm female *Yoldiella ecaudata* from 500m in the Amundsen Sea showing vitellogenic oocytes in the dorsal portion of gonad above the stomach and surrounding digestive gland. **dg** – digestive gland; **st** – stomach; **oc** – oocyte. Scale bar 100µm.

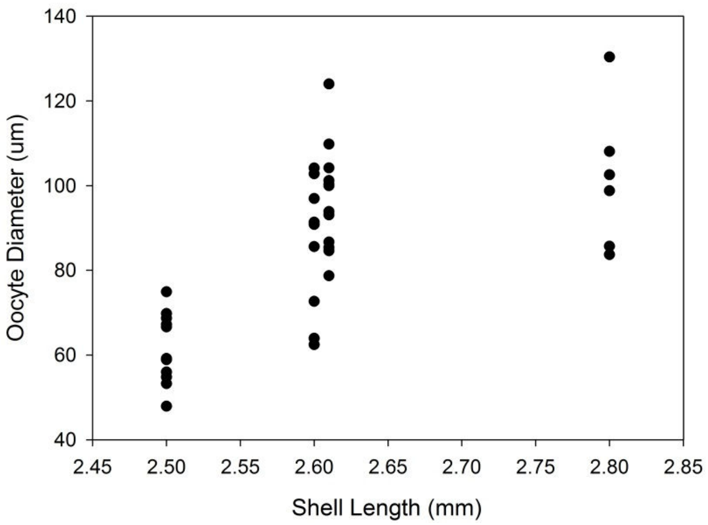

Figure 6.11 Oocyte diameter measured from histology sections against shell length in *Yoldiella ecaudata* from the Amundsen Sea. Two 2.6mm shell length specimens offset for clarity.

Reproductive ecology of *Yoldiella valettei*

In total, 34 specimens from the Scotia Sea were used for histology and 21 were identified with active gonads. The gonad occupied an area dorso-laterally on both the left and right external to the digestive gland, and internally around digestive tissue and hind gut loop (Figure 6.12 for position of hing gut loop; 6.13a, 6.15 for gonad histology). The gonad extends dorsally to join the left and right portions of gonad (Figure 6.13, 6.15). In males, the testis extends anteriorly under the mantle epithelium (Figure 6.12a).

Females were found between 2.2 and 3.2 mm shell length with vitellogenic oocytes observed in the gonad (Figure 6.16). The largest oocyte measured 120.6µm diameter and up to 56 oocytes were counted in a single individual (3.1mm). In one specimen of 3.1mm shell length, both testis and vitellogenic oocytes were observed with spermatozoa accumulation in the lumen (Figure 6.13a, 6.14). Testis in the hermaphrodite was only observed dorso-laterally on the right external to the digestive gland and hind gut loop (Figure 6.14). Male *Y. valettei* were found between 2.3 and 3.2mm shell length with well-developed testis enveloping the bivalve, externally to the digestive glands and along the mantle cavity epithelium (Figure 6.15). Spermatogonia and spermatocytes were observed and accumulations of spermatozoans were visible in some specimens. There was no evidence of protandry or protogyny.

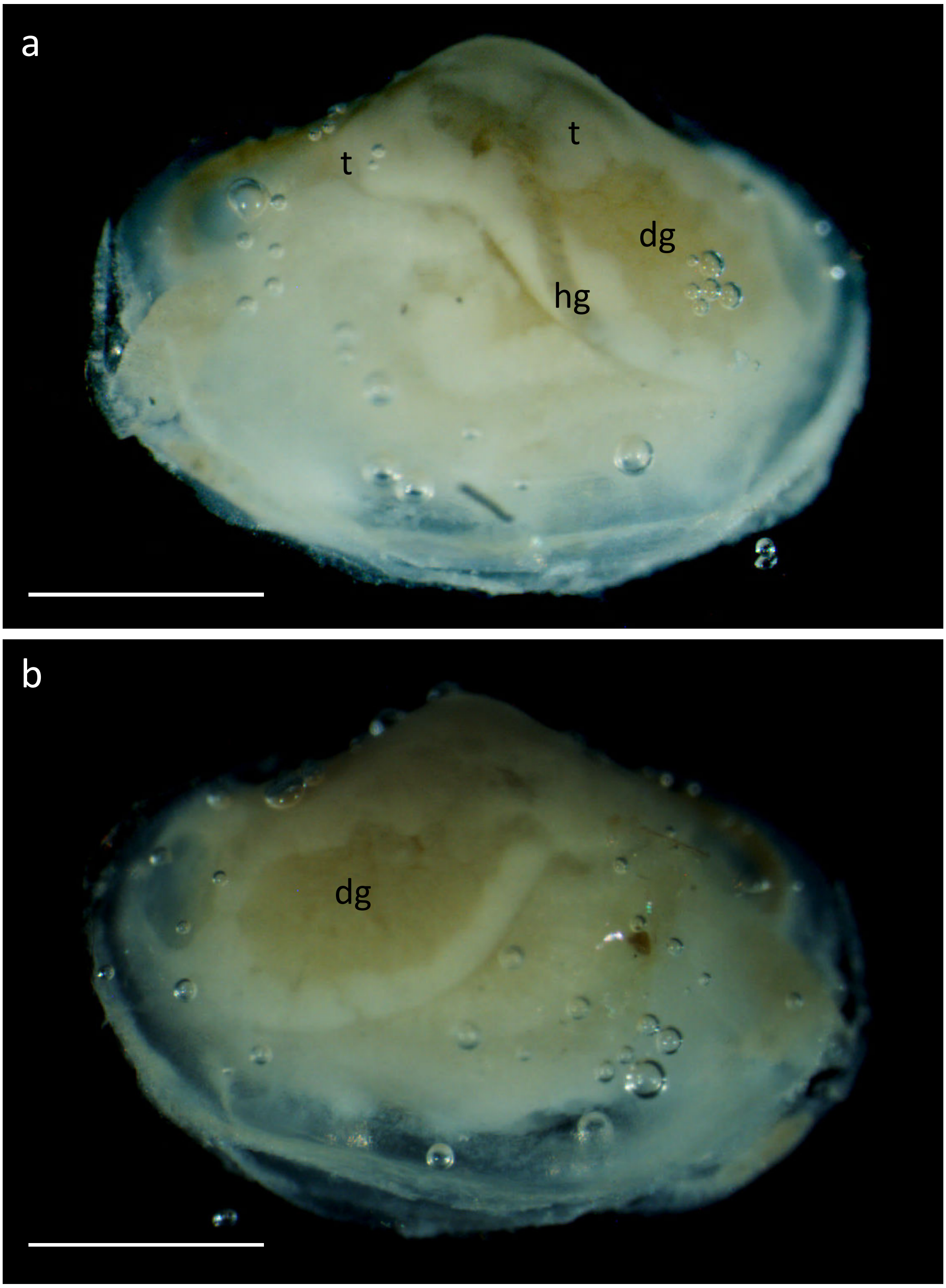

Figure 6.12 Photomicrographs of 3mm decalcified male *Yoldiella valettei* from 1000m in the Scotia Sea. a) right view showing hind gut loop and digestive tissue and gonad; b) left view showing digestive gland and testis (no hind gut loop). **dg** – digestive gland; **hg** – hind gut; **t** – testis. Scale bars = 1mm.

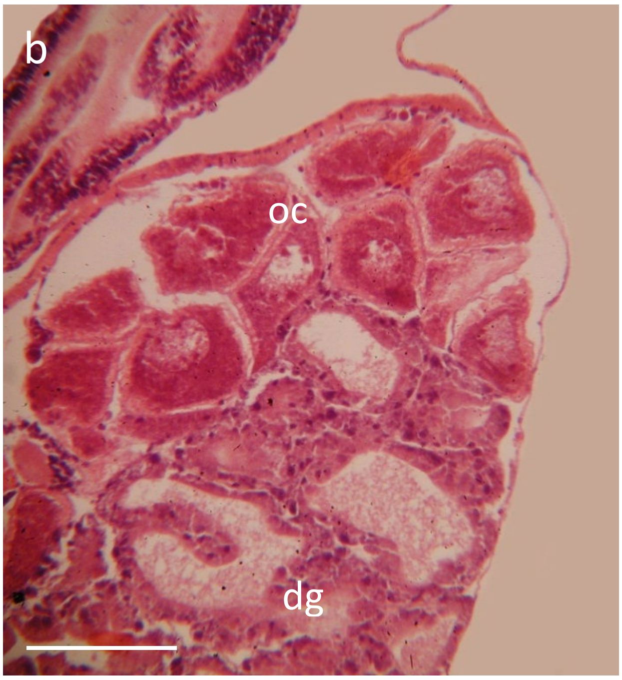

Figure 6.13 Transverse histology sections of 3.1mm hermaphrodite *Yoldiella valettei* from 1000m in the Scotia Sea. a) section of entire animal showing vitellogenic oocytes and testis in relation to digestive gland; b) large vitellogenic oocytes with large nuclei under the digestive gland. **dg** – digestive gland; **oc** – oocytes; **hg** – hind gut; **t** – testis. Scale bars 100µm.

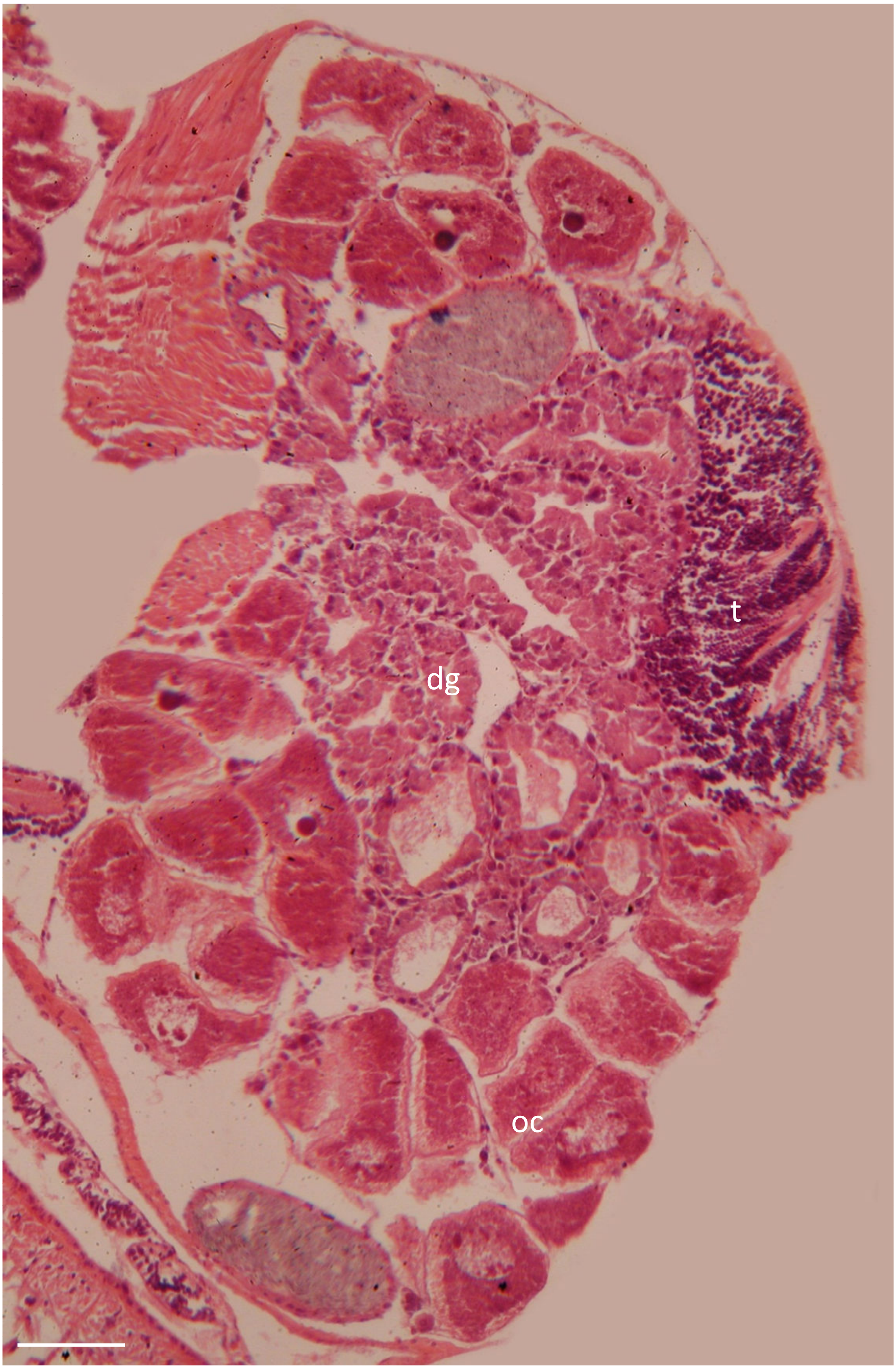

Figure 6.14 Transverse histology sections of 3.1mm hermaphrodite *Yoldiella valettei* from 1000m in the Scotia Sea. **dg** – digestive gland; **oc** – oocytes; **t** – testis. Scale bar = 100µm.

Figure 6.15 Transverse histology section of a 3.1mm male *Yoldiella valettei* from 1000m in the Scotia Sea showing testis enveloping digestive gland and hind gut loop, and extending dorsally above the stomach. **dg** – digestive gland; **t** – testis; **st** – stomach. Scale bar = 100µm.

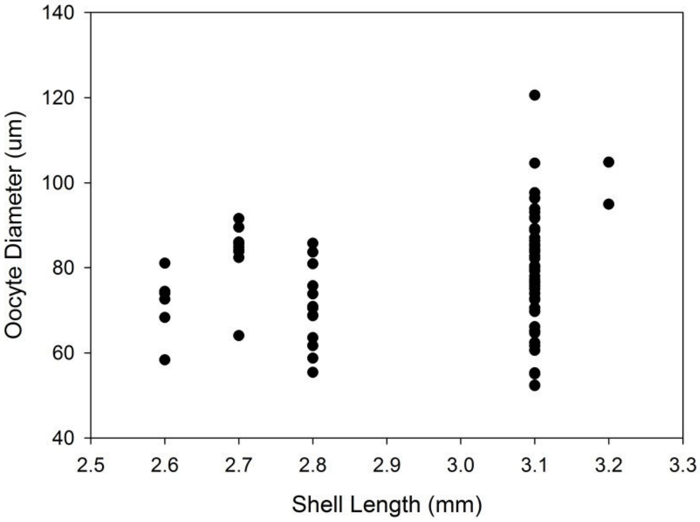

Figure 6.16 Oocyte diameter measured from histology sections against shell length in *Yoldiella valettei* from 1000m in the Scotia Sea.

Prodissoconch Measurements

Prodissoconch was easily identified by a characteristic pitted surface. Both *Y. valettei* and *Y. ecaudata* showed similar prodissoconch lengths of 184µm and 191 µm respectively, with variation of less than 20 µm between specimens (Figure 6.17) (n=15). As common with all protobranch bivalves, no prodissoconch II was observed.

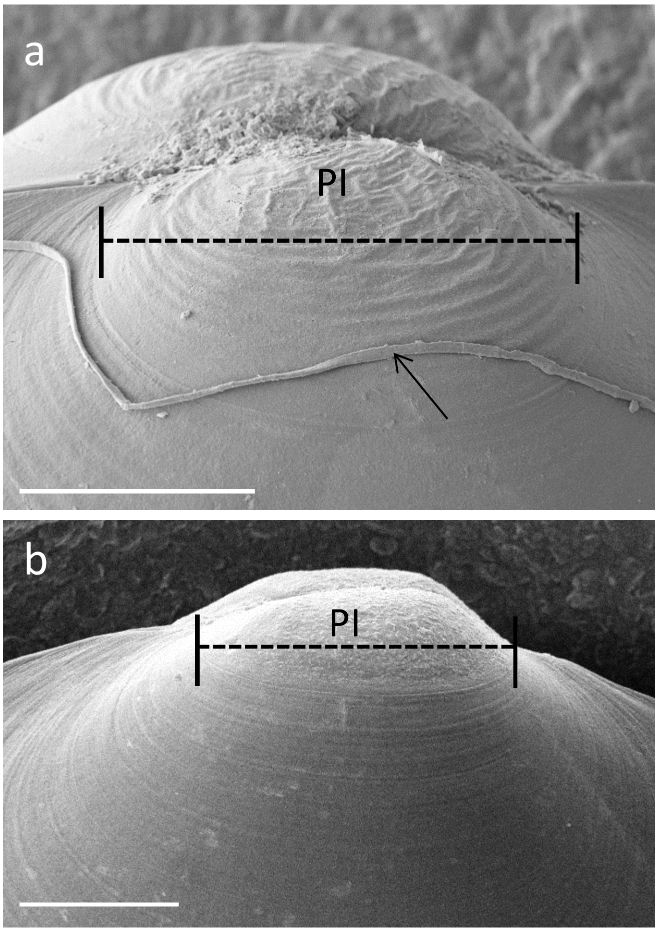

Figure 6.17 Prodissoconch I (PI) images taken under low vacuum Scanning Electron Microscopy. Arrow indicates artefact a) *Yoldiella valettei*; b) *Yoldiella ecaudata.* Scale bars = 100µm.

6.4 Discussion

The deep-water protobranchs *Yoldiella vallettei, Y. ecaudata*, and *Y. sabrina* are most similar to deep-sea *Yoldiella* spp. (Allen et al. 1995), *Ledella* spp. (Allen and Hannah 1989) and *Portlandia* spp. from the Atlantic (Killeen and Turner 2009) respectively, and are likely to represent these three genera in future studies. Reproduction within these genera is not well known; the closest species taxonomically with known reproduction are *Ledella pustulosa* and *Yoldiella jeffreysi* from 2900m in the Rockall Trough, North Atlantic (Lightfoot et al. 1979; Tyler et al. 1992). Gonad morphology and anatomy in all studied species is similar to that described in Tyler et al (1992); external to the digestive gland and hind gut loop, but histology also revealed oocytes and testis formation internally to the digestive gland, and dorsally above the stomach.

Species	Location & Date	Depth (m)	male/female	Max. oocyte size (µm)	n
Yoldiella ecaudata	Amundsen Sea March	500	3/6	130.4	9 (15)
Yoldiella sabrina	Amundsen Sea March	500	3/1	?	6 (10)
Yoldiella sabrina	Scotia Sea February	200-500	6/5	55.68	12 (17)
Yoldiella sabrina	Scotia Sea February	3500	2/1	187.9	3 (5)
Yoldiella sabrina	South Atlantic February	4730	3/1	132.0	4 (5)
Yoldiella valettei	Scotia Sea February	1000	9/12	120.6	21 (34)

Table 6.1 Species collection and reproductive data for *Yoldiella ecaudata, Yoldiella sabrina*, and *Yoldiella valettei* from the Southern Ocean. Braketed 'n' indicates total number of animals used to get sex ratio data (n).

The anatomy of *Yoldiella ecaudata* appears most similar to *Ledella pustulosa* with the characteristic extensive stomach and hind gut coil on both the left and right sides (Allen and Hannah 1989). Gonad morphology and oocyte size is also similar with *L. pustulosa* having a maximum oocyte size of 120µm (Tyler et al. 1992) while *Y. ecaudata* in this study have maximum oocyte size of 130.4µm (Table 1). Likewise, the similar Southern Ocean *Yoldiella valettei* and North Atlantic *Y. jeffreysi* have maximum oocyte sizes of 120.6µm and 120µm respectively. This study only represents a 'snapshot' of the reproduction in these species but these comparisons of maximum oocyte size with regularly sampled material from the North Atlantic may infer that the largest oocytes observed are likely to be mature ova. Prodissoconch measurements of *Y. valettei* and *Y. ecaudata* together with the oocyte sizes between 85 and 140µm suggest a pelagic lecithotrophic development (Ockelmann 1965), as found in other deep-sea protobranch bivalves (Tyler et al. 1992; Scheltema and Williams 2009).

Yoldiella sabrina studied over a range of depths and broader geographic range generally show a higher proportion of males which contrasts to *Y. ecaudata* and *Y. valettei* which have were observed to have one male to two females (Table 1). Although consistent over depth and distribution, the small number sampled make interpretation of this uncertain. Maximum oocyte size for *Y. sabrina* of 187.9µm is larger than observed in *Y. ecaudata* and *Y. valettei* and is close to the predicted range for direct development (Ockelmann 1965). The mean prodissoconch I measurements of *Y. sabrina* from published data suggest a length of approximately 200µm with a barely discernible prodissoconch II (Hain and Arnaud 1992), which would however, support lecithotrophic larval development.

Seasonality in reproduction can be difficult to interpret from samples collected at limited times of the year but given the difficulties in obtaining Southern Ocean deep-sea bivalves in ice-free conditions, any evidence of seasonal or continuous reproduction provide a valuable insight into the wider reproductive picture. Lecithotrophic larval development could be uncoupled from seasonality of food supply in the Antarctic benthos as found in the asteroid *Porania* sp. (Bosch and Pearse 1990). Protobranch bivalves are specially adapted to living in food-impoverished conditions (Allen 2008) reducing the effect of food limitation into the deep-sea (Gage and Tyler 1991). Cold Antarctic deep-sea conditions can leave high levels of organic matter in sediments acting as an annual food supply to deposit feeding fauna (Mincks et al. 2005; Glover et al. 2008), further reducing any effect of seasonality. This study found variation in oocyte size within individuals of *Y. ecaudata* and *Y. valettei*, which may be representative of asynchronous oocyte development within individual specimens, as found for *Y. jeffreysi* in the North Atlantic. The smaller egg size range in *Y. ecaudata* from 500m in the Amundsen Sea between specimens could represent an earlier stage of oocyte development at smaller sizes. Oogenesis is often slow in molluscs taking between 12 and 24 months (Picken 1979, 1980; Richardson 1979; Brey and Hain 1992; Higgs et al. 2009; Reed et al. 2013) and multiple cohorts may exist within individuals. The smaller eggs seen in some individuals may represent a second cohort of eggs being produced simultaneously to the current cohort. The differences observed in egg size and depth in *Y. sabrina* could indicate a difference in seasonality of reproduction with depth or an earlier stage of oocyte development with smaller shell size, however, the bathymetric differences between samples may also have a role in the timing and tempo of reproduction at the benthos.

Pelagic lecithotrophic larval development not only has the advantage of being uncoupled with primary productivity, but also offers the potential for long periods of dispersal, essential for maintaining a homogenous circum-Antarctic distribution. Bosch and Pearse (1990) determined the development period of the demersal and pelagic lecithotrophic asteroids *Porania* sp. and *Acodontaster hodgsoni* to be 78 and 106 days, respectively. A study into the metabolic rates and biomass of larvae and eggs in Antarctic echinoderms suggested larval survival over extended periods in the Antarctic, contrasting to weeks in similar temperate species (Shilling and Manahan 1994). Although untested in the field, the large egg size of the Antarctic protobranch bivalves may also provide the energetic requirements for long periods of dispersal time facilitating the potentially wide dispersal of these species in the Southern Ocean. Deep Antarctic waters have complex currents driven by cold bottom water formation in the Weddell Sea and the flooding of warmer waters from the Pacific. Together with the Antarctic circum-polar current, continental counter currents and large scale eddy formation characteristic of the Weddell and Scotia Seas, larval dispersal has great potential if suspension and survival can be maintained in the pelagic environment.

The occurrence of hermaphroditism is rare in deep-sea protobranchs (Zardus 2002) although not unusual in Southern Ocean molluscs and bivalves, globally. Simultaneous and sequential hermaphroditism has been described in *Laternula elliptica* (Bosch and Pearse 1988), *Thracia meridonalis* (Sartori and Domaneschi 2005), *Mysella charcoti* and *M. narchii* (Passos and Domaneschi 2009), *Lissarca notorcadensis* (Prezant et al. 1992) and *Lissarca miliaris* (Chapter 3, Reed et al. 2013). In the deep-sea context, simultaneous hermaphroditism may have an important role in the successful radiation of *Y. valettei*, increasing the likelihood of successful fertilisation in areas of low species abundance.

Conclusions

Yoldiella ecaudata, *Y. sabrina*, and *Y. valettei* all show evidence of lecithotrophic larval development and gonad morphology consistent with what is known for other species of deep-sea protobranch bivalves in the Atlantic Ocean. Large egg size is likely to facilitate the wide dispersal of larvae in deep-water currents and larvae have the potential to survive for long periods on the energy reserves from the egg. There are no observed differences in egg size to other deep-sea protobranch bivalves. Large egg size, often described in deep-sea benthic invertebrates, enables the uncoupling from primary production, and may be driving continuous or quasi-continuous reproduction. Simultaneous hermaphroditism observed in *Y. valettei* may have an important role in the success of this species in its radiation around the Southern Ocean. From an evolutionary perspective, such reproductive traits will have enabled fast recolonisation of the Antarctic continental shelf during ice retreat and in the presence of changing ice free benthic environments, a non-feeding wide dispersing larval type to disperse. Given the contrasting morphology and anatomy of these three *Yoldiella* species and similarities with different protobranch genera, a thorough review of the protobranch taxa of the Southern Ocean should be completed to understand evolutionary links to other deep-sea basins.

6.5 Chapter Summary

Deep-water Southern Ocean protobranchs demonstrate large egg size indicative of lecithotrophic larval development.

- *Yoldiella ecaudata* appears similar in morphology to *Ledella* spp. and has egg sizes of up to 130.4µm in the Amundsen Sea at 500m, comparable to the North Atlantic *Ledella pustulosa*.

- *Yoldiella sabrina* appears similar in morphology to *Portlandia* spp. and has oocytes up to 187.9 at 3500m in the Scotia Sea, and potential asynchronous development or differences in seasonality between bathymetry.
- *Yoldiella valettei* is appears similar to other *Yoldiella* spp. and has oocytes comparable to *Yoldiella jeffreysi* from the North Atlantic. This species also demonstrates simultaneous hermaphroditism, a rarely described reproductive trait in deep-sea protobranchs.

Chapter 7

Spatial plasticity in shell morphology and growth in the deep-water protobranch bivalves *Yoldiella ecaudata*, *Yoldiella sabrina*, and *Yoldiella valettei* from contrasting Southern Ocean environments

Study Published as: Reed AJ, Morris JP, Linse K, Thatje S (*accepted*) Plasticity in shell morphology and growth among deep-sea protobranch bivalves of the genus *Yoldiella* (Yoldiidae) from contrasting Southern Ocean regions. Deep-Sea Research Part 1

Abstract

The ecology of Antarctic deep-sea fauna is poorly understood in comparison to other deep-sea environments and few studies have gone beyond assessing biodiversity when comparing deep regions of the Southern Ocean. Protobranch bivalves are ubiquitous in the deep ocean and are widely distributed in the Southern Ocean. This chapter examines potential responses to environmental differences in the common protobranchs *Yoldiella valettei*, *Yoldiella ecaudata*, and *Yoldiella sabrina* from contrasting deep-sea environments of the Weddell Sea, Scotia Sea, Amundsen Sea, and South Atlantic. There are significant differences in morphology between deep-sea regions in all species and a significant difference in shell weight in *Y. valettei* between the Amundsen Sea and Weddell Seas. Growth rates of *Y. valettei* and *Y. ecaudata* in the Amundsen Sea are also higher than elsewhere and *Y. valettei* have heaviest shells in the Amundsen Sea, suggesting more favourable conditions for calcification and growth. The plasticity observed among deep-sea regions in the Southern Ocean is likely to be driven by different oceanographic influences affecting temperature and food fluxes to the benthos, and demonstrate the species ability to differentially adapt between stenothermal environments. This study suggests that subtle changes in the environment may lead to a divergence in the ecology of invertebrate populations and showcases the protobranch bivalves as a future model group for the study of speciation and radiation processes through cold-stenothermal environments.

7.1 Introduction

Despite an increase in sampling programmes, Antarctic deep-sea ecosystems remain poorly understood, with few studies investigating the ecology of deep benthic fauna beyond their taxonomic compositions (Brandt et al. 2007). A taxonomic group consistently found from the Antarctic shelf to the deep-sea are the protobranch bivalves (Cattaneo-Vietti et al. 2000; Arnaud et al. 2001; Linse 2004) a subclass with a global distribution to depths below 6000m in all deep-sea basins (Allen 1978; Allen and Saunders 1996; Allen 2008). The protobranchs are of interest to deep-sea biologists as they have a long evolutionary history and physiological characteristics, which enable them to thrive in high abundances and play an important role in the turnover of surface sediments (Allen 1978). Key adaptations are a gill structure exclusively used for respiration, modified palps for feeding and long digestive systems to process huge quantities of fine sediment in areas of impoverished food supply (Zardus 2002; Allen 2008). In the Antarctic, protobranch bivalves are well represented, although the ecology of the deep-water species remain unknown and only the common shallow water *Yoldia eightsii* has been studied in any detail (Davenport 1988; Peck and Bullough 1993; Peck et al. 2004)

Phenotypic plasticity is a common trait in marine intertidal molluscs (e.g. Vermeij 1973; Nolan 1991; Trussell 2000; Bayne 2004; Sousa et al. 2007) where adapting to different environmental conditions may increase their chances of survival and successful dispersal. In the deep sea, morphological analysis has been focussed on gastropods where species identification is often more reliable (Etter and Rex 1990; Rex and Etter 1990; Iguchi et al. 2005). However, the deep-sea protobranch bivalves make a good model group to examine morphological plasticity due to their large geographical ranges. Plasticity in protobranchs has been observed by changes in morphology over short geographical distances and bathymetry in the Rockall Trough (Fuiman et al. 1999) and can be evidence of distinct ecotypes or even cryptic species; however, sample sizes are often too low for detailed morphological studies to be undertaken. The Antarctic remains an interesting place to study plasticity as much of the benthic environment is considered to be extreme in its thermal stability and highly seasonal pulsed food supply from phytoplankton blooms (Clarke 1988; Barnes et al.

2006a; Clarke et al. 2009). Assessing the extent whereby benthic fauna respond to subtle environmental differences between regions is helpful in the understanding of evolutionary processes that have maintained these populations in the Antarctic, and to predict the impacts of future changes.

With the exception of chemosynthetic species, growth data of deep-sea bivalves are also sparse. Some commonly found Atlantic species have been studied, such as *Nuculana pernula* and *Yoldia thraciaeformis* (Hutchings and Haedrich 1984), *Tindaria callistiformis* (Turekian et al. 1975) and *Ledella pustulosa* (Gage 1994), but only species that exhibit seasonal growth can be accurately studied and many deep-sea protobranchs appear to have no obvious seasonal banding of their thin shells (Zardus 2002). Growth estimates from these studies suggest 5-12 years may be the normal lifespan for deep-sea protobranchs, although *T. callistiformis* may grow for up to 100 years (Turekian et al. 1975). Inconsistent food supply is likely to be the limiting factor for growth in many species (Gage and Tyler 1991). The only reported Antarctic protobranch growth data are from the shallow water *Yoldia eightsii*, which can live in excess of 50 years (Nolan and Clarke 1993; Peck and Bullough 1993; Peck et al. 2000).

The protobranch bivalves *Yoldiella sabrina* (Hedley 1916)*, Yoldiella ecaudata* (Pelseneer 1903), and *Yoldiella valettei* (Lamy 1906), are commonly found from the Antarctic shelf to the deep-sea basins with a wide bathymetric range (200 – >4000m) and known distributions from the east Weddell shelf, Scotia Sea, Antarctic Peninsula, and Amundsen Sea. In addition, *Y. sabrina* has a distribution into the South Atlantic (Linse et al. 2007) and all three species are likely to have circum-Antarctic distributions. Although all studied species are officially described as *Yoldiella*, the taxonomy of this genus is complex and has been described as a taxon 'waiting list' requiring revision (La Perna 2004). However, for the purpose of this study, all species will be regarded as *Yoldiella* (see Chapter 1 for discussion).

Aims

This study aims to examine and compare the shell morphology, growth, and weight of *Yoldiella ecaudata, Yoldiella sabrina*, and *Yoldiella valettei* among Antarctic regions. Growth data will provide novel insights into the potential age and growth rates of deep-sea Antarctic protobranchs and morphological plasticity is discussed in relation to the environmental constraints associated with the deep Southern Ocean.

Hypothesis

H_1 *Yoldiella ecaudata, Yoldiella sabrina,* and *Yoldiella valettei* will demonstrate morphological plasticity relating to differing environmental conditions.

H_2 *Yoldiella ecaudata, Yoldiella sabrina,* and *Yoldiella valettei* will demonstrate differences in growth rates and overall growth performance among populations driven by different environmental conditions.

Objectives

O_1 Measure and compare the morphology in shell shape and weight among different populations of *Yoldiella* spp. to determine phenotypic plasticity among regions.

O_2 Measure and compare the growth rates and maximum size among populations of *Yoldiella* spp**.** to infer ecophysiological plasticity.

7.2 Materials and Methods

Sample Collection

Yoldiella valettei, Y. ecaudata and *Y. sabrina* were collected during a number of research expeditions to the Weddell Sea, Scotia Arc, Antarctic Peninsula and Amundsen Sea between 2002 and 2012 as described in Chapter 2.1 and Figure 2.6. Size frequency histograms are plotted for each species in each area. A Kolmogorov Smirnov test was used to identify differences between size frequency distributions.

Morphometric Analysis

Shell length, height, and width of each individual were measured under a stereomicroscope to an accuracy of +/- 0.05mm using a micrometre lens as described in Chapter 2.2.

In total 1050 individuals of *Y. valettei* were measured from the Scotia Sea (189-4679m), Weddell Sea (273-4655m) and Amundsen Sea (477-1473m), 636 *Y. ecaudata* from the Scotia Sea (496-3400m), Weddell Sea (2147m), and Amundsen Sea (477-1495m) and 506 *Y. sabrina* from the Scotia Sea (192-505m), Weddell Sea (600-4900m), Amundsen Sea (500m) and South Atlantic (4552-4730m) (Appendix 3). In all areas under investigation, highest abundances of bivalves were obtained from shelf depths (<500m). Morphology data were pooled between depths within areas when there was no significant difference in any of the characters studied found with depth. When the morphological data showed significant statistical differences across the depth gradient, only shelf depth samples were used for comparisons. Reduced major axis regression of log transformed data was used to identify the relationships between length, height and width. Factorial analysis of covariance (ANCOVA) on normally distributed log transformed data was used to test for differences between areas and a back transformation of mean corrected values used to display morphological differences. A Tukey test was performed to identify differences between populations.

Growth Analysis

Growth data for *Y. valettei* from the Weddell and Amundsen Seas at 500m and *Y. ecaudata* from the Scotia and Amundsen Seas at 500m were collected by counting growth rings under a stereomicroscope as described in Chapter 2.4. To assess fine growth rings, 20 individuals of each species from each area, covering the full size range of the sample, were selected for analysis using a Scanning Electron Microscope (SEM) as described in Chapter 2.3. Prior to the scans, each individual was blotted dry and mounted onto SEM stubs before being pulse sputter coated with a 40nm gold coat. Growth ring counts from SEM analysis closely matched the count taken during stereomicroscope analysis and the rings resolved were assumed to represent annual growth increments (Gage 1994), especially given the highly seasonal variation in food supply in the Southern Ocean (Gutt et al. 2008). No growth data could be obtained for *Y. sabrina* as growth increments could not be counted with confidence. A von Bertalanffy growth function was applied to the count data and overall growth performance (OGP) calculated as described in Chapter 2.4.

Shell Weights

Shell weights were calculated by weighing specimens before and after decalcification in rapid decalcification solution (HCl) for 5 minutes or until effervescence had stopped. Where possible at least 20 individuals were used for each location. *Y. sabrina* from the Amundsen Sea (n=22, depth 500-548m), South Atlantic (n=15, depth 4730-4760m) and

Scotia Sea (n=18, depth 211-3404); *Y. ecaudata* from the Amundsen Sea (n=26, depth 482-1477m) and Weddell Sea (n=22, 2097-3000m); and *Y. valettei* from the Amundsen Sea (n=22, depth 476-492m), Scotia Sea (n=22, depth 307m) and Weddell Sea (n=22, depth 296-910m). Depths were pooled within area where no statistical differences by depth could be found. Reduced major axis regression of log transformed data was used to identify the relationships between length and weight. A factorial ANCOVA on normally distributed log transformed data was used to test for differences between areas and a Tukey test was performed to identify differences between populations where the ANCOVA found a significant result.

7.3 Results

Size Frequencies

The size frequency histograms show variability in population structure between sites (Figure 7.1). Populations of *Y. valettei* in the Scotia and Amundsen Seas were significantly different (k-s, D=0.283, $p<0.05$), have distributions with a peak at around 1mm length, and few specimens above 3.5mm length but maximum sizes of >5mm at each site (Figure 7.1e, f). The Weddell Sea population is dominated by 1.0-1.1mm specimens with few specimens above 2.5mm and no specimens greater than 3mm length (Figure 7.1g). There is a significant difference the Amundsen and Weddell Seas (k-s, D=0.377, $p<0.001$), but not the Scotia and Weddell Seas (k-s, 0.245, p=0.07). *Yoldiella ecaudata* size frequency distributions are significantly different between the Amundsen Sea and Scotia Sea (k-s, D=0.3785, $p<0.001$), and appear similar to *Y. valettei*, dominated by specimens at 1.2-1.3mm shell length (Figures 7.1a, b). The size frequency distributions of *Y. sabrina* show a smaller-sized population in the Amundsen Sea compared to the Scotia Sea (Figures 7.1c, d) although no significant difference in frequency is found (k-s, D=0.173, p=0.19). Few specimens above 4mm are found in the Amundsen Sea samples and only one specimen above 8.5mm compared to specimens up to 11.2mm in the Scotia Sea.

Growth

Yoldiella valettei grow faster than *Y. ecaudata* to a maximum length of 5.2mm with seven growth increments compared to a maximum of 3.3mm with seven growth increments. Assuming growth increments represent a constant time increment, both species demonstrate slower growth than the three filter feeding philobryid bivalves *Adacnarca nitens* (Higgs et al. 2009)*, Lissarca notorcadensis* (Brey and Hain 1992), and *Lissarca miliaris* (Richardson 1979) from the Ross Sea, Weddell Sea and Scotia Sea, respectively (Figure 7.2a). The von Bertalanffy growth function suggest both protobranch species show higher growth rates in the Amundsen Sea (*Y. valettei* S_{oo}=4.87, k=0.293, n=91, *Y. ecaudata* S_{oo}=3.63, k=0.441, n=52) than in the Weddell Sea (*Y. valettei* S_{oo}=3.76, k=0.357, n=17) and Scotia Sea (*Y. ecaudata* S_{oo}=4.70, k=0.184, n=15). The overall growth performance (OGP) for *Y. valettei* and *Y. ecaudata*, derived from the parameters of the von Bertalanffy growth function, is higher in the Amundsen Sea (OGP = 0.154 and 0.204 respectively) than the Weddell Sea and Scotia Sea (OGP = 0.127 and 0.063 respectively) and higher than the Antarctic philobryid bivalves (Figure 7.2b). All OGP values in this study are lower however, than the similar sized North Atlantic, deep-sea protobranch *Ledella pustulosa* (OGP = 0.299; Gage 1994).

Shell Morphology

All three protobranch bivalves showed a significant positive linear relationship between length and height (Figures 7.3a-c) with r^2 values greater than 0.933 ($p<0.001$) and demonstrated positive and negative allometry and isometry (Table 7.1). A preliminary ANCOVA found significant differences with depth within some regions. *Yoldiella valettei* below 1000m in the Amundsen Sea (n=93) were significantly different to those collected at shelf depths above 538m (n=398) for both length/height ($F_{1, 488}$=36.62, $p<0.001$), and length/width ($F_{1, 486}$=37.97, $p<0.001$), and therefore were not used for the morphological comparisons. The deepest samples of *Y. sabrina* below 3404m (n=25) were also significantly different from specimens from the shelf, at 192-505m (n=156) in the Scotia Sea ($F_{1, 178}$=7.52, $p<0.05$) and were not used for length/height comparisons.

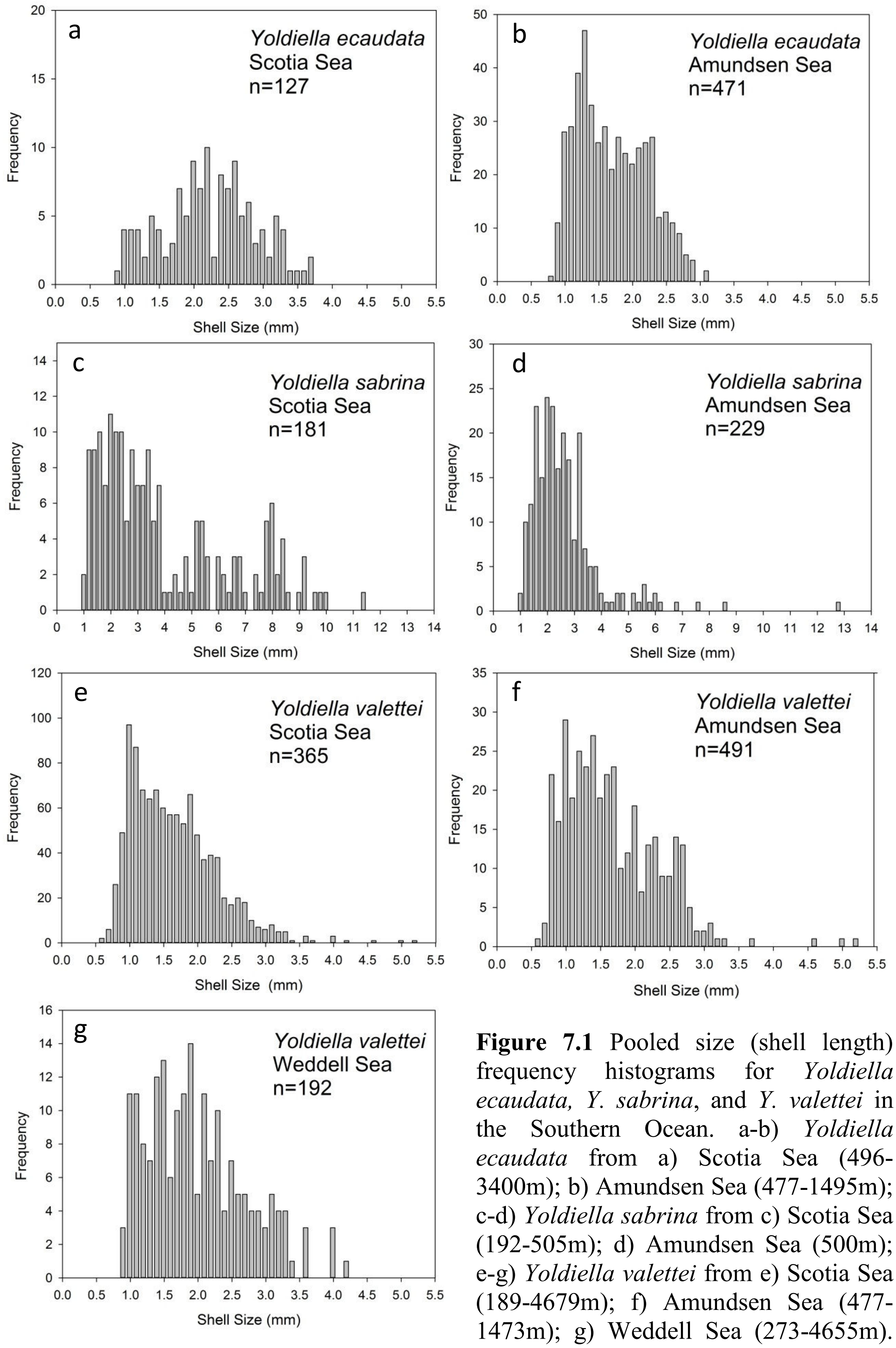

Figure 7.1 Pooled size (shell length) frequency histograms for *Yoldiella ecaudata, Y. sabrina*, and *Y. valettei* in the Southern Ocean. a-b) *Yoldiella ecaudata* from a) Scotia Sea (496-3400m); b) Amundsen Sea (477-1495m); c-d) *Yoldiella sabrina* from c) Scotia Sea (192-505m); d) Amundsen Sea (500m); e-g) *Yoldiella valettei* from e) Scotia Sea (189-4679m); f) Amundsen Sea (477-1473m); g) Weddell Sea (273-4655m). **N.B.** Difference in scale among graphs.

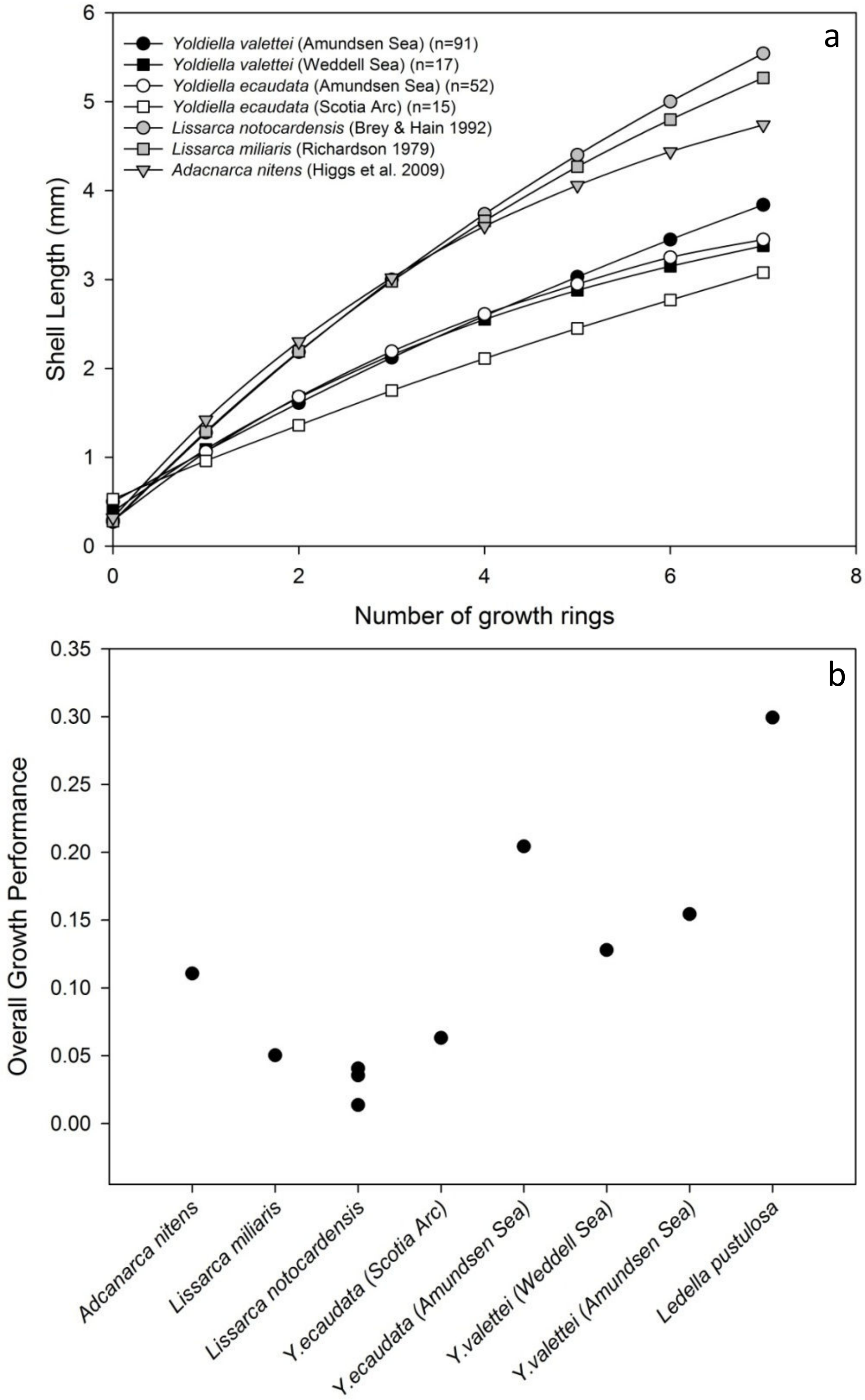

Figure 7.2 Growth and overall growth performance in Southern Ocean protobranchs compared to filter feeding lamellibranch bivalves *Lissarca notorcadensis* (Brey and Hain 1992) *Lissarca miliaris* (Richardson 1979) and *Adacnarca nitens* (Higgs et al. 2009). a) Bertalanffy growth curves for *Yoldiella valettei* from the Weddell Sea (S_∞=3.76, k=0.357, n=17) and Amundsen Sea (S_∞=4.87, k=0.293, n=91), *Yoldiella ecaudata* from the Scotia Sea (S_∞=4.70, k=0.184, n=15) and Amundsen Sea (S_∞=3.63, k=0.441, n=52); b) overall growth performance of *Yoldiella valettei* from the Weddell Sea and Amundsen Sea and *Yoldiella ecaudata* from the Scotia Sea and Amundsen Sea compared to Antarctic lamellibranch bivalves and the North Atlantic protobranch *Ledella pustulosa* (Gage 1994).

An analysis of covariance of length/height found a significant difference between areas for *Y. valettei* ($F_{2,\ 951}$=15.66, p<0.001) and a Tukey multiple comparison test found the Amundsen and Scotia Seas to be significantly different to the Weddell Sea (p<0.05) (Figure 7.3f). Significant differences were also found between areas in *Y. ecaudata* ($F_{2,\ 630}$=26.40, p<0.001) and *Y. sabrina* ($F_{3,\ 453}$=5.22, p<0.001), with the Scotia Sea being distinct from the Amundsen and Weddell Seas in *Y. ecaudata* (p<0.05) (Figure 7.3d), and Weddell Sea being distinct from the Amundsen Sea, Scotia Sea, and South Atlantic in *Y. sabrina* (Figure 7.3e) (p<0.05).

The relationships between length and width also showed positive linear relationships (Figure 7.4a-c). *Y. valettei* length and width showed a significant difference between areas ($F_{2,\ 940}$=3.57, p<0.05) and a Tukey multiple comparison test identifying the Amundsen and Weddell Seas as being significantly different (Figure 7.4f). There was a significant difference between Amundsen Sea, Scotia Sea, and Weddell Sea for *Y. ecaudata* (ANC OVA, $F_{2,\ 629}$=43.21, p<0.001, post hoc Tukey test p<0.05) (Figure 7.4d) and *Y. sabrina* showed a significant difference between the Amundsen Sea, Weddell Sea, and Scotia Sea from the South Atlantic (ANCOVA. $F_{3,\ 477}$=13.10, p<0.001, post hoc Tukey test p<0.05) (Figure 7.4e).

Differences in morphology are visually represented in Figure 7.5 by the corrected mean length/height against corrected mean length/width, derived from residuals of associated ANCOVA, demonstrating variability among populations of species. *Yoldiella ecaudata* and *Y. sabrina* both have the most round and obese shell shape in the Weddell Sea which contrasts to *Y. valettei* with the thinnest elongated shell shape in the Weddell Sea. The Scotia Sea has the thinnest elongate shells of all the *Y. ecaudata* sampled. All *Y. sabrina* in the Southern Ocean are more obese in shell shape than the specimens from the South Atlantic although vary in elongation, while *Y. valettei* from the Scotia and Amundsen Seas are the most rounded and obese.

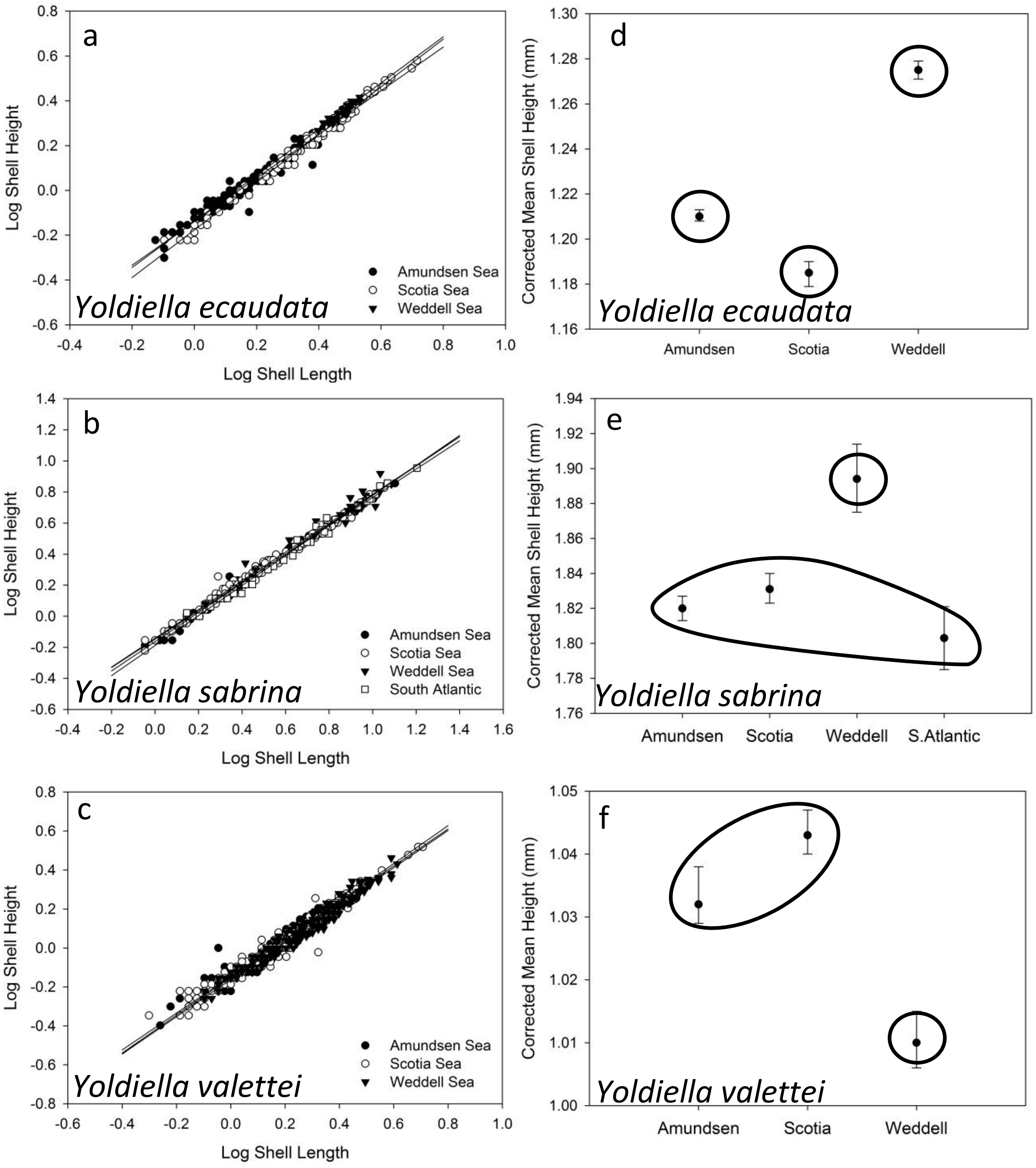

Figure 7.3 a-c) Linear regressions of length and height in *Yoldiella ecaudata* and *Y. valettei* in the Amundsen Sea, Scotia Sea and Weddell Sea, and *Y. sabrina* in the Amundsen Sea, Scotia Sea, Weddell Sea and South Atlantic; d-f) Adjusted means from ANCOVA of length/height in d) *Yoldiella ecaudata* from the Amundsen Sea, Scotia Sea and Weddell Sea; e) *Yoldiella sabrina* from the Amundsen Sea, Scotia Sea, Weddell Sea and South Atlantic; f) *Yoldiella valettei* from the Amundsen Sea, Scotia Sea and Weddell Sea. Circles represent significantly different groups (Tukey test, $p<0.05$).

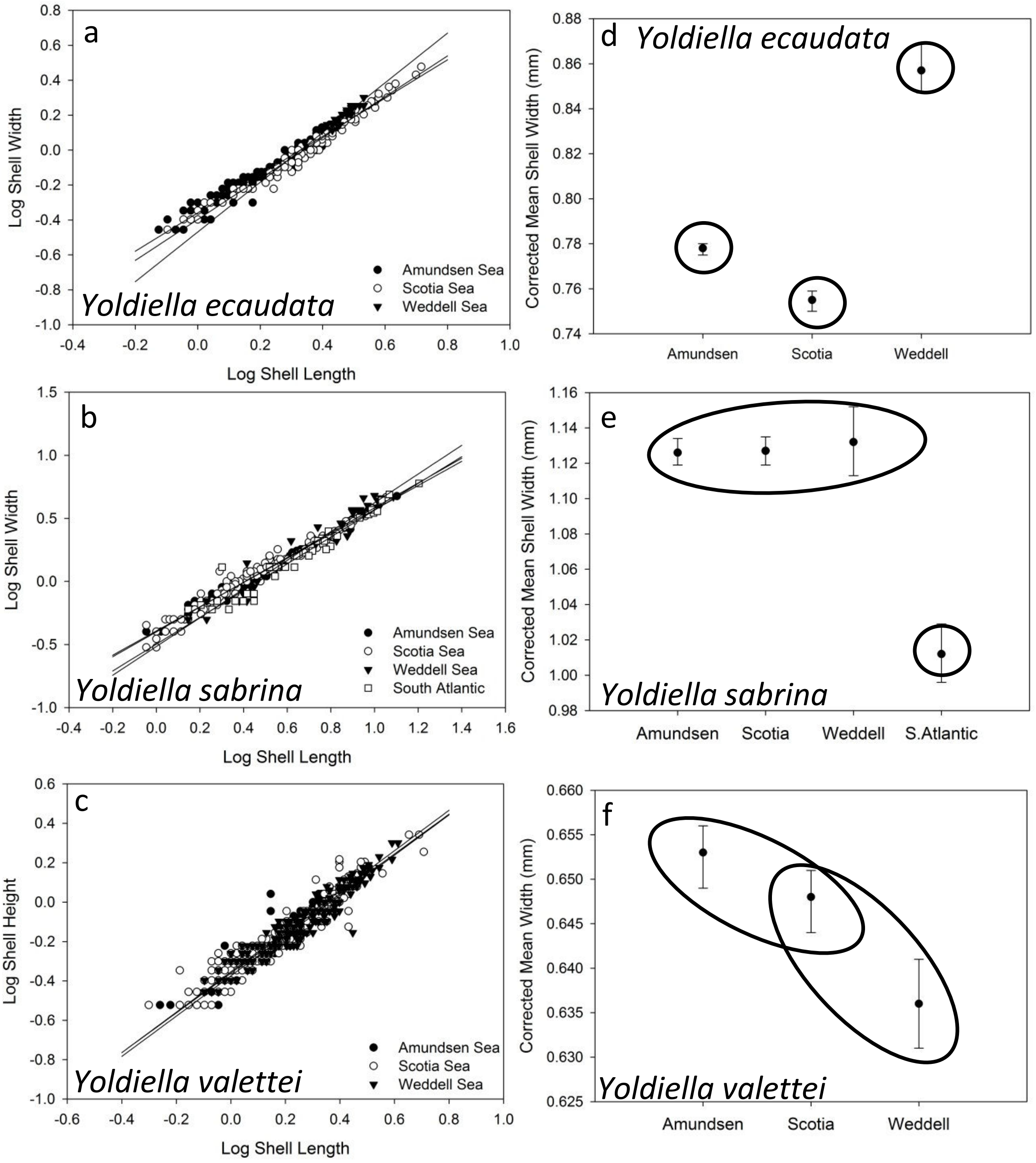

Figure 7.4 a-c) Linear regressions of length and width in *Yoldiella valettei* and *Y. ecaudata* in the Amundsen Sea, Scotia Sea and Weddell Sea, and *Y. sabrina* from the Amundsen Sea, Scotia Sea, Weddell Sea and South Atlantic; d-f) Adjusted means from ANCOVA of length and width in d) *Yoldiella ecaudata* in the Amundsen Sea, Scotia Sea and Weddell Sea; e) *Yoldiella sabrina* in the Amundsen Sea, Scotia Sea, Weddell Sea and South Atlantic; f) *Yoldiella valettei* from the Amundsen Sea, Scotia Sea and Weddell Sea. Circles represent significantly different groups (Tukey test, $p<0.05$).

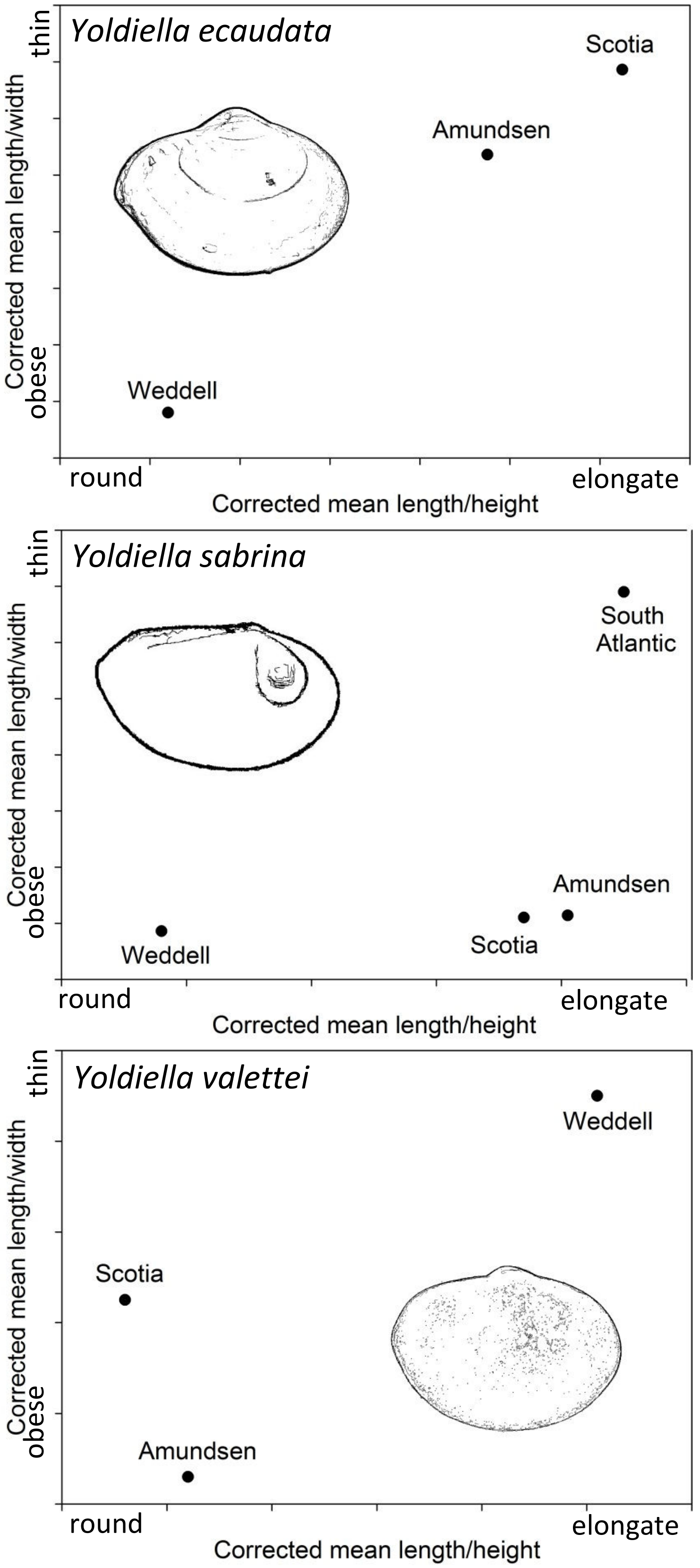

Figure 7.5 Relative morphology of *Yoldiella ecaudata, Yoldiella sabrina*, and *Yoldiella valettei*. Corrected mean length/height v corrected mean length/width among populations identifying different shell shapes.

Species	Area	length/height RMA equation	r^2	allometry/isometry	length/width RMA equation	r^2	allometry/isometry
Yoldiella ecaudata	Amundsen Sea	$-0.13919+0.9739x$	0.975	- allometry	$-0.35962+1.0961x$	0.967	+ allometry
	Scotia Sea	$-0.17637+1.0644x$	0.989	+ allometry	$-0.39712+1.1712x$	0.988	+ allometry
	Weddell Sea	$-0.13816+1.0298x$	0.933	isometry	$-0.46865+1.4237x$	0.900	+ allometry
Yoldiella sabrina	Amundsen Sea	$-0.16355+0.9437x$	0.991	- allometry	$-0.39221+0.9609x$	0.981	- allometry
	Scotia Sea	$-0.1478+0.9107x$	0.994	- allometry	$-0.39978+0.9839x$	0.978	isometry
	Weddell Sea	$-0.14204+0.9271x$	0.974	- allometry	$-0.51555+1.1397x$	0.956	+ allometry
	South Atlantic	$-0.18881+0.9668x$	0.998	isometry	$-0.49699+1.0621x$	0.940	isometry
Yoldiella valettei	Amundsen Sea	$-0.15761+0.9959x$	0.969	isometry	$-0.36431+1.0582x$	0.925	+ allometry
	Scotia Sea	$-0.14549+0.9452x$	0.970	- allometry	$-0.36072+1.0091x$	0.924	isometry
	Weddell Sea	$-0.16197+0.9567x$	0.970	- allometry	$-0.37439+1.0265x$	0.912	isometry

Table 7.1 Morphological parameters from reduced major axis regression showing growth type for *Yoldiella ecaudata*, *Yoldiella sabrina*, and *Yoldiella valettei* from different areas of the Southern Ocean. All r^2 values significant ($p<0.001$).

Shell Weight

Shell weight of *Y. valettei* shows a significant positive relationship between shell length and log weight (Figure 7.6b) (Scotia Sea r^2=0.969, Amundsen Sea r^2=0.916, Weddell Sea r^2=0.944) and have heavier shells in the Amundsen Sea making up 62.1% of total wet weight compared to 52% in the Scotia Sea and 37.1% in the Weddell Sea. Shell weight was isometric in all populations with a RMA slope of 3.19 (Amundsen Sea), 3.02 (Scotia Sea) and 2.74 (Weddell Sea). An analysis of covariance (ANCOVA) shows a significant difference between the regions ($F_{2, 60}$ = 8.40, p=0.001) with a post hoc multiple comparison test showing no significant difference between the Weddell and Scotia Seas while having a significant difference when compared separately to the Amundsen Sea (Figure 7.6d, Tukey test, p<0.05).

Yoldiella ecaudata in the Amundsen Sea were observed to have thicker and stronger sculptures in their shells compared to specimens from the Weddell and Scotia Seas. There was a significant positive relationship between shell length and log weight (Figure 7.6a) (Weddell Sea r^2=0.981, Amundsen Sea r^2=0.984) with both populations showing positive allometric regressions of 3.30 (Amundsen Sea) and 3.61 (Weddell Sea). There was however, no significant difference between the Weddell Sea and Amundsen Sea (ANCOVA $F_{1, 42}$ = 0.51, p=0.479, Figure 7.6e). Scotia Sea specimens were not studied because of lower sample ‘n’ number and unsuitable size distribution of shell lengths for analysis. *Yoldiella sabrina* also show significant positive relationships between shell weight and length (Scotia Sea r^2=0.938, Amundsen Sea r^2=0.950, South Atlantic r^2=0.954) (Figure 7.6b). All populations showed an isometric relationship between length and weight with RMA slope values of 2.98 (Amundsen Sea), 2.81 (Scotia Sea) and 2.88 (Weddell Sea). There was no significant difference between the three areas (ANCOVA $F_{2, 56}$ = 2.31, p = 0.109, Figure 7.6f) despite shell weight in the Scotia Sea made up only 45.3% of total weight compared to 56.6% and 62.0% in Amundsen Sea and South Atlantic, respectively.

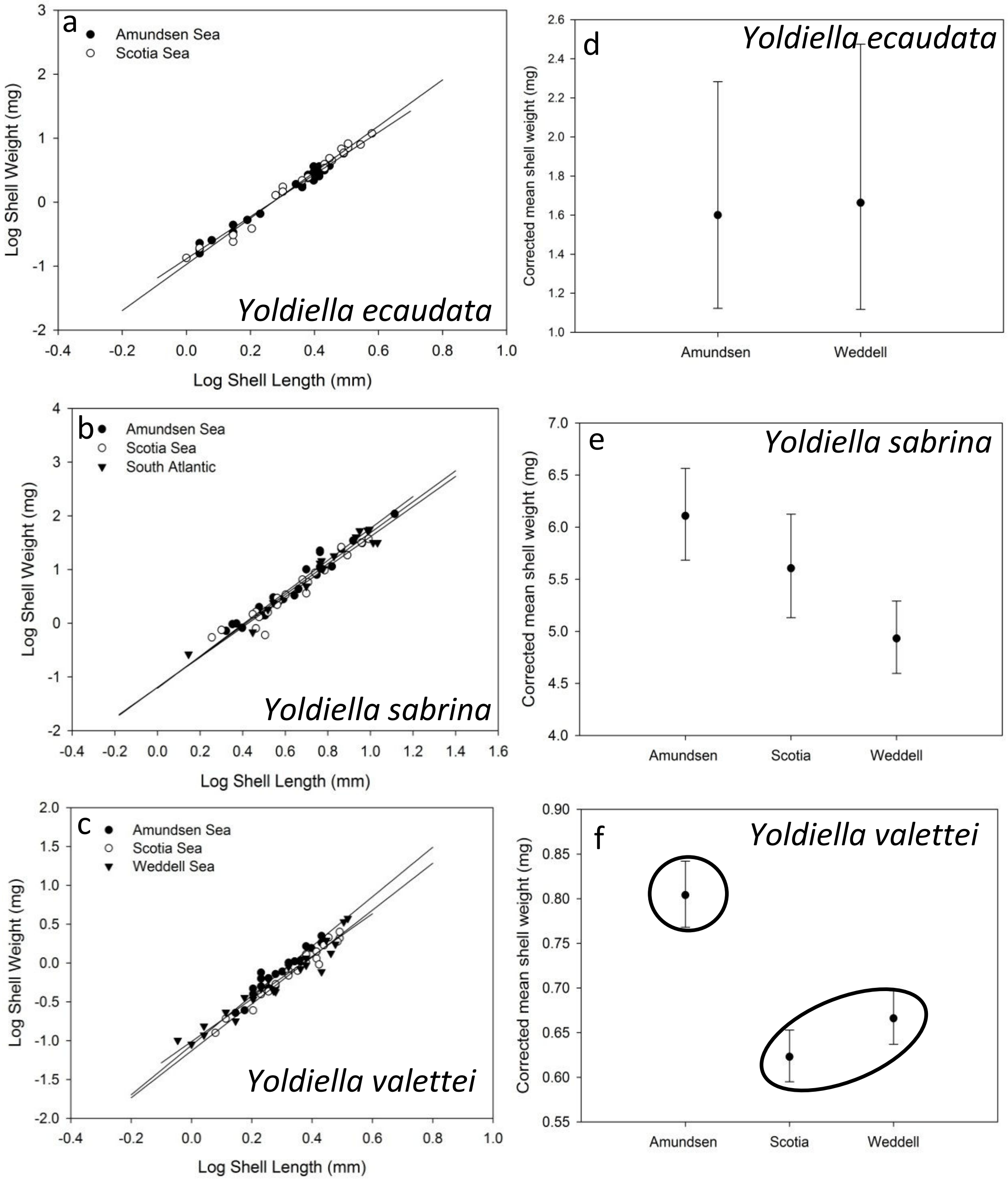

Figure 7.6 Reduced major axis regression of shell length and log shell weight with back transformed mean shell weights. a, d) *Yoldiella ecaudata* from the Amundsen Sea (n=26) and Scotia Sea (n=35); b, e) *Yoldiella sabrina* from the Amundsen Sea (n=16), Scotia Sea (22) and South Atlantic (n=15); c, f) *Yoldiella valettei* from the Amundsen Sea (n=22), Scotia Sea (n=22) and Weddell Sea (n=22). Circles represent significant differences ($p<0.05$).

7.4 Discussion

Phenotypic plasticity is well reported globally in molluscs and has been described in the Southern Ocean lamellibranch bivalve *Lissarca notorcadensis* (Cope and Linse 2006) and deposit feeding *Laternula elliptica* (Harper et al. 2012). In the Antarctic deep-sea, protobranch bivalve plasticity is important for our understanding of how species have come to radiate over large geographic distances and bathymetric ranges. This can also provide evidence of diverging ecologies, distinct ecotypes and give clues to potential speciation processes. The numbers of bivalve specimens used in this study are a considerable collection for the Antarctic deep-sea, representing ten years of deep-sea sampling effort. Representing a wide geographic range, this study highlights three distinct Southern Ocean regions which are under contrasting oceanographic and biological influences (see discussion below). These influences are the likely drivers of observed phenotypic plasticity and ecophysiological responses (through growth and shell production), and represent how the studied species can adapt to contrasting conditions.

Previous genetic studies on small deep-sea protobranchs have uncovered modest genetic structuring over large geographic distances at abyssal depths with evidence of restricted gene flow in the more heterogeneous bathyal depths or vast geographic distances in excess of 8000km (Etter et al. 2005; Zardus et al. 2006; Etter et al. 2011). The unique free-swimming lecithotrophic pericalymma larval form of protobranch bivalves are probably capable of dispersing long distances (Allen and Sanders 1996; Zardus 2002). This may be further pronounced in the cold Antarctic where larval development can be considerably slower (Pearse and Bosch 1986; Bosch and Pearse 1990), allowing longer times for dispersal. Strong currents associated with the Antarctic circum-Polar current and deeper water counter currents, may also enable significant dispersal potential in free-swimming larvae (for discussion see Thatje 2012). However, to fill a new niche and thrive in that environment, it is important to adapt under the selection pressures presented at settlement, and in the Antarctic, contrasting differences in selection may represent only subtle differences in the environment.

Growth and shell development are commonly dependent on food availability, temperature and disturbance events (Appeldoorn 1982; Brey et al. 2011; Harper et al. 2012; Chapter 5), and in the Antarctic deep-sea, the ability to extract calcium from seawater at low temperatures (Harper 2000). Food availability in the deep Antarctic can be highly variable (Gutt et al. 1998) and although data from the sea floor are very patchy there is strong evidence for high levels of seasonal supply to the benthos (Smith et al. 2008). The deep Weddell Sea can have a moderate to high seasonal flux of phytodetritus depending on distance from ice shelf edge and seasonal ice melt (Gutt et al. 1998; Diaz 2004). Open water polynyas are possible sources of deep-water food supply for deposit feeding organisms, especially from the Maudheim polynya (71°01'S, 10° 88°W) in the east Weddell Sea and the Pine Island Bay polynya (73° 16'S, 104° 25'W) in the Amundsen Sea (Arrigo and Dijken 2003; Yager et al. 2012). The Scotia Sea is also variable with regards to phytoplankton on both a spatial and temporal scale and is largely dependent on the mixing of water masses (Holm-Hansen et al. 2004).

Protobranch bivalves are specially adapted to living in food-poor conditions and despite evidence of food being limiting to growth in deep water (Gage and Tyler 1991), the differing fluxes of phytodetritus from the surface to the deep sea are likely to be insufficiently contrasting between regions to affect differences in growth and shell development (Allen 2008). Additionally, cold Antarctic deep-sea conditions can result in the persistence of organic matter in sediments and act as a year round food supply to deposit feeding fauna, dampening the effects of strong seasonal pulses of food (Mincks et al. 2005; Glover et al. 2008). When compared to the similarly sized deep-sea protobranch *Ledella pustulosa* (Gage 1994), the Antarctic species have lower overall growth performances, although this is not expected to be related to food availability. The similarity of growth and OGP to other Southern Ocean bivalves strengthens the assumption of seasonality of growth in the Southern Ocean protobranchs. Assuming food resource to be indistinct between study areas (although perhaps even greater in the Antarctic deep-sea compared to elsewhere), temperature is expected to have the strongest influence on growth and morphology in the deep Antarctic protobranchs.

Oceanographic influences on bottom temperature create different conditions between areas and this can affect the benthic fauna (Clarke et al. 2009). The Antarctic deep-sea is influenced by unique oceanographic conditions that are likely to play key roles in species distribution by larval dispersal, and creating heterogeneity of temperature. As well as feeding the world's oceans by circulating cold bottom water, the Weddell Sea produces some of the coldest deep waters in the world. Weddell Sea Bottom Water is highly influential in the Weddell and Scotia Seas as a stable, dense and extreme cold water mass as low as -1.88°C (Carmack and Foster 1975; Fahrback et al. 1995; Smith et al. 1999). Flowing west into the Scotia Sea, bottom water acts as an important counter current to the powerful Antarctic Circumpolar Current. The lack of differentiation in shell weights between the Weddell Sea and Scotia Sea in *Y. valettei* and *Y. ecaudata* may be a consequence of the similar bottom water temperature effect on calcification and shell formation in these regions. West of the Antarctic Peninsula to the Amundsen Sea, warmer circumpolar deep water (>1°C) can flood the deep (>500m) continental shelf accelerating the melting of ice along the West Antarctic Ice Shelf (Jacobs et al. 1996; Lowe and Anderson 2002; Thorma et al. 2008). These turbulent and complex oceanographic processes increase heterogeneity in a system that may otherwise be considered highly stenothermal and stable (Barnes et al. 2006a; Clarke et al. 2009). Small changes in temperature are known to cause dramatic changes in metabolic rates of Antarctic benthic invertebrates in shallow water (Peck et al. 2002; Peck et al. 2004) but to date there is no published work on metabolic rates of deep-sea Antarctic invertebrates (but see Chapter 8).

The differences in shell weights between the Scotia Sea and Weddell Sea with the Amundsen Sea are also likely to result from spatial heterogeneity in temperature, with the warmer Amundsen Sea temperatures increasing growth and shell weights. Low temperatures increase the cost of calcification and reduce metabolic rates (Peck et al. 2004), and increasing temperatures have been shown to increase growth rates of Antarctic bivalves (Nolan and Clarke 1993; Brey et al. 2011; Chapter 5). The effect of temperature can also be observed with a decrease in shell thickness with latitude in buccinid gastropods and echinoids while increasing shell thickness in the bivalve genus *Laternula* (Watson et al. 2012), where mechanical damage and associated shell repair increases calcification (Harper et al. 2012) at the cost of increased metabolic activity (Phillip et al. 2011). Heavier shells and faster growth observed in *Y. valettei* and *Y.*

ecaudata in the Amundsen Sea may therefore be a consequence of temperature effects on metabolic activity, lower energetic cost of calcification or an adaptation to disturbance events afforded by energy reallocation. Increased shell weights in the Amundsen Sea may also offer extra protection against invasive predators associated with regional warming (Aronson et al. 2007, 2011). No significant differences with depth were found within or between regions, despite comparing shallow shelf depths (from 211m) with abyssal depths in *Y. sabrina* (South Atlantic >4500m) and *Y. ecaudata* (Weddell Sea 2097-3000m).

Conclusions

Significant differences in morphology with depth (*Y. valettei* and *Y. sabrina*) and between regions suggest adaptations to contrasting conditions, which are key to the wide dispersal of a circum-Antarctic species and highlights the success of the protobranch bivalves in deep-sea ecosystems. Increased growth rates and overall growth performance for *Y. valettei* and *Y. ecaudata* in the Amundsen Sea over the Scotia and Weddell Sea are also evidence of environmental conditions being more favourable for growth (Heilmayer et al. 2004; Brey et al. 2011). The morphological variations found in this study demonstrate distinct ecotypes within species and although the degree of connectivity between these deep populations is still unknown, showcase the protobranch bivalves as a group for future molecular and ecological studies in the Antarctic, with special regard to likely cryptic speciation processes. Trade-offs associated with increased growth and calcification rates under different temperature regimes are also important in our understanding of the evolutionary history of the Antarctic benthos and to predict the response to regional warming.

7.5 Chapter Summary

There is evidence of phenotypic plasticity in the deep-water protobranch bivalves *Yoldiella ecaudata, Yoldiella sabrina* and *Yoldiella valettei* from the Southern Ocean. Evidence of divergent ecologies are;

- Significantly different relationships of length/height and length/width between contrasting environments within he Scotia, Amundsen and Weddell Sea resulting in different overall shell shapes.

- Difference in growth rate between contrasting populations of *Yoldiella ecaudata* and *Yoldiella vallettei,* although similar overall to other Southern Ocean bivalves.
- A difference in shell thickness in the Weddell Sea compared with the Amundsen and Scotia Seas in *Yoldiella valettei* suggesting a potential energetic trade-off associated with shell production (See also Chapter 4, Reed et al. 2012).

Chapter 8

Synopsis

8.1 Ecological plasticity of Southern Ocean bivalves from contrasting environments

The investigation presented here is the most extensive study into plasticity and ecological divergence of Antarctic invertebrates to date. Where Antarctic benthic invertebrates have been previously studied, plasticity has only been compared between two populations (Brey and Hain 1992; Cope and Linse 2006), or between populations covering huge geographic areas (Harper et al. 2012). This study has however, compared populations over the Antarctic range of *Lissarca miliaris* (Chapter 4), and for the first time investigated phenotypic plasticity in an Antarctic deep-sea benthic invertebrate, (Chapter 7). The aims of this study were to investigate variations in phenotypic and inferred physiological traits among contrasting environments and over time, using both newly collected and historic archived specimens from the Southern Ocean. This scrutinises past studies, which appropriately for the time, pooled together populations to increase sample sizes, potentially increasing error and missing hidden variability (see Chapter 1 for discussion). Representative species from deep-sea and shallow-water environments have been used to identify these plastic traits, including differences in growth, morphology, and reproduction among different populations. Additionally, temporal changes in a population of *L. miliaris* at Signy Island over 40 years, demonstrates plasticity and ecological divergence occurring since the onset of recent rapid warming at the Antarctic Peninsula (Chapter 5). Despite a long history in a cold stenothermal environment, all the species studied appear to demonstrate plasticity to subtly contrasting environments, which traditionally may have been considered homogeneous.

Species Identification

All species were identified by the anatomical and shell characteristics, which have defined them as a species since their original descriptions were published. Whether the populations of *L. miliaris* and the deep-water protobranchs are one taxonomic species with distinct morphologies or a selection of cryptic species, cannot be determined from this study alone, and would require future molecular analysis. Phenotypic plasticity does not require a change in genotype, although selection over time may result in a distinct lineage, displaying distinctive characteristics (West-Eberhard 1989). Ecological divergence between the populations, evidenced by differences in shell morphology, growth and reproduction, could drive speciation processes throughout the Southern Ocean, especially where populations became established before the formation of physical barriers. It is the common ancestry of these populations which make plasticity significant, as it demonstrates how a species can respond to the environment and expand their geographic range. If speciation processes are occurring, it would strengthen the hypothesis that phenotypic plasticity, and the ecological divergence of populations, allows radiation and speciation within cold stenothermal environments. However, phenotypic plasticity observed over time in a changing environment, within a single population of *L. miliaris* at Signy Island (Chapter 5), demonstrates that plastic traits cannot be used as evidence of speciation, but may represent species resilience to contrasting conditions.

8.2 Phenotypic plasticity in the cold; evidence for ecological divergence?

Antarctic evolutionary history has been structured by late Cenozoic glacial and inter-glacial periods on Milankovitch cycles, and driven by the physiological demands associated with surviving within a cold-stenothermal environment (Zachos et al. 2001; Thatje et al. 2005; see Chapter 1). Past ice sheet advance associated with glacial periods could have forced a shift in the distribution range of benthic species into the deep-sea or into temporary ice-free shelters on the shelf (Thatje et al. 2005, see Chapter 1). Subsequent radiation of surviving taxa from the ice-free pockets, the deep sea, or surrounding sub-Antarctic islands, make up much of the faunal diversity seen today, while evolution *in situ* has specialised many taxa to the cold stenothermal environment which prevails. Phenotypic plasticity is likely to have played an important role in the adaptation of species to these cold stenothermal conditions, and despite many species demonstrating poor acclimation to small increases in water temperature in laboratory controlled experiments (e.g. Pörtner et al. 1999; Peck et al. 2004, 2010), resilience to change on longer timescales may be significant to realistically determine species survival.

Lissarca miliaris is in some respects an unusual species by not showing evidence of the wide bathymetric range of other invertebrates, and being most abundant in shallow water (<30m) or inter-tidal environments (Dell 1964). This may be indicative of its past survival within Antarctic ice-free refuges in glacial periods. Alternatively, the species may have recolonized the Southern Ocean from sub-Antarctic regions where the species can still be found today, including the Straits of Magellan and the Falkland Islands (Huber 2010). Based on geological records, Tevesz (1977) suggested that the origin of *Lissarca* in the Antarctic dates back to the Miocene, which would coincide with the significant cooling step, predicted ~17 Ma (Lawver et al. 1992). As a brooding species, overcoming dispersal limitations is important for successful radiation and colonisation of new environments, and is likely to occur through rafting on macroalage. Rafting, discussed in Chapter 1, is responsible for the dispersal of some benthic taxa among Antarctic islands, and *L. miliaris* can be found at very high abundances in clusters on submerged macroalgae (Richardson 1979), which may be liable to uprooting through ice scour disturbance. If a cluster of adult *L. miliaris* were to raft and settle in a new area, an already reproducing population would be able to settle and make an impact in an environment devoid of duraphagous predators and with few fish predators (Helmuth et al. 1994; Higgs et al. 2009). The resistance which enables similarly small bivalve species to survive ingestion by fish (Domaneschi et al. 2002) and birds (Green and Figuerola 2005), should also be considered a significant method of short-distance dispersal, where oceanographic barriers may otherwise impede dispersal by drifting. Additionally, the congener *L. notorcadensis*, commonly found to shelf depths, have been observed floating in aquaria by the secretion of mucous which enables short distance dispersal of juveniles and adults (Brey et al. 1993), and is also described in the freshwater clam *Corbicula fluminea* (Prezant and Chalermwat 1984). A similar trait was observed in *L. milaris* from Signy Island, whilst in captivity, whereby floating of adults occurred after disturbance, although no study has yet quantified this response (personal observation).

Contrasting to *L. miliaris*, the protobranch bivalves are typically deep-sea (Zardus 2002; Allen 2008), and Southern Ocean populations probably originated from deep-sea environments, although in the Antarctic, *Yoldiella* spp. have a distribution onto the shelf up to 200m. This emergence of species onto the shelf from deep-sea environments is a common occurrence in the Southern Ocean (Brandt et al. 2007). With the exception of the Weddell Sea, the Southern Ocean's deep-sea, below 1000m, is connected to the world's oceans without the barrier of the Polar Front, and limited thermal difference (Clarke and Johnston 2003). It is likely that the low temperatures of the Southern Ocean, and niche availability similar to those found in the deep-sea, have allowed species' range distributions to rise into shallower water in thermally stable regions (Brey et al. 1996). Plasticity in this study is identified between different Southern Ocean shelf and deep-sea regions in three eurybathic protobranch species *Yoldiella ecaudata*, *Y. sabrina*, and *Y. valettei*, which show contrasting morphologies and growth with area, with some evidence of differences in shell weights (Chapter 7).

Role of reproduction

Descriptions of reproductive traits in Chapter 3 and 6, might suggest wide dispersal throughout the Southern Ocean. Although unconfirmed, there is a record of ten specimens of *L. miliaris* collected in the Ross Sea in 1968 at 350m (cat. no. 904397, Smithsonian National Museum of Natural History), suggesting a wider geographic range than has been described to date.

Reproductive traits, previously unknown for the Antarctic protobranchs, revealed similar characteristics to the North Atlantic protobranch species (Lightfoot et al. 1979; Tyler et al. 1992) and suggest the potential for a relatively wide dispersal with large yolky eggs and lecithotrophic development (Chapter 6). Lecithotrophy is a common reproductive trait in the deep-sea protobranchs where larvae drifting in bottom currents for several days (Allen and Sanders 1996; Zardus 2002) to several months (Shilling and Manahan 1994), and for the protobranchs of the Atlantic deep-sea, modelled to disperse between 237 and 749 kilometres (McClain et al. 2012). Molecular evidence would appear to support this, with little genetic structuring at abyssal depths in the North and South Atlantic, although high levels of differentiation at bathyal depths, where complex topography often characterises the benthic environment (Etter et al. 2005, 2011; McClain and Hardy 2010). It appears however, that more genetic work is required on species with known ecologies and dispersal mechanisms to understand dispersal of these deep-sea and Antarctic benthic specialists.

The hermaphrodite reproductive traits observed in *L. miliaris* (Chapter 3) and in a single specimen of *Y. valettei* (Chapter 6) may be a further adaptation, which can facilitate the establishment of populations, or enhance reproduction in areas of low density populations. Although the full reproductive cycle of hermaphroditism in *L.*

miliaris is unclear, the potential to be able to alternate sex throughout a lifetime would maximise the chances of successful reproduction within the population. This may hold true in the eventuality of successful adult dispersal by rafting, where there would be higher level of uncertainty over the availability of a mate. Long periods of brooding (24 months from gametogenesis to larval release; Richardson 1979) may be considered wasteful in a species only predicted to live for up to 7 years (Chapter 4). Sequential protandrous hermaphroditic traits in this setting is, therefore, an appropriate adaptation to maximise the reproductive output over the life of *L. miliaris*, where the females capacity to brood is limited by shell volume, and more efficient at adult shell lengths (Chapter 3). This may have also had an impact on their radiation into the Southern Ocean from sub-Antarctic regions, and the maintenance of small isolated populations. Similarly, simultaneous hermaphroditism in *Y. valettei* would also allow for the species to rapidly colonise new areas by increasing the chance of both sexes being available for fertilisation, or even the possibility of self-fertilisation.

Although not well understood in the deep-sea or Antarctic, the frequency of successful recruitment of a species may be important in maintaining wide connectivity, especially in comparatively short-lived species. While long-lived species may afford episodic successful recruitment with wide dispersal potential, shorter lived species would potentially need to maintain a higher level of recruitment to maintain gene flow. Additionally, seasonal or continuous reproduction may have differing effects on dispersal by the inter-annual variations in physical barriers of ice (Dayton et al. 2013) or known cyclic oceanographic variations (e.g. Quetin and Ross 2003). A two year sampling programme of waters surrounding Signy Island measured abundance of pelagic and demersal larvae, with 131 distinct larval types identified (Stanwell-Smith et al. 1999). Although distinct peaks of abundance of different species were found through the year, there was a low background abundance of all larval types, attributed to the slow and extended larval development in many species. Some larval abundance peaks were also found to be closely linked to local plankton blooms, while other species were found peaking in mid-winter, and variations between the two years studied demonstrated high levels of inter-annual variability in recruitment events (Stanwell-Smith et al. 1999).

Previous settlement plate experiments have, on short timescales, also shown recruitment in shallow water to be largely continuous, with distinct peaks after the onset of winter (Stanwell-Smith and Barnes 1997; Bowden 2005). Species found on these plates over two years of sampling, represented species of lecithotrophic larval development, with very low rates of settlement, and settlement peaks uncoupled to the summer chlorophyll bloom. This uncoupling has been associated with ice break-up disturbance at the Antarctic Peninsula (Bowden 2005), and larval predation by ctenophores (Stanwell-Smith and Barnes 1997). In deeper water, continuous recruitment of benthic polychaetes with mid-summer peaks was found to depths of ~500m; high levels of persistent organic matter in soft sediments dampening the effects of seasonal phytodetritus fall (Mincks and Smith 2007).

There are few long-term studies of recruitment in the Southern Ocean, although regular sampling at Rothera at the Antarctic Peninsula have demonstrated seasonality and high inter-annual variability in reproduction of two planktotrophic benthic species, suggesting variations in recruitment success (Tyler et al. 2003; Grange et al. 2004). Similarly, long-term observations at McMurdo Sound have demonstrated inter-decadal and spatial variations in the recruitment and survival of long-lived sponge communities (Dayton 1989), while a recent study demonstrated episodic rapid growth in sponge species previously thought to be very long lived, associated with iceberg calving and increased primary productivity (Dayton et al. 2013). Episodic recruitment of Antarctic krill at the Antarctic Peninsula over ten years, discussed to be associated with El Niño Southern Oscillation, further demonstrates long term variability in shallow-water species at inter-decadal timescales (Quetin and Ross 2003).

In this thesis, reproduction in *L. miliaris* is known to be distinctly seasonal, with two cohorts developing simultaneously in some females (Richardson 1979; Chapter 3), while the protobranch bivalves appear to show evidence of asynchronous oocyte development (Chapter 6). Although only representing a snap shot in time, the variations in oocyte size in *Y. ecaudata* and *Y. valettei* suggest the potential for continuous recruitment, and like other benthic deposit feeders, may be only weakly coupled with seasonal primary production (Mincks and Smith 2007). The timing of any peak in reproduction may also be different with depth in *Y. sabrina*, possibly demonstrating a mismatch in any seasonality of gamete development between shelf, slope, and deep-sea populations. Continuous background recruitment would avoid seasonal selection pressures on larvae from predation (Stanwell-Smith and Barnes 1997), increasing the likelihood of settlement. Although demonstrating evidence of continuous reproduction, the effects of primary productivity in known productive spots, could also create a stronger seasonal pulse in recruitment compared to deeper sites.

Ecological Plasticity

The morphological plasticity expressed by shell shape in *L. miliaris* and the deep-water protobranchs among populations in the Scotia Sea and Antarctic Peninsula demonstrates the adaptability to environments, which contrast by only subtle differences in temperature range and food availability (Chapters 4 and 7). Geographic distance appears to have no effect on morphological differences between populations of *L. miliaris*, and the only latitudinal trend observed was an inverse ‘u’ shaped relationship with prodissoconch size, indicating that offspring size is smallest at the extreme north (South Georgia) and south (Stepping Stones) of the Antarctic geographic range studied. This links to the morphology, which is also most similar between these two contrasting populations, highlighting the relationship between morphology (as a proxy of volume) and the female’s capacity to brood young (Chapter 3).

The plasticity among populations of *L. miliaris* may be evidence of an ecological divergence, although the extent of reproductive isolation is currently unknown. Populations within enclosed bays, such as Potter Cove (King George Island) may be more physically isolated than an open population at Stepping Stones, but not enough is known regarding the species distributions around islands to infer details of the potential ecological isolation of specific areas. Although morphological plasticity may be forced by environmental constraints at each site studied, the morphological variations in larval shells with increasing latitude may be indicative of ecological divergence. Larval shell development can be environmentally modified (Foighil 1986), and as this is occurring before larval release in brooders, it could be argued that the dispersing young have reduced scope for plastic response to alternative environments, and natural selection against the migrant specimens would result in reproductive isolation (Via et al. 2000; Nosil et al. 2005). However, the likely broadcasting approach to fertilisation by males is unlikely to prevent gene flow between morphologically dissimilar specimens, unless the populations are already significantly genetically distinct to produce hybrids of reduced fitness.

Likewise, the deep-water protobranchs show evidence of ecological divergence between populations, although the effect on offspring size remains unknown. The differences in shell weights between populations of *Y. valettei* suggest that an energetic trade-off is limiting shell thickness in the Scotia and Weddell Seas in comparison to the Amundsen Sea, where physical disturbance through ice scour from the grounding of ice, and warmer water temperatures, may have selected for thicker shells. Such plasticity seen in the protobranchs will have been key to their radiation into the Antarctic, or given similar fossil forms, which date back to the Early Cambrian, approximately 500 Ma (Morris and Fortey 1976), persistence through Antarctic formation, cooling, and climate variability on Milankovitch cycles.

Summary

Lissarca miliaris and the deep-sea protobranchs demonstrate phenotypic plasticity among populations, with only subtle differences in environmental conditions. Rafting as a dispersal mechanism is expected to maintain connectivity among populations investigated, but environmental modification of pre-release larval *L. miliaris* may affect the morphology during early ontogeny, and the species ability to adapt morphologically. The ability to be plastic and respond to new environments will have enabled a wide dispersal of Antarctic bivalves and may explain the frequency of *L. miliaris* in the shallow sheltered environments of the Scotia Sea and Antarctic Peninsula. It would also be expected that such radiation into the Antarctic had come from South American origin where the species is still prevalent (Huber 2010), although only a study on the genetic structuring of the species would reveal if any such biogeographic affinities still, if ever, truly existed. It would be expected that *L. miliaris* in the Antarctic represent overlapping populations, and any speciation processes would be driven by natural selection against migrants. Some populations that are significantly physically isolated, such as Potter Cove, King George Island, which is protected from large icebergs by a shallow sill and is generally untypical with high sedimentation and glacier melt, may have higher reproductive isolation than more exposed areas, and faster rates of ecological divergence. Large, yolky eggs and lecithotrophic development in protobranch bivalves may enable a wide dispersal and potentially high connectivity between regions, although local scale genetic structuring associated with depth or physical barriers may also occur. If this holds true, high dispersal and phenotypic plasticity is likely to counter the effects of apparent ecological divergence and hinder genetic differentiation.

8.3 Phenotypic plasticity in the cold; evidence of plasticity to environmental change?

Phenotypic plasticity to different conditions over a geographic area shows a striking ability to adapt, but does not provide evidence regarding the species resilience over shorter timescales (not geological timescales). In this study however, the response by *L. miliaris* to warming over 40 years, in a single population collected at the same location, highlights the importance of plasticity for survival and adaptation, despite the increased potential for reproductive isolation (Chapter 5). The effects of climate change on marine fauna are hot-topics in the Antarctic marine environment, especially since the onset of significant warming of the surface waters at the Antarctic Peninsula (e.g. Meredith and King 2005). The rate of current atmospheric warming is more rapid than the climate variability that has been measured in the geological records (Turner et al. 2005; Clarke et al. 2007), and ecological responses in the marine environment, as a consequence of warming, have been observed on large scales. Most notable is the decrease in magnitude of phytoplankton blooms by ~12% between 1979 and 2009 (Montes-Hugo et al. 2009) and decrease in algal cell size (Moline et al. 2004). The knock on effect has been a reduction in Antarctic krill (*Euphasia superba*) abundance, an important ‘keystone’ species in the Antarctic food chain, which are not able to feed as efficiently on small celled algae, and generally select for diatom chains (McClatchie and Boyd 1983; Haberman et al. 2003). Salps, meanwhile, have been blooming (Atkinson et al. 2004) with a negative feedback effect with increased predation on larval krill (Nishikawa et al. 1995), and further population reductions or distribution shifts in penguins and other higher predators, which rely on krill as a primary food source (Fraser and Hoffmann 2003; Moline et al. 2004).

At the local scale, there are few studies which have investigated the effects of climate warming on the ecosystem; instead warming is discussed in the context of laboratory experiments on thermal tolerance and acclimation. Although laboratory studies on the short term acclimation to temperature are difficult to interpret on evolutionary timescales, the response to only subtle increases in temperature above the natural environment demonstrate that cold stenothermal tolerance of many species is perhaps limited at the cellular level (Pörtner et al. 1999; Pörtner 2001; Peck et al. 2004, 2010). Although patchy, some intertidal environments are surprisingly more species rich than originally assumed, despite fauna having to survive extreme ranges of temperature (Waller et al. 2006a,b). The fauna found in the shallow sheltered bays of South Georgia are also largely similar to those found around islands nearer to the Antarctic Peninsula (Barnes et al. 2006b), although the water temperature at South Georgia are up to 4°C higher (Barnes et al. 2006a). This represents temperatures beyond the ranges of higher latitude Antarctic waters of the Scotia Sea and the laboratory tested thermal limits of many Antarctic species (Peck et al. 2004, 2010). More recent evidence however, suggests that on longer timescales, there may be more scope for adaptation (Morley et al. 2012), but long-term studies to measure physiological factors need to be conducted.

Temporal v spatial plasticity in L. miliaris

By comparing the growth, shell morphology and larval shell size over time, the phenotypic and ecophysiological response to warming can be identified in *L. miliaris* (Chapter 5), and compared to the spatial variations in plasticity identified in Chapter 4. When plotted together (Figure 8.1), the morphology of the 2011 specimens from Signy Island are morphologically distinct by having the most elongate and thinnest shell shape. This shell shape would offer the lowest internal volume for brood care, affecting larval size, but may offer greater protection from heat associated stress in the intertidal, including desiccation. Expending energy into shell length over shell width, also allows for longer ctenidia which improves respiratory and feeding efficiency during periods of submergence (Franz 1993). Such phenotypic plasticity could therefore affect the ecophysiology of *L. miliaris*. With the smaller offspring sizes inferred from prodissoconch length, this is consistent with the hypothesis that reproduction, limited by the female's capacity to brood, is restricted by decreased volume in response to phenotypic plasticity at Signy Island (Chapter 5, Figure 8.1).

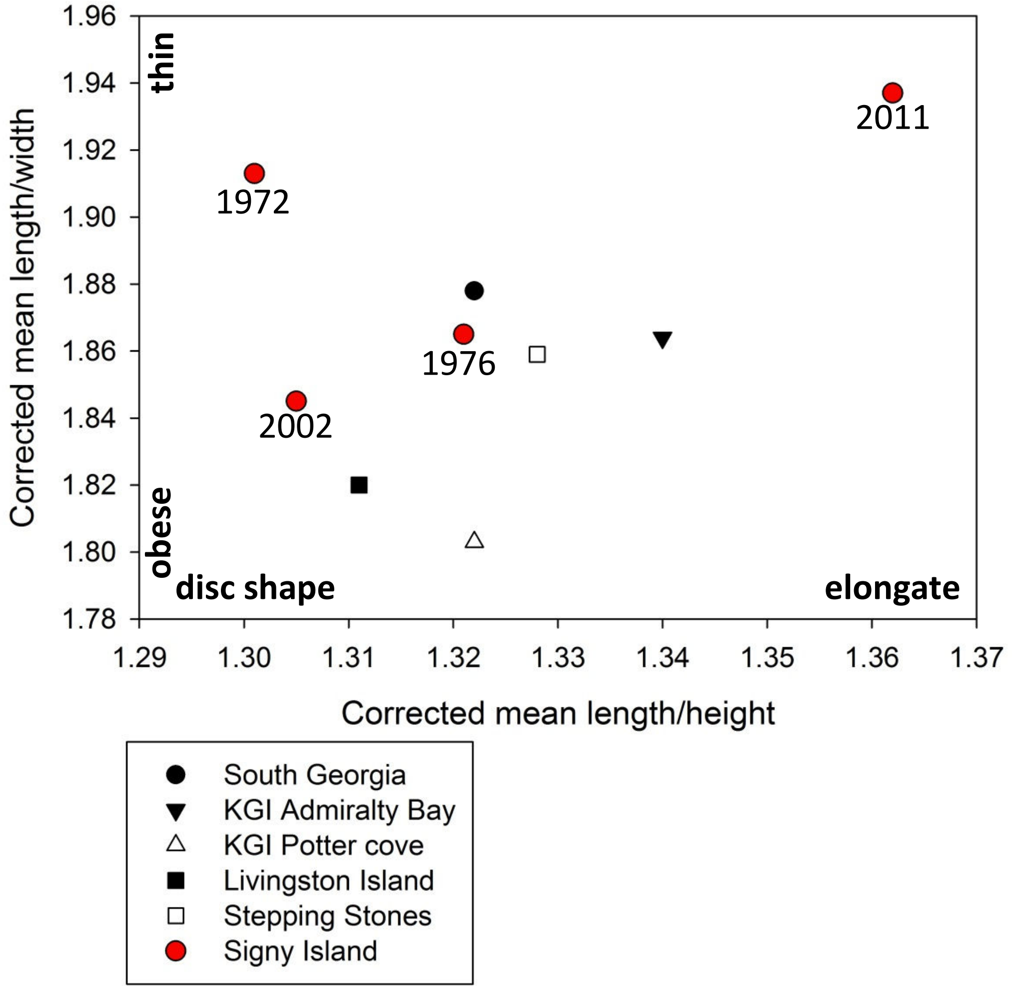

Figure 8.1 Morphology of *Lissarca miliaris* collected at different islands in the Scotia Sea and Antarctic Peninsula and at Signy Island between 1972 and 2011 (shown in red). Shell shape described in the axis. KGI = King George Island.

In comparison to the prodissoconch size measured in other populations, *L. miliaris* from 1976 had the largest prodissoconch length and inferred offspring size. The subsequent decline associated with increasing temperature (Chapter 5) show that the current size and morphology of larval shell, is most similar to the morphology and size of those of the warmer waters in South Georgia (Table 8.1). This comparison further strengthens the hypothesis that warmer temperatures may have a negative impact on reproduction by either the increased costs of faster growth rates and calcification, or higher physiological maintenance at elevated temperatures.

Site/Year	**length**	**height**	**ratio**
South Georgia	687.7	478.7	1.44
KGI Admiralty Bay	701.1	472.5	1.49
Stepping Stones	680.5	402.3	1.70
Signy Island			
1976	714.5	499.0	1.60
2002	699.2	448.0	1.53
2011&2012	694.3	458.9	1.52

Table 8.1 Table of prodissoconch lengths, heights and ratios for *Lissarca miliaris* collected at different islands in the Scotia Sea and Signy Island between 1976 and 2012. KGI = King George Island.

Plasticity and its cost can therefore be observed over generational time periods and provide some evidence that in the face of thermally driven challenges, species show resilience, observable by phenotypic plasticity. The smaller offspring size in South Georgia associated with morphology (Chapter 4) appear to be a trade-off with fecundity, and although not currently known at Signy Island, this may enhance dispersal by providing smaller young that could drift more freely on floating material or by mucous threads. Interestingly, a similar study at Potter Cove, King George Island, used long-lived bivalves to model past growth rates and physiology from 40 year old shells in the circum-Antarctic bivalve *Laternula elliptica*, but found contrasting growth results with a decrease in growth performance (Brey et al. 2011). The distribution of *L. miliaris* north of the polar front may have evolutionary implications to its past and future adaptations to climate variability, when most other Antarctic bivalves have no extant populations beyond South Georgia. Successful dispersal across the polar front may still play an important role in maintaining Southern Ocean populations by reducing reproductive isolation and the devolution of plastic traits.

Summary

From an evolutionary perspective, the adaptability of a species over short periods of time may provide some insight in their past radiation and survival. By using shells collected in the past and present, a direct measure of ecophysiological variables (growth and reproduction) has been taken to show adaptability and evidence of ecological divergence. The current population is beginning to resemble, by offspring size and morphology, specimens from South Georgia, the northerly limit south of the Polar Front. It is worth noting however, that the population at South Georgia in which the comparison was made, were collected in 1979, and atmospheric warming at South Georgia has increased at a similar rate to the Antarctic Peninsula, with negative impacts on krill abundance (Whitehouse et al. 2008). Unfortunately, no significant numbers of *L. miliaris* have been sampled in South Georgia subtidally or intertidally over the past two years, and no comparison to measure the effect of climate warming on the population from 1979 can be made at present. Other pressures associated with warming, for instance the increased pressure of endolithic decay, may have future impacts on growth, shell integrity, and reproduction, especially in light of predicted ocean acidification (McNeil and Matear 2008; Fabray et al. 2009; McClintock et al. 2009) and increased predation from invasive durophagous predators (Aronson et al. 2007, 2011).

8.4 Phenotypic plasticity in the cold; physiological plasticity between areas?

Physiological plasticity in Antarctic invertebrates has rarely been investigated among populations of contrasting environments. This may be important for species to maintain wide geographic and eurybathic range distributions however, especially with our knowledge of the sensitivity of shallow-water invertebrates to increases in temperature (e.g. Pörtner 2001; Peck 2002; Peck et al. 2004, 2010; Aronson et al. 2011). Thermal plasticity of mitochondria in two Antarctic molluscs from contrasting environments demonstrated greater physiological plasticity to warmer waters at populations which experience larger temperature ranges (Morley et al. 2009), while some mollusc species shift their depth distributions to deeper cooler waters at lower latitudes (see Morley et al. 2013 for review). To the author's knowledge, beyond shallow-water bays and coves, the respiration rates of invertebrate fauna living on the deep-Antarctic continental shelf have not been investigated. Additionally, there are no respiration data available to date for deep-sea protobranch bivalves globally and no known studies on physiological plasticity within an Antarctic species over a significant part of its geographic range.

During a recent expedition to the Weddell Sea however, a rare preliminary investigation into respiration rates in Southern Ocean bivalves from shelf depths, revealed physiological plasticity with geography in *Lissarca notorcadensis*, collected from the Weddell Sea and Scotia Sea. It has been demonstrated previously from analysis of COI genes, that populations of *L. notorcadensis* from these regions, which live in sea urchin spines, are reproductively isolated (Linse et al. 2007), and this makes an interesting comparison to the effects of isolation on physiological plasticity. The study on board the RSS James Clark Ross during JR275 in February/March 2012, used specimens collected by Agassiz Trawl from 300m in the Scotia Sea, South of the South Orkney Islands (60°68'S, 44°01'W), and from 600m in the Weddell Sea (74°67'S, 29°43'W). After 48 hours of 'recovery' time and acclimation to laboratory conditions maintained at bottom water temperature at time of collection, the respiration rates were calculated by oxygen consumption against a control, as described by Oliphant et al. (2011). Specimens were exposed to increasing temperatures from -1°C, with six hour acclimation per 1°C increase, which occurred every 24 hours. Isolation periods were 24 hours at the coldest temperatures but this decreased with increasing temperature to ensure oxygen levels did not drop below 50% during experiments. In the Scotia Sea three specimens were used at each temperature with three controls run simultaneously, while in the Weddell Sea 15 specimens were used with five controls. A separate study using specimens from the Weddell Sea tested the effect of starvation and captivity on the respiration rates in *L. notorcadensis* at 0-1°C during the 14 days of experimental treatment, to determine any effects on respiration rates.

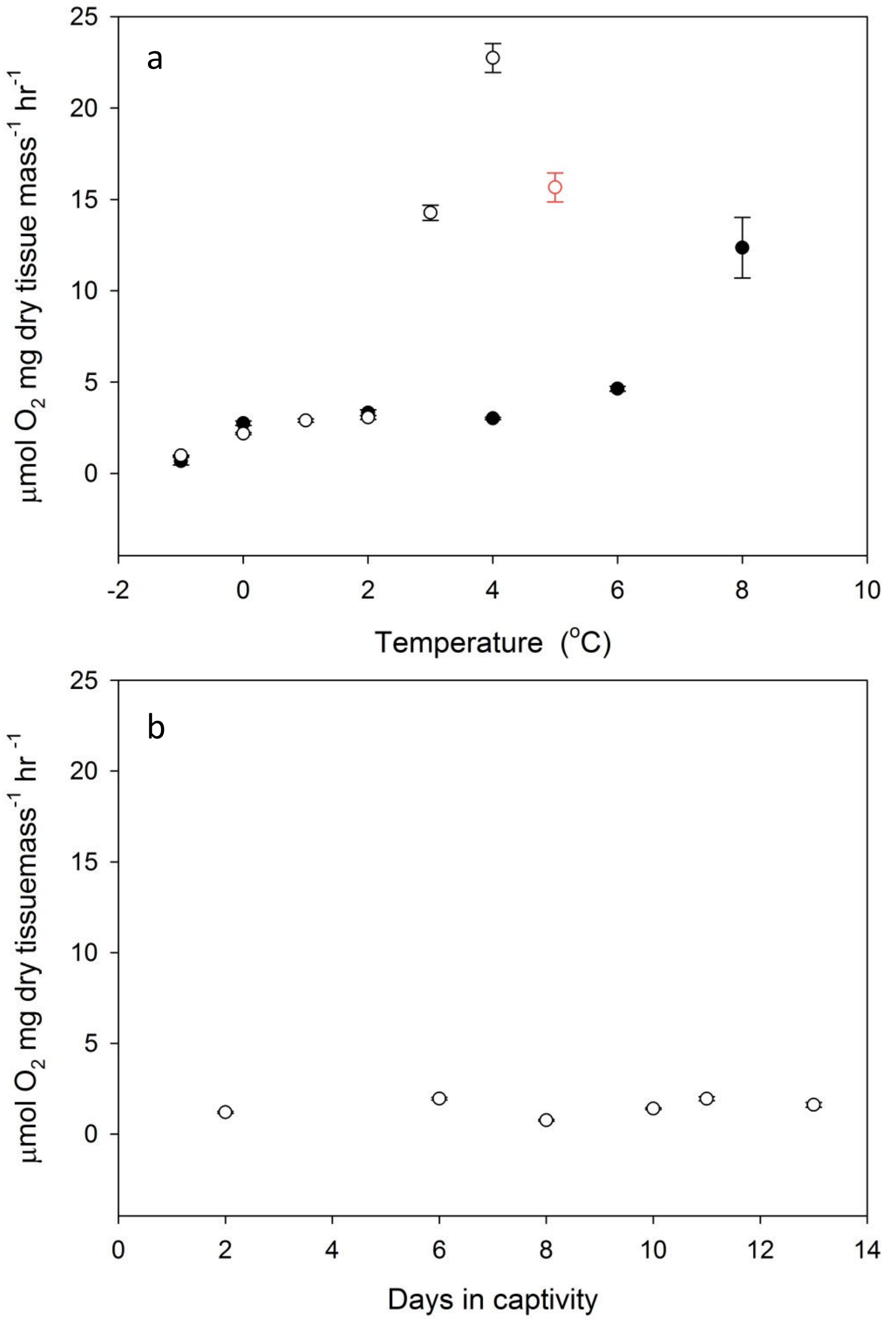

Figure 8.2 Respiration rates and standard error of *Lissarca notorcadensis* under thermally controlled conditions. Closed circle Scotia Sea, Open circle Weddell Sea. a) respiration rates at different temperatures after 6 hour acclimation and maximum 24hr isolation. Red symbol = 50% mortality; b) respiration rates with days since collection under stable conditions. Days 1-10 at 0°C, days 11 and 13 at 1°C.

There was no significant difference in respiration rate between -1°C and 2°C in the Weddell Sea, and between -1°C and 6°C in the Scotia Sea (Tukey test at 99% confidence). The Weddell Sea population show a peak in respiration rate of 22.8 μmol O_2 g^{-1} dry mass hr^{-1} at 4°C, with 50% mortality at 5°C. In the Scotia Sea, respiration rates are significantly higher at 8°C (ANOVA $F_{5,17}$=15.01, p<0.001) peaking at 12.4 µmol O_2 g^{-1} dry mass hr^{-1}, but still only half the respiration rates measured in the Weddell Sea. No Scotia Sea specimens survived the following acclimation to 9°C. There was no effect of captivity and starvation on respiration rate over the 14 day study period (ANOVA $F_{4,34}$=1.88, p=0.140, Figure 8.2b). Respiration rates between 1 – 3 µmol O_2 g^{-1} dry tissue mass hr^{-1} are within the range of other small Antarctic molluscs, but slightly lower than the infaunal bivalves *Yoldia eightsi* (Davenport 1988), *Limopsis marionensis* (Pörtner et al. 1999) and the brooding intertidal bivalve *Gaimardia trapesina* (Ralph and Maxwell 1977) (see Peck et al. 2002 for standardised summary of oxygen consumption rates of Antarctic molluscs).

The Weddell Sea is important for the formation of bottom water, and deep-sea temperatures below -1.8°C have been recorded, which can flood onto the shelf (Robertson et al. 2002), while the Scotia Sea at 300m may have a greater heterogeneity in temperature through the year (Smith et al. 1999; Barnes et al. 2006a). Reproductive isolation of *L. notorcadensis* in the Weddell Sea from the Scotia Sea populations, as evidenced by Linse et al. (2007), suggest that thermal plasticity in an area with an extreme cold stenothermal temperature window has potentially driven ecological divergence between the two regions. The Weddell Sea Gyre may also act as a strong thermal (physiological) and physical barrier to dispersal into the Scotia Sea, or within the Weddell Sea, retaining populations and driving speciation processes by reproductive isolation (Held and Wägele 2005; Raupach and Wägele 2006; Linse et al. 2007). Although the upper temperatures tested exceed the temperatures experienced in the shallowest waters of the Antarctic, they provide evidence of differing extents of physiological plasticity between two contrasting populations of *L. notorcadensis.*

Respiration rates were also measured for two deep-sea protobranch bivalves *Propeleda longicaudata* and *Yoldiella sabrina* from 600m and 500m, respectively, in the Weddell Sea with bottom temperatures of <-1.8°C. Using the same methods described above, eight *P. longicaudata* and six *Y. sabrina* were tested between -1.9 and 3°C, at which point >50% mortality occurred in both species (Figure 8.3). There was no significant difference in respiration rate in either species with increasing temperature (*Propeleda longicaudata* ANOVA $F_{5,42}$=1.03, p=0.412, *Yoldiella sabrina*

ANOVA $F_{5, 79}$=1.94, p=0.118), which may be indicative of a reduced scope for physiological plasticity to temperature variations. Despite having radiated into the Weddell Sea, characteristic of extreme cold stenothermal water temperature, the lowest respiration rates in both species were at 0°C, a temperature characteristic of the world's deep-seas. This could be linked to the past emergence from deep-sea environments and onto the shelf, where temperature remains low enough to support these cold water specialists.

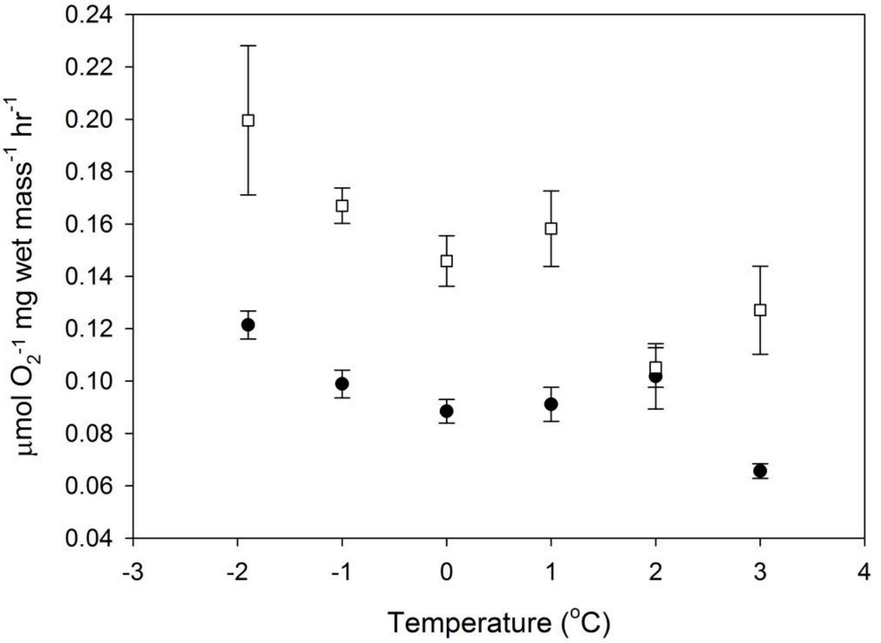

Figure 8.3 Respiration rates and standard error of *Propeleda longicaudata* (closed circle) and *Yoldiella sabrina* (open square) under thermally controlled conditions. N.B. respiration rates displayed as per wet tissue mass.

8.5 Future studies on ecological divergence and phenotypic plasticity

Representing some of the shallowest and deepest Southern Ocean environments, this study has significantly developed our understanding of the processes involved in ecological divergence to minor variations in the environment, evidenced by phenotypic plasticity between populations and over time. It is likely that plasticity in Southern Ocean benthic invertebrates provides a mechanism in which species can persist in contrasting environments, and future studies should explore the evolutionary consequences of plasticity within shallow-water and deep-sea environments. Specifically, future studies should investigate whether expressed plasticity has an effect on speciation processes, either by dampening selection of genetic traits or by enhancing selection of phenotypic traits which have become genetically controlled by extended periods of reproductive isolation.

- An investigation into the genetic divergence between the studied populations, and the extent of connectivity between them, is important in determining whether environmentally induced plasticity in the species studied could result in reproductive isolation. This would test the hypothesis that ecological divergence may drive speciation in the Antarctic by reproductive isolation or natural selection against migrant morphologies.
- Molluscs are frequently used to investigate plasticity, as environmental responses can be measured by analysis of shell characteristics. Other taxa with measureable morphological differences or growth rates would reveal ecological divergence at the ecosystem level however, and this is important in understanding the role of plasticity in a macroecological context. In the Antarctic, a study of such proportions would be challenging as the extensive sampling of a single ecosystem can represent major logistical problems.
- With rapid regional warming at the Antarctic Peninsula and associated pressures, the risk of shifting baselines (highlighted in Chapter 5) is becoming apparent. To assess the impacts of environmental change, future investigations into plasticity should attempt to maximise the use of material from museum and archived collections, to identify changes since the onset of current rapid regional warming at the Antarctic Peninsula. Deep-sea species should also be included, as recent studies have demonstrated the effects of ice calving on productivity to the deep benthos (Dayton et al. 2013).

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Appendices

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Shifting Baselines in Antarctic Ecosystems; Ecophysiological Response to Warming in *Lissarca miliaris* at Signy Island, Antarctica

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Abstract

The Antarctic Peninsula has experienced a rapid increase in atmospheric temperature over the last 50 years. Whether or not marine organisms thriving in this cold stenothermal environment are able to cope with warming is of concern. Here, we present changes to the growth and shell characteristics of the ecologically important, small and short lived brooding bivalve *Lissarca miliaris* from Signy Island, Antarctica. Using material collected from the 1970's to the present day, we show an increase in growth rate and adult shell deterioration accompanied by a decrease in offspring size, associated with an increase in annual average temperatures. Critical changes to the bivalve's ecology seen today evidence the problem of a shift in baseline since the onset of warming recorded in Antarctica. These small bivalves are demonstrating ecophysiological responses to subtle warming that, provided warming continues, could soon surpass a physiological tipping point, adding to warming associated threats such as increased predatory pressure and ocean acidification.

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Introduction

The rate of atmospheric warming at the Antarctic Peninsula has been around 0.56°C decade^{-1} since 1950 [1], higher than the global average and most extreme in winter with an increase of 5–6°C over the past 50 years [2]. Observed glacial retreat [3], reduced sea ice formation [4] and a regional increase of 1°C in the upper ocean layer in summer [5], have all been attributed to this temperature increase. Ecosystem responses can be difficult to identify but to date include changes in plankton biomass [6], penguin distribution and krill abundances [7]. Investigating physiological responses to thermal stress may also be important in understanding ecosystem changes occurring at population levels [8,9].

A problem facing ecologists when studying ecosystem change is finding a baseline in which to measure change against. The term 'shifting baselines' was first used to describe fishery scientists who failed to use historic data to evaluate the status of the ecosystem, instead using the ecosystem status at the start of their career as the baseline for change [10,11]. This concept has since been discussed to include a wide range of ecosystems that are only studied whilst in decline; in rare cases no historic data being available to develop a suitable baseline [12]. This is certainly the situation for many coral reefs [13,14], benthic environments [15] and in rocky shore ecology [16], where often no adequate baselines are currently attainable.

Long-lived marine bivalves are often used to study faunal response to environmental change as different variables may be recorded by growth increments and shell chemistry. A commonly studied species is the sub-Arctic bivalve *Arctica islandica* that can live in excess of 350 years [17], while in the Antarctic, 40 year old *Laternula elliptica* shells have been modelled to infer details into past growth rate and production [18]. While providing an invaluable insight into responses to climatic variability, the slow growth rates of long-lived species may hide inter-decadal variation. Interpretation of results can also be difficult in a macro-ecological context when communities are dominated by comparatively small, short lived and faster growing species. Southern Ocean bivalves are typically thin shelled [19] with calcium difficult to extract from seawater at low temperatures [20], making them potentially vulnerable to changes in temperature, decreasing $CaCO^3$ saturation [21,22] and predation from invasive durophagous predators in the course of warming [23,24].

The philobryid bivalve *Lissarca miliaris* (Philippi 1845) is a small species (typically <5 mm) commonly found in the inter- and sub-tidal regions attached to macro-algae by byssal threads around Signy Island, Antarctica. They are relatively short lived, living up to 7 years, and brood a maximum of 70 young for 18 months [25,26]. Populations occur along the Antarctic Peninsula, Scotia Sea, and sub-Antarctic, often in dense aggregations. Within Borge Bay, Signy Island, *L. miliaris* are the most dominant species of mollusc both by weight and number living on the abundant macroalgae *Desmarestia anceps* [25]. As an ecologically important species with a wide distribution and comparatively short life-span, *L. miliaris* make a good model species for identifying changes in the Antarctic environment. Using published data from 1972 [25], specimens collected in 1976, 2002, 2011 and 2012, we study the effects of regional temperature increase on the growth rates and shell characteristics of *L. miliaris*. This study highlights the importance of historic data in polar areas, the striking response of shelled invertebrates to subtle changes in temperature, and the risk of shifting baselines affecting our perception of the 'pristine' Antarctic ecosystem.

Materials and Methods

Ethics Statement

Collections were not made from any protected or private sites within Antarctica. This study did not involve endangered or protected species. All necessary permits were obtained for the described field collections, within the Antarctic Act (1994).

Collection

We analysed a total of 808 hand-collected intertidal *Lissarca miliaris* near the British Signy Base at Shallow Bay, within Borge Bay, Signy Island (60°42′S, 45°36′W; Figure 1). This was made up of 226 specimens fixed in formalin but stored in ethanol from 1976, 462 specimens from April 2002 in 96% ethanol, 68 specimens in February and March 2011 fixed in 96% ethanol and 52 specimens in March 2012, 10 dried at 30°C, 42 fixed in 96% ethanol. The specimens from 2002 were collected by hand as part of the R/V *Polarstern* LAMPOS (ANT XIX/5) expedition [27].

Growth

Each specimen of *L. miliaris* was measured along the maximum distance across the shell using a stereo-microscope (precision ±0.025 mm). Annual growth measurements were counted by eye and assumed to be annual [25,28]. Size-at-age data were analysed using the von-Bertalanffy growth function (VBGF) [29]:

$$S_t = S_\infty\ 1 - e^{-K(t-t0)}$$

where S_t is length, S_∞ is asymptotic length, K is growth coefficient, t is age and t_0 is age when size equals zero.

Overall growth performance (P) was computed using K and S_∞ derived from the VBGF equation;

$$P = \log(K * S_\infty)$$

Prodissoconch sizes

Prodissoconch sizes were measured by image analysis of micrographs taken with camera mounted stereo-microscope. Only '0 year' and '1 year' specimens with an undamaged prodissoconch were used for this analysis. 2011 and 2012 data were pooled as sampling of these later specimens included low numbers of '0 year' animals. A total of 84 measurements were made (1976 n = 26, 2002 n = 47, 2011/2012 n = 11).

Shell analysis

The right valve from three specimens in each collection with 5 growth rings was used for shell analysis. Shells were embedded into Epoxy resin and were cut with a 100×0.37 mm diamond low speed saw along the longest growing margin from the umbo. Cut blocks were polished with graded diamond-coated sanding cloths to 1 µm and carbon coated before Scanning Electron Microscopy (SEM) analysis.

Energy dispersive spectrometry (EDS) was used to provide qualitative and quantitative measurements of the dominant trace elements in the shell structures. EDS analysis was taken through the shell at transects 600 µm apart from the umbo, representing average growth per year to ensure all years of growth were accounted for. The middle layer showed least variability in shell chemistry (unpublished data) and was used to generate quantitative measurements. Sections were analysed with a Leo 1450 scanning electron microscope with a PGT microanalysis energy dispersive system. Ratios of trace elements with calcium were used to identify changes in chemistry over time.

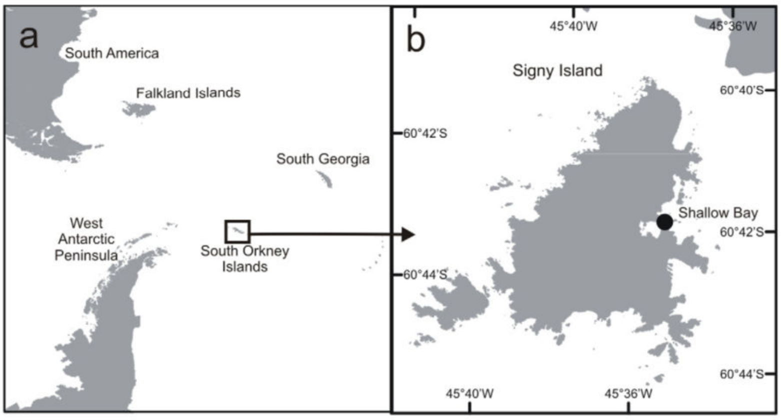

Figure 1. Map of study area a) Antarctic Peninsula showing location of South Orkney Islands; b) Signy Island (62°42′S, 45°36′W) showing location of the intertidal Shallow Bay within Borge Bay.
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Results

Using monthly temperature date from the Argentinean Orcadas research station, an increase in air temperature is observed since the collections of *L. miliaris* began. The increase in air temperature for our collections is described by the number of months a year the average temperature was above 0°C, over the seven-year life span of the specimens collected (Figure 2). Months above 0°C increased from 26 months between 1966 and 1972 to 39 months between 2005 and 2011. Peak frequency of months above 0°C shifted from 1°C to 2°C over the same period. Average summer temperatures between 1970 and 2011 show high inter-annual variability (Figure 3b) but a significant increase in air temperature ($r^2 = 0.402$, $p<0.001$).

The calculated growth constant *K*, representing 'rate of growth', increases from 0.130 in 1972 to 0.208 in 2002 and 0.290 in 2011 while the asymptotic maximum size (S_∞) decreases from 8.61 mm in 1972 to 5.88 mm in 2011 (Figure 3a, Table S1). The overall growth performance (OGP) for each sample shows a five-fold increase from the 1970's samples to 2011 (Figure 3b). Maximum prodissoconch lengths (P1) of *L. miliaris* have decreased from 714.5 µm±5.17 in 1976 to 694.3 µm±7.14 in 2011 and 2012 while the maximum height have increased slightly from 449.0 µm±1.60 to 458.9 µm±1.52 (Table 1). No P1 data were available for the collection in 1972. The resulting decrease in length/height ratio from 1.598±0.017 to 1.516±0.029, was significant (Kruskal-Wallis H = 10.10, df = 2, $p<0.01$).

Shell formation and chemistry have also changed over time. The Strontium:Calcium ratio increased from 0.0012±0.0007 in 1976 to 0.0029±0.0011 in 2011 (Figure 4). Strontium in 2002 was similar to 1976 (0.0017±0.0007) while the dried material from 2012 was highest (0.0063±0.0013). The Phosphorus:Calcium ratio was very low but decreased from 0.0013±0.0003 to 0.0006±0.0002 between 1976 and 2012. Maximum shell thickness increased from 166 µm in 1976 to 276 µm in 2011 and 206 µm in 2012 and is associated with deteriorating shell quality and increased shell repair (Figure 5). The integrity of the shells from 2011 and 2012 are compromised by endolithic decay causing erosion of the upper layer of shell and the subsequent secondary shell deposition results in a thicker shell (Figure 5c&d). Decay is not observed in the 1976 collection (Figure 5a), and cannot be confidently identified in the 2002 collection (Figure 5b). To confirm dissolution was not an artefact of preservation, the 2012

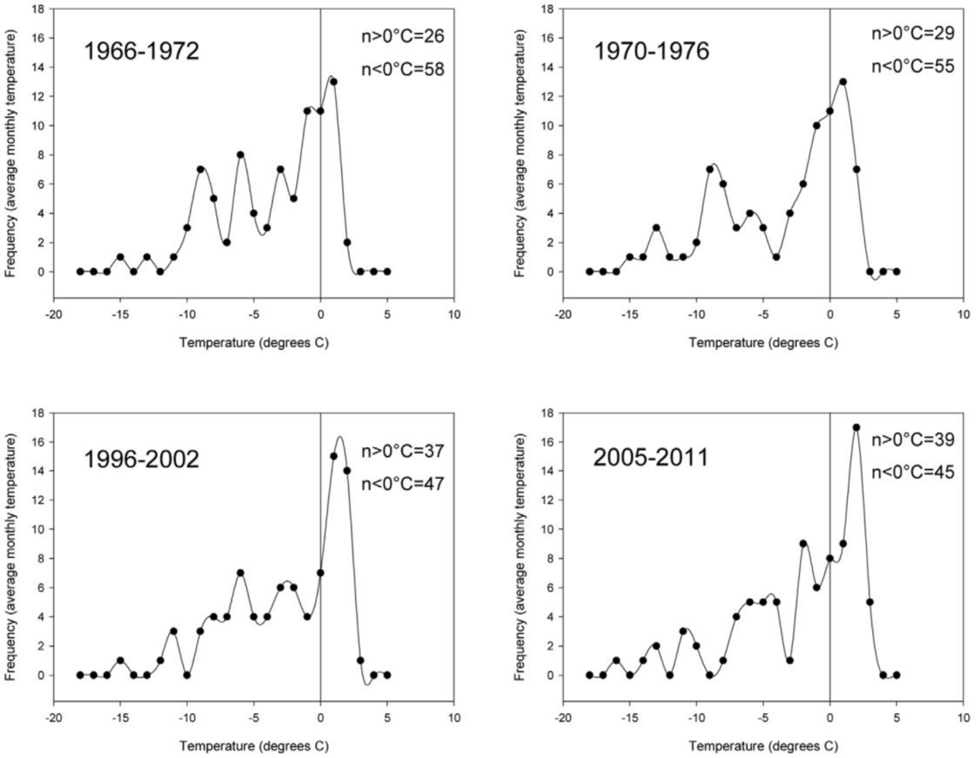

Figure 2. Frequency of mean monthly temperatures at Orcadas Research Station, Laurie Island for the 7 years up to the specimen collection dates, demonstrating the number of months averaging below and above 0°C over the life of the adult *Lissarca miliaris*. Vertical line represents 0°C.

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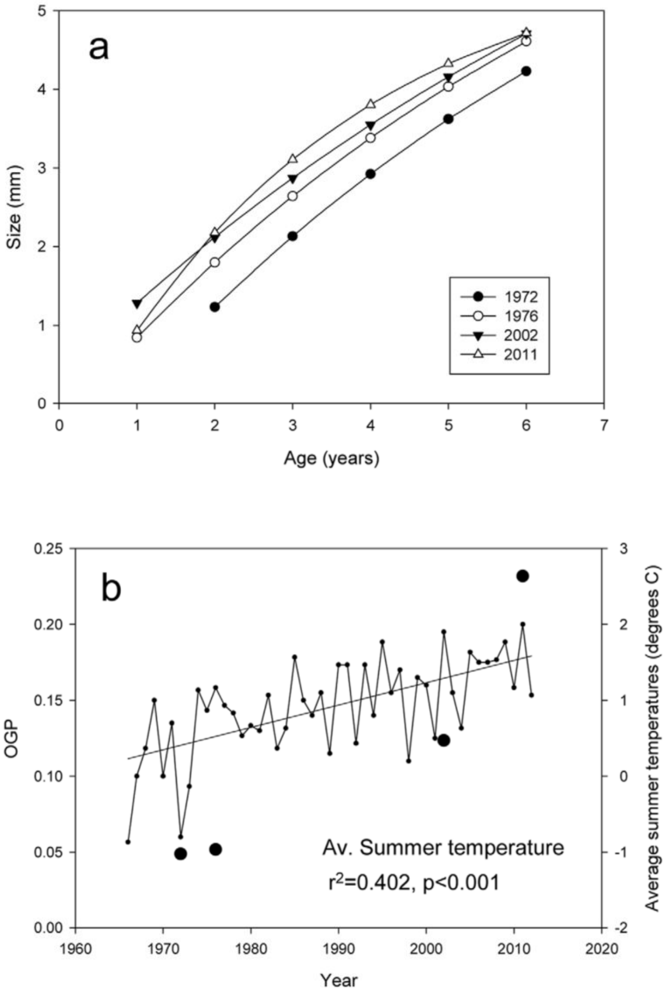

Figure 3. Growth parameters of *Lissarca miliaris* from Signy Island. a) von Bertalanffy growth function from size-at-age data of *L. miliaris* from 1972–2011; b) Overall growth performance of *L. miliaris* from 1972–2011 (large circles) displaying average summer air temperatures from 1966 to 2012 (small circles).
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specimens were dried without contact with formalin/ethanol, and only the 1976 material had been fixed in formalin (subsequently ethanol stored).

Discussion

The South Orkney Islands have one of the longest time series of recorded air temperature, dating back to 1903 at the Argentinean research station. Temperatures recorded fit closely to the monthly air temperature data collected at the British Signy research station from 1945 until 1995 when data collection halted (Figure S1) and have shown an average increase of 0.20°C decade^{-1} over 100 years [1], although much of this changes appears to be in the last 50 years. The increased growth rate of *L. miliaris* over the past 40 years is likely to be a response to this changing temperature in the region. Although no record of sea-water temperature is available for this period at Shallow Bay, the intertidal distribution of *L. miliaris* would make an increase in air temperature a significant factor affecting its physiology. Growth rate is closely linked with two factors, food availability and temperature [18,30]. Chlorophyll

Table 1. Maximum prodissoconch length, height and ratio including standard error for *Lissarca miliaris* collected at Shallow Bay, Signy Island in 1976, 2002 and 2011/12.

Year	Length (μm)	Standard Error	Height (μm)	Standard Error	Length/height ratio	n
1976	**714.5**	5.17	**449.0**	6.31	1.60	26
2002	**699.2**	4.96	**458.0**	3.79	1.53	46
2011&2012	**694.3**	7.14	**458.9**	3.63	1.52	11

The difference in length/height ratio between the collections was significant (Kruskal-Wallace H = 10.10, df = 2, P<0.01).

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a values in Borge Bay show high levels of interannual variability and are considered to be high in the summer while extremely low in the winter [31,32]. The duration of these blooms might be expected to increase with air temperature as nutrients are rarely limiting, although no evidence of this is observed in the water sampling programmes of 1972–1994 [31,32].

L. miliaris has a wide distribution and is commonly found as far north as South Georgia [33]. Air temperatures experienced at Signy Island still remain cooler than the temperatures experienced by other populations in the sub-Antarctic and assuming connectivity between populations, the increase in temperature at Signy Island is unlikely to have reached the species thermal limit. OGP provides a method to compare 'how well' an organism grows [34,35] and a graphical representation of growth, a higher OGP suggesting a less stressful environment in which to grow. The five-fold increase in OGP over 40 years is in contrast to the larger, infaunal bivalve *Laternula elliptica* from King George Island [18] which showed a decrease in OGP over 40 years, inferred from shell growth per year in old specimens. However, the two species differ in their distribution, with *L. miliaris* perhaps favouring the conditions that regional environmental changes have brought.

The most striking change between collections was the condition, and composition of the shells. Strontium in aragonite structures is very well studied and its relationship with temperature is used as a climatic proxy [36,37]. During faster crystal growth rates, near-surface migration of ions, which expel impurities, are less efficient and an increase in strontium replacement of calcium ions might be expected [38]. Strontium has also been found to be correlated with temperature indirectly by increased growth rates in marine gastropods [39]. The increased ratios of strontium in the shells of *L. miliaris* are therefore likely to be linked to the increased growth rates observed, although other abiotic influences may also affect this relationship. Phosphorus is incorporated from the surrounding seawater, suggesting a change in dissolved phosphorus levels.

The shell damage observed in 2011 and 2012 shells resembles the decay caused by photosynthetic bacteria [40,41]. Shell dissolution in the Antarctic is very poorly understood [22] and only one study has described endolithic algae on the shells of the bivalve *Adamussium colbecki* [42], and at Signy Island a single example of macro algae causing shell erosion in the limpet *Nacella concinna* [43]. The dissolution observed appears to be greater on older specimens where the protective periostracum has become eroded; algal cells are observed on the freshly preserved adult 2012 specimens (Figure S2). All *L. miliaris* shells in this study were of the same age, but the degree of shell dissolution was much greater in the 2011/2012 specimens compared to the almost perfect shell surface in 1976 specimens. This is also reflected in the thickening

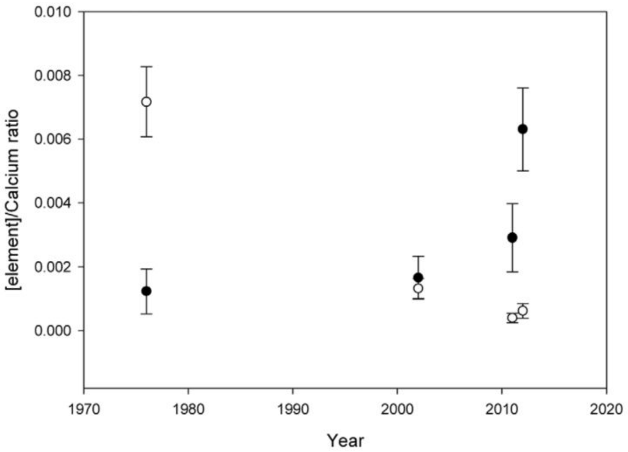

Figure 4. Element/Calcium ratios (mean ± standard error) of *Lissarca miliaris* shells from Signy Island. Strontium/Calcium ratios (filled circle) from 1976–2012 and Phosphorus/Calcium ratio (open circle) from 1976–2012. Error bars represent standard error.

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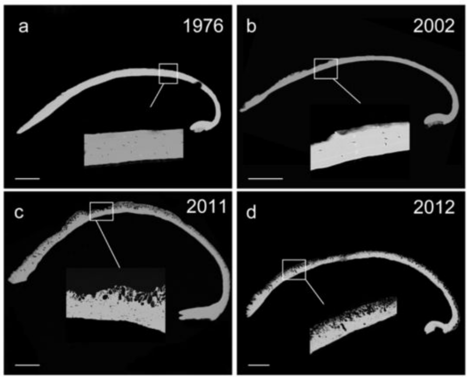

Figure 5. SEM backscatter images of section adult *Lissarca miliaris* shells with higher magnification inlays. a) 1976 section; b) 2002 section; c) 2011 section; d) 2012 section. Scale bars = 500 µm.
doi:10.1371/journal.pone.0053477.g005

of shells as the bivalves attempt to repair their damaged shells with secondary calcification (Figure 5c). The temperature increase in the region may be encouraging faster growth of such endolithic bacteria and the warmer winters experienced over the past decade may be increasing the survival of such organisms during the light and temperature limiting months.

Shell repair is energetically costly and requires an energy reallocation, potentially negatively affecting reproductive output [44,45] and the decrease in Prodissoconch 1 sizes is indicative of this decrease in reproductive effort per egg. Secreted by the bivalve in early development, the prodissoconch size can be directly linked to egg size and differences in egg size as small as 10 µm can be reflected in significant differences in prodissoconch 1 length [46]. Smaller egg sizes in *L. miliaris* might suggest less energy available for development and a higher risk of larval mortality. The observation of thickened shells and reduced reproductive output demonstrates the energetic trade-off involved in maintaining shell integrity. Some brooding bivalves are known to decrease immune responses leaving a greater susceptibility to parasite infection [47]. Many Antarctic invertebrates brood for over a year; if *L. miliaris* have suppressed immunity for 18 months of brooding, their reduced resistance to endolithic parasites and cost of shell repair may impact their survival.

L. miliaris at Signy Island show the vulnerability of Antarctic fauna by rapidly responding to only a subtle change in temperature over four decades, demonstrating the importance of having a realistic baseline for measuring change. The growth rate response already occurring is startling but before a physiological tipping point is reached other factors, including the shell dissolution described in this study, may affect future growth and survival. We highlight the need to investigate other small, shelled organisms that may already be in a highly altered ecological state in polar ecosystems, although only by using historic and archived material can the true extent of environmental change be measured. It is likely too late for realistic baselines to be established for measuring such change and any management approach must therefore be assessed against an already altered ecosystem state. Antarctic ecosystems are often perceived to be pristine [48] but this study demonstrates changes that have been occurring unnoticed for at least 40 years, and other critical changes could be occurring in ecosystems that are assumed to be stable. The immediate concern for *L. miliaris* and the polar shelled organisms it represents is the energetic cost of shell repair affecting reproduction, but also the reduced resilience to the inevitable pressures associated with invasive species, ocean acidification and predictions of further warming of the Southern Ocean [24] that by far exceed the comparatively subtle warming seen to date.

Supporting Information

Figure S1 Mean annual temperature from 1947–1995 from the British Signy Island base (dotted line) and Argentine Orcadas, Laurie Island Base (solid line). Signy Island temperature data collection stopped in 1995.
(PDF)

Figure S2 Adult *Lissarca miliaris* from Shallow Bay, Signy Island collected in 2012. Endolithic algae can still be seen in green, covering the shells shortly after fixation. Scale bars = 2 mm.
(PDF)

Table S1 Parameters of the von Bertalanffy growth function and calculated overall growth performance (OGP) for *Lissarca miliaris* at Shallow Bay, Signy Island.
(PDF)

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Author Contributions

Conceived and designed the experiments: AJR ST KL. Performed the experiments: AJR ST KL. Analyzed the data: AJR ST KL. Contributed reagents/materials/analysis tools: AJR ST KL. Wrote the paper: AJR ST KL.

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ORIGINAL PAPER

An unusual hermaphrodite reproductive trait in the Antarctic brooding bivalve *Lissarca miliaris* (Philobryidae) from the Scotia Sea, Southern Ocean

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Abstract The Antarctic marine environment is extreme in its low temperatures and short periods of primary productivity. Invertebrates must therefore adapt to maximise reproductive output where low temperature and limited food slow larval development. Brooding is a common reproductive trait in Antarctic marine bivalves; larval development occurs within the mantle cavity, and larvae are released as fully developed young. *Lissarca miliaris* is a small, short-lived, shallow-water brooding bivalve of circum-Antarctic distribution and found most abundant in the sub-Antarctic Magellan Region and islands of the Scotia Arc. Here, an unusual hermaphrodite reproductive trait is described for *L. miliaris* from King George Island (62°14′S, 58°38′W) and Signy Island (60°42′S, 45°36′W), Antarctica, using histological and dissection techniques. Specimens demonstrate simultaneous and sequential hermaphrodite traits; male and female gonads develop simultaneously, but the production of oocytes is reduced while testes are ripe. Functional females are more abundant in specimens above 3 mm shell length, although male reproductive tissue persists and functional males are found in all size classes. The number of previtellogenic oocytes produced by far exceeds the number of oocytes extruded and brooded, which may indicate an ancestral link to a planktotrophic past. Hermaphroditism in *L. miliaris* maximises reproductive efficiency in a short-lived species, in which the female's capacity to brood its young is limited, and demonstrates a specialised adaptation to a cold stenothermal and food-limited environment prevailing in the Southern Ocean.

Keywords Antarctica · Invertebrate reproduction · Cold adaption · Hermaphrodite · Early ontogeny

Introduction

Brooding is a common and successful reproductive trait in many Antarctic and deep-sea invertebrates representing an extreme of parental trade-offs between quantity and quality, with lowest mortality and lowest fecundity (Vance 1973; Ripley and Caswell 2008). In some invertebrate groups from the Antarctic, such as echinoids and bivalves, the predominance of brooding traits is striking (for review see Poulin et al. 2002; Thatje et al. 2005), but the suggestion that this has been driven by the current cold stenothermal and stable environmental constraints is often questioned (Strathmann 1978; Poulin et al. 2002; Pearse and Lockhart 2004; Thatje et al. 2005; Pearse et al. 2008). It has been discussed that the phyletic constraints on the reproduction and extinction of many planktotrophic traits during previous glacial periods have driven the evolution of entirely brooding clades of taxa (Strathmann 1978; Emlet et al. 1985; Gallardo and Penchaszadeh 2001; Thatje et al. 2003; Pearse and Lockhart 2004; Pearse et al. 2008). Southern Ocean brooding species may have become isolated in ice-free shelf refuges on multiple occasions during glacial periods, forming fragmented and isolated populations, preventing gene flow and facilitating speciation of

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brooding clades over time (Thatje et al. 2005, 2008; Pearse et al. 2008; Thatje 2012).

Reproduction in polar and deep-sea benthic invertebrates has always been a subject of contrasting opinions and controversy, but there are still few detailed descriptions on the reproductive ecology of many benthic species, especially in the Antarctic. There is a well-documented mismatch between the long developmental times of indirect planktotrophic larvae and the short period of primary production in Polar Regions, potentially selecting for non-feeding larval forms (Thorson 1950; Mileikovsky 1971). Vance (1973) stated that selection must favour the reproductive trait with the greatest efficiency. Lecithotrophy and direct development have higher investment per egg with lower fecundity compared with planktotrophy. This is balanced by lower dependence on external food sources and settlement sites (Strathmann 1974). Recent studies have challenged the early views on the dominance of direct development in the Antarctic after the description of species with successful planktotrophic larval development in the Southern Ocean (Arntz and Gili 2001; Poulin et al. 2002; Raupach et al. 2010; but see Thatje 2012) and the belief that the isolation of the Southern Ocean has driven evolution and endemism in many taxa (Arntz et al. 1997; Clarke and Johnston 2003; Pearse and Lockhart 2004; Thatje et al. 2005).

Philobryidae are a notably speciose and increasingly studied family of bivalves, typical of the Southern Ocean and often occurring in high abundances in shallow bays and the continental shelf (Tevesz 1977; Arntz et al. 1997; Linse 2004). A characteristic important to the success of this group is the ability to brood young to juvenile stages, facilitated by the reduced or absent anterior adductor muscle and strongly constructed modified filibranch ctenidia (Tevesz 1977; Morton 1978; Prezant et al. 1992; Brey and Hain 1992; Hain and Arnaud 1992; Higgs et al. 2009). The philobryid *Lissarca miliaris* (Philippi, 1845) is a small ubiquitous bivalve (<6 mm shell length) often found attached to macroalgae by byssal threads between the intertidal and 270 m (Dell 1964). With a life span of up to 6 years, it is relatively short-lived for a cold-water invertebrate and broods up to 70 young in clusters within the mantle cavity for 17–18 months (Richardson 1979). Populations are known to occur along the Western Antarctic Peninsula, Scotia Sea islands, and in the Ross Sea, as well as reaching into the sub-Antarctic Magellan Region, Falkland and Kerguelen Islands (Huber 2010, www.scarmarbin.be). They are most commonly found in large numbers and dense aggregations in shallow bays (<30 m). The brooding characteristics of *L. miliaris* were described by Richardson (1979) from a population at Signy Island, but no study has described reproduction from gametogenesis. Only few studies have comprehensively described reproduction in any Antarctic philobryid species (Morton 1978; Brey and Hain 1992; Higgs et al. 2009). Philobryids are normally considered to be dioecious (Morton 1978; Richardson 1979; Brey and Hain 1992; Higgs et al. 2009) although a small percentage of *Lissarca notorcadensis* within certain size classes possess both testes and ovaries, indicating protandric hermaphroditism in a small proportion of a population (Prezant et al. 1992). Studies on the reproductive ecology of benthic invertebrates from the Southern Ocean are important for our knowledge of reproductive trade-offs in Polar Regions. This study describes the reproductive biology in *L. miliaris* and discusses the reproductive trade-offs associated with hermaphroditic traits within the species.

Materials and methods

Sample collection

Specimens of *L. miliaris* were collected by divers at 10 m depth in Potter Cove, King George Island (Fig. 1a, c), Scotia Sea (62°14′S, 58°38′W) in December 1993 and January 1994 and fixed in 4 % buffered formalin. A total of 461 specimens were hand-collected from the intertidal of Shallow Bay, Signy Island (Fig. 1a, b), Scotia Sea (60°42′S, 45°36′W) in April 2002 and fixed in 96 % ethanol during the R/V *Polarstern* LAMPOS (ANT XIX/5) expedition (Arntz and Brey 2003).

Histology

Fifty-three individuals from King George Island (Potter Cove) and nineteen specimens from Signy Island (Shallow Bay) between 2.0 and 5.0 mm shell length were used for histological analysis. Maximum shell length, height and width were measured using a stereomicroscope. Whole animals were processed after decalcification (hydrochloric acid). Tissue was processed as described by Higgs et al. (2009) and embedded into wax blocks. Sections of 7 µm were mounted on slides and stained with haemotoxylin and counter-stained in eosin. Reproductive features were captured with a Nikon D5000 mounted on a stereomicroscope and analysed using SigmaScan Pro4 software; Feret diameters used to measure oocyte sizes.

Dissection

A total of 306 specimens from King George Island were selected for dissection. Bivalves were opened and the gonads examined. Separate sexes were easily identified in specimens above 2.5 mm shell length. The vitellogenic eggs of 90 females from 2.5 to 5.8 mm shell length were manually removed from the gonads and counted; 44 of

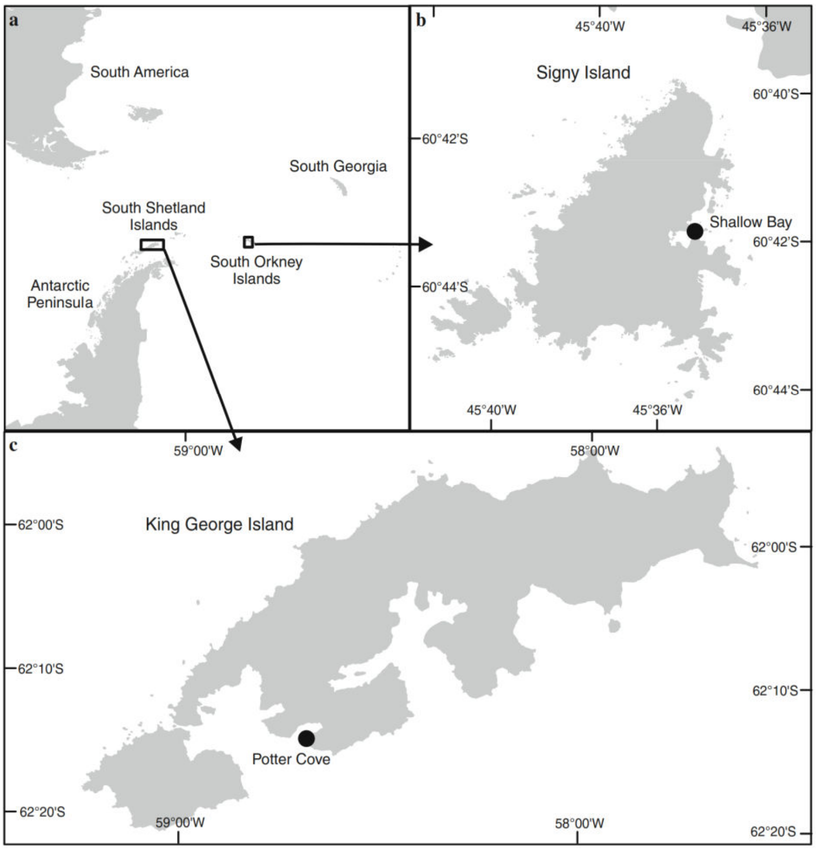


Fig. 1 *Map* showing study areas. **a** Study areas in relation to Antarctic Peninsula, **b** Shallow Bay, Signy Island, **c** Potter Cove, King George Island

these had oocytes photographed with a Leica Microsystem camera and Feret diameter calculated.

Sex ratios

Sex ratios were determined from specimens collected at King George Island. Histology was used to determine functional sex of specimens below 3 mm; males determined by mature ripe testis and females from the presence of previtellogenic oocytes and no testis. Above 3 mm shell length, specimens were dissected to observe functional sex. Hermaphrodite percentage in the population was determined by using histology data of all size classes and is observed as functional males with previtellogenic oocytes developing in a small portion of gonad.

Results

Lissarca miliaris demonstrate traits of both simultaneous and sequential hermaphroditism—sequential by a change in sex that can occur once within their lifetime and simultaneous by the occurrence of both male and female tissue present throughout the life of some specimens. Gametogenesis is observed from 2.00 mm shell length with oogenesis occurring in two small portions of gonad beside the posterior adductor muscle from 2.15 mm. Developing testis is observed from 2 mm as gonad extending from the posterior adductor muscle to the antero-dorsal half on both the left and right sides. Up to and over 200 previtellogenic oocytes develop in both males and females and vary in size between 19 and 50 µm (Figs. 2a, 5a). They appear densely packed or loosely arranged and often exceed the maximum number of eggs that become vitellogenic and brooded.

Vitellogenesis occurs from 2.9 mm length; <35 oocytes from the site of oogenesis develop on each side in dorsal parts of gonad extending from the posterior adductor muscle to the anterior-dorsal half (Fig. 2b). Oocytes are lined up in tightly packed rows in the dorsal parts of the gonad and measure between 200 and 500 µm Feret diameter at King George Island (mean = 368 µm, SD = 44, $n = 396$) depending on degree of vitellogenic accumulation (Fig. 3b). The observed number of eggs per female increased with shell length from 2.9 mm ($r^2 = 0.451$, $F_{1,89} = 72.34$, $p < 0.001$, King George Island) but also showed large variation within size groups (Fig. 4). Between 15 and 69 eggs were found in females of 2.9 to 5.0 mm shell length. Fully developed but unfertilised eggs are extruded into the mantle cavity and are initially held together in two clusters on each side (Fig. 2c) by an unidentified maternal tissue (Fig. 3c, d). This tissue breaks down, probably after fertilisation but the clusters remain loosely held together by thin chords resembling thin strands of byssus (Fig. 3c). Embryos develop larval shells and the large prodissoconch (Signy Island mean length = 699 µm, SD = 33, $n = 47$) often displays a distinct growth disturbance mark. Fully developed but pre-release shelled veliger was only found in specimens from Signy

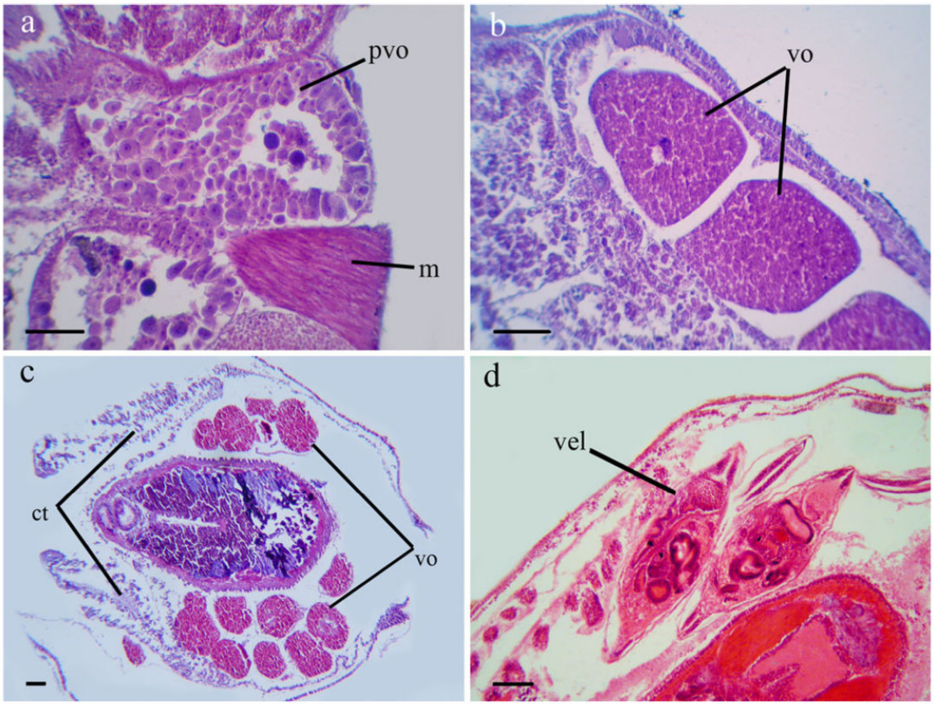


Fig. 2 *Lissarca miliaris*. **a**, **b** King George Island January 1994, **c**, **d** Signy Island April 2002. **a** Section through the posterior part of the body of functional male (3.6 mm shell length) showing histology of previtellogenic oocytes in a small portion of gonad next to posterior adductor muscle; **b** transverse section through dorsal portion of gonad in a functional female (4.2 mm shell length) showing histology of vitellogenic oocytes; **c** transverse section of entire brooding female (4.2 mm shell length) showing histology of brooded ova in mantle cavity; **d** transverse section of mantle cavity of brooding female (3.1-mm shell length) showing histology section of advanced brooded veligers. *pvo* Previtellogenic oocyte, *m* muscle, *vo* vitellogenic oocyte, *ct* ctenidia, *vel* veliger. Wax sections, 7 µm thick, stained with haemotoxylin and eosin. *Scales* 100 µm

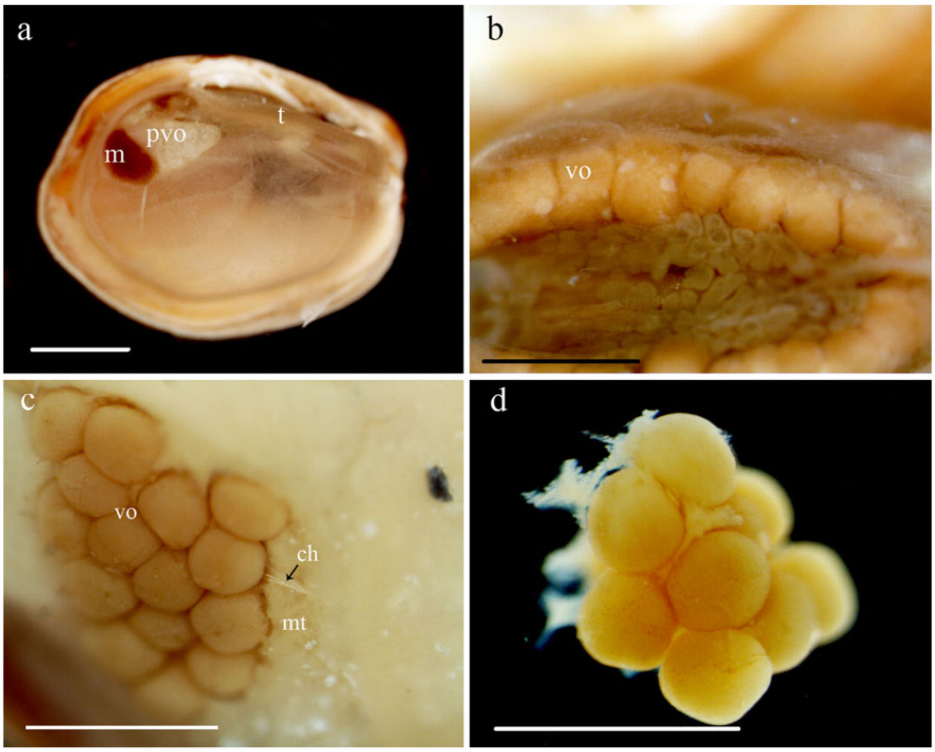


Fig. 3 *Lissarca miliaris*, King George Island January 1994. **a** Micrograph image showing previtellogenic oocytes developing next to posterior adductor muscle and testis in functional male (4.2 mm shell length); **b** micrograph showing dorsal view of vitellogenic oocytes lined up in dorsal portion of gonad (4.9 mm shell length); **c** micrograph showing brooded vitellogenic oocytes in mantle cavity held in place by maternal tissue and thin chords (4.7 mm shell length); **d** micrograph of cluster of vitellogenic oocytes brooded in the mantle cavity (4.7 mm shell length). *m* Muscle, *pvo* previtellogenic oocytes, *t* testis, *vo* vitellogenic oocytes, *ch* thin chords, *mt* maternal tissue. *Scales* 1 mm

Island and were loose in the mantle cavity (Fig. 2d) with a mean shell length of 0.715 mm (SD = 0.04, $n = 13$). The smallest released shelled young from King George Island had a shell length of 0.775 mm.

Hermaphroditism is most commonly observed as functional males with ripe testis and previtellogenic oocytes developing in the small portions of gonad beside the posterior adductor muscle (Figs. 3a, 5a–d). In functional females, hermaphroditism is observed above 2.9 mm shell length with broods or large vitellogenic oocytes and reduced testis at the edges of the dorsal portion of the gonad (Fig. 5e). Sex ratio data from specimens at King George Island demonstrates an increase in functional females from 2.5 mm and a corresponding decrease in male abundance (Fig. 6). The proportion of functional males with previtellogenic oocytes increases in stages from 15 to 17 % between 2 and 2.99 mm, 33–37 % between 3 and 3.99 mm and 83–100 % between 4 and 4.99 mm. The changing ratios, supported by evidence of hermaphrodite stages, suggest that the dominant sex of the species changes with age but both male and female portions of gonad persist. Testis appears to be the dominant functional gonad up to 3 mm shell length, but previtellogenic oocytes are also developing simultaneously. During vitellogenesis, the testis becomes inactive and reduced while the oocytes develop in ovaries that form dorsally, in the position that testis had previously occupied. Brooding females with partly shelled veligers were found to have large (250–500 µm)

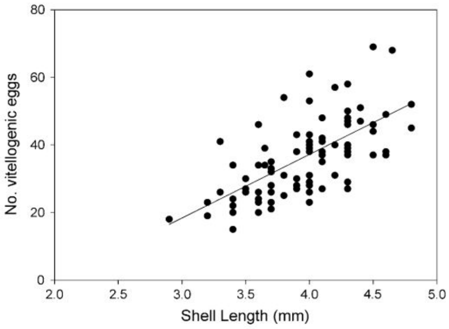


Fig. 4 *Lissarca miliaris* from Potter Cove, King George Island, Dec–Jan 1993/1994. Number of vitellogenic oocytes plotted against maximum shell length. $R^2 = 0.451$, $n = 90$, $p < 0.001$

vitellogenic oocytes developing in the dorsal parts of the gonad, and there is no evidence that suggests the male portions of gonad become active at larger sizes once reduced. No differences in reproductive trait were observed between the two populations studied, and despite the temporal difference between collections, no differences in the hermaphrodite stage were observed between sites.

Discussion

Brooding and gametogenesis

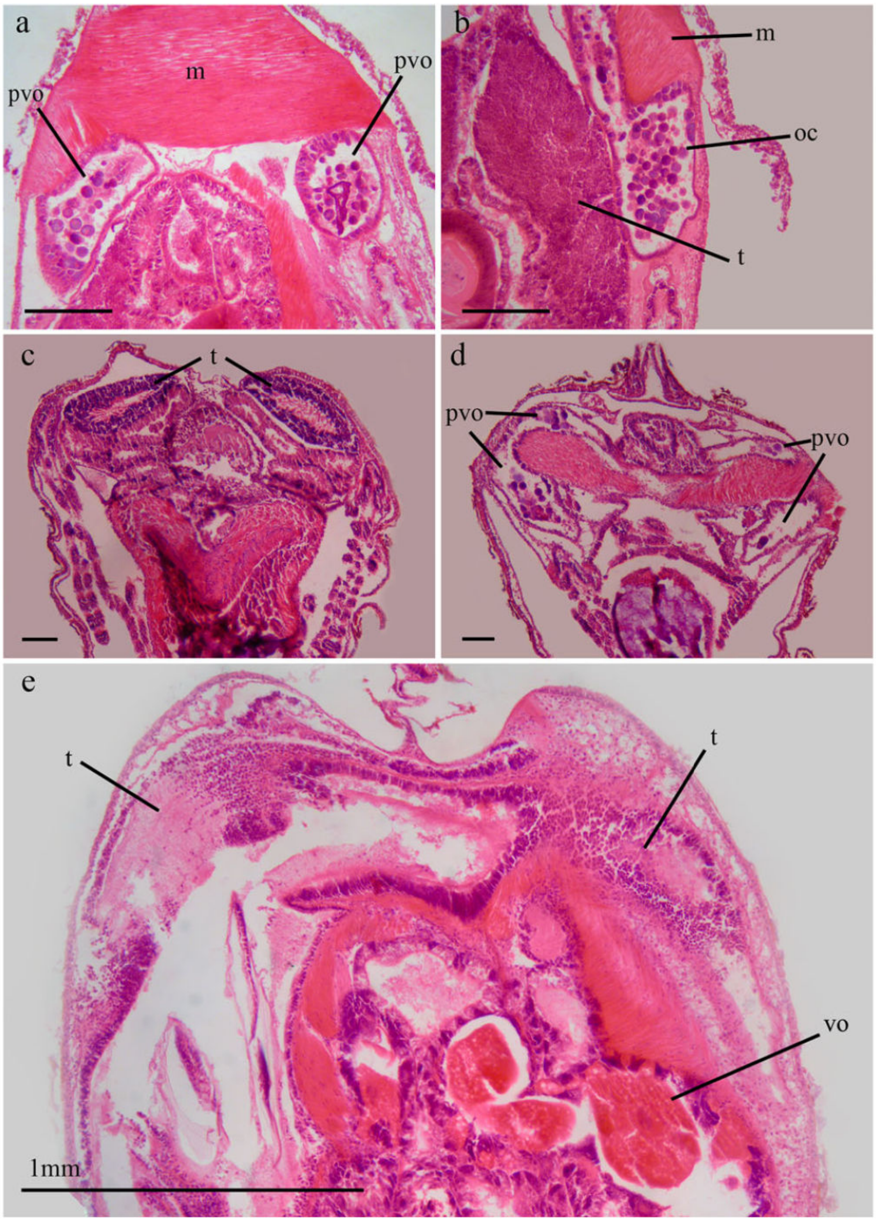
The Antarctic shallow-water environments are dynamic and diverse habitats, disturbed by ice scouring, cold temperatures and high concentrations of short-lived primary production (Gutt 2001; Thatje et al. 2005; Barnes and Conlan 2007). In such limiting conditions, a diverse range of benthic fauna have evolved traits that not only increase the survival of young but also maximise reproductive output. The brooding traits of *L. miliaris* are not unusual for philobryids although there are distinct differences to the described traits of *L. notorcadensis* (Brey and Hain 1992) and *Adacnarca nitens* (Higgs et al. 2009). *L. miliaris* are typically abundant in shallow water, commonly in high abundances in the intertidal and sub-tidal shallow bays in the sub-Antarctic (Dell 1964; Richardson 1979), whereas the previously studied *L. notorcadensis* were found between 80 and 1,108 m in the Weddell Sea and *A. nitens* at 84 m in the Ross Sea (Brey and Hain 1992; Higgs et al. 2009). Different environmental constraints associated with these depths and latitudes may influence reproductive cycles, investment of energy into offspring, and brooding characteristics.

Fig. 5 *Lissarca miliaris*, Signy Island April 2002. **a**, **b** Transverse ▶ section through posterior part of body in functional male (3.5 mm shell length) showing; **a** previtellogenic oocytes developing in two areas of gonad beside the posterior adductor muscle; **b** previtellogenic oocytes developing beside the posterior adductor muscle next to mature testis. **c**, **d** Two *dorsal–ventral sections* from same specimen (3.1 mm shell length) demonstrating (**c**) testis reduced in size and low density in spent male (**d**) oocytes from portion of gonad by posterior adductor muscle spreading into portion of gonad the testis occupies; **e** *Dorsal–ventral section* showing functional female (4.1 mm shell length), which contained shelled brood (not seen) with vitellogenic oocytes and reduced testis. *m* Muscle, *pvo* previtellogenic oocytes, *t* testis, *vo* vitellogenic oocytes. Wax sections, 7 µm thick, stained with haemotoxylin and eosin. *Scales* 100 µm unless stated

Despite a smaller shell size, *L. miliaris* have larger broods than *L. notorcadensis,* but this is balanced by smaller young—*L. miliaris* producing young up to 0.77 mm shell length compared with 1.25 mm in *L. notorcadensis* (Table 1). Both species have seasonal reproduction, but *L. miliaris* have a shorter 18-month reproductive cycle compared with the 24-month cycle of *L. notorcadensis* (Richardson 1979; Brey and Hain 1992). In contrast, *A. nitens* are asynchronous producing few eggs at a time in small numbers continuously through the year (Higgs et al. 2009). Availability of food is the single most likely factor explaining these differences with *L. miliaris* in this study being exposed to the high concentrations of phytoplankton during the summer months at Signy Island (Clarke et al. 1988) and Potter Cove, King George Island (Klöser et al. 1994; Schloss et al. 2002; Tatian et al. 2002) while their deeper relatives may only be exposed to sinking phytodetritus or low concentrations of phytoplankton during blooms.

Egg size of *L. miliaris* are similar to *L. notorcadensis* and *A. nitens*, developing up to 500 µm and as described in *A. nitens*, the eggs appear to have a high yolk content, demonstrating a high investment of energy into each egg (Higgs et al. 2009). It is not clear how fertilisation occurs although it is likely that the unidentified maternal tissue (Fig. 3c, d) may play a role in holding the ova in position or in fertilisation.

A previously unknown trait observed in *L. miliaris* is the large number of previtellogenic oocytes that develop in a small portion of gonad beside the posterior adductor muscle which far exceed the number of young an individual can brood, perhaps in the lifetime of the animal. Brooding traits are considered to have evolved from more primitive planktotrophic traits as a consequence to environmental conditions and once lost, these traits are not likely to be reacquired (Strathmann 1985). These previtellogenic eggs are akin in size (19–50 µm), number (200+) and arrangement to planktotrophic species and may be indicative of a planktotrophic past of an ancestor species.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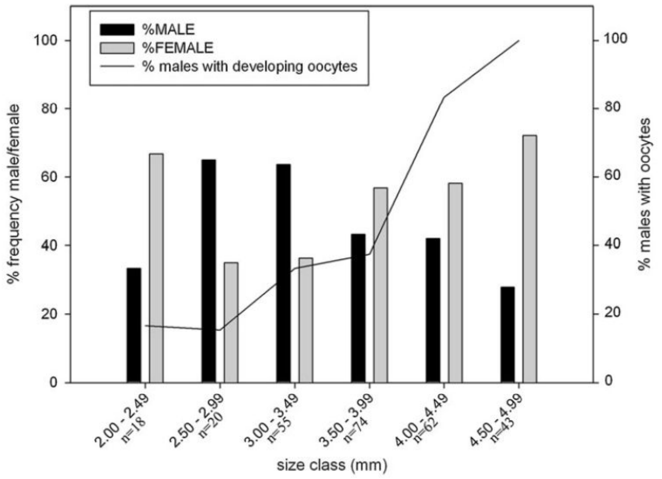


Fig. 6 *Lissarca miliaris* from Potter Cove, King George Island, January 1994. Percentage frequency male:female sex ratios with increasing size classes using histology <3 mm and dissection >3 mm. Percentage frequency of males with developing previtellogenic oocytes is from histology on mature males

Table 1 Brooding traits of Antarctic philobryid bivalves *L. miliaris, L. notorcadensis* and *A. nitens*

Species	Depth range (m)	Brood size (*n*)	Size at release (mm)	References
L. miliaris	0–275	15–69	0.77	Richardson (1979), this study
L. notorcadensis	100–1,000	15–30	1.25	Brey and Hain (1992)
A. nitens	8–2,350	16–55	0.84	Hain and Arnaud (1992), Aldea et al. (2008), Higgs et al. (2009)

Hermaphroditism

Hermaphroditism is not a rare reproductive trait in Southern Ocean molluscs and has been described in the bivalves *Laternula elliptica* (Bosch and Pearse 1988), *Thracia meridonalis* (Sartori and Domaneschi 2005) and the brooding bivalves *Mysella charcoti* and *M. narchii* (Passos and Domaneschi 2009), all demonstrating simultaneous hermaphroditism. Sequential protandric hermaphroditim is also common in molluscs where an organism functions initially as a male and then female at a later stage (Heller 1993) and is potentially advantageous for small brooding species, which are limited by space available to brood their young (Ghiselin 1969; Heath 1977; Wright 1988; Heller 1993). The occurrence of different types of hermaphrodite traits within populations is known to persist in species of *Crassostrea* (Heller 1993). This group of oysters is mostly single sex but also demonstrates protandric and simultaneous hermaphrodite traits, the cues of which are genetically or environmentally driven (Coe 1943; Guo et al. 1998; Ruiz-Verdugo et al. 2000).

Sequential protandric hermaphroditism was described in *L. notorcadensis* when a 5 % of a population from the Scotia Sea was found to possess both testes and ovaries while the proportion of males was highest in the smallest size classes (Prezant et al. 1992). Hermaphroditism in *L. miliaris* is different, however, as it demonstrates both simultaneous and sequential traits. The long retention of male gonads in mature females and development of primary oocytes in mature males are evidence of simultaneous gonad development, but the reduction of one of the gonads is indicative of sequential hermaphroditism. Sex-ratio data in this study support the change of sex from male to female and the proportion of hermaphrodites in the entire population is 30 %, higher than the 5 % observed in *L. notorcadensis* (Prezant et al. 1992). The higher percentage of females in the 2.00–2.49 mm size class may relate to the difficulty of determining immature testis from other tissue types and the development of previtellogenic oocytes may occur before the formation of determinable testis.

The occurrence of reduced testes in brooding *L. miliaris* could also be evidence of the rarer alternating sex hermaphrodite trait where specimens change sex more than once in their lifetime. This is commonly found in temperate and tropical *Ostrea* spp., which change sex seasonally (Asif 1979; Heller 1993) and is supported in this study by the observation of reduced testis in late brooding females, indicating a possible male reproductive stage whilst brooding young. Reduced testis at this stage of reproduction is curious as its position is in the portion of gonad where vitellogenesis of oocytes occurs. Figure 5e, which shows the undeveloped testis in the dorsal portion of gonad and vitellogenic oocytes, may be in a transitional stage where the oocytes are being extruded into the mantle cavity and the testes are re-developing; however, only regular sampling of *L. miliaris* would confirm this reproductive stage. Alternating sexuality would enable females to allocate energy to male gonad production and remain sexually active during long periods of brooding. In a short-lived species with a long period between gametogenesis and larval release, alternating sexuality would maximise the reproductive output of *L. miliaris* that may otherwise only reproduce up to 3 times within their life span. Ripe testis has not, however, been observed in a brooding *L. miliaris,* and larger brooding females have been observed with small vitellogenic oocytes. It is not possible to accurately determine the percentage of functional females with testis in *L. miliaris* as reduced testes are not easily distinguished from surrounding tissue.

Lissarca miliaris may have evolved hermaphrodite brooding traits in response to a number of different constraints including small adult size and isolation. Small size is commonly associated with brooding as larger animals can often produce more young than they can brood (Strathmann and Strathmann 1982; Strathmann et al. 1984). In the Antarctic context, brooding is likely to be so high due to speciation within brooding clades and the possible extinction of non-brooding species during glacial periods (for discussion see Poulin et al. 2002; Thatje et al. 2005). Hermaphroditism can also be linked to small sizes, however, as the female's limited capacity to brood may select for sequential protandric hermaphroditism (Ghiselin 1969; Heath 1977; Strathmann and Strathmann 1982; Wright 1988) and the retention of unused gonad may represent an intermediate stage of development. Isolation and small population size may also select for hermaphrodite traits as this may increase the chances of successful reproduction (Ghiselin 1969; Heller 1993).

Evolutionary consequences

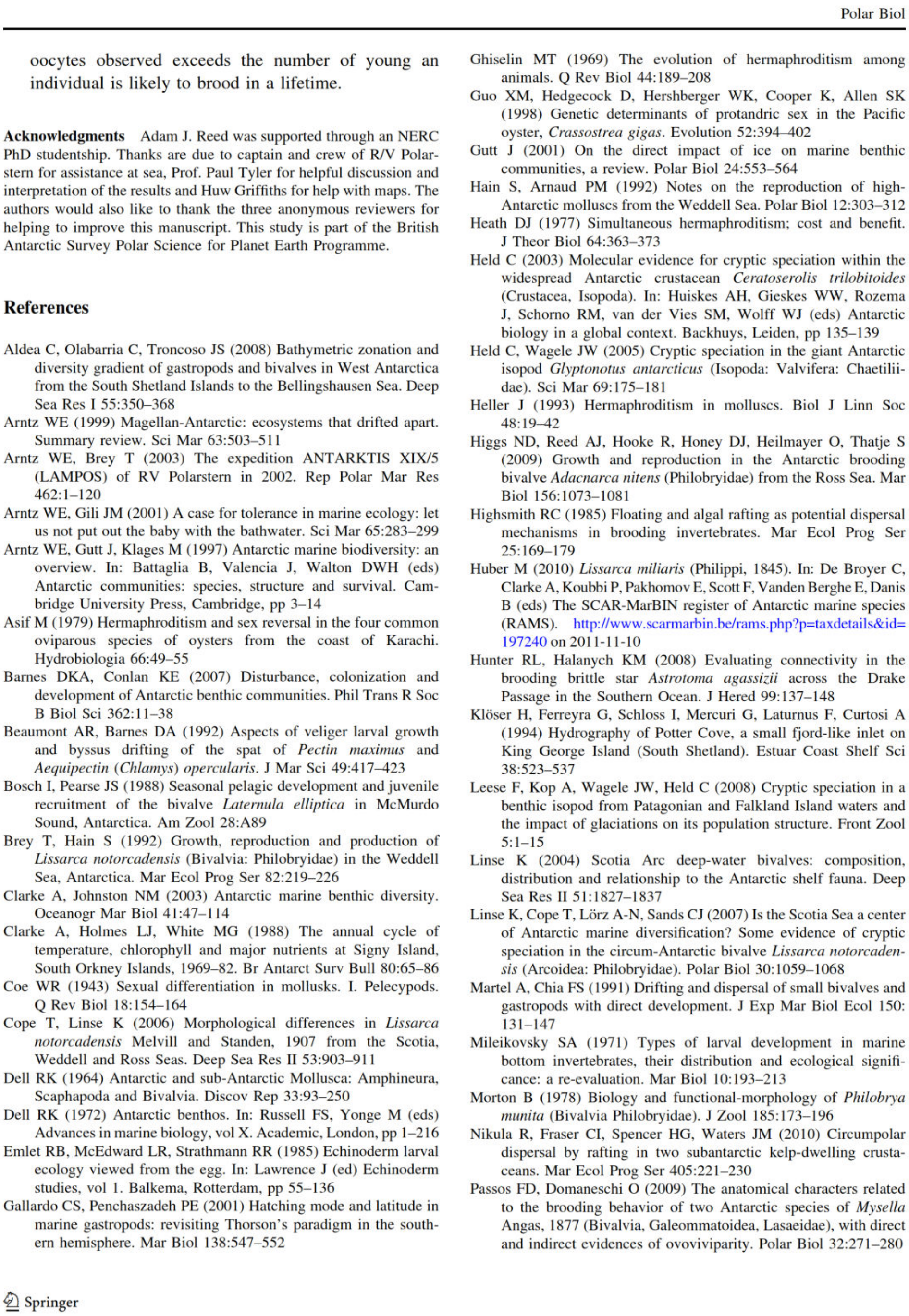
The limited capability for dispersal in brooding species can be overcome by rafting, and the unique oceanographic conditions offered by the Antarctic circumpolar current allow for connectivity between separated island groups (Highsmith 1985; Martel and Chia 1991; Smith 2002; Nikula et al. 2010). It has to be recognised, however, that genetic diversity in brooders appears high, frequently resulting in cryptic speciation and species clusters of circum-Antarctic distribution (Held 2003; Held and Wagele 2005; Cope and Linse 2006; Linse et al. 2007; Hunter and Halanych 2008; Leese et al. 2008; for discussion see Thatje 2012). The distribution of *L. miliaris* in the Scotia Sea is likely to be the consequence of dispersal via macroalgal rafting (Highsmith 1985; Higgs et al. 2009) or juvenile byssus drifting (Sigurdsson et al. 1976; Beaumont and Barnes 1992). Hermaphroditism may have facilitated founding populations and population expansion following habitat contraction during glacial periods (Thatje et al. 2008, Thatje 2012) as small populations in ice-free refugia would be likely to have both sexes for reproduction. Future molecular studies into the origins of *L. miliaris* may provide evidence of the Scotia Sea Islands acting as stepping-stones into the Antarctic from the Magellan region during recolonisation events (Arntz 1999).

Lissarca miliaris demonstrates a specialised reproductive trait in the Southern Ocean and provides more evidence to the hypothesis that brooding in small bivalves may provide an evolutionary advantage over other Antarctic fauna (Dell 1972; Poulin et al. 2002; Thatje et al. 2005, 2008; Higgs et al. 2009). The occurrence of hermaphroditism in this species is a further adaptation that has enabled the species to maximise their reproductive output and perhaps to colonise islands on both sides of the polar front. Philobryid bivalves represent many species of small benthic Antarctic fauna by demonstrating common traits (small size, brooding, and variable Antarctic/sub-Antarctic distributions), and this study highlights the need for further investigation into brooding traits and the processes that may have driven unique characteristics. *Lissarca* spp. also make a good model species for future studies of population genetics, potentially revealing in detail the origins and radiation processes of the philobryid bivalves and increasing our understanding on the processes that have driven the evolution of Antarctic benthic diversity.

Summary

Lissarca miliaris show an unusual reproductive trait that may function to maximise reproduction in a cold stenothermal and food-limited environment and are distinct from other Antarctic bivalves by;

- A transitional hermaphrodite phase in *L. miliaris* from male to female occurring throughout every size class, and functional males are found in the largest size class.
- Male reproductive tissue persisting long after changing sex and observed in late brooding females, 18–24 months after the change in sex.
- The production of a large number of previtellogenic oocytes in functional males, where the number of



oocytes observed exceeds the number of young an individual is likely to brood in a lifetime.

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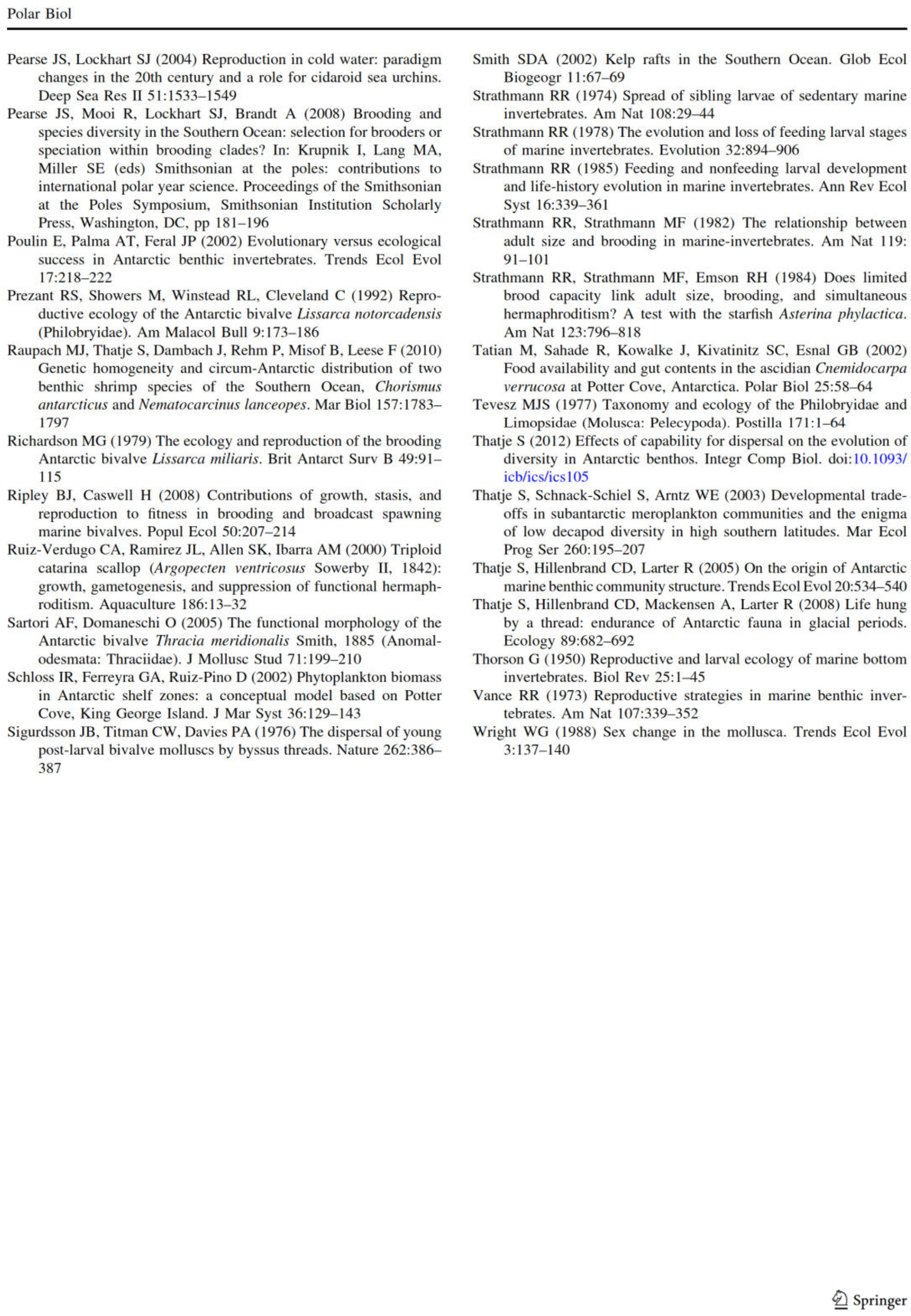

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Species	Research Cruise	Station/sample no.	n	Depth (m)
Yoldiella valettei	LAMPOS 2002	PS61-46-7-E	9	2893
		PS61-133-3	1	1122
		PS61-135-3	2	4679
	BENDEX 2003	PS65-233	1	846
		PS65-232	25	910
		PS65-148	1	290
		PS65-048	40	457
		PS65-145	5	402
		PS65-337	5	273
		PS65-173	5	279
		PS65-284	9	805
		PS65-283	7	554
	ANDEEP III 2005	PS67-059-5-SE	1	4655
		PS67-078-11	7	2186
		PS67-074-6-E	46	1053
		PS67-081-9	4	4526
		PS67-078-9	30	2174
		PS67-080.9	1	3138
		PS67-081-8	3	4427
		PS67-133-2	2	1549
		PS67-150-7	1	1938
		PS67-151-1	3	1188
	BIOPEARL I 2006	ST-EBS-4	175	307
		LI-EBS-3	4	557
		LI-EBS-4	14	189
		EI-EBS-2	6	1000
		EI-EBS-3	1	493
		EI-EBS-4	9	199
		PB-EBS-3	18	505
		ST-EBS-3b	150	500
		ST-EBS-2	32	1019
		ST-EBS-3	20	543
		SR-EBS-5	1	501
		ST-EBS-1	1	1571
		EI-EBS-1	3	1490
	BIOPEARL II 2008	B105 EBS 3F EPI	193	538
		B106 EBS 3F EPI	21	477
		B105 EBS 3F SUPRA	36	538
		B106 EBS 2B EPI	3	1003
		B105 EBS 1B EPI	41	1473
		B106 EBS 2B SUPRA	8	1003
		B105 EBS 1B SUPRA	41	1473
		B106 EBS 3C EPI	148	488
Yoldiella ecaudata	BIOPEARL I 2006	ST-EBS-2	48	1019
		ST-EBS-3	1	543
		SG-EBS-3	54	496

	BIOPEARL II 2008	B106 EBS 1B SUPRA	4	1495
		B106 EBS 2B EPI	9	1003
		B105 EBS 1B SUPRA	13	1473
		B105 EBS 3F SUPRA	13	538
		B105 EBS 1B EPI	48	1473
		B106 EBS 3F SUPRA	50	477
		B106 EBS 3F EPI	83	477
		B106 EBS 3C EPI	78	488
		B105 EBS 3C EPI	79	548
		B105 EBS 3F EPI	96	538
Yoldiella sabrina	BIOPEARL I 2006	BO6 1105	45	211
		BO6 1036	19	197
		BO6 1113	52	211
		BO6 1198	28	505
		BO6 1098	12	504
		BO6 1207	2	1656
	BIOPEARL II 2008	B105 EBS 3F EPI	120	538
		B105 EBS 3C EPI	109	548
	ANDEEP III 2005	05-062	14	4670
		05-724	5	4803
		05-0040	11	4730
		05-723	19	3404
		05-682	1	4700
		05-357	2	4993
		05-417	6	2156
		05-086	1	4580
		05-597	3	4900
		05-460	2	4797
		05-112	7	4578
		05-618	4	2617
		05-135	2	4552
		05-432	4	2156
		05-687	2	4803
		05-587	3	4931
	LAMPOS 2002	02-036	6	3960
		02-076	2	5190
		02-179	1	2307
	BENDEX 2003	03-205	1	
		03-320	2	