The biology, ecology, and taxonomy of sea urchins in the subtropical biogeographic transition zone of southeast Australia

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I live and work on the lands of the Gadigal and Wangal people, in the Eora nation. I would like to pay my respects to their elders past, present and emerging. Sovereignty was never ceded, always was and always will be Aboriginal land.

Statement of originality

This is to certify that to the best of my knowledge, the content of this thesis is my own work. This thesis has not been submitted for any degree or other purposes.

I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

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Author attribution statement

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I co-designed this study with co-authors MB, OB, AK and BS. All imaging (micro-CT) and laboratory work was performed by me. I conducted the data analysis assisted by OB and wrote the manuscript. All co-authors provided feedback on the manuscript.

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I co-designed this study with BS and MB. BS conducted the fieldwork. I collected the data from photographs, analysed the data and wrote the manuscript. All co-authors contributed feedback on the manuscript.

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Chapters 2-4 are formatted in accordance with the respective journal guidelines, including in text citations and I use the collective multi-author pronouns (we, our) to reflect the work of my co-authors. For the remainder of the thesis, the General Introduction (Chapter 1), Chapter 5 and General Discussion (Chapter 6), I use the single author pronoun (I, my). I am the corresponding author on each of the submitted and published manuscripts listed above.

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As supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

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Em

Abstract

Ocean warming is driving the global redistribution of marine ectothermic taxa to higher latitudes and the ecotone between tropical and temperate realms is likely to be at the forefront of this redistribution. In marine ecosystems, herbivory is a significant ecological process, regulating ecosystems through grazing, and sea urchins are among the most important herbivorous taxa across latitudes on tropical coral reefs, temperate rocky reefs and in seagrass meadows. This thesis focusses on how ocean warming will affect sea urchins in the subtropical biogeographic transition zone of eastern Australia, and I aimed to fill knowledge gaps regarding the taxonomy, ecology and biology of echinoids in this unique ecosystem.

The tropical-to-temperate cline along the continent from the Great Barrier Reef to the Great Southern Reef in eastern Australia offers an excellent model system to understand how warm-affinity taxa will affect higher latitude ecosystems into the future. More so, the subtropical biogeographic transition zone of eastern Australia is strongly influenced by the East Australian Current (EAC), a poleward flowing continental boundary current that transports propagules along the east coast of Australia. Ocean warming is driving intensification and poleward encroachment of the EAC and so high latitude ecosystems in southeastern Australia are increasingly receiving low latitude species.

Until recent taxonomic investigation, the presence of *Tripneustes australiae* in southeast Australia was considered to be a poleward range extension of its tropical congener *T. g. gratilla*. To clarify the taxonomy of *Tripneustes* species in the region, morphological and molecular taxonomic investigations were employed. *Tripneustes kermadecensis* was re-

designated as *Tripneustes australiae* based on type material (*Evechinus australiae*) found in the Australian Museum with the type locality being Sydney Harbour (Port Jackson). I highlight that this species is characteristic of the southeast Australian shallow water marine fauna (Chapter 2).

In a 9-year large-scale ecological study of sea urchin abundances and size structure in the subtropical biogeographic transition zone of eastern Australia, I demonstrate that biogeographic affinity alone does not predict shifts in the abundances of echinoids. The density and population size structure of temperate (*Centrostephanus rodgersii, Phyllacanthus parvispinus*), subtropical (*Tripneustes australiae*) and tropical (*Diadema* spp.) affinity species were quantified. Populations of *Diadema* spp. and *Tripneustes australiae* declined over the survey period, while the cool-affinity species *C. rodgersii* remained stable, despite the surveys occurring over a period punctuated by severe marine heatwaves and warming. This contrasts with the paradigm that warm affinity/low latitude species will become more dominant in these transition zones. I highlight that when predicting changes in echinoid abundances, life history and population biology traits such as boom-bust dynamics need to be considered.

Utilising museum collections and citizen science, the distributions of *Tripneustes* species in eastern Australia were resolved and the data used to predict the range extension capacity of *Tripneustes* in this region. *Tripneustes g. gratilla* is a tropical-to-temperate generalist that ranged in the collections from Papua New Guinea (9° 56' 2.4"S) to Jervis Bay (35° 7' 12"S) with a broad realised thermal niche (16.3 - 29.97°C). In contrast, *T. australiae* is a narrow range subtropical-to-temperate specialist restricted to New South Wales and offshore Islands around the Tasman, ranging from Byron Bay (28° 37' 0.12"S) to Narooma (36° 15' 0"S) with a narrow thermal niche (15.3 - 26°C). *Tripneustes australiae* is likely to undergo a poleward range shift by 2100, and *T. g. gratilla* will likely undergo a poleward range expansion into the future. More so, there are important ecological implications for these species living in sympatry across 7° of latitude with respect to their potential to hybridise.

The embryonic and larval thermal tolerances of *T. australiae* show that the optimal window for survival, normal development and developmental progression for this species is between 17 and 23°C. Thus, in both larval and adult life phases this species is a narrow range specialist. This has ecological implications, as this species may disperse further poleward as the oceans warm but will likely be limited to mainland Australia and New South Wales in the near future.

Throughout this thesis, I draw on taxonomic, ecological and biological investigations to address questions regarding the effect of climate change on sea urchins in the subtropical biogeographic transition zone of eastern Australia. In doing so, I highlight that echinoids in this unique ecotone will have varied responses to climate change depending on the taxa and region of interest. Sea urchins are ecologically important grazers globally, and the findings of this thesis have key ecological and management implications for the diverse assemblage of these species in southeastern Australia and beyond.

Chapter 1.

General Introduction

1.1 Species on the move

Environmental conditions such as temperature shape ecological communities, determine species distributions and affect the evolution of marine taxa across different spatial scales (Tittensor et al. 2010; Sunday et al. 2011, 2014; Stuart-Smith et al. 2017; Saupe et al. 2019; Zarzyczny et al. 2023). For example, low latitude tropical regions are dominated by warm-affinity species, while high latitude temperate communities are dominated by cool-affinity species (Sunday et al. 2011; Stuart-Smith et al. 2017). This drives the evolution of species traits and physiologies that confer fitness in these environments (Sunday et al. 2011, 2012, 2019; Stuart-Smith et al. 2013; Sommer et al. 2014). However, climate change is driving a global re-distribution of taxa. As the oceans warm, taxa are moving towards higher latitudes and greater depths to remain within their physiological tolerances (Parmesan 2006; Sunday et al. 2012, 2015; Poloczanska et al. 2016; Pecl et al. 2017; Burrows et al. 2019; Stuart-Smith et al. 2022). This can impact receiving ecosystems as new ecological interactions and novel selective forces emerge that can alter ecological assembly and the evolutionary trajectories of taxa (Parmesan 2006; Zarzyczny et al. 2023).

Globally, ecological communities are homogenising as taxa move across traditional bio-geographic barriers. For example, high latitude or warm-affinity species are moving poleward across the thermal boundary between tropical and temperate realms (Sunday et al. 2012; Poloczanska et al. 2016; Vergés et al. 2019; Burrows et al. 2019; Stuart-Smith et al. 2022; Zarzyczny et al. 2023). As a result, conventional biogeographic paradigms such as the dominance of cool-affinity species at high latitudes are changing (Tittensor et al. 2010; Sunday et al. 2011, 2012; Burrows et al. 2019). This global phenomenon is called 'tropicalisation' and presents unknown and pressing risks to receiving ecosystems, wherein new biotic interactions can re-shape ecosystems and cause widespread biodiversity loss (Vergés et al. 2014, 2019; Wernberg et al. 2016; Zarzyczny et al. 2023). More so, this phenomenon is accelerated at the continental scale in regions with strong poleward flowing boundary currents, such as the east coasts of Japan, South America, Africa and the east and west coasts of Australia (Vergés et al. 2014, 2019; Wernberg et al. 2016).

1.2 Life on the edge: Biogeographic transition zones

Marine biogeographic transition zones represent a dynamic interface between biogeographic realms, such as the ecotone between tropical and temperate ecosystems (Malcolm et al. 2010; Sommer et al. 2014; Horta e Costa et al. 2014; Troast et al. 2020). These subtropical transition zones are characterised by a mixture of tropical, subtropical and temperature species (Malcolm et al. 2010; Beger et al. 2014; Sommer et al. 2014; Malcolm and Ferrari 2019; Smith et al. 2021; Cant et al. 2023) and are dominated by taxa living at their range edges, such as corals living at their cool-range edges and macroalgae living at their warm-range edges (Sommer et al. 2014, 2017; Vergés et al. 2016; Smith et al. 2021). As such, the assembly of these species can be driven by fine scale environmental gradients (e.g. in-shore off-shore gradients), and seasonality (Malcolm et al. 2010; Sommer et al. 2014). More so, they are especially vulnerable to ocean warming and climate extremes such as heatwaves and cold stress (Vergés et al. 2016; Kim et al. 2019, Leriorato et al. 2019; Cant et al. 2021; Lachs et al. 2021; Smith et al. 2021)

There is a prevailing school of thought that tropical-to-temperate transition zones, which host sensitive taxa and are spatially proximate to the tropics, will be the frontline for the range expansion of tropical species (Zarzyczny et al. 2023). More so, they may provide refugia for low latitude species into the future (Beger et al. 2014; Kim et al. 2019; Zarzyczny et al. 2023). As such, these regions are hotspots for biodiversity change and can provide insight into how ecological assemblages and interactions may transform as the oceans warm. This is especially relevant on the east coast of Australia due to the rapid intensification and warming of the East Australian Current that transports biological propagules from the tropics poleward (Ridgway and Godfrey 1997; Suthers et al. 2011; Vergés et al. 2014).

1.3 The East Australian Current and its role in southeastern Australia

The East Australian Current (EAC) is a poleward flowing boundary current that extends from the Coral Sea in the north, and flows south along the east coast of Australia, until it reaches the Tasman Front where it diverges and extends east into the Pacific towards New Zealand (Ridgway and Godfrey 1997). In eastern Australia this current is responsible for the poleward (southern) dispersal of larval marine fishes and invertebrates (Ling 2008; Vergés et al. 2016; Byrne et al. 2017, 2022; Ling et al. 2019; Smith et al. 2021). In addition, ocean warming is resulting in the intensification, increased southern encroachment and overall warming of the EAC (Suthers et al. 2011).

The east coast of Australia is unique as it hosts a tropical-to-temperate temperature gradient along the continental shelf, from the tropical thermal regimes of Cape York in the north, to cold-temperate climates in Tasmania. This creates an ecological cline from coral reefs on the Great Barrier Reef in the north, to the macroalgae-dominated rocky reefs on the Great Southern Reef in the south (Bennet et al. 2015). In combination with the EAC, this makes the subtropical biogeographic transition zone of eastern Australia an excellent model system to investigate how the poleward range extension of marine taxa will affect ecosystems into the future.

1.4 Sea urchins: biology, ecology and taxonomy

1.4.1 Ecology

Herbivory is an important ecological processes in marine systems (Milchunas and Lauenroth 1993; Hawkes and Sullivan 2001; Côté et al. 2004; Ohgushi 2005; Futuyma and Agrawal 2009; Naeem et al. 2016; Bernes et al. 2018), and sea urchins are some of the most significant herbivorous taxa from the poles to the tropics, across habitats including coral reefs, macroalgal forests and seagrass meadows (Larkum and West 1990; Underwood et al. 1991; Alcoverro and Mariani 2002; Ling 2008; Filbee-Dexter and Scheibling 2014; Lessios 2016; Byrne and Andrew 2020; Lawrence and Agatsuma 2020; Steneck 2020; Moreira-Saporiti et al. 2023). For example, on Caribbean coral reefs the echinoid genus *Diadema*, is a grazer that mediates coral success by supressing the colonisation of turfing algae that can outcompete corals (Lessios 2016). However, a disease outbreak caused local extinctions of *Diadema* in the Caribbean, contributing to the collapse of these coral reef ecosystems (Lessios 2016). In contrast, in the Caribbean and globally, sea urchins in the genera *Tripneustes* and *Lytechinus* can overgraze seagrass meadows (Valentine and Heck 1991; Alcoverro and Mariani 2002; Eklöf et al. 2008; Moreira-Saporiti et al. 2023). These studies show the importance of species-specific studies to understand the ecological roles of echinoids across habitats.

1.4.2 Sea urchin barrens

Sea urchin barrens (hereafter 'barrens') are ecological communities dominated by sea urchins and coralline algae in place of macroalgal-beds and turfing algae (Steneck et al. 2002; Filbee-Dexter and Scheibling 2014; Steneck 2020). Barrens are typically mediated by sea urchin grazing and represent a stable alternate state to macroalgal-beds on temperate rocky reefs (Steneck et al. 2002; Filbee-Dexter and Scheibling 2014; Steneck 2020). Barrens are persistent and resilient systems, with positive feedback loops that maintain a low abundance of macroalgae and high abundance of urchins, making them very difficult to restore/reverse to macroalgal systems (Steneck et al. 2002; Filbee-Dexter and Scheibling 2014; Ling et al. 2015; Steneck 2020). Generally, barrens host different and lower biodiversity compared to macroalgal-beds (Filbee-Dexter and Scheibling 2014; Ling et al. 2015; Steneck 2020), but for some taxa they can support higher diversity (Coleman and Kennelly 2019). In coastal communities of New South Wales (southeast Australia), stable sea urchin populations promote a spatial mosaic of barrens and macroalgal-beds that support high biodiversity (Jones and Andrew 1990; Underwood et al. 1991; Kingsford and Byrne 2023). The best studied barren-forming sea urchins are *Centrostephanus rodgersii* in southeast Australia, *Strongylocentrotus purpuratus* in the eastern Pacific, and *S. droebachiensis* in the Atlantic (Steneck et al. 2002; Filbee-Dexter and Scheibling 2014; Ling et al. 2015; Steneck 2020).

1.4.3 Biology

Due to a long and enduring history of embryological and larval research, the larval development, biogeography and life history strategies of echinoids are very well understood (McEdward 1986; Strathmann 1993; McEdward and Miner 2001; Strathmann et al. 2002; Marshall et al. 2012). Sea urchins have a bi-phasic life history, that comprises a mobile larvae stage that occupies the water column and is dispersed by ocean currents, and a benthic adult stage (McEdward and Miner 2001; Strathmann et al. 2002; Byrne et al. 2017). Echinoids have a variety of reproductive strategies that range from brooding of juveniles (e.g. *Abatus cordatus*), non-feeding simple larvae that settle into the benthos within days (e.g. *Heliocidaris erythrogramma*), to long-lived feeding pelagic echinoplueti that are able to disperse extremely long distances (e.g. *Tripneustes g. gratilla* & *Centrostephanus rodgersii*) (McEdward and Miner 2001; Lessios et al. 2003; Byrne et al. 2022). Strong foundational knowledge of larval echinoids has given rise to the field of larval ecology (Byrne 2011a; Byrne 2011b; Byrne et al. 2016, 2017, 2022; Byrne and O'Hara 2017). In particular, studies regarding larval ecology and climate change have advanced our understanding of local invasions, outbreaks and current and future dispersal and distribution patterns (Byrne 2011a; Byrne 2011b Byrne et al. 2016, 2017, 2022; Byrne and O'Hara 2017).

1.4.4 Taxonomy

As the biological field moves away from traditional biodiversity discovery and description of taxa pioneered by early biologists such as Carl Linnaeus, there is an emerging paradigm that the taxonomy of common and abundant taxa is 'complete'. Conspicuous echinoids were described by early taxonomists such as Alexander Agassiz, Hubert Lyman Clark and Theodor Mortensen, the latter being regarded as the pioneer of echinoderm biology whose work is still the foundational reference for many aspects of echinoid taxonomy and larval biology. Many species descriptions and names that were assigned by these early taxonomists are still retained. However, many examples show that taxonomic understanding is far from complete, especially with respect to the discovery of species complexes (Lessios et al. 2003; Bronstein et al. 2017; Addison and Kim 2018; Coppard et al. 2021). Given the prevalence of cryptic species in marine invertebrates and lack of research and funding into species descriptions, we are undoubtably underestimating the extent of biodiversity loss as the biodiversity crisis worsens (Dubois 2003; Bálint et al. 2011)

The emerging field of integrative taxonomy combines molecular phylogenetics, facilitated by the rapid evolution of molecular technology, coupled with traditional morphological taxonomy. Integrative taxonomy has allowed for a greater understanding of biodiversity and particularly cryptic diversity (Dubois 2003; Dayrat 2005; Padial and Miralles 2010). As such, many common and abundant taxa of echinoids have been re-designated or described in recent years (Lessios et al. 2003; Bronstein et al. 2017; Addison and Kim 2018; Coppard et al. 2021). For example, the Atlantic *Strongylocentrotus droebachiensis* is likely a cryptic species complex, and the common east Pacific species *Tripneustes depressus* is not distinct from its western Pacific congener *T. g. gratilla*, or they represent a species complex (Lessios et al 2003; Zigler et al 2003).

1.5 The sea urchins of southeastern Australia

Southeastern Australia is a hot spot for echinoid diversity, where sea urchins are common and abundant in rocky reefs, seagrass meadows and soft-sediment habitats (Larkum and West 1990; Underwood et al. 1991; Byrne and O'Hara 2017). Echinoids in this region have a range of life history strategies and ecological niches (Byrne and O'Hara 2017). For example, some species display long term stable populations, while others form barrens and undergo boom-bust cycles (Valentine and Edgar 2010; Byrne and O'Hara 2017; Byrne and Andrew 2020; McLaren et al. 2024). There are three dominant genera of echinoids in southeastern Australia with different ecological roles, life-histories and distributions: *Heliocidaris, Centrostephanus* and *Tripneustes* (Byrne and O'Hara 2017).

1.5.1 *Centrostephanus*

Centrostephanus rodgersii is the most common and abundant species in southeastern Australia and the dominant barren-forming species on the southeast coast (Jones and Andrew 1990; Underwood et al. 1991; Byrne and Andrew 2020; Kingsford and Byrne 2023). The climate-driven range expansion of *C. rodgersii* across the Bass Strait into Tasmania has transformed the local sea scape from diverse macroalgal-dominated ecosystems to low diversity barrens

(Ling 2008; Ling et al. 2015). More so, the expansion of *C. rodgersii* barrens in northern New Zealand poses a threat to local biodiversity (Balemi and Shears 2023).

However, its ecological role within its native range on the mainland in New South Wales is less studied and has contrasting patterns between its northern and southern range (Glasby and Gibson 2020; Davis et al. 2023). In the northern - mid distribution of its native range, *C. rodgersii* plays an important role in mediating the mosaic system of macroalgal dominated rocky reef, barrens and incipient barrens habitat that is a characteristic and somewhat stable feature of nearshore communities in this region (Jones and Andrew 1990; Underwood et al. 1991; Glasby and Gibson 2020; Kingsford and Byrne 2023). However, barrens appear to increase in extent with increasing latitude (Glasby and Gibson 2020; Przeslawski et al. 2023; Davis et al. 2023). In the south of its native range, the extent and density of *C. rodgersii* barrens appears to have increased and will likely continue to increase (Glasby and Gibson 2020; Przeslawski et al. 2023; Davis et al. 2023). More so, projections suggest that while barrens in the southern range may continue to expand, those in the mid – northern range may decrease in extent and density (Davis et al. 2023). Interestingly, *C. rodgersii* has a congener on the west coast of Australia *C. tenuispinus,* which in contrast to *C. rodgersii* is rare and occurs in low densities (Byrne and O’Hara 2017). This species plays a similar ecological role to other global *Centrostephanus* and Diadematid sea urchins, occurring in low numbers and mediating the dynamics of local turfing algae (Lessios 2016; Byrne and O’Hara 2017; Thilakarathna 2017; Byrne and Andrew 2020).

1.5.2 *Heliocidaris*

There are two sympatric *Heliocidaris* species in our region, *H. e. erythrogramma* and *H. tuberculata*. The reproductive biology and larval development of these species are well described, as they are congeneric species with contrasting modes of development (feeding and non-feeding larvae). This makes them excellent models to study the evolution of developmental modes (Byrne and O'Hara 2017; Wang et al. 2020; Davidson et al. 2022). *Heliocidaris erythrogramma* is a common shallow water species in the intertidal and shallow subtidal and has been known to have localised population outbreaks in estuaries such as Port Phillip Bay and Gamay (Botany Bay) (Larkum and West 1990; Keesing 2020). In contrast, *H. tuberculata* is a long-lived stable species that has been historically overfished and is now subject to fisheries closures to promote population recovery (Byrne and O'Hara 2017).

1.5.3 *Tripneustes*

Tripneustes is a global genus that comprises *T. ventricosus, T. gratilla gratilla, T. gratilla elatensis, T. depressu*s and *T. australiae*. Of these 5 taxa, 3 occur in the Pacific Ocean, with different distributions and range sizes. *Tripneustes g. gratilla* occurs tropically in the Indo-Pacific, from east Africa to the offshore islands of Australia in the Pacific, with occurrences at Lord Howe and Norfolk Island, and on the coast of eastern Australia (Lawrence and Agatsuma 2020). *Tripneustes depressus* occurs primarily in a narrower range along the west coast of America, occurring in the Gulf of California and the west coast of Mexico and the Galapagos (Lawrence and Agatsuma 2020). However, Lessios (2003) concluded that *T. gratilla* and *T. depressus* are the same species or part of a species complex, and that the occurrences of *T. depressus* in the western Pacific are likely *T. g. gratilla*. The Atlantic species of *Tripneustes*, *T. ventricosus,* is a well-studied tropical species found on the west coast of South America from Panama to Brazil and around the Caribbean (Lawrence and Agatsuma 2020). *Tripneustes australiae* (formerly *T. kermadecenis*; see McLaren et al 2023; Chapter 2) is a trans-Tasman endemic that occurs on the southeast coast of Australia, in northern New Zealand, and around the Tasman on Lord Howe, Norfolk and the Kermadec Islands. Both *Tripneustes australiae* and *T. g. gratilla* occur on the southeast coast of Australia (Bronstein et al. 2019) and co-occur throughout part of their distributions. The *Tripneustes* genus is characterised by boom-bust population dynamics that can transform ecosystems (Valentine and Edgar 2010; Lawrence and Agatsuma 2020; Moreira-Saporiti et al. 2023). For example, *Tripneustes* have been observed to have population outbreaks in southeastern Australia around Sydney, Port Stephens and Lord Howe Island (Byrne et al in review; Valentine & Edgar 2010).

Recently *Tripneustes* has undergone phylogenetic revision as mitochondrial and nuclear loci revealed a Red Sea subspecies *T. g. elatensis* and a new species *T. kermadecensis* from the west Pacific (Bronstein et al. 2016, 2017). These studies support that *T. g. gratilla* and T. *depressus* in the west Pacific are the same species or a species complex (Zigler and Lessios 2003; Lessios et al. 2003; Bronstein et al. 2017).

1.6 'Trip ups': *Tripneustes* in Australia

In southeast Australia there are two sympatric species of *Tripneustes*, *Tripneustes australiae* (formerly *T. kermadecensis*) and *T. g. gratilla*. *Tripneustes australiae*, is described in Chapter 2 of this thesis and was designated as *T. kermadecensis* during the inception of this thesis (Bronstein et al. 2017). *Tripneustes kermadecensis* was described in 2017 from type material collected in the Kermadec Islands (Bronstein et al. 2017), a series of remote islands in the subtropical western Pacific, and in 2019 it was shown to be the common species of *Tripneustes* in southeastern Australia (Bronstein et al. 2019). Until this taxonomic clarification, the occurrences of *T. australiae* on the southeast coast of Australia were considered to be a poleward range extension of *T. g. gratilla* (Castro et al. 2020). Therefore, studies of *Tripneustes* in southeastern Australia were attributed to *T. g. gratilla*, but could have been *T. australiae*, or a mixture of both species. The description of *T. australiae* revealed a critical knowledge gap regarding the biology and ecology of *Tripneustes* in southeastern Australia. Much of this thesis focuses on filling this knowledge gap.

1.7 *Evechinus australiae*

Early in the history of biodiversity discovery and description in the colony of Sydney, Reverend Tennison-Woods described a species designated as *Evechinus australiae* in his work 'The Echini of Australia' (Tension-Woods 1878). I discovered this type material in the Australian Museum collections, and realised that it was potential senior type material for *T. kermadecensis (*now *T. australiae,* see Chapter 2*)*. These specimens were collected in Sydney Harbour and have not been considered or redescribed since. Taxonomic investigation into this species comprises the first data chapter of this thesis.

1.8 Thesis overview and aims

The primary aim of this thesis is to build knowledge on the biology, ecology and taxonomy of echinoids in the subtropical biogeographic transition zone of southeastern Australia. I do so with the central question in mind: *how will echinoids in the subtropical bio-geographic transition zone of southeastern Australia respond to climate change?* Specifically, I employ an integrative approach across these fields to answer overarching questions regarding climate change in southeastern Australia. I integrate traditional morphological taxonomic practices, modern molecular taxonomy, large scale ecological surveys, museum collections and larval biology to understand how echinoids are responding to change in the subtropical transition zone of southeastern Australia. In particular I focus on *Tripneustes*, which have received less research attention compared to *C. rodgersii* and *Heliocidaris*, despite having the capacity to transform ecosystems through population outbreaks. More so, there are major knowledge gaps given the recent description of *T. kermadecensis* and mistaken range extension of *T. g. gratilla.* I do so to build knowledge and contribute new data on how these important herbivores are responding to climate change and how they will influence ecosystem dynamics into the future.

This thesis comprises four data chapters (Chapter 2- 5) that are prepared and presented as stand-alone manuscripts formatted for publication. Chapter 2 and Chapter 3 are published in peer-reviewed journals and the chapters are formatted in accordance with journal guidelines. Chapter 4 has been accepted and is also formatted for the target journal. Chapter 5 has not yet been submitted for review.

In Chapter 2, I aimed to resolve the taxonomy of *Tripneustes* in Australia using morphological and molecular taxonomic practices. In doing so I aimed to establish whether the type locality of the *Tripneustes* in southeastern Australia was Sydney Harbour, adding knowledge to the biogeography and distribution of this species. This chapter is published in Invertebrate Systematics (Mclaren et al. 2023).

In Chapter 3, I describe decadal trends in the population dynamics of echinoids in the subtropical biogeographic transition zone of southeastern Australia. I do so by surveying the abundance and size of echinoids at seven sites throughout the biogeographic transition zone from 2010 - 2019. This is the first study to describe temporal patterns of echinoids in the subtropical biogeographic zone of Australia and included a period of significant warming and heatwaves. This chapter is published in Ecology and Evolution (Mclaren et al. 2024).

In Chapter 4, I used museum collections and citizen science initiatives to resolve the distributions and realised thermal niches of *Tripneustes* species in Australia and across the

Tasman Sea. These data were used to predict their future distributions with respect to ocean warming and ‘species on the move’. This is especially important considering that population outbreaks are characteristic of *Tripneustes*. This chapter is under review in the Journal of Biogeography.

In Chapter 5, I investigated the thermal biology of development in *T. australiae* with respect to its warm and cold tolerances to predict future range extensions or local extinctions across its range. I did so to understand the potential future poleward success of *T. australiae* larvae, and in turn predict its future range extent and potential ecological impacts.

Finally in Chapter 6, the General Discussion, I synthesise my key findings throughout this thesis and provide suggestions for future research. I consider the ecological, evolutionary and life history strategies of echinoids in southeastern Australia with respect to their contrasting and distinct biology and ecology. I also highlight the implications that the contrasting biology and ecology of echinoids in southeastern Australia has for management and the future of coastal ecosystems in the region.

Figure 1.1. Images of *Tripneustes australiae* (a & b), and *Tripneustes g. gratilla* (c & d). Photos provided by John Turnbull.

Chapter 2.

Hidden in plain sight: *Tripneustes kermadecensis* (Echinodermata: Echinoidea) is a junior synonym of the eastern Australian sea urchin *Evechinus australiae* described in 1878

2.1 Abstract

Accurate taxonomy and descriptions of species are key to understanding biodiversity. The echinoid genus *Tripneustes* is an ecologically and commercially important taxon that includes the tropical *Tripneustes gratilla gratilla* and the recently described *T. kermadecensis* from Australia and New Zealand. When employing the Australian Museum collections to clarify the distributions of these two species in east Australia we found potential senior type material for *T. kermadecensis*. These specimens from Sydney Harbour were originally described as *Evechinus australiae* by Tenison-Woods (1878), but not figured or redescribed in any subsequent report. We undertook molecular and morphological analysis of these specimens to determine whether *T. kermadecensis* and *E. australiae* represent two distinct taxa or not. This included micro-computed tomography, quantification of test traits, and molecular genetic analysis. The COI sequence and morphology of *Evechinus australiae* matched that of *Tripneustes kermadecensis*. As such, *T. kermadecensis* is a junior synonym of *Evechinus australiae*. The correct designation of this taxon thus is *Tripneustes australiae* (Tenison-Woods, 1878).

2.2 Introduction

The echinoid genus *Tripneustes* is an ecologically and commercially important pan-tropical taxon. *Tripneustes* are important herbivores (Lawrence and Agatsuma 2013) and as fast growing species, are of interest to fisheries and aquaculture (Sonnenholzner-Varas *et al.* 2018; Mos 2019). *Tripneustes* species occur in sea grass, coral, and rocky reef ecosystems and can have boom-bust population dynamics (Valentine and Edgar 2010; Lawrence and Agatsuma 2013). In high densities *Tripneustes gratilla gratilla* can overgraze sea grass (Alcoverro and Mariani 2002; Moreira-Saporiti *et al.* 2023) and in Hawaii, *T. g. gratilla* are used as a biological control measure to limit the growth and impact of invasive algae (Neilson *et al.* 2018).

Globally there are five recognised species-group taxa within the *Tripneustes* genus. *Tripneustes ventricosus*, the Atlantic species, *T. g. gratilla*, the Indo-Pacific species, *T. depressus*, the east Pacific species, *T. kermadecensis*, the west Pacific species and the Red Sea endemic *T. gratilla elatensis* (Lawrence and Agatsuma 2013; Bronstein *et al.* 2016, 2017). Within *Tripneustes*, there is discordance in phylogenetic relationships indicated by data from nuclear and mitochondrial loci, and thus conflicting information with respect to species delimitation (Zigler and Lessios 2003; Lessios *et al.* 2003; Bronstein *et al.* 2016, 2017). In a phylogeographic study of *Tripneustes*, Lessios *et al.* (2003) concluded that the east Pacific *T. depressus* and the west Pacific *T g. gratilla* are not distinct species. This finding was based on data from the mitochondrial locus cytochrome oxidase subunit I (COI) and Zigler and Lessios (2003) came to the same conclusion using the nuclear gene *Bindin*. However, when an integrative approach is used, combining nuclear and mitochondrial genetic markers with morphological traits, the five taxa are distinct and there is genetic evidence that *T. g. gratilla* might be a cryptic species complex (Bronstein *et al.* 2016, 2017). It is hypothesised that introgression of the mitochondrial genome is the driver of the discordance between nuclear and mitochondrial markers (Bronstein *et al*. 2016, 2017). Nevertheless, *T. ventricosus* and *T. kermadecensis* can be discerned from the other members of the genus using COI, but nuclear markers are needed to distinguish between *T. g. gratilla*, *T. g. elatensis*, and *T. depressus* (Bronstein *et al*. 2016, 2017).

In the subtropical-to-temperate transition zone of New South Wales (NSW) in eastern Australia, two species of *Tripneustes* co-occur, the tropical species *T. g. gratilla* and the subtropical species *T. kermadecensis*. Until recent taxonomic clarification (Bronstein *et al*. 2019), the presence of *T. kermadecensis* was misidentified as a range extension of *T. g. gratilla* (Castro *et al*. 2020). This confusion may have been driven by the wide variety of colour morphs that exist within *T. g gratilla* globally (Bronstein *et al*. 2016). The misidentification of the *Tripneustes* species in NSW highlights a knowledge gap in our understanding of the biology and ecology of these species in Australia. *Tripneustes kermadecensis* was first identified as a distinct species by morphological and molecular genetic analysis of specimens from the Kermadec Islands, New Zealand (Bronstein *et al*. 2017). Subsequently, *T. kermadecensis* was confirmed to be the species that commonly occurs also in the subtropical and temperate regions of NSW where it is known locally as the ‘lamington urchin’ (Bronstein *et al*. 2017, 2019).

Due to the previous confusion of *T. kermadecensis* and *T. g. gratilla*, we used the Australian Museum collection of *Tripneustes* to clarify the distributions of these species on the east coast of Australia (McLaren *et al*. in prep.). During our survey of the collection, we discovered juvenile *Tripneustes* specimens that represent type material of an east Australian species originally described by Tenison-Woods (1878) as *Evechinus australiae*, with the type locality Sydney Harbour (Port Jackson). In the present study we combine morphological analyses of a growth series, micro-computed tomography, and genetic tools to test if the type material of *Evechinus australiae* is conspecific with *Tripneustes g. gratilla* or *T. kermadecensis.*

2.3 Material & Methods

2.3.1 *Tripneustes* material in the Australian Museum

The Australian Museum collection contains 232 *Tripneustes* specimens from Australia. Among these specimens we discovered three, partly fragmented juvenile specimens (jointly registered under the inventory number AM J. 1099) that were labelled as type-material of *Evechinus australiae* Tenison-Woods, 1878. The lot was subsequently split into three separate inventory numbers, each containing the remains of a single individual (AMJ. 30960, J. 30961, and J. 1099). According to Ramsey (1885) the type material of *E. australiae* was collected from Port Jackson (Sydney Harbour), New South Wales (NSW) (see below). This material included three dried specimens, one intact (J. 30960) and two fragmented tests (J. 30961 and J. 1099), all of which are juveniles.

2.3.2 Institutional abbreviations

AIM MA – Auckland War Memorial Museum, Auckland, New Zealand
AM – Australian Museum
NIWA – National Institute of Water and Atmospheric Research, Wellington, New Zealand
NHMW-Geo – Naturhistorisches Museum Wien, Geologisch-Paläontologische Abteilung, Vienna, Austria
NHMW-EV – Naturhistorisches Museum Wien, 3. Zoologische Abteilung, Sammlung Evertebrata Varia, Vienna, Austria

2.3.3 Morphological analysis

To examine the morphology of specimens AM J. 30960 and J. 30961 the tests were analysed by micro-computed tomography (μCT) at the Australian Centre for Microscopy & Microanalysis, University of Sydney, Australia. The specimens were scanned using a Bruker SkyScan 2214 system. The J. 30960 specimen scan was performed at 60 kV, 200 μA, with 2,179 ms exposure time, 1×1 binning, and 3,601 projection images, using a 0.25 mm aluminum filter. The J. 30961 scan was performed at 60 kV, 150 μA, with 757 ms exposure time, 1×1 binning, and 1,801 projection images, using a 0.25 mm aluminum filter. *T. kermadecensis* paratype specimen NHMW-GEO-2017-0016-0001 was scanned at the Natural History Museum Vienna, Austria, using a YXLON FF35 CT equipped with a YXLON FXT 225.48 micro-focus directional beam tube and a 4343 CT CsJ flat panel detector. Scanning was performed at 100 kV, 130 μA, with 1,000 ms exposure time, 1×1 binning, and 2,700 projection images, using a 0.4 mm copper filter. Isotropic voxel sizes of the reconstructed datasets were 36.5 to 144 μm, depending on the size of the specimen. Reconstructions of the specimens was performed using the program Dragonfly (v. 2020.2 for Microsoft Windows; Object Research Systems (ORS) Inc, Montreal, Canada, 2020; software available at http://www.theobjects.com/dragonfly).

Traits from these visualisations were compared to those in the type material of *T. kermadecensis* (Bronstein *et al.* 2017) and non-type material of *T. gratilla gratilla.* The analysed traits included test shape, adapical ambulacral primary tuberculation, ambital ambulacral primary tuberculation, occlusion of ambulacral plates from the perradial suture, the size of the interambulacral tubercles, and peristome size (Table 2.1).

The tuberculation patterns of *Tripneustes* change during growth. As the *E. australiae* material are small individuals, to compare this material to other *Tripneustes* species we constructed a growth series of *T. kermadecensis* consisting of cleaned tests obtained from the Sea Urchin Science Centre and Gallery (SUSCG) (SUSCG. 1-8) and from specimens collected from the Solitary Islands (J. 31050, J. 31051, J. 31052) (ranging 20.88 to 101.3 mm in test diameter − TD). To understand the variation in ambulacral tuberculation, both within and between individual specimens, we counted the number of tubercles on 10 random ambulacral plates at the ambitus in both columns of each ambulacrum (n = 5) per specimen (n = 11). Based on the description (Figure 4C in Bronstein *et al*. 2017), *T. kermadecensis* would be expected to have a primary tubercule on every 4th plate at the ambitus and therefore would have three tubercles per 10 plates. When the counts for each individual are pooled across all 5 ambulacra, *T. kermadecensis* is expected to have 25/100 plates with primary tubercles.

2.3.4 Molecular analysis

Historical material (J. 30960, J. 30961, and J. 1099)

Total genomic DNA was extracted in an ultraclean room using the Qiagen Investigator kit with the addition of carrier RNA (at step 5 of the manufacturer's protocol) to enhance the binding of DNA to the spin column. As there was no visible tissue left on the *E. australiae* type specimens, the tests were swabbed with swabs dipped in AL lysis buffer (Qiagen) to collect remaining cells and tissue, followed by extraction according to the manufacturers protocol for extraction from surface and buccal swabs, with minor modifications. Specifically, at the DNA elution step, we applied 25 µl of pre-heated elution buffer (70 °C) and allowed an incubation time of 3 minutes prior to centrifugation. This process was repeated twice for each sample, re-pipetting the eluted DNA from the first round back on the spin-column filter.

Table 2.1. Primary morphological traits of *Tripneustes g. gratilla* and *T. kermadecensis* (from Bronstein *et al.* 2017) and the traits of the *Evechinus australiae* (Tenison-Woods 1878) type specimens.

Trait	*T. gratilla gratilla*	*T. kermadecensis*	J. 30960	J. 30961
Test shape	high (TH > 63%TD)	depressed (mean TH 51%TD)	TH 52% TD	TH 44% TD
adapical ambulacral primary tubercles	every second plate	every second plate	every second plate	every second plate
ambital ambulacral primary tubercles	every second plate	every fourth plate	varies between 3 and 4, see figure 2.5	varies between 3 and 4, see figure 2.6
adoral ambulacral primary tubercles	every third plate	every second plate	every second plate	every second plate
ambulacral plates occluded from perradial suture	none	numerous (2 in 3 at ambitus)	numerous (2 in 3 at ambitus)	numerous (2 in 3 at ambitus)

size of interambulacral tubercles	half of plate height	full plate height	Does not reach full plate height	Does not reach full plate height
mean peristome size	28.1%TD	25.2%TD	38.38% TD	NA
deeply bifurcate compass ends	yes	no	NA	NA
compass ends flat and wide	no	yes	NA	NA
dumb-bell shaped ossicles very abundant in distal tube feet	yes	no	NA	NA

As the *Evechinus australiae* material was highly degraded and poorly preserved for molecular analysis (dried specimens collected >140 years ago) genetic analysis of the mitochondrial marker cytochrome oxidase subunit I (COI) was attempted by a nested PCR design, including an initial amplification of the material with the specific echinoid primers (COI16bf/COIer). We then used the products of the first PCR reaction as template for a second amplification using the universal metazoan primers of Folmer (Figure 2.1) (Folmer *et al.* 1994).

Amplification of the initial COI fragment followed Bronstein *et al.* (2019) using the forward primer COI16bf 5′ AGCCAGGTCAGTTTCTATCT 3′ (Bronstein *et al.* 2019) with the reverse primer COIeR5′ GCTCGTGTGTCTACGTCCAT 3′ (Arndt *et al.* 1996) (flanking a region of ca. 1,000 bp). PCR reactions were done in a total volume of 25 µl and contained 5.0 µl DNA, 2.5 µl 10X CoralLoad PCR Buffer, 2.0 µl of 25 mM $MgCl_2$, 0.5 µl of 10 mM dNTPs, 0.25 µl of each 10 mM forward and reverse primers, 0.25 µl CoralLoad DNA polymerase and brought to 25 µl with nuclease free water. PCR conditions were 3 min at 94 °C followed by 35 cycles of 94 °C for 30 s, 65 °C for 30 s and 72 °C for 70 s, and a final extension step of 10 min at 72 °C.

These PCR products were then PCR re-amplified using the primers LCO1490 5' GGTCAACAAATCATAAAGATATTGG 3′ and HC02198 5' TAAACTTCAGGGTGACCAAAAAATCA 3′ (Folmer *et al.* 1994) to generate a ca. 700 bp amplicon. PCR reactions and thermocycler conditions were repeated as described above. PCR products were then purified using ExoSap. Samples were then sequenced in both directions

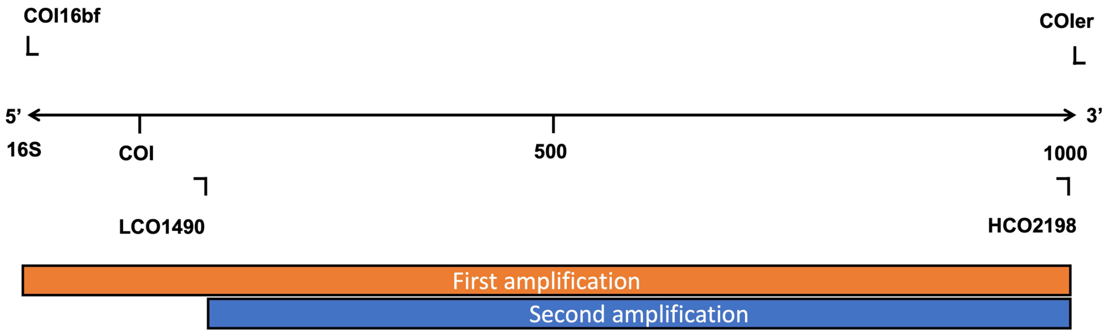

Figure 2.1. Visualisation of the gene regions and primer binding sites used to amplify and re-amplify the mitochondrial cytochrome c oxidase subunit 1 gene (COI).

using the nested PCR primers (i.e., the Folmer primers), at the Australian Genome Research Facility (AGRF). Sequences were deposited in GenBank (Accession numbers: OR038167, OR038168, OR038169, OR038170, and OR038171). Sequence assembly was performed in Geneious Prime (V 11.0.14.1+1). To confirm the identity of the specimens these sequences were subjected to a BLAST search prior to inclusion in a phylogenetic analysis as detailed below.

Additional material from the Australian Museum collection

Additional (juvenile and adult) specimens (AM J. 1117, J. 4250, and J. 5003) from the Australian Museum from the location where the *E. australiae* type material was collected, Port Jackson, as well as newly collected samples from the Solitary Islands (AM J. 31050, J. 31051, and J. 31052) were also included in the genetic analysis. Both wet and dry material were investigated using the mitochondrial marker listed above. Total genomic DNA was extracted from the tube feet and spine muscle, or internal tissue that could be extracted from the dry and wet specimens using the Bioline ISOLATE II Genomic DNA kit (Meridian Bioscience) following manufacturer instructions. For AM J. 1117, J. 4250, and J. 5003 instead of eluting to 100 µl, the DNA was eluted into 50 µl and the final elution buffer was run through the membrane twice and incubated at room temperature for 3 minutes. PCR conditions for each amplification were then performed as outlined above, however 2 µl of DNA template was used in the PCR protocol. For the newly collected samples, AM J. 31050, J. 31051, and J. 31052 the DNA was eluted 100 µl and only one PCR amplification was performed with the COI16bf/COIer primers and PCR conditions as outlined above. All sequences were deposited in GenBank (Accession numbers: OR144098, OR144099, OR144100) (Supplementary Material, Table A1).

2.3.5 Molecular computational analysis

Phylogenetic analysis was performed using the COI sequences generated for the *Evechinus australiae* type material (AM J. 30961), the successfully sequenced *T. kermadecensis* material from AM (J. 1117, J. 23530, J. 24345, J. 4250, and J. 5003), and the Solitary Islands material (J. 31050, J. 31051, and J. 31052). These sequences were complemented with publicly available data from the original description of *T. kermadecensis* and *T. g. elatensis* (Bronstein *et al.* 2016, 2017). We then created a COI dataset with these 69 sequences and our 9 novel sequences, including the *Evechinus australiae* material. As sequences varied in length, they were trimmed to align with the shortest sequence generated to maximise overlap (J. 30961, 283 BP). Raw sequences were edited and aligned using Geneious Prime and phylogenetic reconstruction was done using both Maximum Likelihood (ML) and Bayesian Inference (BI) analysis. ML analysis was performed in IQ tree and BI was performed using MrBayes (Version v3.3.7a) (Ronquist *et al.* 2012; Nguyen *et al.* 2015; Chernomor *et al.* 2016). MrBayes analysis was run for 10 million generations, sampling every 100th tree and discarding the initial 25% of data as burn-in. For all phylogenetic analysis the outgroups used were *Toxopneutes pileolus* (GenBank Accession No. MK084954.1, MK084955.1) and *Lytechinus variegatus* (GenBank Accession No. MG676469, MG676468). (Bronstein *et al*. 2019).

2.3.6 Haplotype network analysis

Median-joining networks were calculated for the 283 BP alignment of all *Tripneustes* spp. used for the phylogenetic analysis, including *T. kermadecensis* from NSW, New Zealand and Solitary Islands, following morphological and phylogenetic species assignment (see Supplementary Material, Table A1.) (See also Bronstein *et al.* (2016, 2017, 2019), using PopArt with default settings (Leigh and Bryant 2015). The 76 sequences used for the alignment (excluding the outgroups) yielded 25 unique haplotypes across the *Tripneustes* genus for COI.

The 26 NSW, New Zealand, and Solitary Islands sequences, collapsed into five unique haplotypes and we added biogeographic provinces to the individual sequences to determine unique haplotypes by provinces, or genetic connectivity between these provinces. The locations were Port Stephens (32° 42′ 38.1″ S 152° 11′ 11.9″ E), the Solitary Islands (29° 55′ 53″ S 153° 23′ 34″ E), Norfolk Island (29° 02′ 60″ S 167° 59′ 13″ E), Sydney (33° 49′ 13″ S 151° 17′ 12″ E), Port Jackson (33° 49′ S, 151° 16′ E), and Kermadec Islands (29° 4′ 40″ S 178° 09′ 14″ W).

2.3.7 Statistical analysis

To determine the variability of tuberculation within the growth series, a Kolmogorov-Smirnov test was performed to determine if the observed tuberculation differed from a normal distribution with a population mean of 25 tubercules per 100 plates. To test if this trait varied with size, we performed a linear regression between the number of tubercles per 100 plates and test diameter. The model was visually checked for normality and equal variance by inspecting Q-Q plots and the spread of residuals with a residual's vs fitted plot in R Studio (R Core Team, V 4.1.0 (2021-05-18)). The variability within an individual was analysed by a Chi-Square goodness of fit test with the expected and observed values of tubercles per 100 plates. Each individual was tested for deviance from the expected value of 25 tubercles per 100 plates counted (Supplementary Material, Table A2).

2.4 Results

2.4.1 Morphological analysis

The main diagnostic features for the two species are listed in Table 2.1. Comparing *T. kermadecensis* individuals across their size range, from juveniles to adults (22.8 to 101.3 mm test diameter) reveals that ambulacral tuberculation varies between and within individuals (Supplementary Material, Table A2, Figure 2.2, Figure 2.3 & 2.4). The observed tuberculation values of the growth series were significantly different to a normally distributed population with a mean of 25 ($D = 0.9$, $df = 11$, $p < 0.001$). The number of primary tubercles also varied within an individual, some ambulacra having four tubercles per 10 plates, and others having three (Figure 2.4, Supplementary Material, Table A2). Of the 11 individuals examined, six significantly deviated from the expected value of 25/100 (Supplementary Material, Table A2). These individuals had TD ranging from 91.1 mm to 22.8 mm (Table 2.2). There was also no significant relationship between the test diameter of specimens and number of tubercles ($F = 0.019$, $df = 1,9$, $p = 0.89$).

When comparing the type material of *T. kermadecensis* (Bronstein *et al.* 2017) with that of *E. australiae*, the specimens are similar with respect to most of the diagnostic traits described by Bronstein *et al.* (2017). However, as the *E. australiae* material are juveniles, some of the traits were not evident (Table 2.1). The *E. australiae* specimens share the depressed test characteristic of *T. kermadecensis*, a similar pattern of adapical and adoral ambulacral plating, and occlusion of plates from the perradial suture. However, the size of the interambulacral tubercles did not reach the full plate height and the ambitally ambulacral plating varied between every 3rd and 4th plate (Figure 2.5 & 2.6). The peristome size also seems to not align with the description in Bronstein *et al.* (2017), showing a proportion of 38.38% of the TD, as opposed to the 25.2% TD noted in the original description of *T. kermadecensis*, which was based on specimens larger than those of *E. australiae* (Table 2.2). Our examination of test tuberculation in an ontogenetic series of *T. kermadecensis* revealed that the ambulacral tuberculation varied both between and within individuals. This trait may not be as useful a diagnostic trait as previously thought and ontogenetic stage/test sizes has to be considered. It is important to note

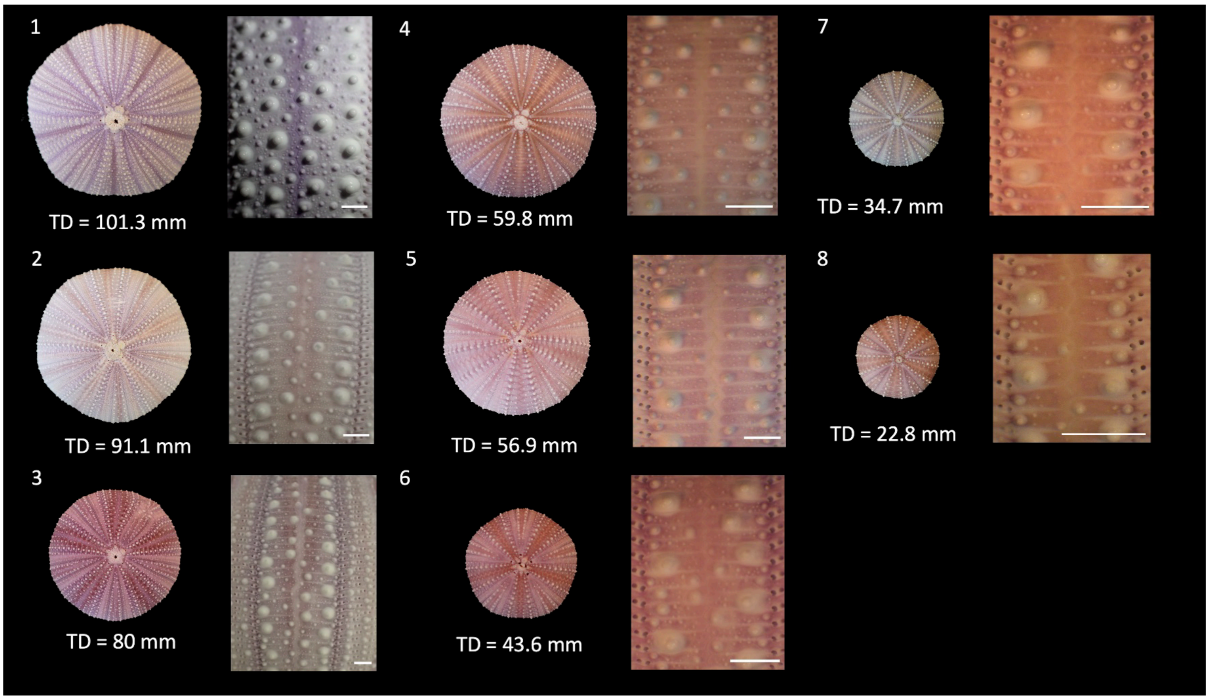

Figure 2.2. Tests and tuberculation of *Tripneustes kermadecensis* growth series constructed from specimens from The Sea Urchin Science Centre and Gallery. Numbers (1-8) represent the SUSCG identifier (SUSCG. 1-8). Scale bar represents 2 mm.

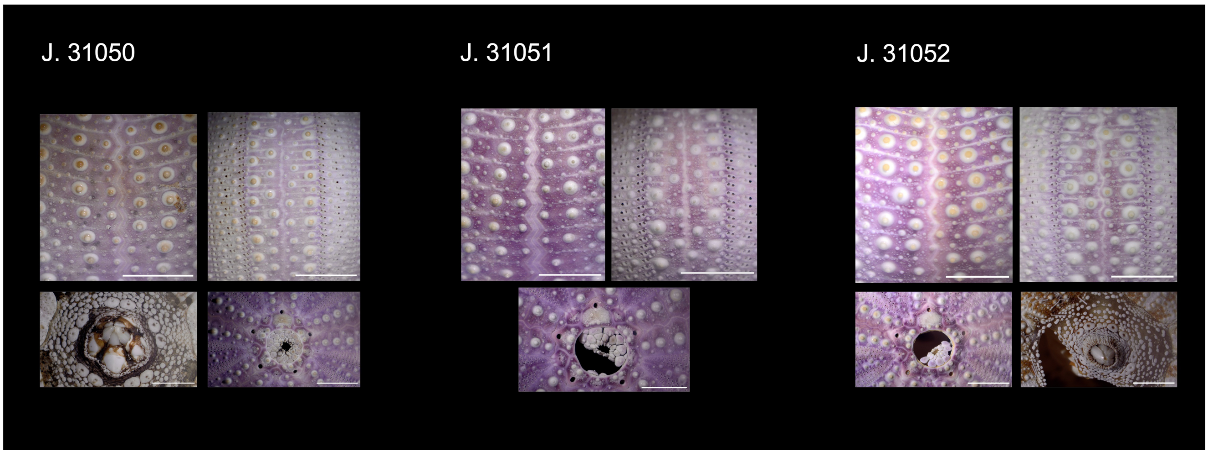

Figure 2.3. Tuberculation, peristome, and apical disc of the Solitary Islands specimens. Scale bar represents 2 mm.

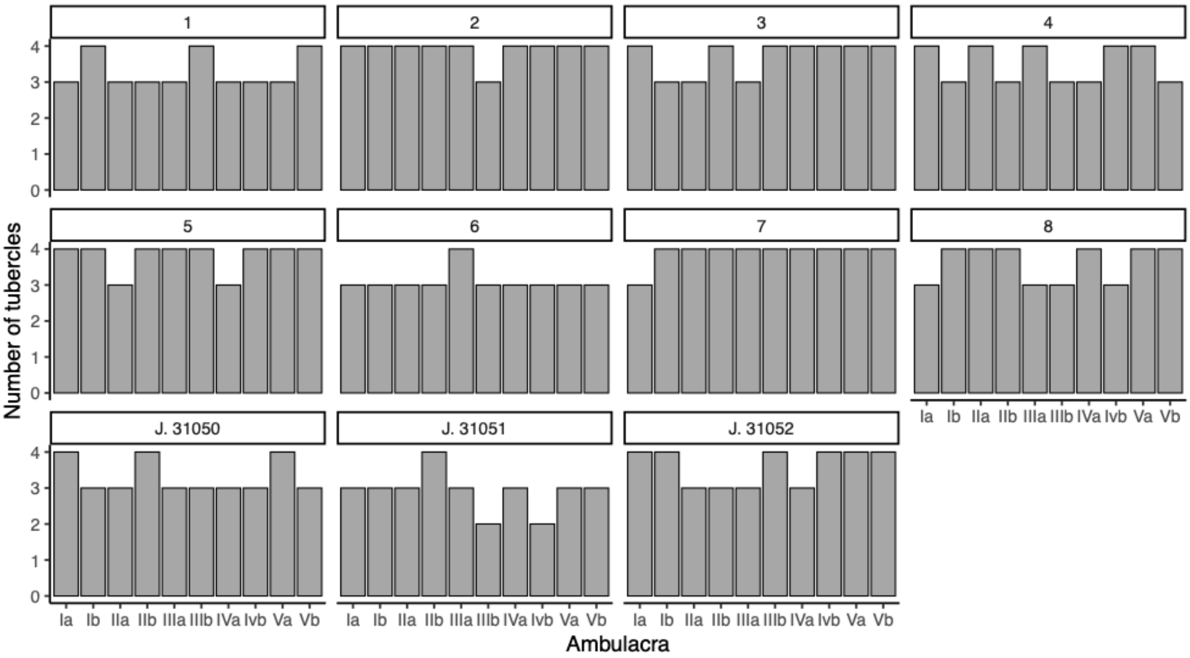

Figure 2.4. Between and within individual variation of tuberculation patterns in eleven *Tripneustes kermadecensis* across a growth series 22.8 – 101.3 mm test diameter. X – axis lettering denotes the ambulacra of each an individual in accordance with Lovén plane. Specimen numbers refer to Figs. 2 and 3.

Table 2.2. Morphological features of the *Tripneustes kermadecensis* growth series. Data that are significant are denoted by an asterisk (*).

Individual	Test diameter (mm)	Height (mm)	Height (%TD)	Peristome (mm)	Peristome (%TD)
SUSCG. 1	101.3	57.8	57.06	22.3	22.01
SUSCG. 2	91.1	54.7	60.043	20.2	22.17
SUSCG. 3	80	46.1	57.63	18.6	23.25
SUSCG. 4	59.8	33.5	56.02	15.8	26.42*
SUSCG. 5	56.9	33.5	58.88	15.8	27.77*
SUSCG. 6	43.6	23.5	53.9	13.6	31.19*
SUSCG. 7	34.7	19.3	55.62	11	31.7*
SUSCG. 8	22.8	11.9	52.19	8.4	36.84*
J. 31050	63.1	33.1	52.46	17.2	27.26
J. 31051	59	30.8	52.2	16.7	28.31
J. 31052	72.3	33.3	46.06	20.8	28.77
J. 30960	27.1	14.1	52.03	10.4	38.38*
J. 30961	35.9	16	44.57	NA	NA

that Bronstein *et al.* (2017) remains the key diagnostic resource for identification of this species and to differentiate from *T. gratilla gratilla*.

We recommend using the following morphological traits to diagnose *Tripneustes australiae* and to assist in the identification of this species in the field.

1. Depressed test: test height between ~45 to 60 % of the test diameter (TD)
2. Large peristome/mouth: between 22 - 27 % of the TD
3. Black interambulacral/pedicellariae
4. White spines
5. Dark grey, red/orange, or white tube feet
6. Absence of banded or greenish tube feet (as seen in *T. g. gratilla*)

We encourage those identifying these two species in the field to primarily rely on the shape of the test as there can be large variation in colour within *T. gratilla gratilla* and *T. australiae*. In particular, there are two common colour morphs of *T. australiae* on the east coast of Australia, the lamington morph with black interambulacral/pedicellaria and white or dark tube feet, and another morph with orange/red tube feet, which was noted to occur on Norfolk Island (Bronstein *et al.* 2019) but has also been collected in Port Stephens and Sydney on the NSW mid coast (McLaren, personal obs.).

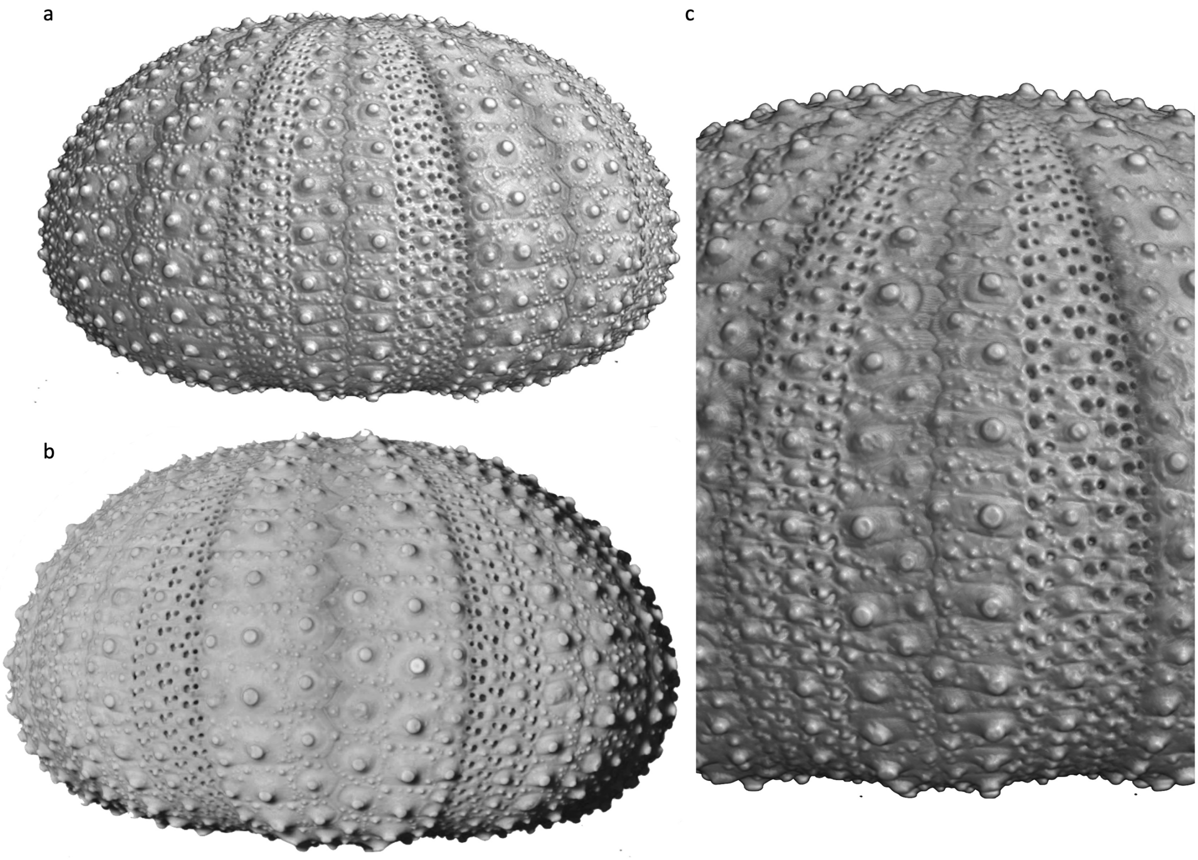

Figure 2.5. Micro Computer Tomography reconstruction of *Evechinus australiae* lectotype AM J. 30960; **a.** lateral view focusing on an ambulacrum, **b.** lateral view focussing on an interambulacrum; **c.** detail of the ambital region in another ambulacrum.

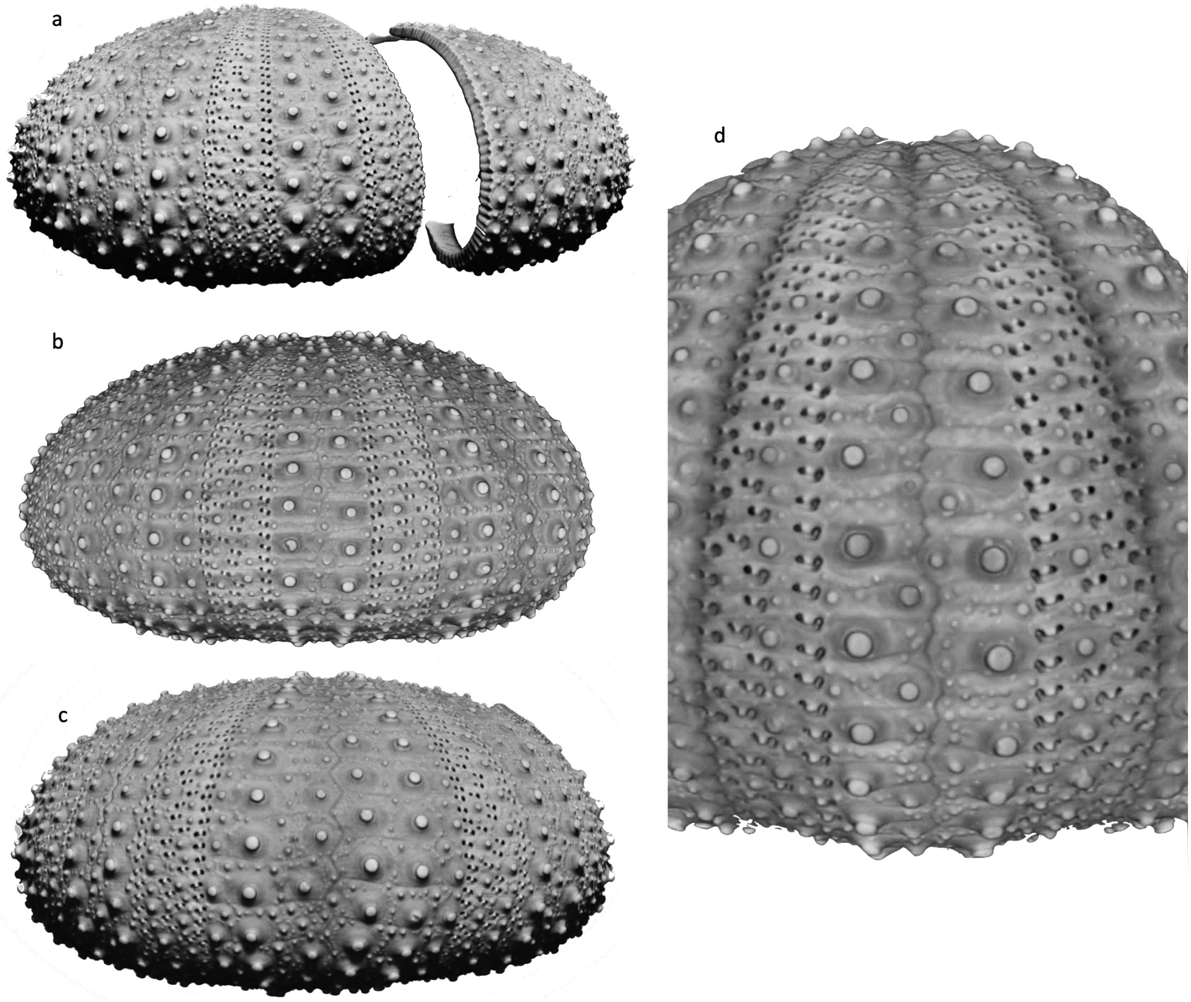

Figure 2.6. Micro Computer Tomography reconstruction of *Evechinus australiae* paralectotype AM J. 30961; **a.** lateral view of two fragments; **b.** lateral view focusing on an ambulacrum of the largest fragment, **c.** lateral view focussing on an interambulacrum of the largest fragment; **d.** detail of the ambital region in another ambulacrum.

2.4.2 Molecular diagnosis of the type material of *Evechinus australiae*

DNA extraction and PCR amplification was successful in one of the three syntypes of *Evechinus australiae*. COI sequences of specimen J. 30961, despite the DNA being highly degraded, matched those of *T. kermadecensis*. The sequence produced was 283 BP and a BLAST search identified the sequence as *T. kermadecensis* (query cover 100%; E Value = 0; Accession no. MK084948.1).

2.4.3 Molecular analysis

Both ML and BI analyses produced similar phylogenies for all clades of *Tripneustes* (Figure 2.7). As expected with mitochondrial markers, the three major clades recovered were *T. kermadecensis, T. ventricosus*, and a mixed clade consisting of *T. g gratilla*, *T. g. elatensis*, and *T. depressus* samples. All of the Australian Museum material identified as *T. kermadecensis* from the type region (Supplementary Material, Table A3), from which DNA could be extracted (Table 2.3), as well as the *Evechinus australiae* type material, belong to the *T. kermadecensis* clade. Specimens from the Solitary Islands (AM: J. 31050, J. 31051, and J. 31052, GenBank: OR144098, OR144099, and OR144100) (Figure 2.7), likewise fell in the *T. kermadecensis/australiae* clade.

Our haplotype joining network for COI for *Tripneustes* showed similar groupings to the phylogenetic trees. *Tripneustes australiae* and *T. ventricosus* cluster separately and have unique haplotypes, while *T. g. gratilla*, *T g. elatensis* and *T. depressus* group together and share

Table 2.3. Australian Museum specimens of *Tripneustes kermadecensis* from the type locality of *Evechinus australiae* and surround from which DNA was successfully extracted.

Registration Number	Collection Location	Year Collected	Preservation Method
J. 30961	Port Jackson	1878	Dry
J. 1117	Port Jackson	NA	Dry
J. 4250	Port Jackson	1923	Ethanol
J. 23530	Port Stephens	1998	Ethanol
J. 5003	Port Jackson	1926	Dry

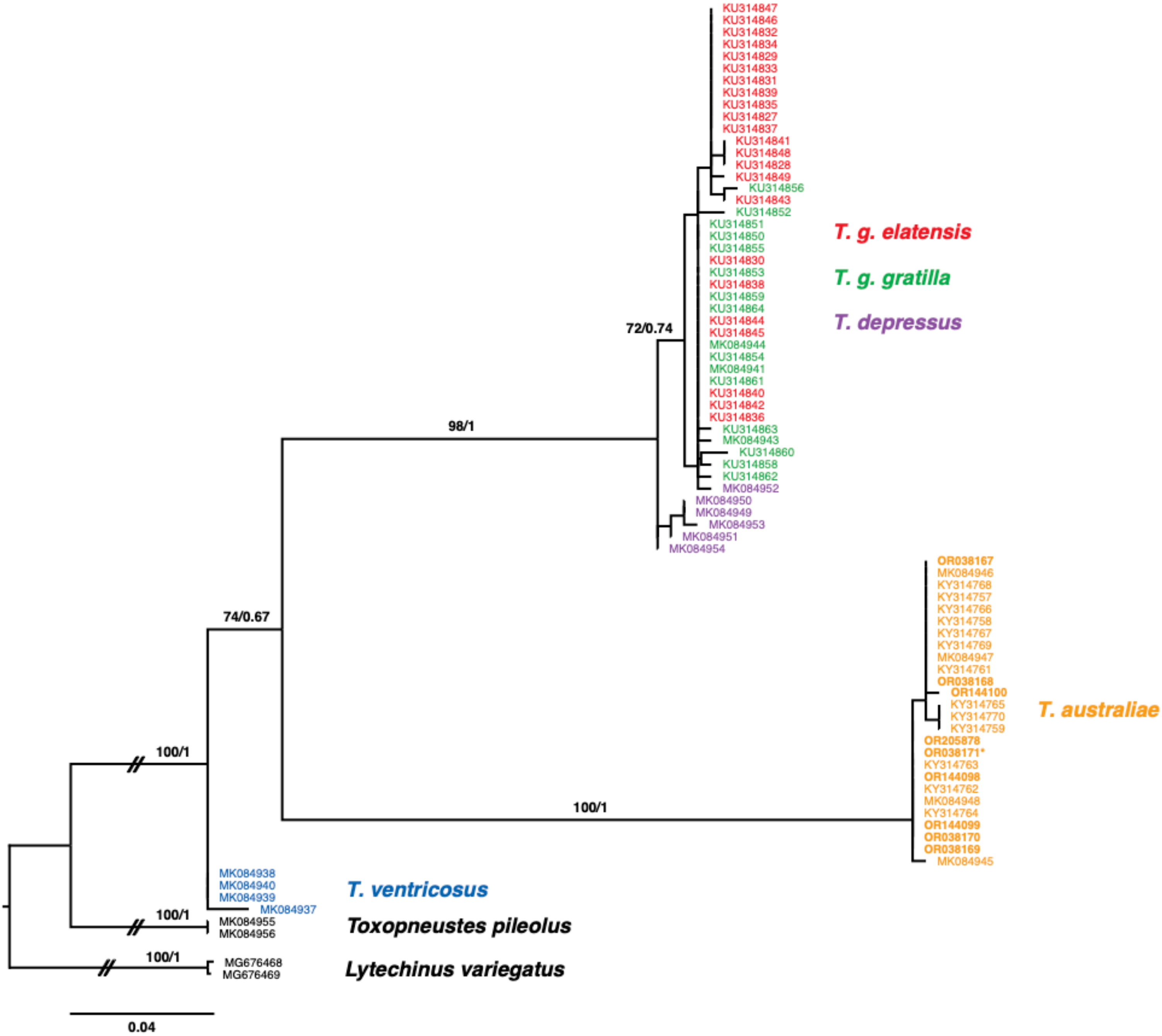

Figure 2. 7. Phylogenetic tree of *Tripneustes* based on 80 partial sequences (283 bp) of the mitochondrial COI gene. *Toxopneustes pileolus and Lytechinus variegatus* were used as outgroups. Bootstrap support values and posterior probabilities from both maximum likelihood and Bayesian inference analyses are reported above the respective nodes, before and after the slash, respectively. *Evechinus australiae* type specimen AM J. 30961 (OR038171) falls into the *T. australiae* clade. All sequences generated for this study are in bold and the type specimen is marked with an asterisk (*) (AM J. 30961 (OR038171).

haplotypes (Figure 2.8a). With respect to *T. australiae*, the two most frequent COI haplotypes occur from the Solitary Islands to Sydney, suggesting the populations of *T. australiae* on the east coast of Australia and those on offshore islands are genetically similar. However, there is one haplotype that occurs exclusively in the Kermadec Islands, one that occurs exclusively in the Solitary Islands, and one that only occurs in Sydney (Sydney) (Figure 2.8b).

2.4.4 Systematics

Classification follows Kroh (2020), for the changed attribution of Echinoidea to Schumacher (1817) see Stokes and Kroh (2022). As the morphological description of *Evechinus australiae* is based on juvenile specimens and due to the fact that many echinoids show allometric growth (e.g., Mooi *et al.* 2014) and ontogenetic increase of tubercle number, additional *T. kermadecensis* from NSW (J. 31050, J. 31051, and J. 31052) and *T. gratilla gratilla* from various Indo-West Pacific localities were examined in a size series in order to facilitate comparison of similarly sized specimens.

Class Echinoidea Schumacher, 1817
Order Camarodonta Jackson, 1912
Family Toxopneustidae Troschel, 1872
Genus *Tripneustes* L. Agassiz, 1841

Tripneustes australiae (Tenison-Woods, 1878) nov. comb.

*1878 *Evechinus australiæ* Tenison-Woods: 167.
1885 *Evechinus australiæ* – Ramsay: 23 [type locality information provided]
1889 *Evechinus australiæ*, Ten.-Woods – Whitelegge: 204. [citing Tenison-Woods]

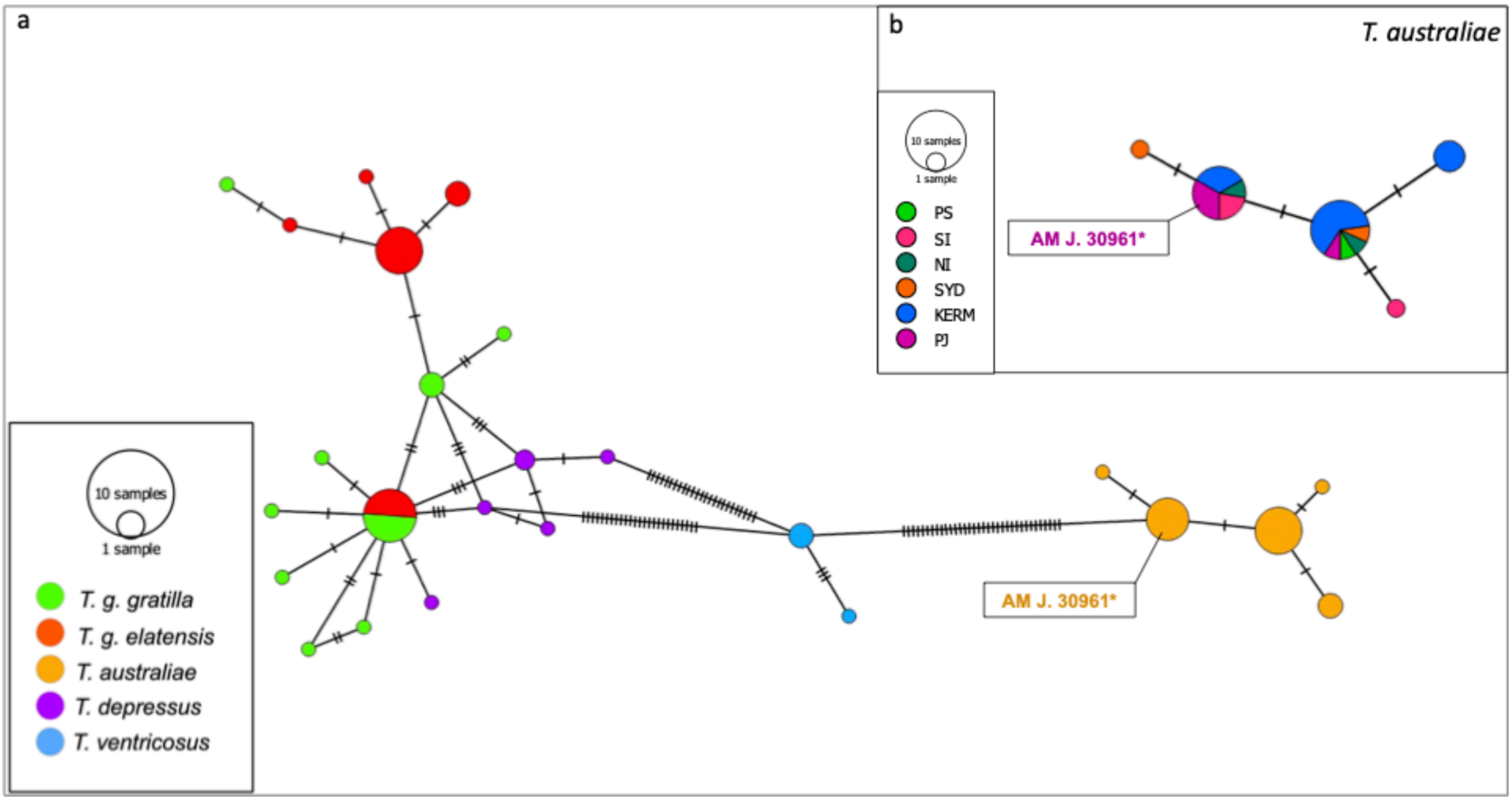

Figure 2.8. Median-joining haplotype networks for COI sequences of *Tripneustes* (**a**) and *T. australiae* (**b**). *Tripneustes* and *T. australiae* network consists of 76 and 26 sequences respectively that are 283 bp long. The bars represent the number of substitutions between the nodes and haplotype frequency is represented by the size of the nodes. Our geographical categories were Port Stephens (PS), the Solitary Islands (SI), Norfolk Island (NI), Sydney (SYD), and Port Jackson (PJ). Details on the sequences used are given in Supplementary Table A1. The position of the type specimen J. 30961 is highlighted and marked a with an asterisk (*) in 2.8a and 2.8b.

1891 *Euechinus* [sic!] *australiae* Ten. Woods – Meissner: 166. [summary of Whitelegge's report]

1894 *E. australe* – Farquhar: 195 [misspelling].

1943 *Evechinus australiæ* – Mortensen: 298. [mentioned as synonym of *Tripneustes gratilla*]

2017 *Tripneustes kermadecensis* Bronstein *et al.*: 7–13; figs 2A–M, 3A–E, 4C, 10A, B, 11A–C, 12A, D, E, H

Type material of *Evechinus australiae*: Lectotype, designated herein: Australian Museum (AM) J. 30960, 1 Intact specimen (Figure 2.5) (https://sydney.pedestal3d.com/r/5UJwvpuVld). Paralectotypes: two fragmentary specimens: AM J. 30961, AM J. 1099 (Figure 2.6).

Type material of *Tripneustes kermadecensis*: Holotype: Auckland War Memorial Museum, Auckland, New Zealand (AIM MA) specimen AIM MA73563 (isolate nr. Ker907), a cleaned corona, spines and lantern, collected by J. David Aguirre and Libby Liggins on 31/10/2015 at a depth of 0 to 10 m. Paratypes: two cleaned coronas (AIM MA73564 and NHMW-Geo 2017/0016/0001; isolate Ker903 and Ker910 respectively) and five intact, formalin-fixed specimens preserved in ethanol (AIM MA73565, AIM MA73566, NIWA 116558, NHMW-EV 20452, and NHMW-EV 20453 [= isolates Ker902, Ker904, Ker911, Ker901, and Ker906 respectively]); collection details as for holotype.

Type locality of *Evechinus australiae*: "small sea beach about three miles north of Port Jackson heads" (Ramsay 1885: footnote on p. 23); Sydney Harbour, NSW, Australia; no depth recorded. As described by Ramsey, a small sea beach 3 miles north of the Port Jackson Heads could refer to Collins Beach (33° 48′ 31.0″ S, 151° 17 ′ 28.6″ E), Store Beach (33° 48′ 42.1 ″ S, 151° 17′ 22.9″ E), or Little Manly (33° 48′ 24.6″ S, 151° 17″ 14.1″ E). To provide as close a location as possible we use the co-ordinates of Port Jackson (33° 49′ S, 151° 16′E), as the type locality.

Type locality of *Tripneustes kermadecensis*: West of Meyer Islands (29° 14′ 39.06″ S, 177° 52′ 46.56″ W), near Raoul Island, Kermadec Islands, New Zealand; 0–10 m depth.

Additional genetically confirmed material of *T. australiae* has been deposited in the Australian Museum (AM J. 31050, J. 31051, and J. 31052). These were collected from North Solitary Island (29° 55′ 40.8″ S, 153° 23′ 31.7″ E) on 01/09/2022; GenBank Accession numbers: OR144098, OR144099, and OR144100.

Distribution: The subtropical and temperate coast of East Australia and off-shore Islands in the Pacific (Lord Howe Island, Norfolk Island, and the Kermadec Islands), to the west coast of New Zealand (McLaren *et al.* in prep.).

Emended diagnosis: A species of *Tripneustes* with ambulacral primary tubercles occurring typically on every third to fourth plate ambitally (see Fig. 2.2; very rarely there are cases where four subsequent ambulacral plates without primary tubercle occur at the ambitus, e.g., Fig. 2.6, left column of ambulacrum); flattened test ranging from ~45 % to 60 % height of the test diameter; large peristomal opening (ca. 25 % of TD) without sunken margin; presence of one to two plates for every four ambulacral plates occluded from perradial suture, giving the perradial suture a unique irregular zig zag (except in specimens < 80 mm TD); primary series of interambulacral tubercles continuous from peristome to apex; tubercles large; secondary interambulacral tuberculation reduced above ambitus.

Description of the lectotype (AM J. 30960): Micro computed tomography (μCT) imagery of the lectotype was used to generate a 3D render of the specimen (Figure 2.5) (https://sydney.pedestal3d.com/r/5UJwvpuVld). The lectotype (J. 30960) is 27.1 mm TD, with a depressed test. The test height is 52% of the TD (Figure 2.5). The apical disc and genital plates are not preserved. The ambulacral plating is trigeminate and the pores form three vertical columns separated by columns of tubercles. The inner- and outermost of these are almost straight, while the middle part is less linear and wiggles from the aboral to oral side. Close to the peristome and apical disc, primary tubercles occur on each or every 2nd ambulacral plate, and ambitally tubercles occur every 3rd plate. Some plates are occluded from the perradial suture, but not as numerously as is seen in adult individuals. This feature of juveniles was also noted by Bronstein *et al.* (2017). Along the ambulacra, some plates are larger and more asymmetric than others, as such the perradial suture is irregular and zig zags, a typical trait of this species (Bronstein *et al*. 2017). From the adoral to adapical side the interambulacral plates have one column of primary tubercles running through the middle of the plates, and one column of secondary tubercles on either side of these primary tubercles. Towards the adoral side these plates can have up to four tubercles per single plate (Figure 2.5). Unlike the holotype assigned by Bronstein *et al*. (2017), in J. 30960, the areoles of the tubercles do not take up the majority of the height of the interambulacral plates. However, this may be a juvenile feature. In other small genetically confirmed specimens, similar tubercule style occurs (see below) (Figure 2.3). The peristome (10.4 mm diameter) is 38% of TD which is larger than reported in Bronstein *et al*. (2017) and again typical for the differences observed between juveniles and adults in camarodont echinoids. Specimen colour of the 140+ years old specimen has faded, but the test still has the typical light pink/violet hue. See Bronstein *et al.* (2017) for a description of recently collected specimens.

Description of the paralectotypes: Specimen AM J. 30961 has the characteristic depressed test (Figure 2.6, Table 2.2). The test is 35 mm in diameter, with a test height of 16 mm (44% TD). This specimen shares the same traits of ambulacral and interambulacral tuberculation as in AM J. 30960, however, fewer ambulacral plates are asymmetric and occluded from the perradial suture, meaning the perradial suture is more symmetric, but still has the distinct zig zag. Specimen AM J. 1099 is a fragmented specimen of between 20–30 mm TD. Its morphology agrees with that of the other specimens in the type series of *T. australiae*, but it offers no additional detail.

Remarks: Although the trait of one primary tubercule on every 4th ambulacral plate around the ambitus is suggested to be diagnostic (Bronstein *et al.* 2017), comparison of a larger size range including specimens as small as 22.8 mm revealed that this trait is variable (see above).

2.5 Conclusions

Considering the weight of evidence; locality of the specimen, its depressed test morphology and irregular zig zag of the perradial suture, we conclude that the type material of *Evechinus australiae* Tenison-Woods, 1878 is conspecific with *Tripneustes kermadecensis* described by Bronstein *et al.* (2017). The apparent differences between the two nominal taxa are solely caused by the fact that the former is based on juvenile specimens. Both our novel morphological and genetic data support the synonymy of *T. kermadecensis* and *Evechinus australiae*, wherefore *australiae* is transferred to the genus *Tripneustes* and *T. kermadecensis* placed into the synonymy of *T. australiae.*

Genetic evidence based on the mitochondrial gene COI supports the synonymy of *T. kermadecensis* and *T. australiae*, which share haplotypes (Figure 8) that do not occur in and are very distinct from those of other *Tripneustes* species. Mitogenome introgression which drives discordance between nuclear and mitochondrial markers in this genus (Bronstein *et al.* 2016, 2017), was thus far only observed between *T. gratilla gratilla*, *T. gratilla elatensis*, and *T. depressus*. As such, because this study solely employed mitochondrial loci (due to the age and condition of the specimens), it is important to recognise that nuclear molecular evidence would be required to completely delineate species of *Tripneustes*. However, as previously established by Bronstein *et al.* (2017), *T. kermadecensis* (now *T. australiae*) can be unambiguously discerned from *T. g. gratilla* using COI.

With clarification of the taxonomy of the Australian subtropical and temperate *T. australiae* the Australian Museum collections indicated that the current distribution of this species in NSW extends from Byron Bay (28° 37′ S) in the north to Narooma (36° 15′ S) in the south, over 8 degrees of latitude and over 900 kilometres. *Tripneustes australiae* has a trans-Tasman distribution of 17 degrees and over 1,800 km to Norfolk Island and Lord Howe Island in Australia, and in New Zealand to the Kermadec Islands and the west coast of the North Island.

Our haplotype network indicates genetic similarity between the populations of *T. australiae* on the east coast of Australia and the offshore islands in the Tasman Sea and New Zealand. It seems likely that the larval supply of *T. australiae* from the east coast of Australia to the Kermadec Islands and other off-shore Islands in the Tasman such as Lord Howe and Norfolk Island is facilitated by transport in the Tasman Front. This is a major current that crosses from the mid north coast of NSW to northern New Zealand (Ridgway and Godfrey 1997). Interestingly, there are also haplotypes unique to the east coast of Australia that are not shared with the offshore islands and there is also a unique haplotype in the Kermadec Islands, although the latter may be an artefact of a low sample size (n = 25) of individual sequences. Future studies are needed to expand the collection effort of *T. australiae* in locations across its distribution to understand how well these populations are connected thorough population genetics analyses. Sea urchins sympatric with *T. australiae* in eastern Australia, the diadematoid *Centrostephanus rodgersii* and the echinometrid *Heliocidaris tuberculata* have similar cross Tasman distributions. For both of these species, populations genetic analysis indicate that populations on the east coast of Australia colonised across the Tasman, facilitated by the Tasman Front (Thomas *et al*. 2021; Gall 2016).

It is important to highlight the type locality of *T. australiae* being Sydney Harbour (Port Jackson) and that this species has been present at this location at least since its description by Tenison-Woods in 1878. *Tripneustes australiae* is an ecologically important species in Australia and should be considered in regional conservation and management. For a species with a planktotrophic larva, *T. australiae* has a relatively limited latitudinal distribution along the coast of Australia, despite the potential for a greater distribution facilitated by the southerly flowing East Australia Current (EAC). In this regard *T. australiae* is similar to *H. tuberculata* which has a similar limited poleward distribution that is thought to be due to the cold intolerance of its larvae (Byrne *et al.* 2022).

The sea scape of the southeast coast of Australia is dynamic, and is undergoing tropical/sub-tropicalisation, as the poleward flow and temperature of the EAC increase with ocean warming (Suthers *et al*. 2011; Vergés *et al.* 2014). This is resulting in poleward range extension of tropical and subtropical species with dispersive larvae. Tropical species are expanding while the distribution of temperate species is contracting (Gervais *et al.* 2021). The fate of species such as *T. australiae* which appear limited to the subtropical and warm temperate regions of Australia and New Zealand is uncertain in the face of changing climate.

Data availability: CT scanning datum generated for this study has been deposited in the NHMW research data repository (https://doi.org/10.57756/a1x6kf, https://doi.org/10.57756/5zzydy, and https://doi.org/10.57756/7pkzpx) and the genetic datum has been uploaded to GenBank (Supplementary Material, Table A1). Data related to the phylogenetic analysis and haplotype networks have been uploaded to FigShare (doi: 10.6084/m9.figshare.23699997.)

Chapter 3.

Taxon dependent temporal trends in the abundance and size of sea urchins in subtropical eastern Australia

3.1 Abstract

Subtropical reefs host a dynamic mix of tropical, subtropical, and temperate species that is changing due to shifts in the abundance and distribution of species in response to ocean warming. In these transitional communities, biogeographic affinity is expected to predict changes in species composition, with projected increases of tropical species and declines in cool-affinity temperate species. Understanding population dynamics of species along biogeographic transition zones is critical, especially for habitat engineers such as sea urchins that can facilitate ecosystem shifts through grazing. We investigated the population dynamics of sea urchins on coral-associated subtropical reefs at seven sites in eastern Australia (28.196°S to 30.95°S) over nine years (2010 – 2019), a period impacted by warming and heatwaves. Specifically, we investigated the density and population size structure of taxa with temperate (*Centrostephanus rodgersii, Phyllacanthus parvispinus*), subtropical (*Tripneustes australiae*) and tropical (*Diadema* spp.) affinities. Counter to expectation, biogeographic affinity did not explain shifts in species abundances in this region. Although we expected the abundance of tropical species to increase at their cold range boundaries, tropical *Diadema* species declined across all sites. The subtropical *T. australiae* also showed decadal declines, while populations of the temperate *C. rodgersii* were remarkably stable throughout our study period. Our results show that temporal patterns of sea urchin populations in this region cannot be predicted by bio-geographic affinity alone and contribute critical information about the population dynamics of these important herbivores along this biogeographic transition zone.

3.2 Introduction

Marine biogeographic transition zones represent a gradient between tropical and temperate ecosystems (Horta e Costa et al. 2014) and feature a mix of tropical, subtropical and temperate species arrayed along gradients of environmental conditions (Beger et al. 2014; Sommer et al. 2014). These transition zones are often influenced by western boundary currents that transport propagules of tropical and subtropical species poleward, as seen in Australia and Japan (Vergés et al. 2014). In these regions, many species live at the edges of their warm or cool thermal ranges, making them highly vulnerable to climate driven thermal anomalies (Beger et al. 2014; Poloczanska et al. 2016; Mulders et al. 2022; Stuart-Smith et al. 2022; Zarzyczny et al. 2023).

Globally, there has been a shift from the dominance of cool to warm affinity species in marine transition zones, a phenomenon called tropicalisation (Vergés et al. 2014; Mulders et al. 2022). In a warming ocean, range shifts are often explained by biogeographic affinity, with warm-affinity species increasing in abundance and diversity at their leading edges and cold-affinity species decreasing at their warm-range margins (Horta e Costa et al. 2014; Poloczanska et al. 2016; Mulders et al. 2022; Stuart-Smith et al. 2022; Zarzyczny et al. 2023). Along with the direct effects of heat stress on temperate species, such as cold-water kelps, tropicalisation is often characterised by changes in biotic interactions, including marked changes in herbivory due to the range extension and increased abundance of tropical herbivorous fishes (Vergés et al. 2014, 2016; Wernberg et al. 2016).

Herbivory is amongst the most important ecological interactions in marine ecosystems (Milchunas and Lauenroth 1993; Hawkes and Sullivan 2001; Côté et al. 2004; Ohgushi 2005; Futuyma and Agrawal 2009; Naeem et al. 2016; Bernes et al. 2018). In particular, sea urchins are important habitat engineers and can play a pivotal role in mediating benthic dynamics in warming seas (Lessios et al. 2001; Ling 2008; Lessios 2016; Ling et al. 2018; Zarzyczny et al. 2022). A long history of exclusion experiments and die-off events show the ecological role of sea urchin herbivory in marine ecosystems (Steneck 2020; Sweet 2020). For example, in the Caribbean, herbivory by the diadematid sea urchin *Diadema antillarum*, was key in maintaining the health of coral reefs by controlling turfing algae until its die-off from an unknown pathogen precipitated a phase shift from a coral dominated to a turf dominated ecosystem in the 1980s (Lessios et al. 2001; Hughes et al. 2007; Lessios 2016). In temperate ecosystems, high sea urchin abundance can result in phase shifts from macroalgae dominated to sea urchin and coralline algae dominated systems, termed 'barrens' (Steneck et al. 2002; Pederson and Johnson 2008; Ling 2008; Eklöf et al. 2008; Filbee-Dexter and Scheibling 2014; Ling et al. 2019). Although sea urchins can also be abundant along subtropical transition zones, where they are hypothesised to accelerate tropicalisation through grazing (Schuster et al. 2022), the population ecology of sea urchin species with different thermal affinities is not well understood.

On the east coast of Australia, the biogeographic transition zone is centred around the Solitary Islands Marine Park (SIMP, 30°S), which hosts a high diversity of tropical, subtropical and temperate species, including endemic species and corals at their poleward range limits (Malcolm et al. 2007, 2010; Sommer et al. 2014, 2017; Baird et al. 2017; Ferrari et al. 2018; Malcolm and Ferrari 2019; Smith and Peregrin 2020; Mizerek et al. 2021). The ecological dynamics of this region are influenced by the East Australian Current (EAC) and south-eastern Australia is considered a global warming hot spot where increasing flow of the EAC is driving the poleward range extensions of many marine species (Ridgway and Godfrey 1997; Suthers et al. 2011; Hobday and Pecl 2014; Vergés et al. 2014, 2016).

Ecological dynamics along this biogeographic transition zone vary among taxa and are mediated by latitudinal and seasonal variation in temperature, ongoing ocean warming and heatwave anomalies (Malcolm et al. 2011; Bates et al. 2014; Sommer et al. 2018; Lachs et al. 2021). For example, patterns in coral community composition are distinct from those occurring in the Great Barrier Reef (Sommer et al. 2014, 2017) and the abundance of tropical corals has remained remarkably stable since the 1990s (Mizerek et al. 2021). However, in 2016 a significant heatwave resulted in the mass bleaching of coral including the endemic subtropical coral *Pocillopora aliciae* resulting in high mortality with limited recovery of this species in subsequent years (Kim et al. 2019; Lachs et al. 2021). Recently, kelp cover in the Solitary Islands region has declined in response to warming in parallel with an increase in tropical fishes, including tropical herbivorous fishes (Vergés et al. 2016; Smith et al. 2021).

While there have been extensive investigations of the population dynamics of corals and fishes in the east Australian transition zone with respect to regional warming (Malcolm et al. 2010; Sommer et al. 2014, 2018; Vergés et al. 2016; Malcolm and Ferrari 2019; Cant et al. 2021, 2023; Lachs et al. 2021), less is known about sea urchins and other benthic species. The subtropical reefs around the SIMP have high diversity of sea urchins, with 26 species recorded to date (Shaw 2023), including tropical (e.g. *Diadema*, *Pseudoboletia*, *Tripneustes*) and temperate (*Heliocidaris*, *Centrostephanus*) species. The abundant *Tripneustes* species in this region and in more temperate latitudes was previously considered to be a range extension of the tropical species *T. g. gratilla* (Castro et al. 2020), until recent taxonomic studies showed that this species, T. *australiae*, is a subtropical species endemic to south-east Australia and the west coast of New Zealand (Bronstein et al. 2019; McLaren et al. 2023).

Ocean warming and changes to the EAC have driven the poleward range expansion of *Centrostephanus rodgersii* from New South Wales (NSW) to Tasmania, with major impacts on receiving ecosystems resulting in the shift from macroalgae reefs to 'barrens' habitats (Ling 2008; Byrne and Andrew 2020). Within its native distribution in NSW, *C. rodgersii* contributes to the formation of a mosaic of 'barren' and kelp forest habitats characteristic of the region and is important for local biodiversity (Underwood et al. 1991; Curley et al. 2002; Kingsford and Byrne 2023). Understanding spatial and temporal dynamics of common sea urchin species including *C. rodgersii* on subtropical reefs in NSW is critical to understanding future trajectories of associated ecosystems.

Here we investigated the population dynamics of sea urchins on subtropical reefs of east Australia over a nine-year period of warming and heatwaves that impacted coral, kelp, and fish assemblages in the region (Vergés et al. 2016; Malcolm and Ferrari 2019; Kim et al. 2019). We quantified the abundance and population size structure of four sea urchin species to assess population dynamics and potential recruitment and mortality (Meesters et al. 2001; Anderson and Pratchett 2014; Ebert et al. 2019). As the region has been warming for decades (Vergés et al. 2016; Malcolm and Ferrari 2019), we also aimed to understand the potential influence of temperature on sea urchin population dynamics. Overall, we hypothesised that biogeographic affinity predicts patterns in sea urchin populations over time and warm affinity/tropical species would increase throughout the duration of the study, while cool tolerant/temperate species would decrease.

3.3 Methods

3.3.1 Study region and species

This study determined the temporal and thermal pattens in the abundance and size structure of sea urchin populations in subtropical New South Wales (NSW), east Australia. We measured the size and densities of all sea urchin species encountered in surveys from 2010 – 2019 across seven sites (Fig. 3.1) spanning a latitudinal gradient along the subtropical-to-temperate transition zone (28.196°S to 30.95°S). We also recorded whether the sea urchin species were tropical, subtropical, or temperate (Supporting information, Table B1).

3.3.2 Sea urchin surveys and image analysis

Sea urchin surveys in coral associated habitat were conducted at the study sites (Fig. 3.1) during August to October in 2010, 2012, 2016 and 2019; except Julian Rocks which was sampled in March 2016, and South Solitary Island which was sampled in March 2011. Black Rock could not be accessed in 2012. At each sampling time, five replicate 30m long belt transects (8-12m depth) were laid at least 10m apart and downward facing photographs were taken at each meter interval. Each photograph captured a 1m^2 area, resulting in a 30 by 1m^2 belt transect (see Sommer et al. 2014) (Supporting information, Fig. B1). Sites were chosen due to occurrences of Scleractinian corals and were located on the leeward side of headlands or islands. All transects were laid randomly in the same habitat at each site at each sampling timepoint.

The sea urchins observed in the 1m^2 images were identified to species where possible, counted, and outlined in ImageJ to determine test diameter. Urchins were only outlined if more than 50% of their body was present in the image so that an accurate diameter could be extracted. Each sea urchin was outlined using the SizeExtractR workflow in ImageJ and data were extracted using the SizeExtractR R package (Lachs et al. 2022). Ferets' diameter, or the

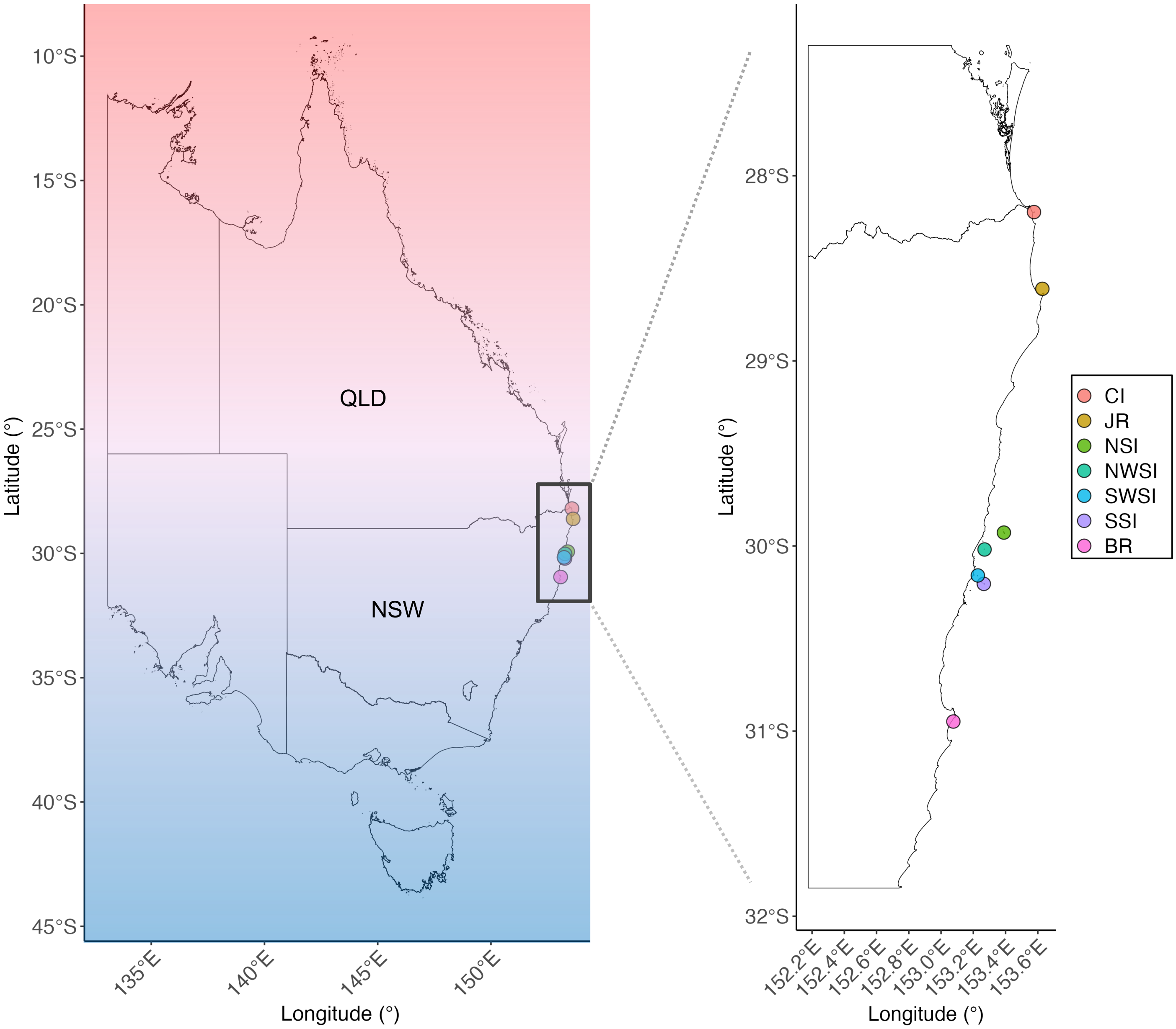

Figure 3.1. Location of the seven study sites across the biogeographic transition zone (28.196°S to 30.95°S) of eastern Australia: Cook Island (CI), Julian Rocks (JR), North Solitary Island (NSI), Northwest Solitary Island (NWSI), Southwest Solitary Island (SWSI), South Solitary Island (SSI), and Black Rock (BR). Red to blue gradient represents the thermal gradient along the east coast of Australia from tropical to temperate thermal regimes

maximum calliper length, which is the longest distance between the outlined boundaries, was determined for each sea urchin. We were unable to consistently distinguish between *D. setosum* or *savignyi* from the images and both could possibly occupy the study sites, so these two species were grouped as *Diadema* spp.

3.3.3 Sea Surface Temperature analysis

Monthly average sea surface temperature (SST) data were extracted for all seven sites from the Modis Aqua satellite (Parkinson 2003) at 1km spatial resolution for each of the seven sites for 2003-2019 (Supporting information, Fig B2., Table B2). From these data we calculated the annual mean, minimum and maximum temperatures (±SEM) (°C), temperature ranges for each site and year, and the mean annual mean, minimum and maximum temperature across all years and sites (Supporting information, Table B3, Table B4).

3.3.4 Temporal patterns in the size structure of sea urchin populations

Counts and test diameter (i.e., the diameter at the widest point of the sea urchin skeleton in the photograph, not including spines) data were used to determine the size frequency distribution and density of sea urchins (m^{-2}) in 2010/11, 2016 and 2019 and to determine the temporal dynamics of the populations. These data were used to infer population size structure through time. For 2012 only counts and not measures of test diameter were conducted and population size structure analyses therefore do not include data for the year 2012.

3.3.5 Statistical analysis

Thermal and spatial trends in sea urchin densities

All statistical analyses were performed in R (R Core team 2021). Of the eight species observed in the transects, only *Centrostephanus rodgersii, Tripneustes australiae*, *Diadema* spp. and *Phyllacanthus parvispinus* were sufficiently abundant for species analysis. To test if the density of these species (*Centrostephanus rodgersii, Tripneustes australiae*, *Phyllacanthus parvispinus* and *Diadema* spp.) varied with thermal conditions we used separate Generalised Linear Mixed Model (GLMM) with a Poisson distribution and a log link function for each species, with mean annual temperature (for each site) as a fixed effect and the random effects of site and transect (transect nested in site) ($n = 5$) in the glmmTMB package (Brooks et al. 2017). Due to multi-collinearity (Pearsons $r > 0.7$, Supporting information, Fig. B3) of the mean, minimum and maximum SST parameters, we used mean SST for all models examining the density of sea urchins and thermal conditions. There was also negative correlation between latitude and temperature, temperature decreasing with increasing latitude ($r > 0.7$, Supporting information, Fig. B4). The distributions of *Diadema* spp. and *P. parvispinus* were zero-inflated, and required a zero-inflated Poisson model (Brooks et al. 2017). Assumption and model checks were undertaken visually using Q–Q plots and the spread of residuals was assessed using residuals vs. fitted values. Model significance was tested with Type II Wald Chi-square Tests using the *Anova* function in the car package (Fox and Weisberg 2019). Post-hoc interpretation of models was done using the emmeans package (Lenth 2024) to back-transform model coefficients. As temperature and latitude are highly correlated, relationships between density and temperature would also apply to latitude, but for the purpose of this study we used temperature as a predictor of density.

Temporal trends in sea urchin densities

To examine whether the density of these species varied among years we used a separate GLMM with a Poisson distribution and a log link function for each species, with year as fixed effect and the random effects of site and transect (transect nested in site) (n = 5) in the glmmTMB package (Brooks et al. 2017). The distributions of *Diadema* spp. and *P. parvispinus* were zero- inflated, and required a zero-inflated Poisson model (Brooks et al. 2017). Pairwise post-hoc comparisons between years were made with Tukey's Tests in the multcomp package (Hothorn et al. 2008). Assumption and model checks were undertaken visually using Q–Q plots and assessing the spread of residuals using residuals vs. fitted values. Model significance was tested with Type II Wald Chi-square Tests using the *Anova* function in the car package (Fox and Weisberg 2019).

Temporal patterns in sea urchin population size structure

Size Frequency distributions (SFDs) and pairwise Two Sample Kolmogorov-Smirnov Tests (K-S tests) were used to compare SFDs of *C. rodgersii, T. australiae*, *Diadema* spp. and *P. parvispinus*. Specifically, SFDs were compared across years to infer temporal trends and were interpreted using descriptive statistics (see below). Pairwise K-S tests between each combination of year (2010, 2016 and 2019) were used to test for differences in SFDs between years. Using a series of K-S tests, we determined if SFDs came from the same distribution, or differed from each other (Meesters et al. 2001; Anderson and Pratchett 2014). The SFDs were used to infer population dynamics including measures of size, skewness, kurtosis, and the coefficient of variation. We used the false discovery rate procedure (Benjamini et al. 1995; Pike 2011) to adjust p-values and minimise Type 1 error from multiple comparisons.

3.4 Results

3.4.1 Sea urchin species

We recorded eight sea urchin species on subtropical reefs in the subtropical-to-temperate transition zone of east Australia. The most abundant species were the subtropical/temperate species *Centrostephanus rodgersii* and *Tripneustes australiae* (Table 3.1). The temperate pencil sea urchin *Phyllacanthus parvispinus* and the tropical *Diadema* spp. (*Diadema savignyi* and/or *D. setosum*) were comparatively less abundant. We also recorded the tropical sea urchin *Tripneustes gratilla* in low numbers in 2019 and the temperate *Heliocidaris* species, *H. erythrogramma* and *H. tuberculata*, at very low densities across all years. The temperate pencil sea urchin *Prionocidaris callista* was rarely observed, with only 5 observations in 2016 (Table 3.1).

3.4.2 Sea surface temperature (SST)

All sites in the region showed strong seasonal variation in temperature (Fig. 3.2, Supporting information Fig. B2) with the highest range in mean annual temperature from 2010-2019 at the most poleward site, Black Rock (18.7 – 26.76°C), and the lowest range in mean temperature at Julian Rocks (19.46 – 26.71°C), one of the most northern sites (Fig. 3.1, Supporting information, Table B3). Across the region, mean annual temperature was highest in 2015 (23.14°C) (Supporting information, Table B4) and lowest in 2012 (22.57 °C) (Supporting information, Table B4). The lowest minimum average monthly temperature recorded in the study period was in 2012 (18.699°C), at Black Rock and the highest recorded mean monthly temperature in the region was at Cook Island (26.93°C), the most northerly site (Figs. 3.1 and 3.2). Overall, the site with the highest level of mean annual variation was Cook Island (SEM = 0.2011), and the site with the least variation was South Solitary Island (SEM = 0.194) (Supporting information, Table B3).

Table 3.1. Summary of the descriptive statistics for sample size (n), density (m-^{2}), Ferets Diameter (mean), coefficient of variation (CV), skewness (g_1) and kurtosis (g_2) for all sea urchin species observed in the biogeographic transition zone of coastal New South Wales, Australia in 2010, 2016 and 2019.

Species	Year	n	Density (m^{-2})	Mean (cm)	CV	g_1 (skew)	g_2 (kurt)
Centrostephanus rodgersii							
	2010	3199	3.18	10.93	0.19	0.16	3.53
	2012	2282	2.61				
	2016	2093	2.46	11.63	0.18	0.315	3.47
	2019	3002	2.92	11.37	0.18	0.34	3.50
Tripneustes australiae							
	2010	358	0.35	7.65	0.21	0.94	6.14
	2012	194	0.13				
	2016	21	0.025	9.1	0.15	-0.11	1.95
	2019	18	0.018	8.68	0.16	0.25	4.13
Diadema spp. (D. savignyi or D. setosum)							
	2010	79	0.079	9.98	0.1	-0.018	3.05
	2012	58	0.066				
	2016	21	0.025	10.81	0.23	-0.28	1.17
	2019	18	0.018	11.08	0.23	0.08	1.72
Phyllacanthus parvispinus							
	2010	57	0.057	6.72	0.22	0.42	2.5
	2012	18	0.021				
	2016	43	0.051	6.83	0.17	-0.42	2.71
	2019	35	0.034	6.24	0.18	0.66	2.97
Tripneustes gratilla							
	2010	0		-	-	-	-
	2012	0					
	2016	0		-	-	-	-
	2019	4		-	-	-	-
Heliocidaris erythrogramma							
	2010	1		-	-	-	-
	2012	0					
	2016	0		-	-	-	-
	2019	0		-	-	-	-

Heliocidaris tuberculata						
	2010	0	-	-	-	-
	2012	0				
	2016	0	-	-	-	-
	2019	1	-	-	-	-
Phyllacanthus callista						
	2010	0	-	-	-	-
	2012	0				
	2016	5	-	-	-	-
	2019	0	-	-	-	-

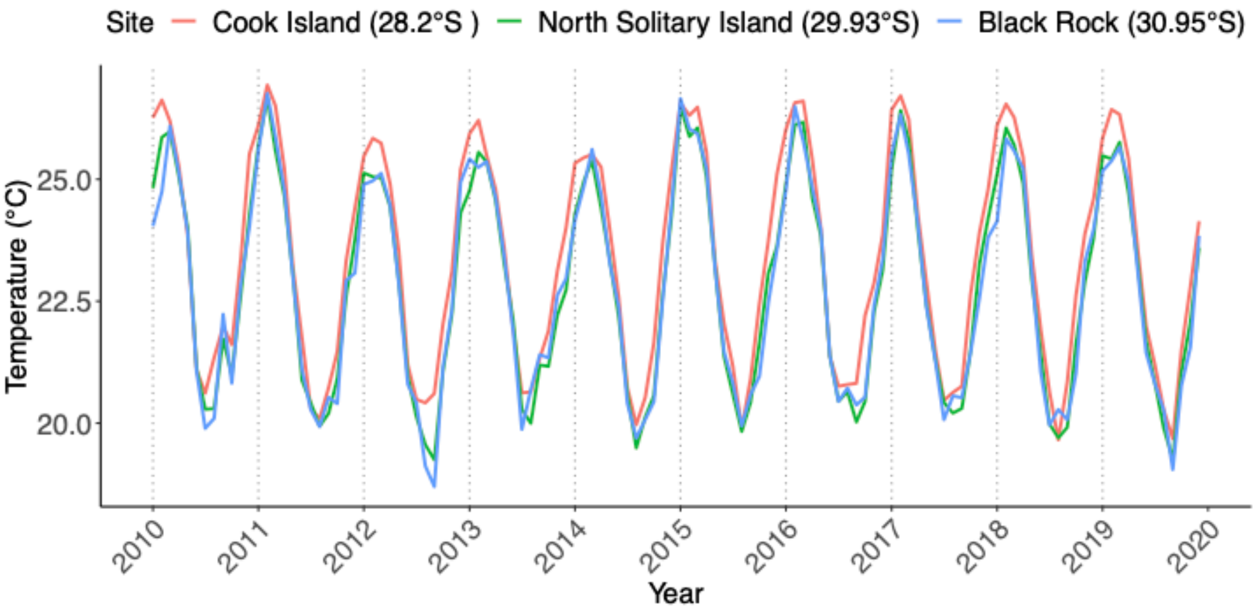

Figure 3.2. Monthly mean temperature (°C) from the Modis Aqua Satellite Parkinson (2003) for three sites (Cook Island, North Solitary Island, and Black Rock) in subtropical eastern Australia from 2010-2019.

3.4.3 Thermal and spatial trends in sea urchin densities

There was no relationship between the density of *Centrostephanus rodgersii* (χ^2 = 0.7978, df = 1, p = 0.37) or *Diadema* spp. (χ^2 = 0.8039, df = 1, p = 0.3699) and mean temperature in our study region in NSW (Fig. 3.3). The density of *Tripneustes australiae* (χ^2 = 130.19, df = 1, p <0.001) and *Phyllacanthus parvispinus* varied significantly with mean temperature (χ^2 = 130.19, df = 1, p =0.01) (Fig. 3.3) at our study sites in NSW. For every 1°C increase in temperature, *T. australiae* density increased by 0.00126 $m^{-2,}$ and *P. parvispinus* density increased by 0.045 m^{-2}.

3.4.4 Temporal trends in sea urchin densities and population size structure

Centrostephanus rodgersii

The largest and most abundant sea urchin was *Centrostephanus rodgersii* (test diameter (TD): 2.7 – 18.9 cm), with over 4000 individuals measured. Densities of *C. rodgersii* varied through time (χ^2 = 51.5114, df = 3, p <0.001) but were the most stable of all the species (Table 3.1) (Fig. 3.4). Size-frequency distributions of *C. rodgersii* populations differed significantly between years (K-S test 2010 vs 2016, D = 0.15, p = 0.0015; 2010 vs 2019, D = 0.088, p = 0024; 2016 vs 2019; D = 0.07, p = 0024). *Centrostephanus rodgersii* was the largest size in 2016 (Mean TD: 11.63cm), and the smallest in 2010 (Mean TD: 10.93cm) (Table 3.1, Fig. 3.5). However, this species was relatively stable in density and size over the study, as indicated by similar kurtosis, skew and CV values across years (Table 3.1, Fig. 3.5).

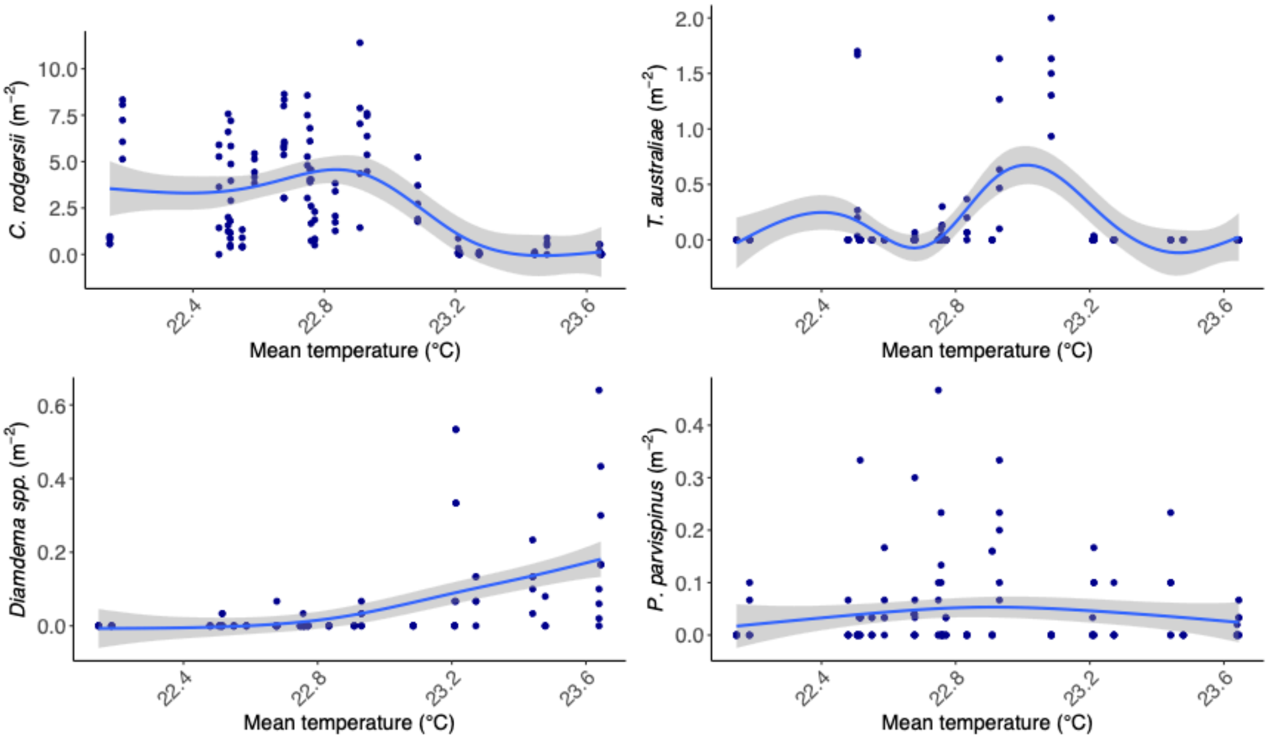

Figure 3.3. Scatter plots between mean annual temperature (means of monthly means for each year) and the mean density (m^{-2}) (per site) of *C. rodgersii, T. australiae, Diadema* spp. and *P. parvispinus* from 2010-2019 in the biogeographic transition zone of New South Wales, eastern Australia. Trend lines are General Additive Model predictions between mean temperature and density for each species, the shaded areas show the standard error from the mean.

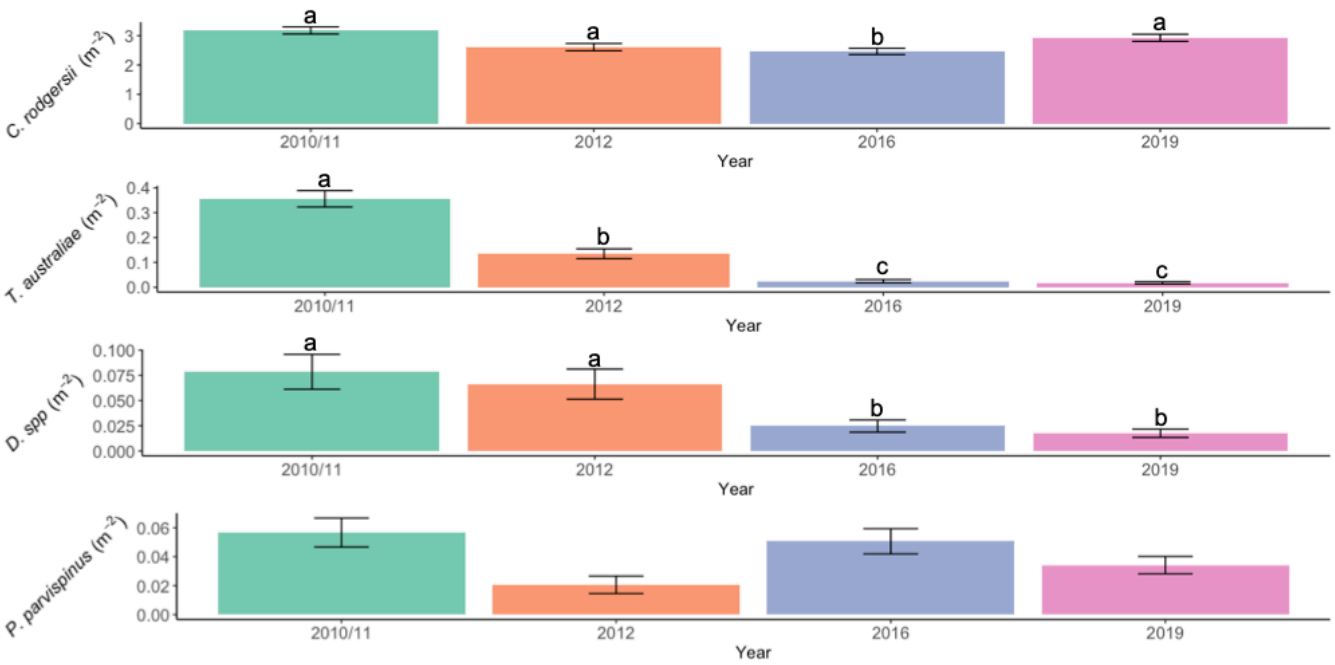

Figure 3.4. Mean densities (±SEM) of *C. rodgersii*, *T. australiae*, *Diadema* spp. and *P. parvispinus* from 2010-2019 in the biogeographic transition zone of New South Wales, eastern Australia. Letters indicate Tukeys post-hoc groups. Groups that do not share a letter are significantly different.

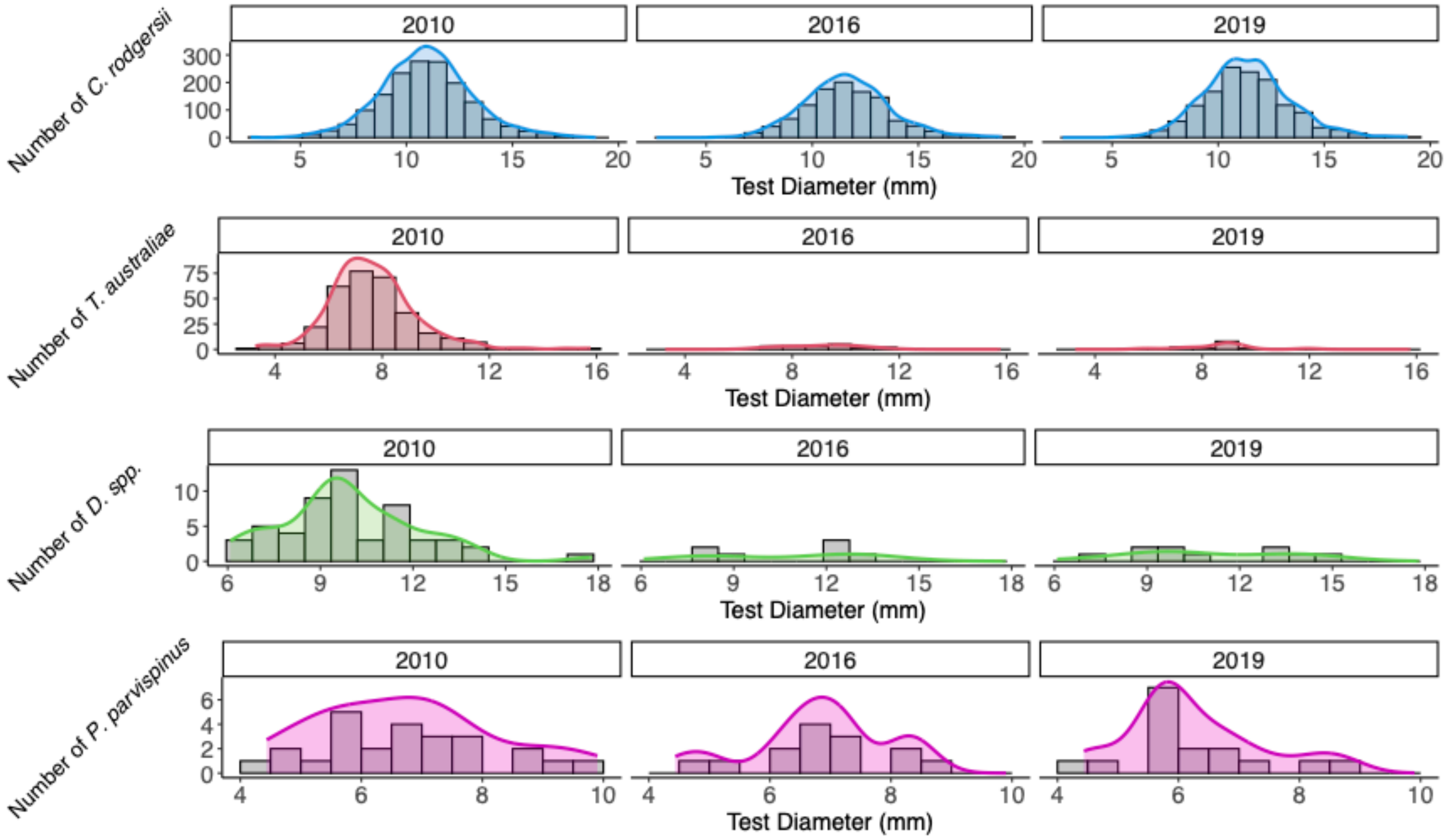

Figure 3.5. Temporal patterns in the population size structure of *C. rodgersii*, *T. australiae*, *Diadema* spp. and *P. parvispinus* from 2010-2019 in the biogeographic transition zone of New South Wales, eastern Australia. Curves overlying the histograms are smoothed density estimates computed using kernel density estimates.

Tripneustes australiae

Tripneustes australiae was the second most abundant sea urchin species, with over 600 individuals observed. The density of *T. australiae* declined markedly during the study period (χ^2 = 304.9966, df = 3, p <0.001) (Table 3.1, Fig. 3.4). The population size structure of *T. australiae* (TD:3.3 – 15.8cm) significantly differed between 2010 and 2016 (K-S test: D = 0.48, p = 0024) and 2010 and 2019 (D = 0.51, p = 0024) but not between 2016 and 2019 (D = 0.3, p = 0.312). Between 2010 and 2016, the *T. australiae* populations declined more than 20-fold and had a low number of small individuals in 2016, as indicated by negative skew (Table 3.1). In 2019 some small individuals remained after the population decline and higher kurtosis compared to 2010 and 2016 indicated that there may have been some recruitment (Table 3.1, Fig. 3.5). However, the abundance of *T. australiae* was significantly lower than in 2010 (Fig. 3.4).

Diadema spp.

Diadema spp. were the third most common (n = 176) and the second largest sea urchin taxon (TD: 6.1 – 17.8cm) (Table 3.1). *Diadema* density varied significantly with time (χ^2 = 35.2437, df = 3, p <0.001) and declined throughout the study period (Fig. 3.4). The population density of *Diadema* spp. was the lowest in 2019, suggesting that the population did not recover between 2012 and 2019 (Fig. 3.4). The SFDs of *Diadema* spp. did not differ among years (K-S test 2010 vs 2016, D = 0.42, p = 0.27; 2010 vs 2019, D = 0.32, p = 0.312; 2016 vs 2019, D = 0.33, p = 0. 312) (Fig. 3.5). There was a higher representation of smaller individuals in 2019, as indicated by positive skew suggesting some recruitment (Table 3.1, Fig. 3.5).

Phyllacanthus parvispinus

Phyllacanthus parvispinus was the least abundant species (n = 153) (TD: 4.4 – 9.9cm) and the density of this species did not vary over time ($\chi^2 =$ 7.0481, df = 1, p =0.07) (Fig. 3.4), nor did SFDs (K-S test 2010 vs 2016; D = 0.42, p = 0.312; 2010 vs 2019; D = 0.32, p = 0.62; 2016 vs 2019; D = 0.33, p = 0.62) (Fig. 3.5, Table 3.1). Given the low abundance of this species in the study region, higher sampling effort may be required to detect changes in the density and population size structure of this species over time.

3.5 Discussion

Across subtropical reefs in the biogeographic transition zone of east Australia, sea urchin density and population size structure varied among species. This result is consistent with observations for other taxa that have exhibited variable population patterns on these subtropical reefs during the study period. For example, in SIMP there are remarkably stable communities of some fishes and corals (Malcolm and Ferrari 2019; Mizerek et al. 2021), increases in tropical herbivorous fishes (Smith et al. 2021), declines in kelp (Vergés et al 2016) and an overall higher diversity of tropical and subtropical fish species than would be expected based on latitude alone (Miller et al. 2023). Our results show that counter to the expectation that cool tolerant/temperate species would decrease and warm affinity/tropical species would increase due to warming in the region, the abundance of tropical *Diadema* spp. and subtropical *Tripneustes australiae* declined, while populations of the temperate species *Centrostephanus rodgersii* were stable.

Over this nine-year study, *Tripneustes australiae* experienced a 20+ fold decrease in numbers. The genus *Tripneustes* exhibits boom-bust population cycles and experiences die-offs in heatwave and decreased salinity conditions (Lawrence and Agatsuma 2020). For example, at subtropical Lord Howe Island, *T. australiae* (then called *Tripneustes gratilla*) established conspicuous aggregations in coral habitat, hypothesised to be driven by anomalous oceanographic events, or favourable effects of predator removal on recruitment and survival of juveniles (Valentine and Edgar 2010). Valentine and Edgar (2010) note rapid increase of *T. australiae* which changed from rare to >1.3 m^{-2} over a two-year period at Lord Howe Island, likely due to major sporadic recruitment, subsequently followed by their disappearance. Similarly, abundant populations of *T. australiae* further poleward along the east coast of Australia, in Port Stephens and Sydney in the 1990's (Bové 2004) did not return over the following 20+ years (authors, pers. obs). Notably, T. *australiae* recently increased in local abundance in Port Stephens and Sydney in 2023, following what appears to be a similar sporadic recruitment event (author et al. in prep.).

Along the east Australian biogeographic transition zone, *T. australiae* are living at their warm range-edges (author et al. in prep.), and the rapid die off of *T. australiae* followed by population decrease could represent the first two stages of population contraction as described by Bates et al. (2014). Our results show that the abundance of *T. australiae* may be linked to temperature, with increases in density with warmer mean temperature, and a density peak around 23°C. The highest mean temperature, and the highest recorded temperature in the study period were in 2011. This may have influenced the population decrease in 2012, suggesting that *T. australiae* may be more sensitive to temperature variation at its warm range edge, especially when compared to the co-occurring temperate sea urchin *C. rodgersii.*

Species are expected to perform at a lower capacity at their warm range edges (Zarzyczny et al. 2023). As the physiological tolerance limits of *T. australiae* have not been described, and *Tripneustes* are known to experience die-offs in heatwave conditions and salinity decreases (Lawrence and Agatsuma 2020), marine heatwaves and storms during our survey period (Lachs et al. 2021) may have contributed to their decline and limited recovery in this region. However, as *T. australiae* sea urchins have been observed in the Solitary Islands region after our study period (author, pers. obs), the population dynamics of *T. australiae* recorded here appear to reflect a boom-bust pattern influenced by environmental drivers other than temperature.

Tripneustes australiae and *Diadema* spp. exhibited dramatic declines across all sizes, which may explain the limited recovery of both species during our study. *Diadema* spp. are living at their cool range edge in this region, likely at the limits of their physiological tolerances and with limited propagules to supply recruitment. This might make them more susceptible to physiological stress and disease (Rowley et al. 2022) and these factors could have contributed to their population decline during the 10-year period. Although *T. australiae* and *Diadema* spp. populations showed some indications of smaller individuals consistent with recruitment in 2019, their abundances were still much lower than in 2010. Both species are likely to rely on external larval supply to re-seed their populations in this region. However, it is worth noting that the *Diadema* spp. in this study may have cryptic juveniles and recruits would not be detected by our survey method. In contrast, *T. australiae* are generally in the open, defended by their cover of shells and other items as camouflage and venomous globiferous pedicellariae (Sheppard-Brennand et al. 2017; Lawrence and Agatsuma 2020). Thus, we are confident that the smaller size classes of this species would have been captured by our photographic survey method.

The barren forming sea urchin *C. rodgersii* had the most stable population through time, and there was no influence of temperature on population densities which ranged between 2.46 and 3.17 m-^{2} over the study period. All sites included in this study were in Marine Protected Areas (MPAs) and similar stability has been observed in other MPAs in NSW, where *C. rodgersii* populations were stable through time despite increases in urchin predators such as snapper (*Chrysophrys auratus*), eastern blue grouper (*Achoerodus viridis*) and eastern rock lobster (*Sagmariasus verreauxi*) (Glasby and Gibson 2020, summarised by Przeslawski et al. 2023). In contrast to the range expansion and proliferation of *C. rodgersii* into Tasmania (Ling 2008), our study highlights that the population dynamics of this sea urchin on subtropical reefs are stable in its historic range in NSW, as noted elsewhere along the NSW coast (Glasby and Gibson 2020; Davis et al. 2023). In fact, Davis et al. (2023) suggests that *C. rodgersii* populations may begin to decrease in their northern range (including SIMP) with warming. However, we did not see a decline in *C. rodgersii* populations during our study period, which experienced a 0.16°C increase in mean annual mean temperatures between 2003 and 2019.This is consistent with the thermal range of *C. rodgersii* (19.6 – 26.5°C in its northern distribution (Byrne et al. 2022)) and suggests that *C. rodgersii* is still within its thermal limits in our study region. Other studies have documented declines in kelp (Vergés et al. 2016) and coral populations (Kim et al. 2019, Sommer et al. 2024) in response to marine heat waves in the region (e.g., up to 14 Degree Heating Weeks in 2016; Lachs et al. 2021) and our results suggest that *C. rodgersii* populations in northern NSW were unaffected by these heat stress events.

Our size frequency data suggest that the populations of *C. rodgersii* were stable over the nine-year study period and that they were dominated by large individuals. This is similar to patterns observed for *C. rodgersii* populations elsewhere in NSW (Andrew and Underwood 1989; Andrew and O'Neill 2000). The literature on *C. rodgersii* suggests sporadic recruitment events, with the possibility of density-dependent recruitment, as the largest observation of juvenile *C. rodgersii* followed mass mortality of adult conspecifics after large storms (Andrew 1991). *Centrostephanus rodgersii* juveniles are very cryptic, and rarely observed even in visual census surveys (Andrew and Underwood 1989). Notably, a recent study observed juvenile *C. rodgersii* emerging at night (Smith et al. 2024) highlighting that nighttime surveys are required to detect small individuals.

Diadematoid sea urchins can facilitate the proliferation of corals on coral and rocky reefs by grazing turfing algae (Sammarco 1980; Lessios et al. 2001; Lessios 2016; Ling et al. 2018). The present study revealed sizeable and stable *C. rodgersii* populations in association with corals on subtropical reefs of east Australia. Given the stability of the coral assemblages in the SIMP over 23 years (Mizerek et al. 2021) and the role that sea urchins, particularly diadematids can play in mediating coral success by reducing habitat competition from algae, *C. rodgersii* may mediate the success of corals in these subtropical coral habitats (Sammarco 1980; Lessios et al. 2001; Lessios 2016).

This study adds to the growing body of knowledge regarding *C. rodgersii* within its historic range in NSW (Glasby and Gibson 2020; Przeslawski et al. 2023; Kingsford and Byrne 2023; Davis et al. 2023) and shows that that *C. rodgersii* populations in this coral associated habitat in NSW are currently stable (Glasby and Gibson 2020, summarised by Przeslawski et al. 2023). Although the poleward range expansion of *C. rodgersii* has altered the sea scape in Tasmania (Ling 2008), in NSW the barrens-macroalgae mosaic is a natural and stable part of the ecosystem that promotes local biodiversity, and is characteristic of this coastal ecosystem (Glasby and Gibson 2020; Przeslawski et al. 2023; Kingsford and Byrne 2023). Any management action in our study region, such as culling or expansion of the *C. rodgersii* fishery in NSW, must be carefully considered and informed by research (Kingsford and Byrne 2023). Further, it should be a research priority to understand the ecological role of the barren forming sea urchin *C. rodgersii* within its pre-industrial range, including its interaction with corals and other organisms on subtropical reefs in the biogeographic transition zone.

3.6 Conclusion

Subtropical reefs are important ecosystems, and this study is the first to investigate the long-term temporal dynamics of sea urchin populations in this region, adding to similar long-term studies of other taxa such as corals, kelps and fishes (Vergés et al. 2016; Sommer et al. 2018; Malcolm and Ferrari 2019; Cant et al. 2023). Sea urchins are important herbivores, and we show that population dynamics of sea urchins in these subtropical reefs vary among taxa and cannot be predicted by biogeographic affinity alone. Specifically, the most abundant species, the temperate species *C. rodgersii* were stable through time, while the subtropical and tropical species *T. australiae* and *Diadema* spp. declined across all size classes during the 10-year survey period. Niche availability in a receiving ecosystem is crucial for the establishment of an invading or range extending population (Bates et al. 2014; Miller et al. 2023; Zarzyczny et al. 2023). It is thus possible that subtropical reefs in this region may not experience an influx of tropical sea urchins, while the populations of *C. rodgersii* are dominant and stable, and that *C. rodgersii* mediates ecological dynamics of other taxa in this region.

Chapter 4.

Searchin' for urchins: Utilising museum collections and citizen science to assess species on the move in the genus *Tripneustes*

4.1 Abstract

Abstract

Aim: With the global redistribution of species due to warming, accurately quantifying species distributions is critical to understand patterns in biodiversity and range shift trajectories. The genus *Tripneustes* comprises globally important sea urchin taxa that graze seagrass and macroalgae and have the potential to transform ecosystems. In eastern Australia, there are two *Tripneustes* taxa, the tropical *T. g. gratilla*, and the subtropical-temperate *T. australiae*. The temperate distribution of *T. australiae* was considered to be a climate driven range extension of *T. g. gratilla* until recent taxonomic clarification. We quantified the present and potential future distributions of these taxa in eastern Australia, a global warming hot spot.

Location: Eastern Australia and the Tasman Sea including Lord Howe Island, Norfolk Island and the north of New Zealand

Taxon: *Tripneustes* (Echinodermata: Echinoidea: Toxopneustidae)

Methods: We reassessed specimen identifications across four museum collections to synthesize distribution data and citizen science observations, determining the biogeographic range and realized thermal niches of *Tripneustes*. Habitat suitability models were used to determine current suitable habitat and predict future distributional change.

Results: Whilst *T. g. gratilla* has a wide tropical-temperate distribution in the region from Papua New Guinea (9° 56' 2.4"S) to Jervis Bay (35° 7' 12"S) and a broad realized thermal niche (16.3 - 29.97°C), *T. australiae* has a subtropical-temperate distribution (Byron Bay, 28° 37'

0.12"S to Narooma, 36° 15' 0"S) and narrower thermal niche (15.3 - 26°C). *Tripneustes* densities were highest in the subtropical ecoregions, where the two taxa co-occur. Habitat suitability modelling indicated that *T. australiae* is a narrow range thermal specialist that will likely undergo a poleward range shift by 2100. In contrast, *T. g. gratilla* is a generalist that appears capable of occupying a broader range of conditions.

Main conclusions: Despite their similar ecological roles, congeneric *Tripneustes* in eastern Australia and the Tasman region have contrasting distributions and realized thermal niches, with distinct implications for their future distributions as the ocean warms.

4.2 Introduction

Temperature gradients structure marine communities across broad latitudinal scales (Tittensor et al. 2010; Stuart-Smith et al. 2015, 2017; Saupe et al. 2019). As a result, ocean warming is driving global biodiversity redistributions (Burrows et al. 2019). Shallow-water marine ectotherms can be broadly classified into two biogeographic groups based on their temperature preferences, warm and cool affinity. This is reflected in their realized thermal niches, the temperature ranges species experience across their distribution (Tittensor et al. 2010; Sunday et al. 2011; Stuart-Smith et al. 2015, 2017). Generally, this manifests as low and high latitude ecological communities being dominated by warm and cool affinity species, respectively (Stuart-Smith et al. 2015). Further, the thermal niches of marine ectotherms tend to widen with increasing latitude away from the equator (Sunday et al. 2011; Stuart-Smith et al. 2017). Irrespective of taxa, animals living closer to the equator have narrower thermal windows than animals living closer to the poles (Addo-Bediako et al. 2000; Sunday et al. 2011; Stuart-Smith et al. 2017). This is hypothesised to be driven by lower seasonal temperature variation in the tropics (Addo-Bediako et al. 2000; Sunday et al. 2011; Stuart-Smith et al. 2017). Cosmopolitan and generalist species can be broadly distributed across tropical and temperate realms (Sunday et al. 2015; Platts et al. 2019), whereas subtropical specialists typically succeed in narrow ranges at the interface of these realms (Sommer et al. 2014, 2018; Cant et al. 2021; Broitman et al. 2021).

As the oceans warm, it is becoming increasingly important to understand the thermal tolerance ranges of marine ectotherms with respect to their present and potential future distributions, and extinction risk (Sunday et al. 2015; Burrows et al. 2019; Stuart-Smith et al. 2022; Edgar et al. 2023). Globally, marine taxa are undergoing poleward range expansions as they track their thermal niches through space, driving re-assembly of receiving ecological communities (Vergés et al. 2014, 2019; Wernberg et al. 2016; Pecl et al. 2017; Stuart-Smith et al. 2022). For example, the range extension of sea urchins in temperate regions are of particular interest, as they can cause widespread biodiversity loss due to grazing (Ling 2008), facilitate colonization of other species such as corals (Ling et al. 2018), and accelerate a shift towards warm-affinity fish species on temperate reefs (Schuster et al. 2022).

Eastern Australia is a climate change hot spot, where poleward range extension of many species mediates flow-on effects for species interactions and ecological dynamics (Hobday and Pecl 2014; Vergés et al. 2014, 2019; Wernberg et al. 2016; Schuster et al. 2022). Sea urchins are important grazers on temperate, subtropical and tropical reefs (Filbee-Dexter and Scheibling 2014; Lessios 2016). In the shallow rocky reefs of New South Wales, Australia, sea urchins mediate a mosaic of barren and kelp forest habitat (Jones and Andrew 1990; Underwood et al. 1991; Kingsford and Byrne 2023).

Tripneustes sea urchins are common shallow water taxa that inhabit seagrass meadows, rocky reefs and coral reefs (Lawrence and Agatsuma et al. 2020). In eastern Australia there are two *Tripneustes* taxa with contrasting and overlapping distributions, the tropical *T. gratilla gratilla* and the subtropical-temperate *T. australiae*. *Tripneustes gratilla* underwent recent phylogenetic revision whereby mitochondrial and nuclear loci revealed a Red Sea subspecies *T. g.* elatensis in addition to the Indo-Pacific *T. g. gratilla* (Bronstein et al. 2016, 2017). *Tripneustes g. gratilla* is an ecologically and commercially important sea urchin that occurs tropically in the Indo-Pacific, from east Africa to the offshore islands of Australia in the Pacific, with occurrences at Lord Howe Island, and on the coast of eastern Australia (Lawrence and Agatsuma 2020). Until recent taxonomic clarification (Bronstein et al. 2019; McLaren et al.

2023), the subtropical-temperate occurrences of *T. australiae* were considered a climate driven range extension of *T. g. gratilla* (Castro et al. 2020). The recent clarification of the presence of two *Tripneustes* taxa in the region highlights a knowledge gap in our understanding of the fundamental biology and ecology of these sympatric taxa, including their geographical distributions and realized thermal niches. Notably, *T. australiae* is only known from Australia and New Zealand, and knowledge of its biology and ecology is limited.

Congeneric taxa with contrasting distributions, niches or zonation, such as *T. g. gratilla* and *T. australiae*, can provide important insights into the relationship between thermal biology and biogeography and differential trajectories of distributional change in a warming ocean (Halsband-Lenk et al. 2002; Stenseng et al. 2005; Oliver et al. 2010; Prusina et al. 2014; Popovic and Riginos 2020; Redfern et al. 2021). Thus, determining the distributions of the ecologically important *T. australiae* and *T. g. gratilla* in eastern Australia is key for predicting their potential future trajectories in a warming ocean. In addition, as *Tripneustes* are known for their boom-bust population dynamics (Uthicke et al. 2009; Valentine and Edgar 2010; Lawrence and Agatsuma 2020; McLaren et al. 2024), with *T. australiae* currently in what appears to be a boom phase (Byrne et al. 2024) delineating the distributions of these echinoids is of particular interest due to their important roles as herbivores (Valentine and Edgar 2010).

Given the recent taxonomic revision of *T. australiae*, we sought to clarify the distributions of *T. g. gratilla* and *T. australiae* in eastern Australia and across the Tasman Sea, as they may influence ecological communities differently, especially considering the boom-bust dynamics of *T. australiae*. For this study, we therefore define our region of interest as the east coast of Australia, Lord Howe Island, Norfolk Island and the north of New Zealand. We used natural history collections and citizen science initiatives to maximise the observations in our region of interest for the two taxa through time. To do so, we first identified all specimens held across four museums to the taxon level following the taxonomic description in McLaren et al. (2023). Reassessment of museum collections was important as historically these were all identified as *T. gratilla*.

We addressed the hypothesis that based on museum collections, the distribution of *T. g. gratilla* would reflect a tropical distribution, while *T. australiae* would be restricted to subtropical and temperate latitudes in our region of interest. The density of *Tripneustes* in the region was expected to be highest in subtropical ecoregions, where *T. australiae* is known to reach high densities (Valentine and Edgar 2010, McLaren et al. 2024). We used the distribution datasets to estimate the realized thermal niches of *T. g. gratilla* and *T. australiae* in eastern Australia and the Tasman to conduct habitat suitability modelling to investigate the range shift potential for the two taxa under climate change. Consistent with the pattern observed for other animals (Addo-Bediako et al. 2000; Sunday et al. 2011; Stuart-Smith et al. 2017) we expected that the tropical species *T. g. gratilla* would have a narrower realized thermal niche than *T. australiae*. Specifically, we predicted that suitable habitat would decline at the warm-range edge of the subtropical/temperate *T. australiae* in the future, while suitable habitat would increase at the cool-range edge of the tropical *T. g. gratilla.*

4.3 Methods

4.3.1 Distributions of *Tripneustes* from museum collections

Tripneustes material examined

We examined all (n = 330) *Tripneustes* material collected from Australia at the Australian Museum (AM, Sydney), the Natural History Museum (NHM, London), the Sea Urchin Science Centre and Gallery (SUSCG, Sydney), and the Museum Victoria (MV, Melbourne). These institutions held specimens from our region of interest, which was mainland eastern Australia and around the Tasman Sea. At the time of examination in 2023, all *Tripneustes* material across these institutions was identified as *T. gratilla*. To investigate differences in the geographical distributions of tropical and subtropical *Tripneustes* taxa, we identified the specimens as *T. australiae* or *T. g. gratilla* based on the morphological traits used to differentiate these taxa (Table C1) (Bronstein et al. 2017; McLaren et al. 2024). To incorporate the latitudinal distribution of the *Tripneustes* material just to the north of eastern Australia (equatorward) we included the collections of *T. g. gratilla* from Papua New Guinea. We considered that the material from this location in the collections represented the low latitude thermal regime for our region of interest.

4.3.3 Associated metadata

All metadata associated with the collection event for each specimen lot were extracted from the specimen labels or from museum data management systems. Most specimen lots (n = 227) had associated geographical data. This was either exact latitude and longitude, or precise descriptions of collection locations. This information was used to compile the collection locations of these specimens (Appendix C).

4.3.4 Sea surface temperature analysis and construction of realised thermal niches

The geographical data associated with the specimens was used to describe the distributions of *T. australiae* and *T. g. gratilla* in Australia and estimate their thermal ranges. Based on the latitudinal extent of the collected specimens, the realized thermal niche width of the two taxa was determined (Stuart-Smith et al. 2017; Collin et al. 2021). The upper-most, lower-most and mid-range points of each taxon's geographical ranges were aligned with remotely sensed sea surface temperature (SST°C) data extracted from the marine data-repository Bio-Oracle (Assis et al. 2024).

We extracted the 2000-2010 and 2010-2020 summary data sets from Bio-Oracle SST records (Assis et al. 2024) and took the average of the long-term average of the yearly maxima and minima for each of the decades (i.e. the mean of the mean temperatures for the warmest or coolest months of each decade) and the average maximum and minimum SST for each decade to determine overall extremes in the 2000-2020 period. From this we determined the temperature ranges at the northern-most, southern-most and mid-point of the distributions of each taxon. This information was used to determine the realized upper and lower thermal limits. We used the equatorial occurrences of *T. g. gratilla* around Papua New Guinea to determine its upper thermal limits.

4.3.5 Distribution and abundance of *Tripneustes* in Australia from Reef Life Survey

Data extraction and summary

Abundance data for the genus *Tripneustes* on the southeast coast of Australia were obtained from benthic survey data from the long-term monitoring program Reef Life Survey (RLS) for the period 2003 – 2023 (Edgar et al. 2020). We extracted the data for all *Tripneustes* spp. from the database, as the taxonomy of *Tripneustes* in eastern Australia has only been recently clarified (Bronstein et al. 2017; McLaren et al. 2023). Prior to the revision of the subtropical-temperate taxon as *Tripneustes kermadecensis* (Bronstein et al. 2017) and later *Tripneustes australiae* (McLaren et al. 2023) all identifications were attributed to be *Tripneustes gratilla.* Thus, we grouped abundance at the genus level for the purpose of this study. Abundance data for sea urchins were collected following the standard RLS method 2 (Edgar et al. 2020). For these surveys, multiple 50 x 2 m transects were deployed at depths between 0.4 – 22 m. Depths varied depending on extent of the reef as delimited by sandy bottom. Mobile macro-invertebrates including sea urchins were counted along these transects and for two meters below. Full details of the RLS methods are reported by Edgar et al. (2020). To control for potential observer or unconscious bias of the RLS citizen science dataset, we summarized the data at the ecoregion level (see below) to complement the primary analysis of this study based on verified museum specimens.

Data on the abundance of *Tripneustes* spp. were used to calculate frequency of observations at any given latitude and longitude within northern and eastern Australia (including the offshore territories Lord Howe Island and Norfolk Island). This was then used in addition to the museum collection data to map frequency of observation in the tropical, subtropical and temperate regions.

In addition, the abundance data for the *Tripneustes* taxa were summarized by ecoregion (n = 7, Figure 3), survey year (n = 19), location (n =18) and site (n = 107). The ecoregions of eastern Australia from the Torres Strait in the north to Cape Howe in the south are defined by Spalding et al. (2007). Within a location, multiple sites and multiple depths were surveyed (n = 74, 0. 4 – 31m) and so we averaged density across depth (n= 74) and site (n = 107), into average densities (m^{-2}) for each year, ecoregion and location. These data were used to determine how the density of *Tripneustes* spp. varies across ecoregions.

4.3.6 Statistical analysis

To test if the density of *Tripneustes* spp. varied among ecoregions in Australia we used a Generalised Linear Mixed Model (GLMM) with ecoregion as a fixed effect and the random effects of site (nested in location and ecoregion) and year (Density ~ Ecoregion + (1 |

Ecoregion/ Location/ Site) + (1 | Year)) using the glmmTMB package (Brooks et al. 2017). The nested model structure was used to reflect the sampling methodology of RLS (Edgar et al. 2020) and to account for spatial and temporal variation in our analyses. Assumption and model checks were undertaken visually using Q–Q plots and the spread of residuals was assessed using residuals versus fitted values. We tested for overdispersion and outliers in the R package DHARMa ($p > 0.05$) (Hartig et al. 2024). The model showed some deviation from normality, so we log-transformed the response variable, which improved the model fit. Model significance was tested with Type II Wald Chi-square Tests using the *Anova* function in the car package (Fox and Weisberg 2019). Post-hoc pairwise comparisons between ecoregions were performed using the emmeans package (Lenth 2024). All statistical analyses were performed in R (R Core team 2021).

4.3.7 Habitat suitability modelling

Environmental predictors

Distribution data for the two *Tripneustes* taxa were derived from the museum collections data outlined above. Mean sea surface temperate (SST), sea water surface velocity (SWV), mean pH (pH), mean sea surface salinity (SSS) and minimum depth (MD) were extracted for the period from 2010-2020 from Bio-Oracle for the area of interest (*T. australiae:* -44 to -20 S and 140.075 to 179.075 E; *T. g. gratilla:* -44 to -5 S and 140.075 S to 179.975 E). These variables were included in the models due to their importance in the biology and ecology of sea urchins (Watson et al. 2012; Byrne and O'Hara 2017; Lawrence and Agatsuma 2020; Collin et al. 2021; Davis et al. 2022). *Tripneustes* are shallow water species that feed on algae and seagrass, which are limited to shallow regions by light availability (Lawrence and Agatsuma 2020). Therefore, we used the MD and SST data available from Bio-Oracle (0.05° resolution). Any model parameters that were important predictors for the distributions of *Tripneustes* from the museum collections in step one above, were then also extracted from Bio-Oracle for future model projections for the 2090-2100 period under the IPCC concentration pathway SSP2-4.5 to estimate potential future distributions of the two taxa. The SSP2-45 pathway was chosen as this represents the "middle of the road" estimates for climate change, assuming a 2.7°C rise in global temperatures by the end of the century (IPCC) 2023). As the Kermadec Islands are an important part of the subtropical distribution of T. *australiae*, we also modelled habitat suitability for *T. australiae* in this region, although the data were not extensive (see Appendix D).

Models and model validation

Habitat suitability modelling was undertaken using a Maximum Entropy Model (Maxent) implemented through the R package *dismo* (Hijmans et al. 2010). Maxent models are machine learning models that model species distributions using presence only data (Phillips 2006). We performed model selection using the *glm* function in ENMTools (Warren et al. 2021) to determine the Akaike Information Criterion (AIC) of different predictor combinations (Warren & Seifert 2011). The predictor variables were checked for collinearity ($p < 0.7$). Maximum Entropy Models are robust to collinearity and as such only highly correlated variables were removed (Feng et al. 2019). No variables were collinear in the *T. australiae* model. However, for the *T. g. gratilla* model pH and SST were collinear, and as such pH was removed from the model. All environmental predictor layers were projected using GDA94 using the R package *Raster* (Hijmans 2010). Initially, a full model was constructed for both taxa using all environmental variables (SST, SWV, pH, SSS and MD) and presence data for *T. australiae* or *T. g. gratilla* determined from museum collections as outlined above. Model selection was then performed by stepwise selection of variables (both forward and backward) and selecting the model with the lowest Akaike Information Criterion (AIC) (Table C7). The model with the lowest AIC value was used to create a Maxent model for analysis and predictions. For *T. australiae* these were MD, SST, SWV and pH (AIC = 141.49, Table D7). For *T. g. gratilla* only MD and SST were in the best model (AIC = 103.8, Table C7). To account for any sampling bias across the museum institutions in the modelling process, a 'bias layer' was constructed for both taxa using the kernel density estimation in *MASS* (Ripley and Venables 2009) and used in the final models. All predictor layers were projected using GDA94 using the R package *Raster* (Hijmans et al. 2010).

The MaxENT models were trained on 75% of the presence data set with the final model variables as predictors and the remaining 25% were used for model validation. The models were 5-fold cross validated, resulting in 5 replicate models. The area under the curve (AUC) from a receiver operating characteristic (ROC) plot was averaged over the replicate runs to give a global AUC to determine the predictive accuracy of the models.

Habitat suitability for current-day conditions were then predicted from the results of the final MaxENT model for each taxon. The average habitat suitability values from the 5 replicated models were then mapped onto the area of interest. The results of the final MaxENT model were then used to predict habitat suitability for the 2090-2100 period using the predicted future conditions under SSP2-4.5. These average habitat suitability values from the 5 replicated models for 2090-2100 were then mapped onto the area of interest.

4.4 Results

4.4.1 Distributions of *Tripneustes* from museum collections

***Tripneustes* material examined**

We examined 227 lots of *Tripneustes* across the Australian Museum (AM), Natural History Museum London (NHML), Sydney Sea Urchin Science Centre and Gallery (SUSCG), and Museums Victoria (MV) collected from 1878 to 2024. The number of specimens within these lots ranged from 1 – 11, with a total of 330 examined individuals. Collections of both taxa were highest around 31° 33 ’S and 159° 4' 58.8 "E, from Lord Howe Island where *T. g. gratilla* and *T. australiae* co-occur (Figure 4.1). Multiple collections events occurred at the same location, such as Lord Howe Island. Examination of these specimens revealed that 211 were *T. australiae* (McLaren et al. 2023) and 119 were *T. g. gratilla* (Bronstein et al. 2017). The earliest collection of *T. australiae* was from Port Jackson (Sydney Harbour), in 1878 held in the AM collections, while *T. g. gratilla* was first collected from the Frankland Group on the Northern Great Barrier Reef in 1924 by the NHML.

4.4.2 Geographical ranges and realised thermal niches

T. g. gratilla

In our region of interest, Eastern Australia and across the Tasman Sea*, Tripneustes g. gratilla* in museum collections demonstrated a largely tropical-to-temperate distribution. The distribution of this taxon ranged from Papua New Guinea (5° 13' 30 "S) to Jervis Bay (New South Wales, southeastern Australia, 35° 7' 12 "S), encompassing ~30° of latitude. The mid-point of its geographic range in the region investigated was at 20° 10' 30 "S, around the Whitsundays in Queensland (Figure 4.1). The furthest east specimen of *T. australiae* in our region of interest was collected from subtropical Lord Howe Island (31° 32' 36.6 "S, 159° 03' 06.6 "E) in the Pacific Ocean (Tables C2 & 3). The long-term temperature range across this distribution is 16.3 - 29.97 °C, a realized thermal width of 13.67 °C. The realized upper and lower limits of *T. g. gratilla* are 14.7°C and 30.4°C, which represent the average yearly minima and maxima experienced by this taxon at its respective upper and lower range edges (from 2000-2020) (Figure 4.2 & Table 4.1).

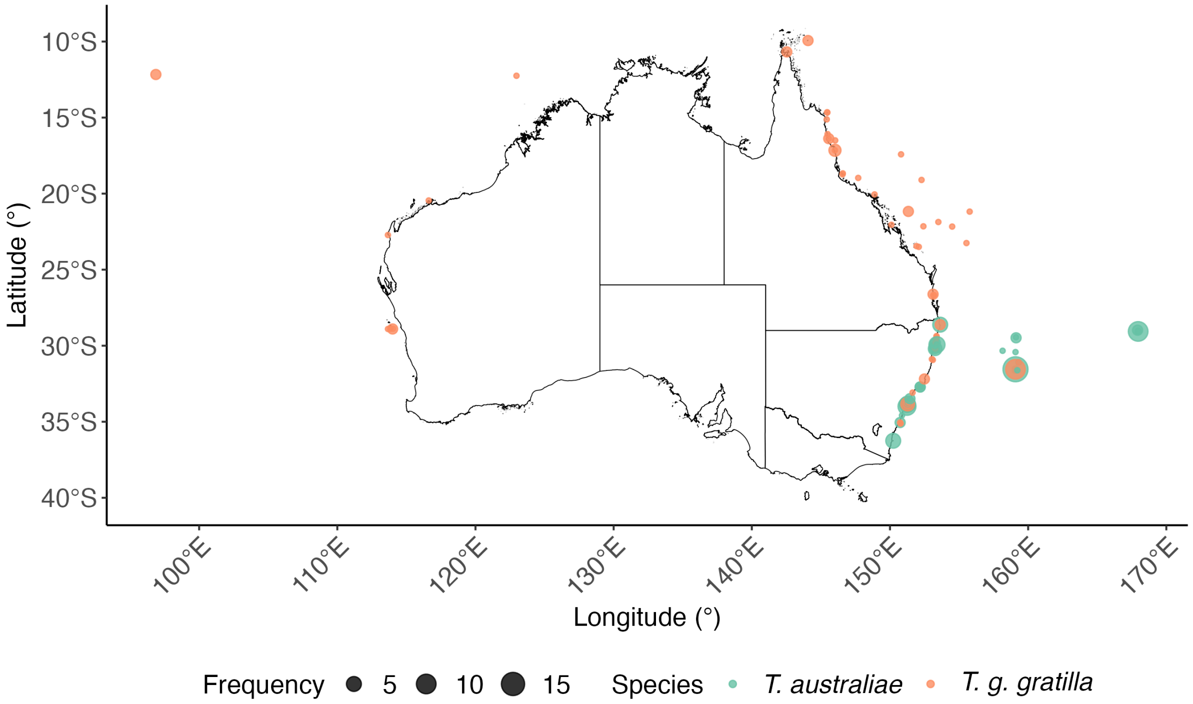

Figure 4.1. The distributions of *Tripneustes g. gratilla* and *T. australiae* in Australia, assembled from the Australian Museum, Museums Victoria, Sea Urchin Science Centre and Gallery and Natural History Museum collections. Size of circle represents the frequency of collection / number of specimens collected at a particular latitude and longitude

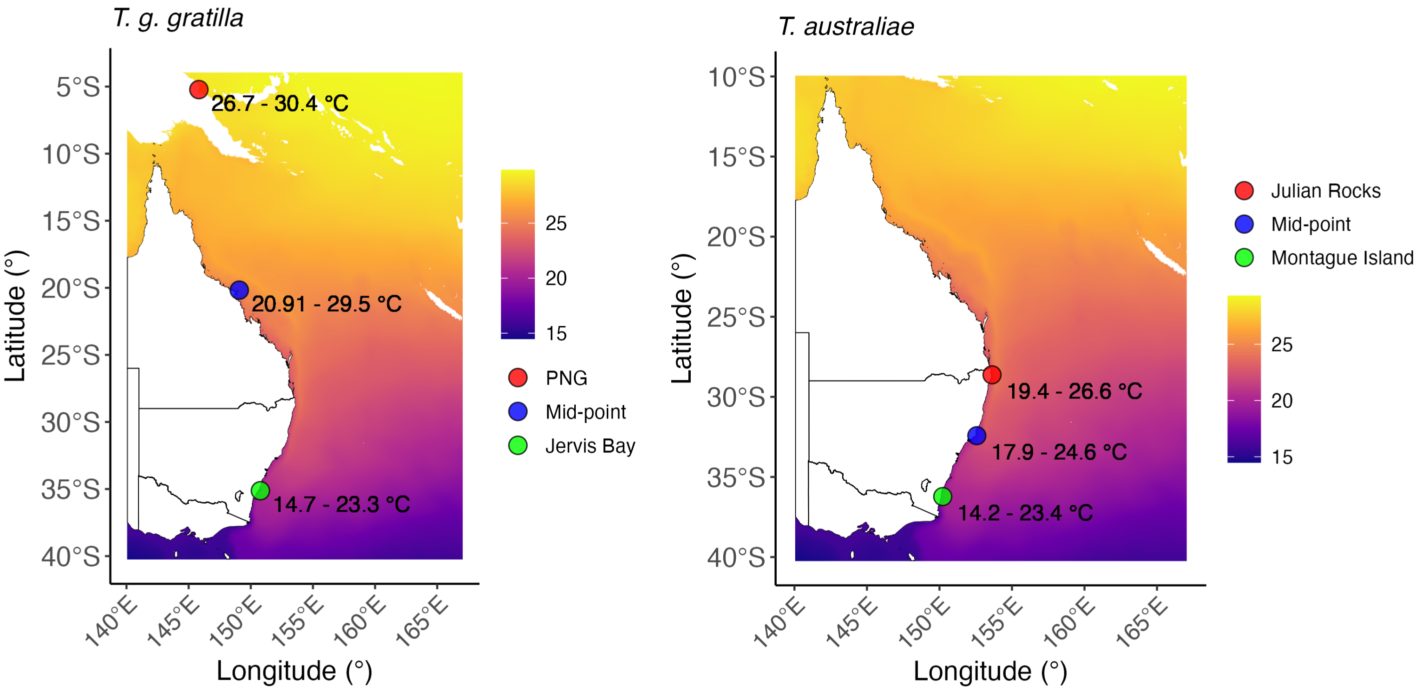

Figure 4.2. The east coast of Australia with the upper, lower and mid-range points of *Tripneustes australiae* and *T. g. gratilla*. Distributions were constructed from Australian Museum, Museums Victoria, Sea Urchin Science Centre and Gallery and Natural History Museum collections. Background colours represent the mean SST from 2000-2020 extracted from Bio-Oracle SST (Assis et al. 2024). Points are accompanied by the mean maximum and minimum SST records from 2000-2020 for that location.

Table 4.1. Realized thermal niches from distributions constructed from Australian Museum, Museums Victoria, Sea Urchin Science Centre and Gallery and Natural History Museum collections. Long-term average range is an average of the yearly maxima and minima for each decade (e.g. the average of the coolest month for the period of 2000-2010 and 2010-2020). Average range values represent the average highest and lowest temperature records and their average (°C) for 2000-2020. These metrics are given for the upper-most, lower-most and mid-range points of *T. g. gratilla* and *T. australiae* and based on morphologically confirmed specimens.

	Site	Latitude	Long-term range (°C)	Range (°C)
T. g. gratilla				
	Papua New Guinea	5° 13' 30" S	29.97 – 27.43	28.8 (30.4* - 26.7)
	Mid-point	20° 10' 30" S	28.8 – 21.8	25.5 (29.5 – 20.91)
	Jervis Bay	35° 7' 30" S	22.5 -16.3	19.6 (23.3 - 14.7*)
T. australiae				
	Julian Rocks	28° 37' 30" S	26 - 20.2	23 (26.6* - 19.4)
	Mid-point	-36° 13' 12" S	24.0 - 18.7	21.4 (24.6 - 17.9)
	Montague	32° 25' 30" S	22.5 - 15.3	18.98 (23.4 - 14.2*)

* realised upper thermal limit
* realised lower thermal limit

T. australiae

Tripneustes australiae has a comparatively narrower subtropical-to-temperate distribution and thermal niche (Figures 4.1 & 2, Table 4.1). *Tripneustes australiae* has a latitudinal range of 8° from Julian Rocks (28° 37' 30 "S) in the north to Barunguba (Montague Island) (36° 13' 12 "S) in the south, with the mid-point at 32° 25' 30 "S near Forster in NSW (Figure 4.2) (Figure 4.1, Table C2). *Tripneustes australiae* is limited to the south western Pacific in the Australia-New Zealand region. The long-term temperature range across this distribution was 15.3 – 26 °C, a realized thermal range of 10.7 °C. The realized upper and lower limits of *T. australiae* are 26.6°C and 14.2°C, respectively (Figure 4.2 & Table 4.1). Outside our region of interest, *T. australiae* extends to the Kermadec Islands, northern New Zealand in the Pacific (29° 42 ’ 29.0 "S, 178° 08' 23.1 "W) (Table C3).

The distributions of *T. australiae* and *T. g. gratilla* overlap in eastern Australia and the Tasman Sea from 35° 03 ’S to 28° 37 ’S and 150° 13' 48 "E to 159° 19' 1.2 "E (Figure 4.1). Based on the distribution dataset, *T. g. gratilla* has a thermal range of 16.3 – 29.97°C, while *T. australiae* has a range of 15.3 – 26 °C. The thermal range of *T. g. gratilla* is 2.97 °C wider than that of *T. australiae,* and its realized upper limit is 3.8 °C higher than *T. australiae (T. australiae*: 26.6°C; *T. g. gratilla*: 30.4°C, Table 4.1). However, despite the contrasting thermal range and upper thermal limits of the two taxa, their realized lower thermal limits are remarkably similar. The realized lower thermal limit of *T. australiae* and *T. g. gratilla* were 14.2°C and 14.7°C, respectively, just a 0.5 °C difference (Table 4.1).

4.4.3 Abundance and distribution of *Tripneustes* in Australia

The RLS survey data for the two *Tripneustes* spp. were from 7 ecoregions from the Torres Strait in the north to Cape Howe in the south, at 18 locations and 107 sites from 2003 - 2024.

The density of *Tripneustes* spp. varied significantly among Australian ecoregions ($\chi^2 = 26.145$, df = 6, p <0.001). Peak densities of *Tripneustes* spp. were in the Lord Howe and Norfolk Island ecoregion, a subtropical ecoregion (Figure 4.3). Post-hoc pairwise comparisons showed that densities in the Lord Howe and Norfolk Islands bioregion were significantly higher ($p > 0.05$) than densities in other ecoregions (Figure 4.3, Table C6). Densities of *Tripneustes* spp. were higher in the Tweed-Moreton and Lord Howe and Norfolk Island ecoregions compared to other regions (Figure 4.3). Consistent with this, frequency of observation of *Tripneustes* spp. was lower in the tropical regions of Australia (Figure 4.4).

4.4.4 Model performance and habitat suitability maps

T. australiae

The best habitat suitability model (variables: MD, SST, SWV and pH) for *T. australiae* had a high predictive capacity (AUC = 0.996 ± 0.001 SD), indicating the model prediction was reliable. Depth was the best predictor of *T. australiae* habitat suitability (57.9%), followed by SST (39.7%), SWV (2.4%) and pH (0.1%). *Tripneustes australiae* habitat suitability was limited to the subtropical and temperate regions of Australia, including the offshore Islands Lord Howe and Norfolk (Figure 4.5). This suggests that depth and temperature act to limit the distribution of *T. australiae* within these regions of Australia. When future habitat suitability was projected using SPP2-4.5 for the period 2090-2100, the latitudinal range of suitable habitat for *T. australiae* narrowed and shifted poleward (Figure 4.5). Suitable habitat also decreased on the offshore islands and increased in the northern part of New Zealand (Figure 4.5).

T. g. gratilla

The final habitat suitability model for *T. g. gratilla* (variables: MD and SST) had a high predictive capacity (AUC = 0.926 ± 0.022 SD), indicating the model prediction was reliable. Depth was the dominant predictor of *T. g. gratilla* habitat suitability (97.1 %) and there was some influence of SST (2.9%). This suggests that future habitat suitability for *T. g. gratilla* will be primarily limited by the availability of habitat at a shallow depth such as rocky reef or seagrass meadows (Figure 4.6).

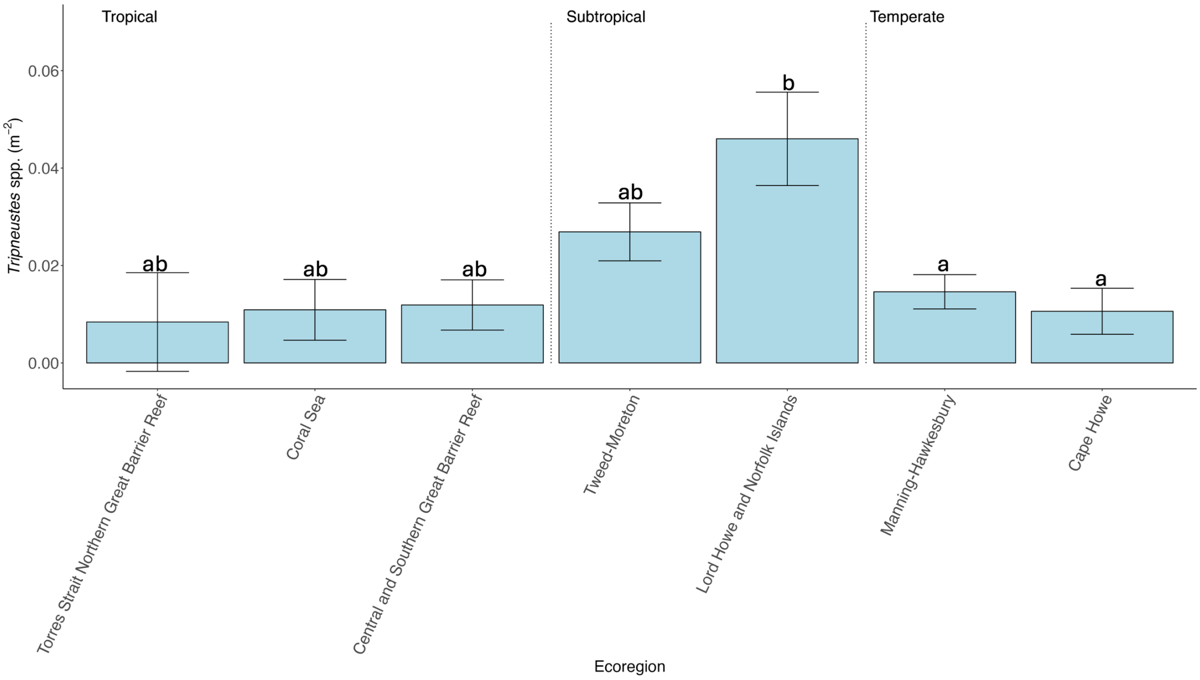

Figure 4.3. Model predicted densities (±SEM) of *Tripneustes* spp. for each ecoregion (n = 7) in Australia. Data are pooled across year (n = 19, 2003 – 2024), location (n =18, 14.66 S - 36.25 ºS, 145.45 E - 159.28 ºE), site (n = 107, Watsons-Turtle Reef, Northern Great Barrier Reef - Montague Island, Cape Howe) and depth (n = 74, 0. 4 – 31m). Letters indicate Tukeys post-hoc groups. Groups that do not share a letter are significantly different. If two or more means share the same grouping symbol, then they are not significantly different based on given sample sizes.

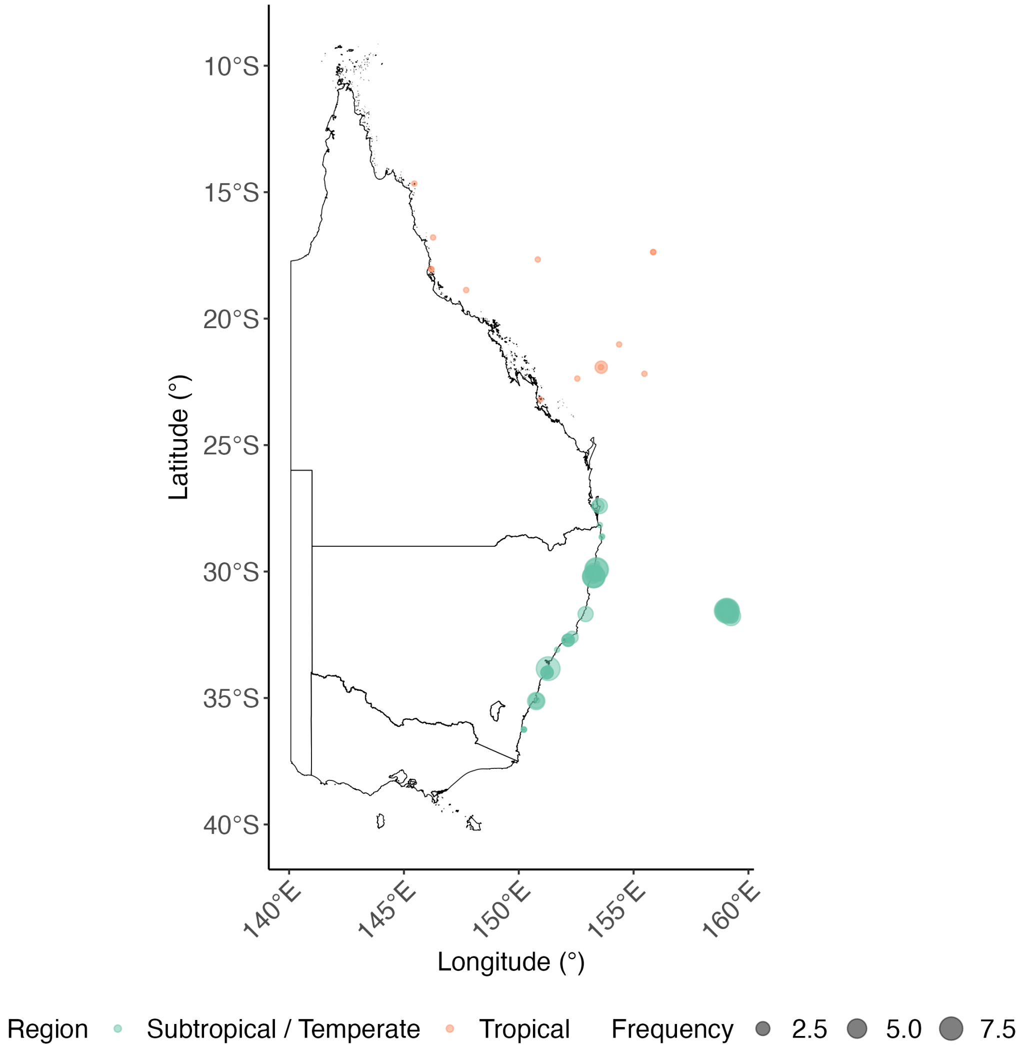

Figure 4.4. The distributions of *Tripneustes* spp. in the tropical, subtropical and temperate regions of Australia, assembled from Reef Life Survey data (2003 – 2024). Size of each circle represents total frequency of observation by RLS at a particular latitude and longitude from 2003- 2024.

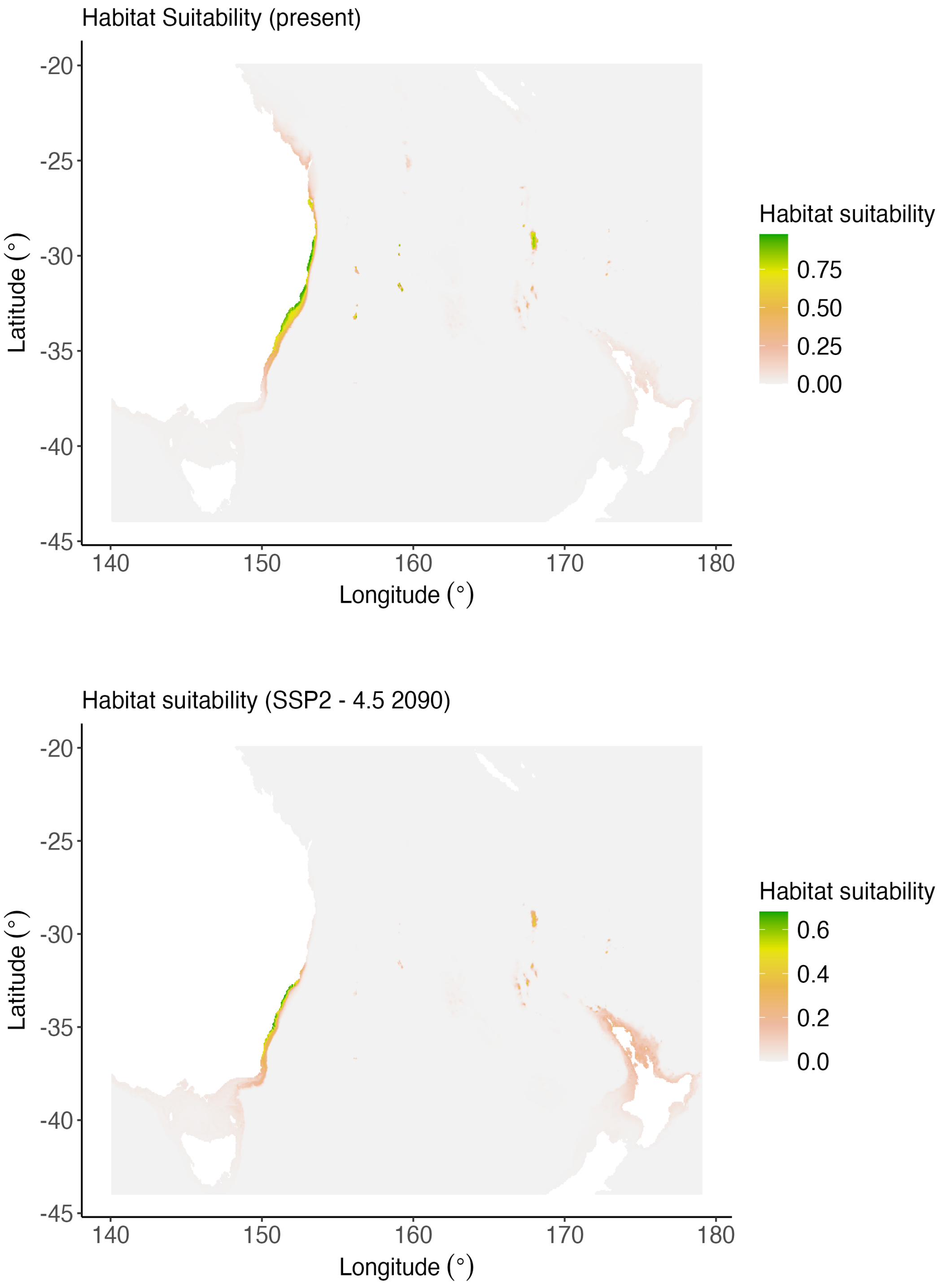

Figure 4.5. Habitat suitability for *T. australiae* in eastern Australia and the Tasman Sea based on model predictions using minimum depth, current day sea surface temperature and current day sea water velocity (top) and minimum depth, sea surface temperature (SSP2-4.5) and sea water velocity (SSP2-4.5) projections for the period between 2090 and 2100 (bottom).

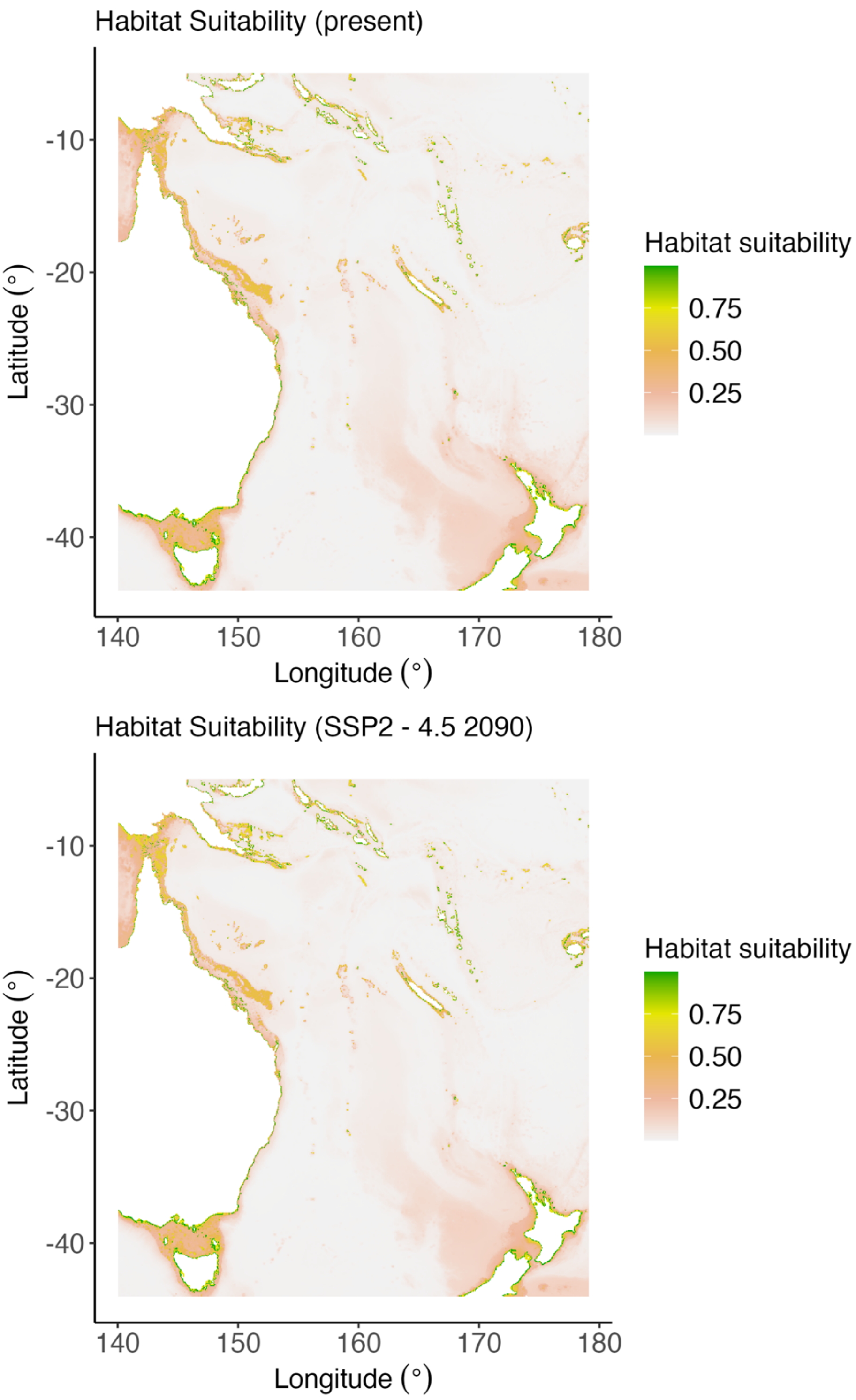

Figure 4.6. Habitat suitability of *T. gratilla gratilla* in the northwestern Indo-Pacific, eastern Australia and the Tasman Sea based on model predictions using minimum depth and current sea surface temperature (top) and minimum depth and sea surface temperature (SSP2-4.5) projections for the period between 2090 and 2100 (bottom).

4.5 Discussion

Determining the distributions of species with contrasting thermal affinities, particularly congeneric species, provides insight into the drivers of distributional limits, and how these are changing and will continue to change in the future as the oceans warm (Halsband-Lenk et al. 2002; Stenseng et al. 2005; Oliver et al. 2010; Prusina et al. 2014; Popovic and Riginos 2020; Redfern et al. 2021). Here we show that the congeneric *Tripneustes* sea urchin taxa in eastern Australia and the trans-Tasman region have contrasting distributions and realized thermal niches. As the thermal niches of marine ectotherms tend to widen at higher latitudes (Sunday et al. 2011; Stuart-Smith et al. 2017), we hypothesised that the subtropical-temperate, *T. australiae*, and the broadly distributed tropical *T. g. gratilla,* would have wider and narrower realized thermal windows, respectively. However, we found the opposite as *T. g. gratilla* appears to be a generalist and *T. australiae* a narrow range specialist in our region of interest.

Globally *Tripneustes* are largely known from tropical and subtropical regions (Lawrence and Agatsuma 2020) and here we show that taxa in this genus can also be common and locally abundant at temperate latitudes (Valentine and Edgar 2010, McLaren et al. 2024). We found that *Tripneustes australiae* is a narrow range thermal specialist restricted to the subtropical and temperate latitudes of eastern Australia and New Zealand. We show that habitat suitability of *T. australiae* is influenced by temperature and that this taxon is vulnerable to a warm-edge range contraction in the coming decade and a potential cool range extension. Our habitat suitability modelling also suggests that the offshore islands in the Tasman Sea (Lord Howe Island and Norfolk Island), which currently support large populations of *T. australiae,* may become unsuitable for this taxon in the next decade due to warming. As such, the trans-Tasman distribution of *T. australiae* (McLaren et al. 2023) and other echinoids with similar thermal profiles (e.g. *Heliocidaris tuberculata*) (Liggins et al. 2014; Thomas et al. 2021; Byrne et al.

2022; Gall), may change in the near future. However, we note that the Kermadec Islands are an important part of the distribution of *T. australiae* (Bronstein et al. 2017). Our supplementary analysis (Appendix D) identified depth as the only predictor of habitat suitability in this region, possibly due to small sample sizes. Future studies would greatly benefit from more extensive collections from the Kermadec Islands for inclusion in habitat suitability modelling.

In contrast to *Tripneustes australiae*, we found that *Tripneustes g. gratilla* is a generalist in our region. Contrary to expectations, this taxon did not reflect the distribution and realized thermal niche of a warm-affinity species. Notably, this taxon is known to contradict biogeographic hypotheses (Lessios et al. 2003). For example, biogeographic barriers such as the eastern Pacific barrier of deep cold water have not limited dispersal of *T. g. gratilla,* and there is little genetic separation between populations of *T. g. gratilla* throughout the Pacific and in the Indian ocean (Zigler 2003; Lessios et al. 2003). Habitat suitability of *T. g. gratilla* was primarily predicted by shallow depth (97.1%), with some influence of sea surface temperature (SST 2.9%), as opposed to *T. australiae*, which was influenced meaningfully by depth (MD 57.9%) and other factors such as temperature (SST 39.7%; SWV 2.4%; pH 0.1%). Our finding that temperature has limited influence on the habitat suitability of *T. g. gratilla* in our study region suggests that this taxon may be relatively robust to climate change in the coming decade. However, we note that the predictive capacity of these models does not reflect the entire distribution of *T. g. gratilla*. Incorporation of collection data across the broad Indo-Pacific distribution of *T. g. gratilla* would be needed to determine future habitat suitability across the entire range of this taxon.

As organisms track spatial shifts in their thermal niches, climate change is driving marine species re-distributions globally towards higher latitudes or greater depths (Parmesan 2006;

Poloczanska et al. 2016; Pecl et al. 2017; Stuart-Smith et al. 2017; Day et al. 2018; Burrows et al. 2019). Notably, there is a shift toward higher representation of warm-affinity species across biogeographic provinces globally (Burrows et al. 2019). This poses a risk to biodiversity, as receiving ecosystems are subject to new biotic interactions (Vergés et al. 2014, 2019) and species close to the poles or with narrow thermal ranges have limited refugia (Sunday et al. 2015; Edgar et al. 2023; Byrne and Lamare 2024).

Across marine taxa, thermal and ecological generalists have a higher likelihood of colonising suitable habitat at their cool-range edges and undergoing range expansions compared to specialists (Sunday et al. 2015; Platts et al. 2019; Kingsbury et al. 2020; Zarzyczny et al. 2023). Similar to invasive species, it is expected that generalists with a large dispersal capacity, the ability to overcome dispersal barriers and physiological tolerance will expand their ranges (Angert et al. 2011; Bates et al. 2013, 2014; Sunday et al. 2015; Zarzyczny et al. 2023). *Tripneustes g. gratilla* is a well-known Indo-Pacific taxon (Lawrence and Agatsuma 2020), and here we report the furthest poleward occurrence of this taxon to date, at 35° 03' S in temperate eastern Australia. Given its widespread distribution and dispersal throughout the Indo-Pacific and its realized lower thermal limit similar to that of *T. australiae*, we posit that *T. g. gratilla* has the potential to undergo and is likely currently undergoing, a poleward range extension in eastern Australia, albeit at low numbers. Faunal poleward range expansion is characteristic of this region, as warming and intensification of the East Australian Current is facilitating the extension of many marine species across taxa (Ling 2008; Suthers et al. 2011; Wernberg et al. 2016; Vergés et al. 2019).

The availability of niche space and competitive dynamics among species likely mediate range dynamics (Bates et al. 2014; Miller et al. 2023; Zarzyczny et al. 2023). For example, for fishes in southeastern Australia, trophic generalism allows for tropical species to persists in temperate systems without competing with residents for trophic niche space (Kingsbury et al. 2020). However, local species can still outcompete the tropical species due to higher foraging and physiological performance (Coni et al. 2021). While some sympatric echinoid species are known to compete for resources (Vanderklift and Kendrick 2004; Rodríguez-Barreras et al. 2020; Silva et al. 2022), other sympatric echinoids have well partitioned foraging niches (Keller 1983; Vanderklift and Kendrick 2004; Privitera et al. 2008; Cabanillas-Terán et al. 2016; Westlake et al. 2021). Interestingly, in seagrass systems in the Caribbean, *Tripneustes ventricosus* and *Lytechinus variegatus* are sympatric and do not seem to compete for food, but grazing and incidental predation of *T. ventricosus* can limit the recruitment of *Lytechinus variegatus* (Keller 1983).

Temperate Australia has a high biodiversity of echinoids, and many have functional niches similar to *T. g. gratilla* in seagrass and rocky reef habitats (Burnell et al. 2013; Byrne and O'Hara 2017; Glasby and Gibson 2020). As such, competition with resident local echinoid taxa in southeast Australia may limit the range expansion of *T. g. gratilla* because there may not be adequate niche availability to support colonisation. It is not known how the two *Tripneustes* taxa interact where they exist in sympatry with respect to potential niche partitioning. Understanding adult biological and ecological traits, is therefore crucial to determine the factors that influence the distributions and limits of these taxa. For instance, *T. australiae* has a well-developed armoury of toxic pedicellaria that defend against predatory fishes (Sheppard-Brennand et al. 2017, as *T. gratilla*). It is not known if the pedicellaria of *T. g. gratilla* possess the same extent of defensive capacity. Future research is needed to determine how niche availability may influence range dynamics of *Tripneustes* and other sea urchin taxa in this region.

The contrasting distributions of *T. g. gratilla* and *T. australiae* raise the question of what factors limit the distribution of *T. g. gratilla* in the region investigated here. Dispersal is a well-recognized driver of distributions for marine ectotherms with a biphasic life-history, and larval thermal niches often match adult distributions in echinoids (Collin et al. 2021; Alzate and Onstein 2022). *Tripneustes g. gratilla* and *T. australiae* have similar planktotrophic larval development, planktonic durations (*T. g. gratilla* 20 – 30 days; *T. australiae* 30 - 35 days) (Dworjanyn and Pirozzi 2008; Juinio-Meñez and Bangi 2010; author pers. obvs.), and egg size (*T. g. gratilla* 82µm; *T. australiae* 84µm) (McEdward 1986; Byrne et al. 2008a). However, *T. g. gratilla* is much more widely distributed (Lawrence and Agatsuma 2020). The eggs of *T. australiae* have a higher content of energetic lipid compared to the eggs of other echinoids with feeding larvae, which supports an extended period of larval development without feeding (Byrne et al. 2008a, b as *T. gratilla*). This larval trait could serve to extend the dispersal potential of this taxon. As *T. australiae* is a basal split in the *Tripneustes* phylogeny (Bronstein et al. 2017, 2019), this may be an ancestral trait that is also present in *T. g. gratilla*. A long larval facultative feeding period may contribute to the wide dispersal capacity of *T. g. gratilla.* Regardless, questions remain about the thermal and dispersal limits of the larvae of both taxa. In particular, comparative data on the energetics and thermal performance of their larvae in eastern Australia are needed to determine what larval traits may influence their distributions.

In sympatric sea urchins, hybridisation has been observed in nature and produced in the laboratory (Lessios and Pearse 1996; Zigler and Lessios 2004; Lessios et al. 2007; Lamare et al. 2018; Lessios 2024). For example, the congeners *Pseudoboletia indiana* and *P. maculata* readily hybridise in temperate Australia and have an established climate induced hybrid zone around Sydney (Zigler et al. 2012; Lamare et al. 2018). Until the description of *T. australiae* (then *T. kermadecensis*) in 2017, and the subsequent confirmation that this was the taxon that occurs on the southeast coast of Australia, all taxa of *Tripneustes* globally were considered to be allopatric (Lessios et al. 2003; Zigler 2003; Bronstein et al. 2017, 2019). Importantly, we show that *T. g. gratilla* and *T. australiae* are sympatric across > 7° of latitude along the southeastern coast of Australia and on Lord Howe Island. This highlights the possibility of a hybrid zone between the two *Tripneustes*.

A hybrid zone for the two *Tripneustes* taxa could have a range of consequences, including successful hybridisation, extinction of the receiving specialist, species reinforcement, or hybrid introgression (Chunco 2014; Pfennig et al. 2016). However, gaps in the current knowledge of the biology of these taxa limit our ability to predict these outcomes. For example, in echinoids the nuclear gene *bindin* codes for a gamete recognition protein that can be used to predict gamete compatibility, and generally diverges rapidly in sympatric, but not allopatric sea urchins (Zigler 2003; Zigler et al. 2005; Lessios 2007, 2024). Congeners with highly divergent *bindin* sequences cannot cross-fertilise (Zigler et al. 2005). However, there are exceptions to this (See Lessios 2024). The *bindin* locus of *T. australiae* diverges sufficiently from that of other *Tripneustes* to support *T. australiae* as a monophyletic novel species (Bronstein et al. 2017). However, the observation of hybridisation between *T. australiae* and *T. g. gratilla* (Bronstein pers. comms) indicates that *bindin* divergence is not a complete method to predict reproductive compatibility for this genus.

Hybridisation can facilitate successful range expansions (Pfennig et al. 2016; Zarzyczny et al. 2023). The hybrid offspring of a range expander and local species may be more adapted to local conditions than a non-hybrid range expander (Pfennig et al. 2016; Zarzyczny et al. 2023). As such, a range expansion of *T. g. gratilla* may be accelerated due to hybridisation with its subtropical congener. These two taxa co-occur in close proximity around Sydney during the spawning period of *T. australiae* (author pers. obvs). However, the reproductive period of *T. g. gratilla* at their cool-range edge is not known. Considering that *Tripneustes* exhibit quick growth to reproductive maturity and can produce gametes year-round (Lawrence and Agatsuma 2020), hybridisation in these two taxa is a possibility. To begin to understand potential hybridisation of these congeners, future research should examine if these taxa synchronously produce gametes where they co-occur and if so, whether they can produce fit hybrids.

Our habitat suitability models suggest that *T. australiae* may migrate poleward as far as the northern border of Victoria in the next 100 years as the region warms. *Tripneustes* sea urchins are important herbivores on tropical, temperate and subtropical reefs (Alcoverro and Mariani 2002; Valentine and Edgar 2010; Lawrence and Agatsuma 2020; Moreira-Saporiti et al. 2023) and their poleward migration could thus present a threat to receiving ecosystems that are already at risk from the intense grazing pressure of *Centrostephanus rodgersii* and ocean warming (Ling 2008; Ling and Johnson 2009; Smale 2020). Notably, *Tripneustes* may also act as ecosystem engineers due to their boom-bust population dynamics (Alcoverro and Mariani 2002; Uthicke et al. 2009; Valentine and Edgar 2010; Lawrence and Agatsuma 2020; Moreira-Saporiti et al. 2023). This trait is likely due to a combination of anomalous recruitment events and a 'live fast die young' life history strategy. For instance, in its first year, *T. g. gratilla* achieve a test diameter of between 60-80 mm, and in the Philippines have been recorded to grow to 60 mm in 6 months (Lawrence and Agatsuma 2020). On Lord Howe Island, population booms of *T. australiae* resulted in decreased macroalgae abundance (Valentine and Edgar 2010) and similar boom and bust patterns have also been recorded around the Solitary Islands Marine Park (McLaren et al. 2024) and Sydney (Byrne et al. 2024) along the east coast of mainland Australia. Considering their fast growth, pulsed recruitment and grazing pressure, poleward migration of *T. australiae* has the potential to affect biodiversity and ecosystem dynamics in southeastern Australia.

4.6 Conclusion and future directions

Biodiversity research and the identification of species that are potential range extenders must be underpinned by accurate taxonomic knowledge. Natural history collections are powerful and often underutilised tools to understand past and present biodiversity distributions. Our study showcases the importance of museum-based studies in the context of species re-distributions. Biological collections contain a wealth of information and can be used to detect species range shifts, invasive species dynamics, phenology, and as we show here should not be overlooked to address biogeographic and climate change questions (Dornburg et al. 2009; Pyke and Ehrlich 2010; Saarinen and Daniels 2012; Meineke et al. 2019; Sonamzi et al. 2019).

Echinoderms are decreasing in abundance along the east coast of Australia, especially cool-affinity species with limited refugia (Edgar et al. 2023) and here we show that *T. australiae* is a narrow range endemic that is vulnerable to ocean warming. This is consistent with observations for other high latitude specialists and endemics in this region such as corals (Kim 2019; Cant et al. 2021; Lachs et al. 2021; Kim et al. 2023). We echo the calls of Bronstein et al. (2019) to prioritize research into the management needs for this thermal specialist sea urchin. Moving forward, we also recommend that the potential for hybridisation of these two *Tripneustes* taxa and potential ecological consequences be a research priority.

Chapter 5.

Larval thermal tolerances of the boom-bust subtropical-to-temperate sea urchin *Tripneustes australiae*

5.1 Abstract

The distribution and dispersal of marine ectotherms are governed by their thermal tolerances, and as the oceans warm species are dispersing differently and re-distributing. Developmental thermal tolerances of marine invertebrates can predict local extinctions and range extensions. As such, embryonic and larval thermal tolerances are especially important to understand future distribution and dispersal trajectories. In southeastern Australia, *Tripneustes australiae* is an ecologically important species that undergoes periodic outbreaks and can transform ecosystems. The cold and warm developmental tolerances of *T. australiae* were investigated with respect to its current distribution (Chapter 4), to predict its future dispersal capacity. The optimal thermal window for survival, normal development and developmental progression of *T. australiae* was between 17 and 23°C. This suggests that this species is a narrow range thermal specialist that will persist at its warm-range edge into the future and may disperse further poleward than its current southern distribution limit in the coming decades.

5.2 Introduction

Species distributions are mediated by their thermal tolerances (Sunday et al. 2011; Stuart-Smith et al. 2017). As the oceans warm, marine communities are reorganising as species move to track changes in their thermal environment (García Molinos et al. 2016; Poloczanska et al. 2016; Pinsky et al. 2020; Stuart-Smith et al. 2022). This global phenomenon is being accelerated in regions with strong continental boundary currents that transport high latitude propagules poleward (Vergés et al. 2014, 2019; Zarzyczny et al. 2023). Species with generalist traits, such as broad thermal tolerances that inhabit a wide range of habitats, are more likely to undergo range expansions (Bates et al. 2014; Sunday et al. 2015; Byrne et al. 2022). More so, the cool thermal tolerance of a species predicts their ability to move poleward, and thermal minima tend to be plastic (Sandblom et al. 2016; Byrne et al. 2017).

Most marine invertebrates have a bi-phasic life history that comprise a dispersive larval stage and benthic adult stage (Strathmann 1993; McEdward and Miner 2001). These larval stages vary in larval body form, feeding strategies and planktonic duration across taxa (Strathmann 1993; McEdward and Miner 2001; Marshall et al. 2012). Marine invertebrate development from fertilisation through metamorphosis can be affected by ocean warming, and this can differ between developmental stages (Byrne 2011a, 2012; Byrne and Przeslawski 2013; Przeslawski et al. 2014; Karelitz et al. 2016, 2019; Balogh et al. 2020; Gall et al 2021).

Embryonic and larval thermal tolerances can be used to predict species distributions, range extension capacity and local extinction risk (Byrne 2011b; Byrne et al. 2016, 2022; Collin et al. 2021). The upper limit of a species thermal biology provides insight into extinction risk at its warm range edge. This is the focus of many studies in the context of climate change as marine heatwaves and ocean warming become more prevalent (Byrne 2011a; Byrne and Przeslawski 2013; Tuckett et al. 2017; Gall et al. 2021; Lang et al. 2023; Edgar et al. 2023). However, cool-tolerances can help to predict cool-edge range extensions (Bates et al. 2014; Sunday et al. 2015; Byrne et al. 2017, 2022; Stuart-Smith et al. 2017, 2022; Edgar et al. 2023), but receive less attention. This is particularly important to understand for taxa such sea urchins, which have long planktonic durations and the ability to transform ecosystems through concentrated herbivory and poleward range extension (Ling 2008; Valentine and Edgar 2010).

Echinoids are important herbivores that contribute to the barren-kelp forest habitat mosaic dynamics as characteristic of nearshore marine communities in southeast Australia (Jones and Andrew 1990; Underwood et al. 1991; Kingsford and Byrne 2023). This region is a hot spot for echinoid diversity, with diverse sea urchin families occupying rocky reef, seagrass and soft-sediment habitats (Byrne and O'Hara 2017). This region is also an ocean warming hot spot, (Hobday and Pecl 2014) and increased poleward flow of the East Australian Current is intensifying ocean warming in the region (Suthers et al. 2011), facilitating poleward range extensions across many taxa (Ling 2008; Vergés et al. 2014, 2019).

The poleward range extension of the diadematid sea urchin *Centrostephanus rodgersii* from New South Wales (NSW) to Tasmania across the Bass Strait has transformed the receiving seascape from kelp forests to sea-urchin barrens (Ling 2008). This is due to increasingly favourable thermal conditions for *C. rodgersii* larval dispersal and post larval success in Tasmania and across the Bass Strait (Byrne et al. 2022). In contrast, other sympatric echinoids with dispersive larvae that are common and abundant on the southeast coast of Australia, such as *Heliocidaris tuberculata* and *Tripneustes australiae*, have not range extended to Tasmania. For *H. tuberculata* this is due to thermal intolerance of larvae to cold high latitude climates

(Byrne et al. 2022). In contrast, the lower cold tolerance and phenotypic plasticity of *C. rodgersii* larvae has facilitated its successful colonisation of Tasmania as the ocean has warmed (Byrne et al. 2022).

Tripneustes are globally important herbivores in seagrass and rocky reef habitats and this sea urchin genus is characterised by boom-bust population dynamics that can transform ecosystems (Uthicke et al. 2009; Valentine and Edgar 2010; Lawrence and Agatsuma 2020; Moreira-Saporiti et al. 2023; McLaren et al. 2024). In southeastern Australia, the local *Tripneustes* is *T. australiae*. Until recent clarifications (Bronstein et al. 2017, 2019; McLaren et al. 2023 & Chapter 4), occurrences of *T. australiae* in southeast Australia were thought to be a range extension of its tropical congener *T. g. gratilla* (Castro et al. 2020). *Tripneustes australiae* is an ecologically important species in southeastern Australia with marked boom—bust population dynamics, known to have population outbreaks that reduce macroalgae cover in the subtropics (Valentine and Edgar 2010; McLaren et al. 2024). These outbreaks may have the capacity to impact already vulnerable ecosystems in southeastern Australia, such as those transformed by *C. rodgersii*.

This study investigates the larval thermal tolerances of *T. australiae*, with respect to its realised thermal niche. The realised thermal niche of *T. australiae* was determined from adult distributions with data derived from museum collections (see Chapter 4). The range of *T. australiae* extends from Byron Bay, 28° 37' 0.12"S to Narooma, 36° 15' 0"S in NSW Australia, and its estimated realised thermal niche is 15.3 - 26°C. More so, based on climate projections *T. australiae* has potential to range extend poleward by 2100 (see Chapter 4). Studies of larval thermal tolerance in *T. australiae* (then called *T. gratilla*) have focussed on warm but not cool tolerances (Sheppard Brennand et al. 2010; Karelitz 2019). As such, data on the lower thermal limits of *T. australiae* embryos and larvae are lacking, which is critical for understanding the poleward range extension potential of this species.

I designed an experiment to understand the larval thermal tolerances of *T. australiae* with respect to both the embryonic and larval stages. Specifically, embryo/larval survival and development 1 – 7 days post-fertilisation at 5 temperature treatments (between 14-26°C) was investigated. I predicted that given the estimated realised thermal niche (15.3-26. 2°C) (Chapter 4), larval survival and development would not be successful at 14 °C and that 26°C would approximate the upper thermal tolerance. *Tripneustes australiae* spawns in winter (Bové 2004) and so the larvae are unlikely to experience 26°C. Understanding larval thermal tolerances is a key in predicting poleward range extensions in marine ectotherms (Bates et al. 2014; Sunday et al. 2015; Byrne et al. 2017, 2022; Stuart-Smith et al. 2017, 2022; Edgar et al. 2023), and this is particularly important for sea urchins in southeastern Australia (Ling 2008; Byrne et al. 2022).

5.3 Methods

5.3.1 Specimen collection, spawning and larval rearing conditions

Specimens of *Tripneustes australiae* were collected from Port Stephens (32° 42' 52.2 "S 152° 10' 55.8 "E) (2-5m) in June 2023 (Test diameter: 63 mm – 83mm). The species identity was confirmed following methods described by McLaren et al. (2023). At the time of collection, the ambient sea water temperature was 18.4°C (±0.4) (Beachwatch 2024). Animals were transported to the University of Sydney and maintained at 20°C in aquaria and fed *Sargassum ad libitum* for ~30 days until spawning.

Spawning was induced by gentle manual manipulation (oral side up to oral side down). The larval cultures were generated from two independent fertilisations each using 1-3 females and 2-3 males. Sperm was collected by a pipette from the gonopores, pooled equally from each male into plastic tubes and stored at 4°C until fertilisation. Egg and sperm quality were checked microscopically. Eggs from the females were pooled into 1000ml beakers of 1μm filtered sea water (FSW) (20 °C), fertilised with sperm (~10^4 ml^{-1}) and fertilisation success (90%) was checked microscopically for the presence of the fertilisation envelope. Eggs were rinsed to remove excess sperm and the FSW was renewed. Density of the embryos was determined by taking the average of five 10 μL aliquots and FSW was added to the beakers to achieve a larval density of 15 embryos/larvae ml^{-1}. This stock was used to load the replicate vials (35mL scintillation vials) for the experiment. Cleavage stage embryos (2-4 cell stage) were transferred into the vials (15 embryos ml^{-1}) and placed in the experimental temperatures (see below). Vials with embryos from the two fertilisations were randomly interspersed throughout the experimental treatments (see below).

The embryos and larvae were reared in the vials at their experimental treatment temperatures. Every 48 hours half of the FSW in the vials was replaced with new FSW at the experimental temperature. The larvae were not fed for the duration of the experimental period, as *T. australiae* is known to have a long facultative feeding period (~ 8 days) reared in ambient temperature 19-20 °C (Byrne 2008a, 2008b). Salinity and dissolved oxygen (DO) were monitored regularly (WTW Multi 3420; Thermo Fisher Scientific Eutech DO 6+) and remained at ~35 PSU and >90% DO.

5.3.2 Experimental design, treatments and set up

The effect of temperature and time on survival, normal development and developmental stage of *T. australiae* was assessed in five temperature treatments (14°C, 17°C, 20°C - control, 23°C and 26°C), at seven time points (1-, 2-, 3-, 5- and 7-days post fertilisation) to capture the early (e.g. gastrulae/early larvae days 1-2) and later (echinoplutei day 3+) stages of development. Each treatment temperature had four independent replicates (vials) for each time point (n = 120), scored at one time point and then discarded to avoid repeated measures and effects of handling. Water bath temperature was measured at each scoring time point (n = 7) to ensure they remained stable (13.9°C ± 0.16; 16.96°C ± 0.10; 19.98°C ± 0.33; 22.91°C ± 0.73; 25. 89°C ± 0.04). To examine absolute upper and lower thermal limits embryos were also placed in 28°C and 7°C treatments post fertilisation. However, 100% mortality occurred within 24 hours and these treatments were not incorporated into the experiment.

5.3.2 Larval scoring and survival

Prior to scoring the embryos/larvae were dispersed in the vial by gentle inversion and a random sample of the first 30 embryos/larvae were scored microscopically for survival (dead or alive), abnormality (normal or abnormal) and developmental stage. For each time point, 4 replicate vials for each temperature treatment were scored. Across the treatments and time points, development was scored according to stage as cleaving (2-cell – morula), blastulae (unhatched and hatched), gastrulae (early through late), prism or pluteus (2-arm echinoplutei) (Figure 5.1). Survival was determined by the presence of motile embryos/larvae. Dead embryos/larvae were immotile or disintegrated. Abnormal embryos and larvae had a range of phenotypes including specimens with an incomplete archenteron or abnormal larval profile (Figure 5.1). As the larvae may be beyond their facultative feeding period by day 9, data past day 7 were not used in the normality or survival analysis. However, day 9 data were used to construct thermal performance curves (TPCs).

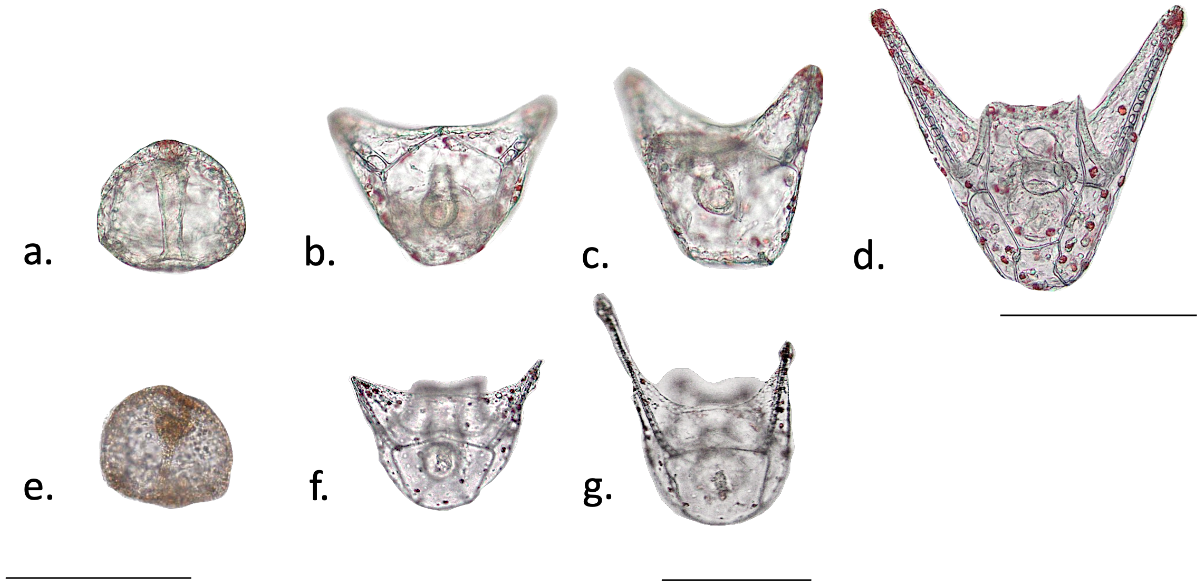

Figure 5.1. Normal embryo/larval profiles of *T. australiae* for gastrula (a.), prisms (b. & c.) and 2-arm plutei (d.). Abnormal embryo/larval profiles of T. *australiae* for gastrula (e.), late prisms/2-arm plutei (f.) and later stage 2-arm plutei (g.). Scale is equal to 500µm for a. – g. and 200µm for e.

5.3.3 Statistical analysis

Survival

The data on survival and normality were analysed by two Generalised Linear Mixed Models (GLMM) with timepoint, temperature and their interaction as a fixed effects and the random effect of replicate to account for any variation among the vials. The response variable was proportion of alive or normal larvae for the respective models, and these were arcsine transformed during the analysis (Proportion alive or normal ~ Temperature * Day + (1| Replicate)). Assumption and model checks were undertaken visually using Q–Q plots and the spread of residuals was assessed using residuals versus fitted values. Model significance was tested with Type II Wald Chi-square Tests using the *Anova* function in the car package (Fox and Weisberg 2019). Post-hoc comparisons between temperatures and timepoints were performed using the emmeans package (Lenth 2024) on back-transformed estimated marginal means from the model.

Thermal performance curves (TPCs)

Data on percentage survival at days 5, 7, and 9 were analysed using a general additive model (GAM) in the *mgcv* package (Wood et al. 2012) to create thermal performance curves (TPCs) and to determine the upper and lower lethal temperature for 50% mortality (LLT_{50} and ULT_{50}) on these days. The percentage of live larvae was explored as a function of a spline smoothed term for temperature with *k* (basis dimension) set to 4 for day 5 and 9, and 3 for day 7. Both GAMs were fit using the Restricted Maximum Likelihood (REML) method. Model fit was checked using the *gam.check* function and visually inspecting the Q–Q plots and the spread of residuals was assessed using residuals versus fitted values. TPCs with 95% confidence intervals were then constructed from the predicted values of the GAMs and LLT_{50} and ULT_{50} were determined from these predicted values.

Developmental thermal tolerances

To examine how developmental stage differed across the temperature treatments and timepoints I used a multivariate approach which allowed developmental stage to be treated as multivariate response data. I did so to test the multivariate hypothesis that the number of larvae in respective developmental stages would vary across the temperatures and timepoints, and to test for an interactive effect between these factors. To do so I fit multiple Generalised Linear Models (GLMs) simultaneously using the mvabund package (Wang et al. 2012). The model had the same variable structure as above, but with the number of larvae at each developmental stage as the response variable and a negative binomial distribution (No. of larvae at developmental stage ~ Temperate*Day, + (1| Replicate)). Assumption and model checks were undertaken by visually inspecting the spread of residuals using a residuals versus fitted values plot. I used the *anova* function in the mvabund package to test the significance of this model which performs a likelihood ratio test and reports resampled p-values. To examine my hypothesis further and understand which developmental stages are likely to be at each timepoint at different temperatures I used a series of univariate GLMs within the package mvabund (Wang et al. 2012). Each of these univariate GLMS had the same variation structure as above, but was performed for each developmental stage (embryos, blastula, prism or pluteus). I used the adjusted re-sampling method in the *anova* function to calculate p-values, which accounts for any correlation between the developmental stages in the vials. All statistical analyses were performed in R (R Core team 2021).

5.4 Results

5.4.1 Survival

There was a significant interaction between temperature and day on the proportion of live *T. australiae* embryos/larvae (χ^2 = 87.462, df = 16 p = <0.001). Survival over time depended on rearing temperature (Figure 5.2). For larvae reared at 14°C, survival was over 90% until 5 days post-fertilisation, when survival decreased to 44% and 7 days after fertilisation all larvae were dead. This suggests that 14°C is lethal to *T. australiae* larvae. This is supported by the post-hoc tests, wherein the 14°C rearing temperature at days 1-3 were significantly different to days 5 ($p < 0.05$, Table E1) and 7 ($p <0.05$) (Figure 5.2). There was no significant variation in survival through time ($p < 0.05$, Table E1) for the 17 °C, 20°C, and 23 °C treatments, with survival remaining above 90% for the entire sampling period. Larvae reared at the warmest temperature 26°C survived at 90% until day 5. Thereafter, these larvae had significantly lower survival, decreasing to 64% by day 7 ($p < 0.05$) (Figure 5.2).

5.4.2 Normal development

Similar to survival, there was a significant interaction between day and temperature on the proportion of normal larvae ($\chi^2 = 28.4141$. df = 16, $p =0.0282$). The proportion of embryos/larvae with a normal profile varied through time depending on temperature. At the coolest rearing temperature 14°C, all larvae were dead by day 7, however until that point there was no significant deviation ($p <0.05$, Table E2) from normal development, and 95% of the larvae were normal. However, this may be due to arrested/slow development in response to the cold (see below). Days 1 through 7, the number of normal larvae was above 95% in the 17-23 °C treatments, and within these rearing temperatures there were no significant variation in the number of normal larvae through time (Figure 5.3, Table E2). After 2 days in 26°C only 70% of the larvae were normal and by 7 days, this reduced to 63% (Figure 5.3). Therefore, successful normal development for this species seems to occur between 17 and 23°C.

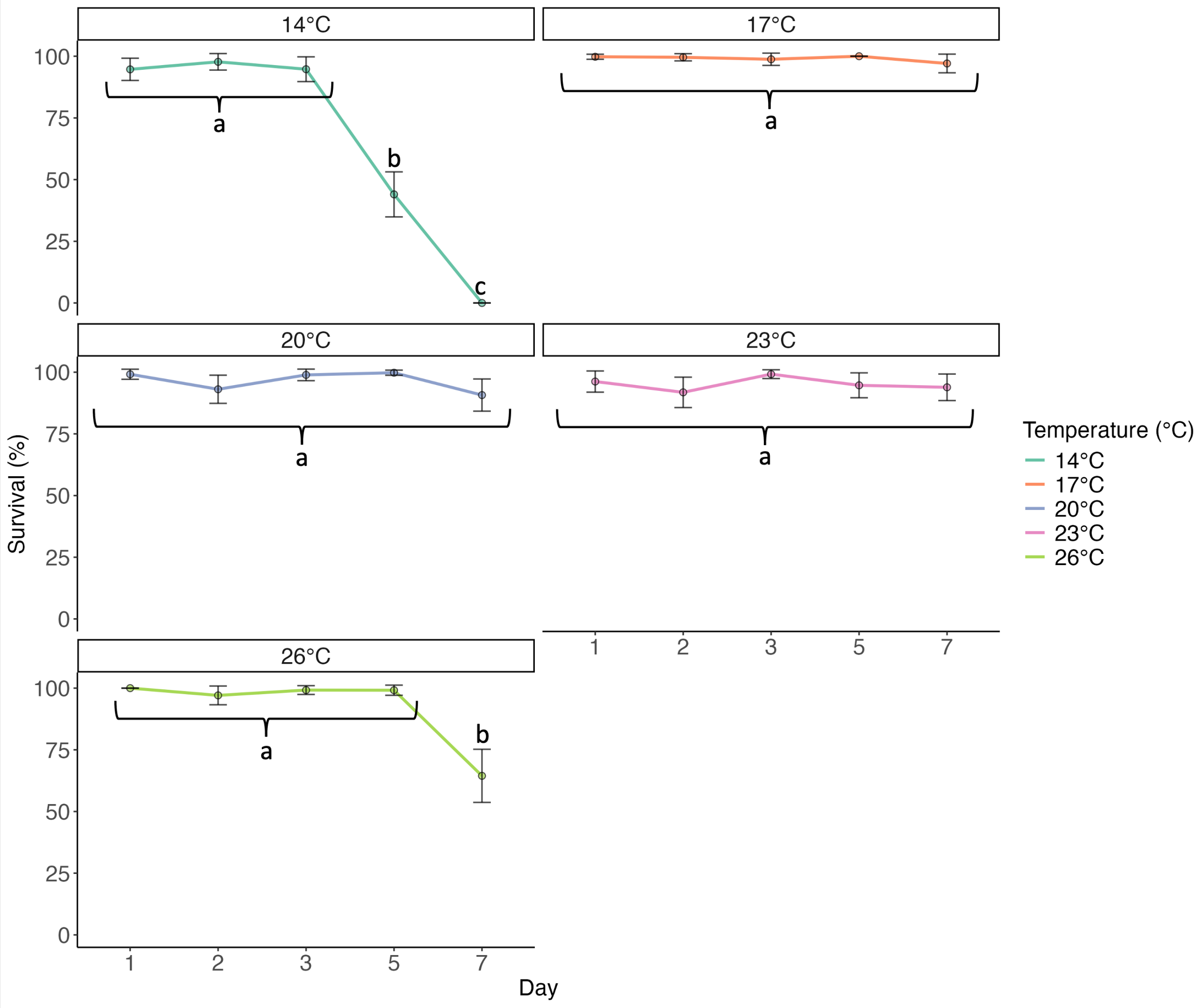

Figure 5.2. Model predicted estimated marginal means for survival (%) (±SEM) of *Tripneustes australiae* larvae for each time point (n = 6), and rearing temperature (n = 5). Letters indicate Tukeys post-hoc groups. Groups that do not share a letter are significantly different. If two or more means share the same grouping symbol, then they are not significantly different based on given sample sizes.

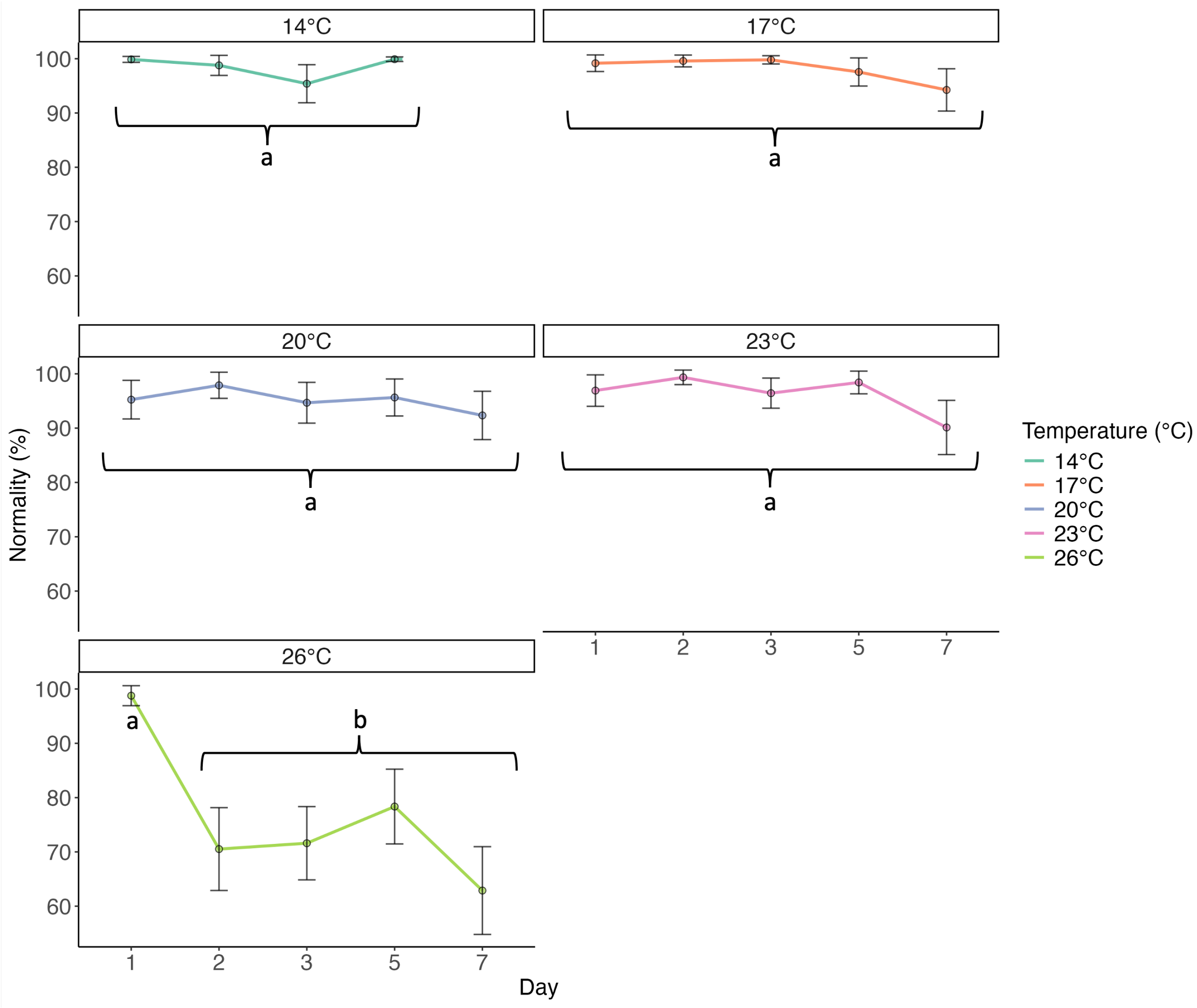

Figure 5.3. Model predicted estimated marginal means for normality (%) (±SEM) of *Tripneustes australiae* larvae for each time point (n = 6), and rearing temperature (n = 5). Letters indicate Tukeys post-hoc groups. Groups that do not share a letter are significantly different. If two or more means share the same grouping symbol, then they are not significantly different based on given sample sizes.

5.4.3 Thermal performance curves (TPCs)

There was a significant relationship between larval survival and temperature on day 5 ($F = 5.5$, $edf = 2.231$, $p = 0.0143$). The lower lethal temperature (LLT_{50}) was 14°C, but only 43% of the variation was explained by the model, suggesting a weak relationship between temperature and survival at this time point. There was a significant relationship ($F = 16.25$, $edf = 1.958$, $p < 0.001$) between temperature and the survival of *T. australiae* at day 7, with 66.9% of the variation explained by temperature. Between days 5 and 7 there was an increase in the predicted temperature at which 50% of the larvae were dead from 14°C to 15.94°C (Figure 5.4). On day 9, there was a strong significant relationship between temperature and survival ($F = 58.92$, $edf = 2.915$, $p < 0.001$; 92.1% variation explained), and the LLT_{50} decreased to 15.81°C. Conspicuous mortality was observed in the upper temperature treatments on day 9, with 50% lethality (ULT_{50}) being 23.7°C. Thus, survival of *T. australiae* through time narrowed as temperature stress accumulated. By day 9 the optimal survival occurred between 17 and 23°C.

5.4.4 Developmental stage progression

With respect to larval stage there was a significant interaction between temperature and time ($LRT = 318.9$, res $df = 75$, $df = 16$, $p < 0.001$). Embryos/larvae were at different developmental stages at different time points, and this varied depending on rearing temperature, with embryos reared at warmer temperatures progressing through developmental stages faster (Figure 5.5).

The number of cleavage stage embryos present depended on the sampling day, with no influence of temperature ($LRT = 40.2$, $df = 16$, $p = 0.001$) and they were only observed in the 14°C group at 24 hours. With respect to number of blastulae, gastrulae, prisms and plutei, there was a significant interaction between day and temperature (blastula: $LRT = 118.86$, $p < 0.001$;

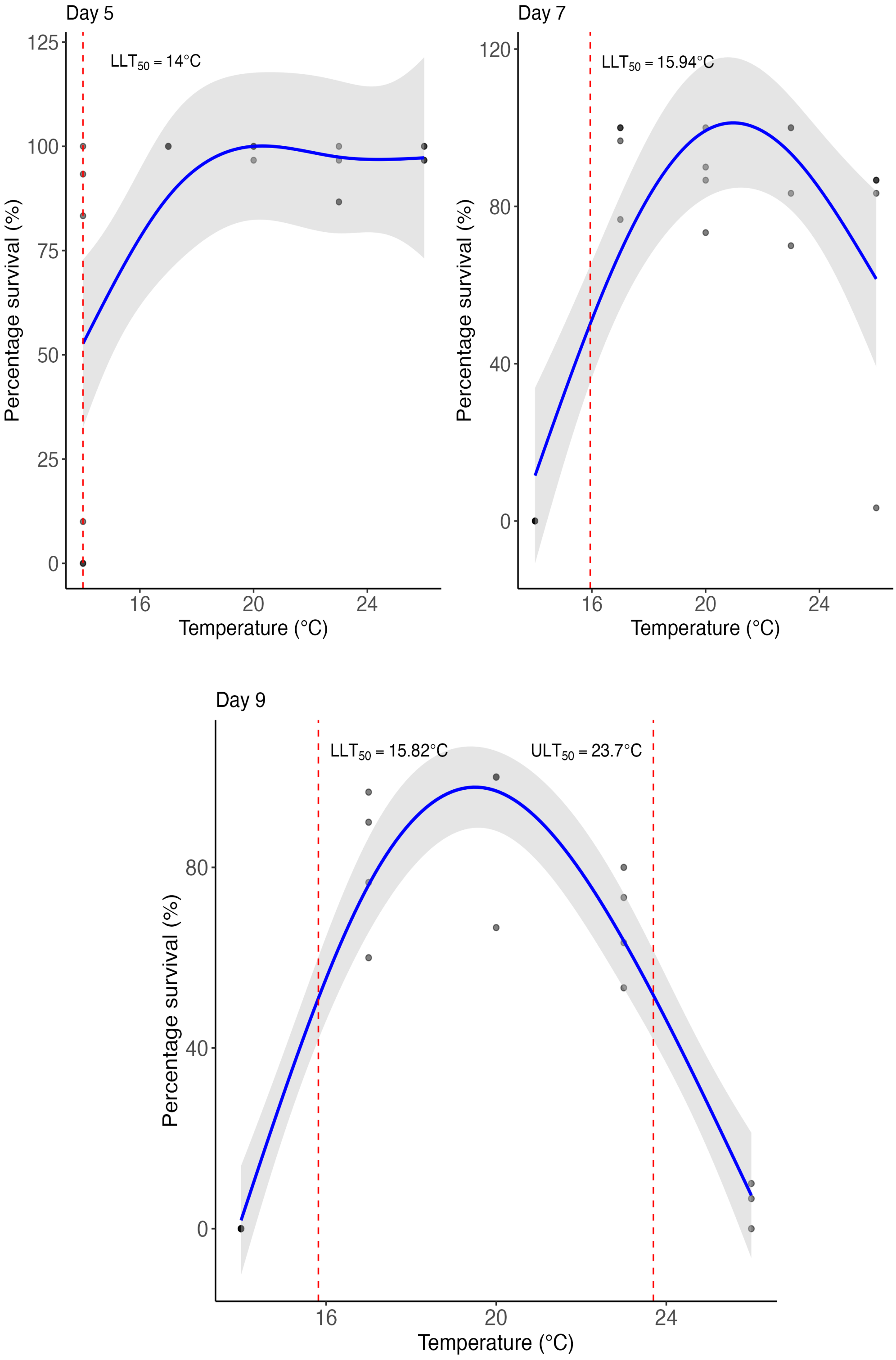

Figure 5.4. Thermal performance curves (95% CI) for *T. australiae* larvae constructed from general additive models (GAMs), for 5-, 7- and 9-days post-fertilisation. LLT_{50} (the temperature at which 50% survival occurs) is indicated by the red dashed line.

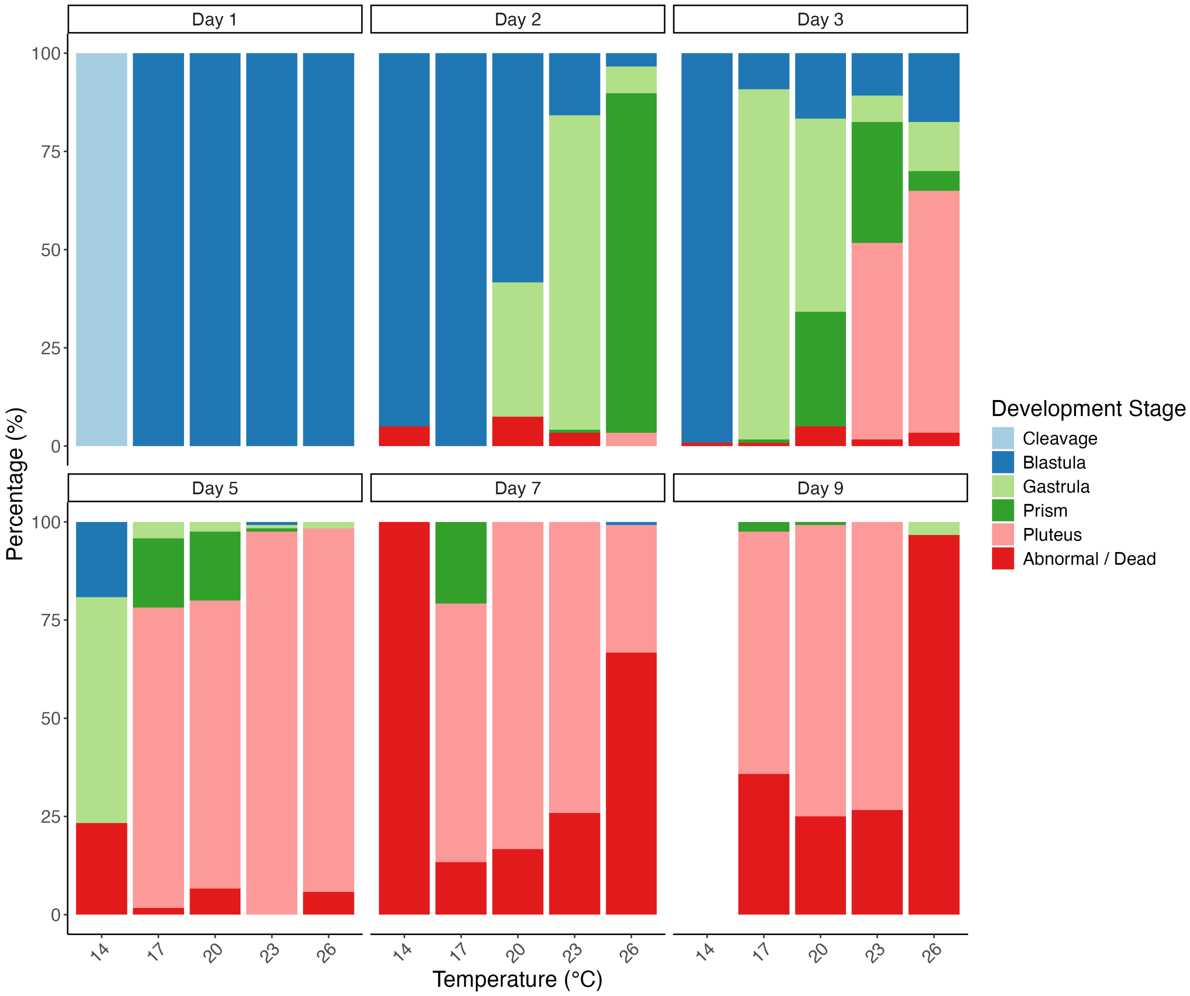

Figure 5.5. The percentage of different developmental stages of *Tripneustes australiae* larvae for each time point post-fertilisation (n = 6), and rearing temperature (n = 5). Means and standard error for the respective treatments and timepoints can be found in Table D3.

gastrula: LRT = 76.705, p = 0.001; prisms: LRT = 47.588, p= 0.002; plutei: LRT = 56.295, p = 0.001). At day 1, temperature groups between 17°C and 26°C were blastulae, and on day 3, only the 14°C degree group were primarily blastulae (Figure 5.5, Table E3). Gastrulae first appeared on day 2 in the 20-26°C temperature treatments with the highest proportion of this stage in the 23°C group (Figure 5.5, Table E3).

In the warmest treatment (26°C) on day 2, the cultures were at the prism stage, having completed gastrulation by 48 hours post-fertilisation (Figure 5.5). In comparison, on day 2 controls were primarily blastulae, and some had gastrulated (Figure 5.5). By day 3, the 23°C and 26°C treatments had 50% plutei and by day 5 the 17-26°C treatments were dominated by plutei (Figure 5.6, Table E3). The 14°C group never achieved the pluteus stage and 26°C group had near total mortality at day 9. Overall, for unfed larvae high survival and normal development was observed at temperatures between 17°C and 23°C, which appears to be the thermal range for development of *T. australiae*.

5.5 Discussion

Here I present the first study to examine both cold and warm tolerances of *Tripneustes australiae* the boom-bust sea urchin that can transform ecosystems in southeastern Australia (Valentine and Edgar 2010; McLaren et al. 2024). Understanding the thermal tolerances of marine ectotherms and especially the thermal tolerances of their dispersive larvae can help predict species redistributions in a warming ocean (Sunday et al. 2012; MacLean and Beissinger 2017; Stuart-Smith et al. 2017; Byrne et al. 2022; Zarzyczny et al. 2023). More so, the upper thermal tolerances of ectotherms can predict local extinction risk, while lower limits provide insight into poleward range shift potential (Bates et al. 2014; Sunday et al. 2015; Byrne et al. 2016, 2017, 2022). With respect to survival, normal development and development to the echinopluteus larva the optimal thermal window of *T. australiae* is between 17 and 23°C. This is in agreement with the thermal range determined from adult distribution data (Chapter 4). It thus appears that *T. australiae* is a narrow range thermal specialist in both the planktonic and benthic life phases.

This study suggests that the lower thermal limit of 50% survival (LLT_{50}) for *Tripneustes australiae* larvae is 15.82°C. In comparison to other sympatric echinoids in NSW with similar planktonic larval duration (PLD) and feeding strategies this is somewhat cold intolerant (Gall et al. 2021; Byrne et al. 2022). For example, the lower limit of 50% survival in *Centrostephanus rodgersii* is 12.6°C, while *Heliocidaris tuberculata* is 14.8°C. Both *Centrostephanus rodgersii* and *H. tuberculata* have adult distributions similar to *T. australiae* in subtropical New South Wales (NSW). This includes dispersal across the Tasman onto the offshore Lowd Howe and Norfolk Islands (Byrne et al. 2022, Chapter 4). The southernmost observations of this species occur around Narooma where the long-term temperature range is 22.5 - 15.3°C (Chapter 4). As such, the cold waters of southeast Australia seem to currently limit the dispersal of *T. australiae* to ~ 36° 15' S (Montague Island, Narooma) (Chapter 4). The waters off southeastern Australia are warming at 0.2°C per decade and so it is likely that *T. australiae* will extend poleward beyond Narooma by 2050 (CSIRO 2022). However, under projected warming, the dispersal of this species and range extensions will likely be limited to southern NSW and mainland southeastern Australia. It is unlikely that temperatures across Bass Strait and into Tasmania will warm sufficiently by 2100 to facilitate local invasion of *T. australiae* (Ridgway and Ling 2023). This species will have a similar dispersal and distribution trajectories to that suggested for H. *tuberculata*, remaining primarily within its native range as the ocean warms in the next century (Byrne et al. 2022).

The warm tolerance of *Tripneustes australiae* larvae suggests this species is likely to persist at its warm range edge into the future. This species was tolerant to at least 3°C degrees above its parents' spawning temperature (Figure 5.2 & 5.3). Considering time of collection and spawning, *T. australiae* is unlikely to experience temperatures above 23°C in the water column. However, there is some discordance regarding the spawning periodicity and timing of this species (O'Connor et al. 1978; Bové 2004; Lawrence and Agatsuma 2020). Some studies of *T. australiae* (then *T. gratilla*), suggest that populations in Solitary Islands Marine Park (SIMP), in the north of its distribution have mature gonads late autumn – summer (O'Connor et al. 1978). Other studies of populations further south in Port Stephens report mature gonads in April, while populations in Sydney begin to develop mature gonads in April developing into winter (Bové 2004). More so, I have observed mature gonads in specimens collected in Sydney in December 2023. As such, there may be asynchrony in the timing of its spawning across its range in NSW, which has also been recorded for the sympatric sea urchin *C. rodgersii* (Byrne et al. 1998).

Tripneustes australiae may transition from spawning in summer – autumn in the north to winter in the south, or they may produce mature gonads throughout the year, which has been noted for other *Tripneustes* species (Lawrence and Agatsuma 2020). As such, populations of *T. australiae* in the north that spawn in summer may experience temperatures above 23°C, as temperatures around SIMP can exceed 24°C in the summer (Malcolm et al. 2011; Lachs et al. 2021). That said, it remains a possibility that *T. australiae* is a continuous spawner or has a prolonged spawning season in some parts of its range. This suggestion needs to be addressed through studies of the annual gonad cycle in populations located along its distribution.

Parent conditions, and where in a species' distribution propagules originate can determine their thermo-tolerance (Byrne 2011b; Pecorino et al. 2014; Ross et al. 2016; Minuti et al. 2022). Propagules of sea urchins from different regions of their range can therefore display contrasting thermo-tolerance (O'Connor et al. 2007; Pecorino et al. 2014). For example, propagules from parents residing at warm range edges are more thermo-tolerant than propagules from cold range edges (Byrne 2011b; Pecorino et al. 2014). The parents collected for this experiment were collected around the mid-point of this species' distribution (Chapter 4), and so are a representation of the thermo-tolerances of larvae from the mid-range of this species. However, considering the potential different timing in spawning periods between northern and southern populations, future studies should establish larval thermal tolerances from the northern and southern populations of *T. australiae*. This would allow a better understanding of cool-tolerance in the southern populations with respect to potential range extensions and how warm-tolerant northern populations are to establish persistence at these latitudes.

As is typical for larval echinoids (Byrne 2011a; Byrne et al. 2016, 2022; Karelitz 2019; Balogh et al. 2020; Gall et al. 2021; Collin et al. 2021), progression through the developmental stages was faster at warmer temperature for *T. australiae*. This has important implications for potential range extensions and local extinctions as it can affect the amount of time spent in the plankton at respective cool and warm range edges (Byrne et al. 2017; Karelitz 2019). Propagules produced at higher latitudes in colder waters will develop at a slower rate and will spend longer in the plankton (Byrne et al. 2017; Karelitz 2019). As such, larvae from cool-range edges may have the capacity to disperse further in the water column. There was a 2-day developmental lag between the warm and the cold treatments. Notably, larvae in the 23°C rearing temperature were plutei by day 3, whereas larvae in the 17°C treatment were not plutei until day 5. As such larvae in the water column between 16 and 17°C such as those between

Sydney and Narooma during winter (Huang et al. 2017), may have the capacity to disperse further.

Larval thermal windows of *T. australiae* narrowed through time, suggesting the further through development the larvae progressed, stress accumulates, narrowing the thermal window. This is typical of marine invertebrates, and the thermal windows of local echinoderms typically narrow through development into settlement (Przeslawski et al. 2014; Balogh et al. 2020; Gall et al. 2021). Understanding the temperature limits within which juveniles recruit and survive into the benthos, where they can begin to impact ecosystems, is important to predict how this boom-bust species may impact vulnerable ecosystems into the future and begin to predict outbreak patterns of this species. As such, future studies should focus on late stages of development and metamorphosis into the juvenile. More so, as this study only considered larval thermal tolerances of unfed *T. australiae* larvae within its facultative feeding period (Byrne et al 2008a; 2008b), future studies need to consider fed larvae, especially as different feeding regimes can impact echino-plutei profiles in *Tripneustes* (Byrne et al. 2008b; Soars et al. 2009) and offset thermal stress (Sokolova et al. 2012).

5.6 Conclusions

Here I established the larval thermal tolerances of *Tripneustes australiae* and suggest it is a narrow range thermal specialist, a trait that is also reflected in its adult distributions (Chapter 4). As this species has a narrow thermal window compared to other sympatric sea urchins, it is unlikely to disperse and colonise poleward in a similar fashion to *C. rodgersii*, which is characterised by generalist traits that predict successful invasion and colonisation at poleward edges (Bates et al. 2014; Sunday et al. 2015; Byrne et al. 2022). However, *T. australiae* may still undergo a poleward range extension in the coming decades into macroalgal-habitats that are already vulnerable due the barren-formation of *C. rodgersii* in southern NSW (Glasby and Gibson 2020; Davis et al. 2023). More so, its persistence in its northern range may have impacts on temperate kelps at their warm range edges that are at risk from warming (Vergés et al. 2016).

Chapter 6.

General Discussion

As the oceans warm marine taxa are redistributing to higher latitudes and greater depths to stay within their physiological tolerances (Parmesan 2006; Sunday et al. 2012, 2015; Poloczanska et al. 2016; Pecl et al. 2017; Burrows et al. 2019; Stuart-Smith et al. 2022). Marine biogeographic transition zones at the interface between tropical, subtropical and temperate ecosystems will likely be the frontline for environmental change into the near future (Sommer et al. 2014; Horta e Costa et al. 2014; Wernberg et al. 2016; Vergés et al. 2019; Troast et al. 2020). The subtropical biogeographic transition zone in southeastern Australia provides an excellent natural laboratory to understand how the redistribution of warm-affinity taxa will affect ecosystems into the future. This is due to the tropical-to-temperate ecological and climate gradient along the east coast of Australia, and the poleward flow of the East Australian Current (EAC) that transports biological propagules poleward towards cooler regions (Ridgway and Godfrey 1997; Ling 2008; Byrne et al. 2022). More so, the EAC is warming and intensifying at a rapid rate (Suthers et al. 2011). Herbivory is a key ecological process in marine systems, and sea urchins are dominant herbivorous taxa across latitudes in habitats such as coral reefs, rocky reefs, seagrass meadows and soft-sediment ecosystems (Larkum and West 1990; Underwood et al. 1991; Alcoverro and Mariani 2002; Ling 2008; Filbee-Dexter and Scheibling 2014; Moreira-Saporiti et al. 2023). This thesis aimed to build knowledge on the biology, ecology and taxonomy of these key herbivores in the subtropical biogeographic transition zone of southeastern Australia.

6.1 Summary and key findings

In this thesis I integrate taxonomic (Chapter 2), ecological (Chapters 3 and 4) and biological (Chapter 4 and 5) approaches to address the overarching question: *how will echinoids in the subtropical biogeographic transition zone of southeastern Australia respond to climate change?* Overall, I found that existing paradigms in ecology and biogeography are not reliable in predicting how sea urchins will respond to environmental change in these dynamic ecotones. Specifically, I found that climate change responses of echinoids in this transition zone will likely vary among taxa, highlighting the importance of regional scale species-specific studies. The findings presented here are relevant for future taxonomic, ecological and biological studies of echinoids globally and have management implications for sea urchins in southeastern Australia.

Four key findings in this thesis contribute novel knowledge of echinoids in the subtropical biogeographic zone of eastern Australia: **(1)** Multiple lines of evidence across life history stages demonstrate that *Tripneustes australiae* is a narrow range subtropical specialist (Chapters 2, 4 and 5). Using integrative taxonomy, I show that this species has been present in temperate Australia for at least 146 years and I have generated new evidence across adult and larval stages on the warm and cool tolerances of this species (Chapters 4 and 5). **(2)** I present the first major temporal study of sea urchins in eastern Australia's biogeographic transition zone and show that echinoid taxa in this region have contrasting population dynamics through time. Biogeographic affinity did not predict changes in population dynamics of sea urchins (Chapter 3), despite capturing a period of warming and significant heatwaves (Kim et al. 2019; Cant et al. 2021; Lachs et al. 2021), in contrast to that found for herbivorous fishes (Vergés et al. 2016; Malcolm and Ferrari 2019; Smith et al. 2021). **(3)** I resolve the distributions of the genus *Tripneustes* in eastern Australia, corroborating that *T. australiae* is a narrow range specialist that will likely undergo a poleward range shift by the year 2100. **(4)** *Tripneustes g. gratilla* appears to be a thermal generalist in our region that ranges from the equatorial tropics to high latitude temperate reefs across ~30° of latitude and will likely undergo a poleward range extension by 2100.

6.2 Novel insights into the taxonomy, ecology and biology of echinoids in the subtropical biogeographic transition zone of eastern Australia

6.2.1 Taxonomy

Accurate taxonomy is essential in studies of biodiversity, and in Chapter 2 I showcase this. I synonymised *Evechinus australiae* (Tenison-Woods 1878) and *Tripneustes kermadecensis* (Bronstein et al. 2017) using an integration of morphological and molecular taxonomy. A compelling weight of evidence was assembled using micro-computed tomography, quantification of test traits through ontogeny, and phylogenetic analysis of DNA extracted from 146-year-old specimens to re-assign the taxa *E. australiae* to the genus *Tripneustes* and re-designate this taxon as *Tripneustes australiae*, type locality Sydney Harbour (Chapter 2, McLaren et al. 2023). In doing so, I demonstrate that this species is an important component of the local marine fauna in southeast Australia and should be managed as such.

6.2.2 Ecology

Chapter 3 demonstrates that despite the paradigm that warm-affinity species increase in transition zones (Poloczanska et al. 2016; Burrows et al. 2019; Stuart-Smith et al. 2022; Zarzyczny et al. 2023), tropical and subtropical echinoids decreased in abundance in the subtropics of eastern Australia over the 9-year survey period, despite considerable warming and heatwaves (Kim et al. 2019; Cant et al. 2021; Lachs et al. 2021). I showed that populations of the tropical *Diadema* spp. and subtropical *Tripneustes australiae* declined, while the cool-affinity species *C. rodgersii* was stable. I highlight that other traits of echinoid population biology such as boom-bust dynamics need to be considered in predicting how these taxa will respond to climate change and predictions based on biogeographic affinity alone are not reliable for echinoids in this dynamic ecotone.

6.2.3 Biology

In Chapter 4, museum collections and citizen science were used to resolve the distributions of *Tripneustes* in eastern Australia and across the Tasman Sea. I did so to predict present and future habitat suitability for *T. australiae* and *T. g. gratilla* in our region of interest. *Tripneustes* are commercially and ecologically important echinoids around the globe, and capable of transforming ecosystems through outbreaks and grazing (Valentine and Edgar 2010; Neilson et al. 2018; Lawrence and Agatsuma 2020; Moreira-Saporiti et al. 2023). More so, until recent clarification, the occurrences of *T. australiae* on the southeast coast of Australia were considered to be a range extension of its tropical congener *T. g. gratilla* (Castro et al. 2020).

I showed that *T. g. gratilla* is a tropical-to-temperate generalist that ranges from Papua New Guinea (9° 56' 2.4"S) to Jervis Bay (35° 7' 12"S) with a broad realised thermal niche (16.3 - 29.97°C). In contrast, *T. australiae* is a narrow range subtropical-to-temperate specialist that is restricted to New South Wales and offshore Islands around the Tasman, ranging from Byron Bay (28° 37' 0.12"S) to Narooma (36° 15' 0"S) with a narrow thermal niche (15.3 - 26°C). Habitat suitability modelling suggests that both *T. australiae* and *T. g. gratilla* are likely to undergo a poleward range shift by the year 2100. I also note the potential hybrid zone of these congeners across 7° of latitude in subtropical Australia. This has novel ecological implications as introgressive hybridisation may facilitate the range expansion of the generalist *T. g. gratilla* or result in genetic swamping and loss of the narrow range specialist *T. australiae*.

In Chapter 5 I documented the embryonic and larval thermal tolerances of *T. australiae* and generated a concurrent line of evidence to Chapter 4 that supports *T. australiae* as a narrow range thermal specialist. I present data on survival, normal development and developmental progression that suggest the optimal window for success in the embryonic and larval stages of this species is between 17 and 23°C. Thus, *T. australiae* is a narrow range specialist across its life history stages. This has interesting ecological implications for management of this species. Like the sympatric echinoid *Heliocidaris tuberculata, T. australiae* may disperse further south into the future, but will likely be limited to NSW and mainland Australia (Byrne et al. 2022).

6.3 Synthesis: *how will echinoids in the subtropical biogeographic transition zone of southeastern Australia respond to climate change?*

The overarching finding of this thesis is that with respect to the common echinoids of the subtropical biogeographic transition zone of eastern Australia, responses to environmental change will be taxa dependent. Considering the wide range of life history strategies, ecological roles and population dynamics across echinoids globally, this is not a surprise (Strathmann 1993; McEdward and Miner 2001; Ling 2008; Valentine and Edgar 2010; Lessios 2016; Moreira-Saporiti et al. 2023). Importantly, this highlights that the ecological and management implications presented in this thesis will depend on the species and region in question.

6.3.1 *Tripneustes*

Narrow range subtropical and temperate specialists and especially echinoderms, are at risk in a warming climate due to limited refugia (Lang et al. 2023; Edgar et al. 2023). It seems that *T. australiae* is particularly cold intolerant compared to other sympatric echinoids (Gall et al. 2021; Byrne et al. 2022) and so may have limited suitable habitat into the future, as also suggested by habitat suitability modelling (Chapter 4). This presents an interesting dichotomy for management and future research, because this endemic narrow range specialist has on-going population outbreaks that can transform seascapes. Future research needs to balance the concept of conserving this species, while also considering management of outbreaks. I propose that research needs to focus on when and where population outbreaks of *T. australiae* will occur and what environmental and biological factors drive these outbreaks. This is especially important for biologically significant ecosystems such as the subtropical coral reefs of Lord Howe Island (LHI) and the temperate macroalgae dominated systems on the southeast coast of Australia that are at risk due to ocean warming and *C. rodgersii* overgrazing.

In contrast, *T. g. gratilla* is a generalist species (Chapter 4) that is generally rare in the subtropics (Chapter 3) and has traits that may facilitate a range extension, including wide dispersal and thermal generalism (Lessios et al. 2003; Bates et al. 2014; Sunday et al. 2015). Evidence generated here suggests that this species is either a tropical-to-temperate generalist, or that the subtropical-to-temperate distribution of *T. g. gratilla* in eastern Australia is a product of a historic or ongoing range extension. Moreover, if the Australian mainland population of *T. g. gratilla* acts as a source population as it does for *T. australiae* and other trans-Tasman echinoids (Chapter 2) (Byrne & O'Hara 2017), *T. g. gratilla* may extend its range into the Tasman to Norfolk Island and northern New Zealand. An investigation of cool tolerance in *T.*

g. gratilla larvae from parents residing in the subtropics and the southern extent of its range is needed to further understand the cool range limits and dispersal capacity of this generalist.

6.3.2 *Centrostephanus rodgersii*

In Chapter 3, I demonstrate that *C. rodgersii* populations are stable in coral associated habitat in the subtropics of eastern Australia. In Tasmania *C. rodgersii* has facilitated the expansion of local corals (Ling et al. 2018), and the grazing of sea urchins is proposed to accelerate tropicalisation (Schuster et al. 2022). It appears that the extent of *C. rodgersii* barrens increases with latitude, and stable populations of this species have been observed by other studies in its northern range (Glasby and Gibson 2020; Davis et al. 2023). This may have ecological implications into the future, as *C. rodgersii* may be filling a characteristic diadematid role on the subtropical reefs of NSW, mediating the success of corals, similar to other coral reef ecosystems such as in the Caribbean (Lessios 2016). The potential competitive release of corals from algal overgrowth facilitated by *C. rodgersii* grazing may become evident at higher latitudes if corals expand their ranges poleward.

In contrast to my findings, it has been suggested that as the oceans warm *C. rodgersii* will decrease at its warm range-edge in northern NSW (localities north of 36.6 °S) (Davis et al. 2023). However, the survey period presented in Chapter 3 was punctuated by significant marine heatwaves (Kim et al. 2019; Lachs et al. 2021), demonstrating these northern populations of *C. rodgersii* were resilient to these thermal stress events for the period 2010-2019. As such, *C. rodgersii* may not decrease in the north as the ocean warms. The role of *C. rodgersii* on these subtropical reefs requires research attention, as it may mediate ecological dynamics along the subtropical-to-temperate transition zone and may be important for the resilience of these dynamic ecosystems into the future.

6.3.3 A possibility for facilitation

The cumulative effect of *T. australiae* outbreaks and expanding *C. rodgersii* barrens on the far south coast of NSW has the potential to rapidly transform macroalgal-beds to barrens. I propose that outbreaks and feeding fronts of *T. australiae* (see Valentine & Edgar 2010) may accelerate the expansion of *C. rodgersii* dominated barrens in southeastern Australia. For example, if *T. australiae* recruit into macroalgal-habitats with *C. rodgersii* present, overgraze macroalgae and die off, recruitment of macroalgae back into these habitats could be limited if *C. rodgersii* persist and form barrens. Research into the ecological role of *T. australiae* and its interaction with other ecologically important grazers should therefore be a research priority. In contrast, in the event of coral bleaching in coral dominated habitats like Lord Howe Island, *T. australiae* may control turfing algae and promote the recovery of coral to prevent a phase shift to turf dominated reefs, similar to their use as invasion biocontrol in Hawaii and co-culturing in aquaculture to promote coral growth (Neilson et al. 2018; Neil et al. 2024).

6.4 Future directions

6.4.1 Taxonomy

HL. Clarke and T. Mortensen commented on the morphological similarities of species in the genus *Tripneustes*, and despite the taxonomy of *T. australiae* being resolved, there is still some discordance remaining. Primarily, the resolution of the *T. depressus/g. gratilla* species complex in the Indo-Pacific. I reiterate hypotheses stated by Lessios et al. (2003), and Bronstein et al. (2017), that the Indo-Pacific likely holds a species complex, masked by introgressive hybridisation, cryptic speciation and morphological similarities, similar to the Red Sea subspecies *T. g. elatensis* (Bronstein et al. 2016). More so, the wide distribution and generalist traits of *T. g. gratilla* suggest a potential species complex or cryptic species, as true generalists/cosmopolitan species are rare (Hutchings and Kupriyanova 2018).

Phylogenetically, *Tripneustes australiae* and *T. ventricosus* are likely resolved monophyletic clades, however the *T. depressus/g. gratilla* clade is polyphyletic (Bronstein et al. 2017; Lessios et al. 2003). As suggested by Bronstein et al. (2017), Hawaiian populations of *Tripneustes* are excellent candidates to start searching for hidden diversity in this genus, as they are very isolated. Despite the success of mitochondrial and nuclear loci in describing echinoid diversity to date, to resolve the taxonomy of this genus, higher resolution phylogenomic techniques, such as the use of single nucleotide polymorphisms, is necessary to detect speciation masked by hybridisation. These methods are common in the description of coral taxa, which are often challenged by introgressive hybridisation and morphological similarities (Forsman et al. 2017; Oury et al. 2023; Bridge et al. 2023). Although phylogenomic tools can likely resolve the *Tripneuste*s genus, it is important to integrate this with morphological descriptions to ensure future ecological surveys can accurately identify any newly described species.

6.4.2 Biology

This thesis highlights the need to consider biology and ecology when making predictions regarding species on the move. For example, in Chapter 3, I assumed based on the global increase in warm-affinity taxa (Poloczanska et al. 2016; Burrows et al. 2019; Stuart-Smith et al. 2022), thermal tolerances would predict changes in abundance. Further, in Chapter 4, I assumed based on the the climate variability hypothesis (CVT), that *T. g. gratilla* would have a narrow range. The climate variability hypothesis (CVT) predicts a positive relationship between a species geographic range and latitude, positing that over evolutionary time, stable climates such as the tropics do not select for large thermal niches (Stevens 1989; Addo-Bediako et al. 2000; Sunday et al. 2011; Stuart-Smith et al. 2017; Saupe et al. 2019). Despite the wide acceptance of this paradigm across marine ectotherms at global scales (Sunday et al. 2011, 2015; Stuart-Smith et al. 2017), echinoids in subtropical eastern Australia do not conform. However, after considering biological factors such as life history traits and dispersal capacity (Chapters 3 and 4), the findings from Chapter 3 and Chapter 4 are understandable in a broader context.

6.4.3 Hybridisation

As highlighted in Chapter 4, the potential for the two *Tripneustes* species to hybridise in NSW needs to be investigated as this would have ecological and management implications. Many foundational questions need to be addressed to discern the likelihood of hybridisation or past hybridisation. First, markers of introgressive hybridisation should be investigated in specimens from the subtropical biogeographic zone in southeastern Australia following the methods in Lessios (2024). This should be complimented with application of phylogenomic techniques to determine the extent of genetic material exchange between these congeners and at what rate between generations. Secondly, these genetic investigations should be supplemented by investigations of gametic compatibility and cross fertilisation of the two species as done for the toxopneustid sea urchins *Pseudobolitia indiana* and *P. maculata* (Zigler et al. 2012). More so, if hybrid larvae are generated, they should be reared into juveniles to examine if juvenile hybrids succeed, because this is the life stage that can affect ecosystems through grazing.

6.5 Ecological and management implications

6.5.1 The contrasting ecology of *C. rodgersii* across its native range and implications for management

The contrasting population dynamics of *C. rodgersii* and potential contrasting ecology across its native and poleward advancing range has interesting management implications for New South Wales. The populations of *C. rodgersii* in the north of its native distribution in northern NSW seem to be stable compared to those in the south (Chapter 3; Glasby and Gibson 2020; Davis et al. 2023). As a result, future management of this species along the NSW coast may need to differ depending on the region. For example, in the north of NSW conservative management may be needed, while in the south extractive management such as increased recreational and commercial harvest and culling may be required.

6.5.2 Boom-bust dynamics of *T. australiae*

As shown in Chapter 3, *T. australiae* has population booms and busts on subtropical reefs in southeastern Australia. As highlighted by Strayer et al. (2017), boom-bust species have periods where they are ‘harmful’, and ‘harmless’ and as such tend to not be managed, or managed minimally, because they eventually return to a ‘harmless’ state. However, environmental factors such as temperature anomalies and oceanic currents may affect population outbreaks of *T. australiae* and these are being altered by climate change. Understanding what drives where and when these outbreaks occur is critical as the oceans warm. On the far south coast of eastern Australia, where ecosystems are already under pressure from *C. rodgersii* overgrazing and above-average warming, considering the boom-bust dynamics of *T. australiae* is essential in future management.

6.6 Concluding remarks

The findings in this thesis are key to understanding the ecology and management of echinoids in subtropical southeastern Australia now and into the future. I have contributed novel information on the ecology, taxonomy and biology of these important herbivores in this unique ecosystem. Importantly, I have presented myself (and hopefully others) with many key questions for future research regarding sea urchins in this dynamic ecotone.

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Appendix A | Chapter 2 supplementary material

Table A1. List of species, GenBank accession numbers, sample names, locality, use (network or phylogeny) and source of sequences used in the current study. Py – Phylogeny; Nt – Network; MA – Morphological Analysis.

Species	Accession number	Sample ID	Locality	Dataset (Py/Nt/ MA)	Source
T. kermadecensis	OR038171	J. 30961	Port Jackson, NSW	Py + Nt	Current study
T. kermadecensis	OR038169	J. 1117	Port Jackson, NSW	Py + Nt	Current study
T. kermadecensis	OR038167	J. 23530	Port Stephens, NSW	Py + Nt	Current study
T. kermadecensis	OR205878	J. 24345	Cape Three Points, NSW	Py + Nt	Current study
T. kermadecensis	OR038168	J. 4250	Port Jackson, NSW	Py + Nt	Current study
T. kermadecensis	OR038170	J. 5003	Port Jackson, NSW	Py + Nt	Current study
T. kermadecensis	OR144098	J. 31050	Solitary Islands, NSW	Py + Nt	Current study
T. kermadecensis	OR144099	J. 31051	Solitary Islands, NSW	Py + Nt	Current study
T. kermadecensis	OR144100	J. 31052	Solitary Islands, NSW	Py + Nt	Current study
T. kermadecensis	NA	SUSCG. 1	Port Jackson, NSW	MA	Current study
T. kermadecensis	NA	SUSCG. 2	Port Jackson, NSW	MA	Current study
T. kermadecensis	NA	SUSCG. 3	Port Jackson, NSW	MA	Current study

T. kermadecensis	NA	SUSCG.4	Port Jackson, NSW	MA	Current study
T. kermadecensis	NA	SUSCG.5	Port Jackson, NSW	MA	Current study
T. kermadecensis	NA	SUSCG.6	Port Jackson, NSW	MA	Current study
T. kermadecensis	NA	SUSCG.7	Port Jackson, NSW	MA	Current study
T. kermadecensis	NA	SUSCG.8	Port Jackson, NSW	MA	Current study
T. kermadecensis	KY314758	Ker901	Kermadec Islands	Py + Nt	Bronstein et al. 2017
T. kermadecensis	KY314765	Ker123	Kermadec Islands	Py + Nt	Bronstein et al. 2017
T. kermadecensis	KY314757	Ker001	Kermadec Islands	Py + Nt	Bronstein et al. 2017
T. kermadecensis	KY314767	Ker218	Kermadec Islands	Py + Nt	Bronstein et al. 2017
T. kermadecensis	KY314769	Ker243	Kermadec Islands	Py + Nt	Bronstein et al. 2017
T. kermadecensis	KY314759	Ker904	Kermadec Islands	Py + Nt	Bronstein et al. 2017
T. kermadecensis	KY314770	Ker378	Kermadec Islands	Py + Nt	Bronstein et al. 2017
T. kermadecensis	KY314766	Ker124	Kermadec Islands	Py + Nt	Bronstein et al. 2017
T. kermadecensis	KY314761	Ker906	Kermadec Islands	Py + Nt	Bronstein et al. 2017
T. kermadecensis	KY314768	Ker219	Kermadec Islands	Py + Nt	Bronstein et al. 2017
T. kermadecensis	KY314763	Ker910	Kermadec Islands	Py + Nt	Bronstein et al. 2017

T. kermadecensis	KY314764	Ker911	Kermadec Islands	Py + Nt	Bronstein et al. 2017
T. kermadecensis	KY314762	Ker909	Kermadec Islands	Py + Nt	Bronstein et al. 2017
T. kermadecensis	MK084947	MB011	Norfolk Island	Py + Nt	Bronstein et al. 2019
T. kermadecensis	MK084946	MB010	Sydney, Australia, NSW	Py + Nt	Bronstein et al. 2019
T. kermadecensis	MK084945	MB009	Sydney, Australia, NSW	Py + Nt	Bronstein et al. 2019
T. kermadecensis	MK084948	MB012	Norfolk Island	Py + Nt	Bronstein et al. 2019
T. g. gratilla	KU314856	Pi7.0	Luzon, Philippines	Py + Nt	Bronstein et al. 2016
T. g. gratilla	KU314860	Pi187157.1	Luzon, Philippines	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314830	RS8.1	Gulf of Aqaba, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314836	RSJo1	Gulf of Aqaba, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314838	RS4.2	Gulf of Aqaba, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314840	RS6.2	Gulf of Aqaba, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314842	RS10.1	Gulf of Aqaba, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314844	RSEQ1	El Quseir, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314845	RSEQ2	El Quseir, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. gratilla	KU314859	Pi187156K1	Luzon, Philippines	Py + Nt	Bronstein et al. 2016

T. g. gratilla	KU314861	Pi187157.2	Luzon, Philippines	Py + Nt	Bronstein et al. 2016
T. g. gratilla	KU314864	Pi187197	Luzon, Philippines	Py + Nt	Bronstein et al. 2016
T. g. gratilla	KU314854	ZnTg104	Zanzibar	Py + Nt	Bronstein et al. 2016
T. g. gratilla	KU314863	Pi187193	Luzon, Philippines	Py + Nt	Bronstein et al. 2016
T. g. gratilla	KU314862	Pi187157.3	Luzon, Philippines	Py + Nt	Bronstein et al. 2016
T. g. gratilla	KU314858	Pi187147	Luzon, Philippines	Py + Nt	Bronstein et al. 2016
T. g. gratilla	KU314853	ZnTg103	Zanzibar	Py + Nt	Bronstein et al. 2016
T. g. gratilla	KU314852	ZnTg102	Zanzibar	Py + Nt	Bronstein et al. 2016
T. g. gratilla	KU314851	ZnTg101	Zanzibar	Py + Nt	Bronstein et al. 2016
T. g. gratilla	KU314855	KenTrip1	Kenya	Py + Nt	Bronstein et al. 2016
T. g. gratilla	KU314850	ZnTg100	Zanzibar	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314841	RS6.3	Gulf of Aqaba, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314847	RSEQ18	El Quseir, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314837	RS4.1	Gulf of Aqaba, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314849	RSEQ20	El Quseir, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314843	RS10.2	Gulf of Aqaba, Red Sea	Py + Nt	Bronstein et al. 2016

T. g. elatensis	KU314834	RSJo2d	Gulf of Aqaba, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314831	RS8.2	Gulf of Aqaba, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314828	RSEi2	Gulf of Aqaba, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314848	RSEQ19	El Quseir, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314827	RSEi1	Gulf of Aqaba, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314829	RS7	Gulf of Aqaba, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314832	RS8.3	Gulf of Aqaba, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314833	RSJo1d	Gulf of Aqaba, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314835	RSJo2	Gulf of Aqaba, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314839	RS6.1	Gulf of Aqaba, Red Sea	Py + Nt	Bronstein et al. 2016
T. g. elatensis	KU314846	RSEQ7	El Quseir, Red Sea	Py + Nt	Bronstein et al. 2016
T. ventricosus	MK084937	RT001	Roatan, Honduras	Py + Nt	Bronstein et al. 2019
T. ventricosus	MK084938	RT008	Roatan, Honduras	Py + Nt	Bronstein et al. 2019
T. ventricosus	MK084939	RT014	Roatan, Honduras	Py + Nt	Bronstein et al. 2019
T. ventricosus	MK084940	RT017	Roatan, Honduras	Py + Nt	Bronstein et al. 2019
T. depressus	MK084954	Dep006	Baja California, Mexico	Py + Nt	Bronstein et al. 2019

T. depressus	MK084951	Dep003	Baja California, Mexico	Py + Nt	Bronstein et al. 2019
T. depressus	MK084953	Dep005	Baja California, Mexico	Py + Nt	Bronstein et al. 2019
T. depressus	MK084950	Dep002	Baja California, Mexico	Py + Nt	Bronstein et al. 2019
T. depressus	MK084949	Dep001	Baja California, Mexico	Py + Nt	Bronstein et al. 2019
T. depressus	MK084952	Dep004	Baja California, Mexico	Py + Nt	Bronstein et al. 2019
T. g. gratilla	MK084943	MB006	Cairns, QLD, Australia	Py + Nt	Bronstein et al. 2019
T. g. gratilla	MK084941	MB001	Kurnell, NSW, Australia	Py + Nt	Bronstein et al. 2019
T. g. gratilla	MK084944	MB007	Cairns, QLD, Australia	Py + Nt	Bronstein et al. 2019
Toxopneustes pileolus	MK084955	Toxo004	Kunduchi, Tanzania	Py	Bronstein et al. 2019
Toxopneustes pileolus	MK084956	Toxo005	Kunduchi, Tanzania	Py	Bronstein et al. 2019
Lytechinus variegatus	MG676469	LVAR.00	NA	Py	Bronstein & Kroh 2019
Lytechinus variegatus	MG676468	Lv4	NA	Py	Bronstein & Kroh 2019

Table A2. Expected and observed number of tubercles on 100 plates in the *T. kermadecensis* growth series and Chi-square goodness of fit values, degrees of freedom and p-values.

Individual	$\chi^2 H_0$	E_i no. tubercles	O_i no. tubercles	χ^2	df	p
SUSCG. 1	H_0: *T. kermadecensis*	25/100	33/100	0.42	1	0.06
SUSCG. 2	H_0: *T. kermadecensis*	25/100	39/100	3.86	1	0.001*
SUSCG. 3	H_0: *T. kermadecensis*	25/100	37/100	2.3	1	0.005*
SUSCG. 4	H_0: *T. kermadecensis*	25/100	35/100	1.19	1	0.3
SUSCG. 5	H_0: *T. kermadecensis*	25/100	38/100	3.048	1	0.002*
SUSCG. 6	H_0: *T. kermadecensis*	25/100	31/100	0.0476	1	0.1
SUSCG. 7	H_0: *T. kermadecensis*	25/100	39/100	3.857	1	0.001*
SUSCG. 8	H_0: *T. kermadecensis*	25/100	36/100	1.71	1	0.01*
J. 31050	H_0: *T. kermadecensis*	25/100	33/100	0.423	1	0.06
J. 31051	H_0: *T. kermadecensis*	25/100	29/100	0.048	1	0.35
J. 31052	H_0: *T. kermadecensis*	25/100	36	1.71	1	0.01*

Table A3. Other *Tripneustes australiae* (formerly known as *T. kermadecensis)* material from the type locality Port Jackson in the Australian Museum collections.

Specimen	No.	Collected from	Longitude	Latitude	Collection date
J. 11125	3	Port Jackson, Sow and Pigs Reef	33° 50′ 18″ S	151° 16′ 12″ E	05 Apr. 1958
J. 5003	1	Port Jackson, Bottle and Glass Rocks	33° 50′ 54″ S	151° 16′ 12″ E	
J. 8825	3	Port Jackson, Bottle and Glass Rocks	33° 50′ 54″ S	151° 16′ 12″ E	Feb. 1961
J. 9892	1	Port Jackson, Bottle and Glass Rocks	33° 50′ 54″ S	151° 16′ 12″ E	23 Apr. 1976
J. 11843	1	Port Jackson, Bottle and Glass Rocks	33° 50′ 54″ S	151° 16′ 12″ E	1978
J. 11858	1	Port Jackson, Bottle and Glass Rocks	33° 50′ 54″ S	151° 16′ 12″ E	1978
J. 1116	1	Port Jackson	33° 51′ S	151° 16′ E	
J. 4250	1	Port Jackson	33° 51′ S	151° 16′ E	Jun. 1923
G. 1138	1	Port Jackson	33° 51′ S	151° 16′ E	
J. 2478	1	Port Jackson	33° 51′ S	151° 16′ E	
J. 2479	1	Port Jackson	33° 51′ S	151° 16′ E	

Appendix B | Chapter 3 supplementary material

Table B1. Species of sea urchins that were observed across the biogeographic transition zone in coastal New South Wales, Australia, in 2010, 2012, 2016 and 2019, and their typical distributional range.

Species	Range
Centrostephanus rodgersii	Northern New South Wales – Southern Tasmania
Tripneustes australiae	Northern New South Wales – Southern New South Wales (Montague Island)
Tripneustes gratilla	Pan-tropical
Diadema spp. *(Diadema savignyi or D. setosum)*	Pan-tropical
Phyllacanthus parvispinus	North Queensland – Southern New South Wales
Prionocidaris callista	North Queensland – Southern New South Wales
Heliocidaris erythrogramma	Southern Queensland, New South Wales, South Australia, and southern Western Australia
Heliocidaris tuberculata	Southern Queensland, New South Wales

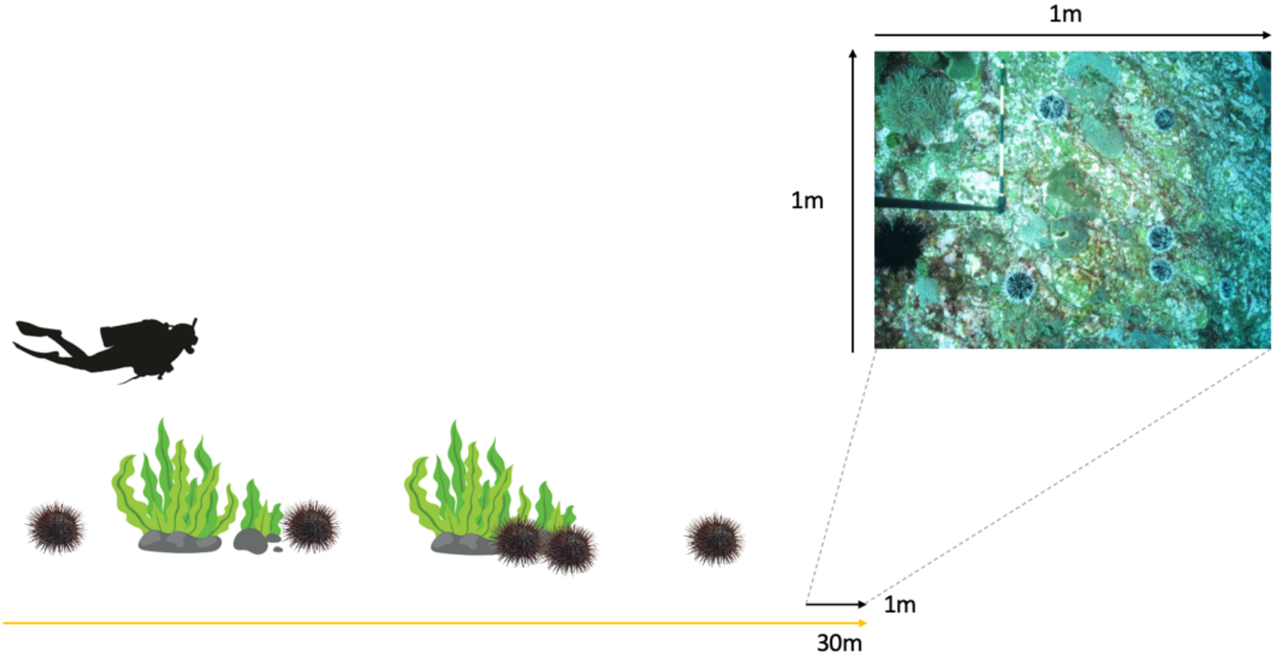

Figure B1. Visualisation of the survey method used. Photographs were taken at a consistent depth (8-12m) and distance from sea floor (1m) for a 30m long by 1m wide belt transect.

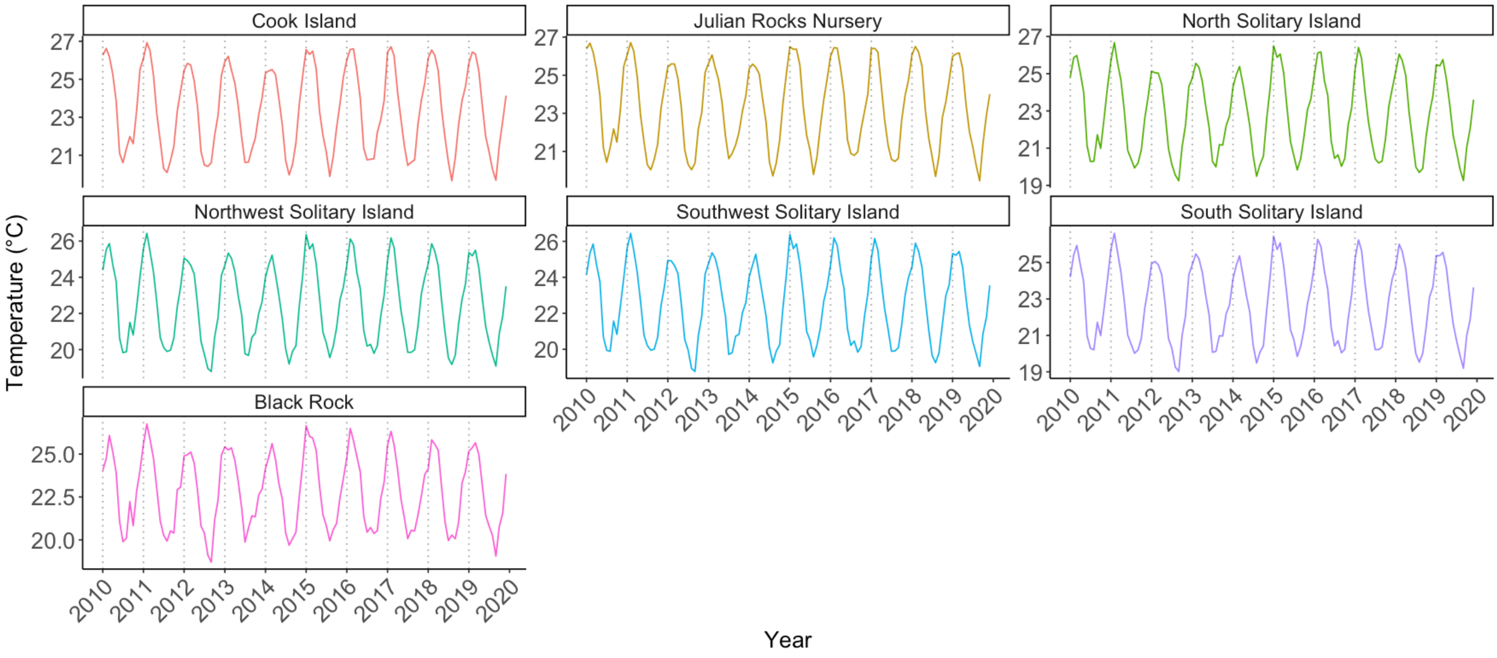

Figure B2. Monthly mean sea surface temperature (°C) from the Modis Aqua Satellite (Parkinson 2003) for all subtropical sites of eastern Australia from 2010-2020. Sites arrayed along increasing latitude (28.196°S to 30.95°S) from left to right in each row.

Table B2. The mean annual temperature and standard error (°C, ±SEM) for all sites arrayed from low to high-latitude from 2010-2019 (January – December). Red asterisk (*) marks the highest/hottest values and blue asterisk (*) marks the lowest/ coolest.

Site	Latitude	Year	Mean temperature (°C)	SEM
Cook Island	-28.19627, 153.57632			
		2010	23.64425*	0.653457
		2011	23.33375	0.717553
		2012	23.212*	0.632587
		2013	23.29833	0.583967
		2014	23.32542	0.617113
		2015	23.627	0.680988
		2016	23.44108	0.663352
		2017	23.44092	0.667823
		2018	23.499	0.685676
		2019	23.27192	0.689328
Julian Rocks Nursery	-28.61087, 153.62809			
		2010	23.639*	0.666457
		2011	23.19042	0.706471
		2012	23.08067*	0.642607
		2013	23.26458	0.554666
		2014	23.21442	0.635086
		2015	23.52242	0.690323
		2016	23.47867	0.635684
		2017	23.37217	0.666683
		2018	23.45375	0.68589
		2019	23.20842	0.682087
North Solitary Island	-29.927723, 153.389619			
		2010	23.085	0.622292
		2011	22.85875	0.693013
		2012	22.50717*	0.653346
		2013	22.76658	0.561477

		2014	22.69058	0.595378
		2015	23.10117*	0.681262
		2016	22.8335	0.657081
		2017	22.9215	0.639734
		2018	22.84067	0.667732
		2019	22.75992	0.657947
Northwest Solitary Island	-30.018969, 153.269667			
		2010	22.771	0.636073
		2011	22.624	0.692397
		2012	22.14667*	0.690337
		2013	22.49308	0.581324
		2014	22.39067	0.613635
		2015	22.82317*	0.691265
		2016	22.55025	0.663087
		2017	22.658	0.659485
		2018	22.547	0.685761
		2019	22.51392	0.662943
Southwest Solitary Island	-30.159215, 153.22809			
		2010	22.75667	0.616772
		2011	22.66892	0.688906
		2012	22.1855*	0.682118
		2013	22.50708	0.583701
		2014	22.41917	0.610091
		2015	22.84267*	0.688252
		2016	22.58758	0.661797
		2017	22.67208	0.647179
		2018	22.58025	0.671596
		2019	22.51542	0.661908
South Solitary Island	-30.20478, 153.26515			
		2010	22.9305	0.59465
		2011	22.82825	0.679919
		2012	22.40233*	0.660952
		2013	22.69692	0.565802
		2014	22.6065	0.593678

		2015	23.01858*	0.673844
		2016	22.74858	0.651197
		2017	22.84217	0.627153
		2018	22.76775	0.654827
		2019	22.67825	0.653097
Black Rock	-30.948371, 153.076078			
		2010	22.90892	0.597918
		2011	22.84942	0.702599
		2012	22.47933*	0.694391
		2013	22.90208	0.55749
		2014	22.7175	0.609029
		2015	23.06642	0.691458
		2016	22.92342*	0.64644
		2017	22.84558	0.627427
		2018	22.79383	0.641966
		2019	22.67667	0.663756

Table B3. The mean annual mean, minimum and maximum temperatures, standard error (°C, ±SEM), and temperature range for all subtropical sites in eastern Australia from 2010-2019. Black asterisk (*) marks the site with the highest variation in the study period.

Site	Mean	SEM	Min	Max	Range
Cook Island	23.40937	0.2011405*	19.657	26.932	7.275
Julian Rocks Nursery	23.34245	0.2006128	19.461	26.713	7.252
North Solitary Island	22.83648	0.1964133	19.248	26.671	7.423
Northwest Solitary Island	22.55177	0.2009287	18.788	26.433	7.645
Southwest Solitary Island	22.57353	0.1989232	18.765	26.448	7.683
South Solitary Island	22.75198	0.1941202	19.018	26.608	7.59
Black Rock	22.81632	0.1965385	18.699	26.756	8.057

Table B4. The mean annual mean, minimum and maximum temperature, standard error (°C, ±SEM), and temperature range for all years (January-December) (across sites). Red asterisk (*) marks the highest/hottest values, blue asterisk (*) marks the lowest/ coolest and black asterisk (*), marks the year with the highest variation.

Year	Mean	SEM	Min	Max	Range
2003	22.64585	0.2211444	18.983	25.899	6.916
2004	22.57379	0.2237762	19.45	26.481	7.031
2005	23.03	0.2261151	19.756	26.463	6.707
2006	22.83646	0.2215143	19.846	26.601	6.755
2007	22.53848	0.2349314	18.972	25.979	7.007
2008	22.41929	0.2142782	19.083	25.578	6.495
2009	22.66174	0.2301746	19.007	26.28	7.273
2010	23.10505	0.2316519	19.83	26.685	6.855
2011	22.90764	0.2552618	19.893	26.932*	7.039
2012	22.57338*	0.2459644	18.699*	25.841	7.142
2013	22.84695	0.2101528	19.676	26.209	6.533
2014	22.76632	0.2254355	19.203	25.613	6.41
2015	23.14306*	0.2515467*	19.557	26.654	7.097
2016	22.93758	0.2412529	19.791	26.603	6.812
2017	22.96463	0.238139	19.845	26.713	6.868
2018	22.92604	0.2473801	19.173	26.543	7.37
2019	22.8035	0.2450272	19.046	26.431	7.385

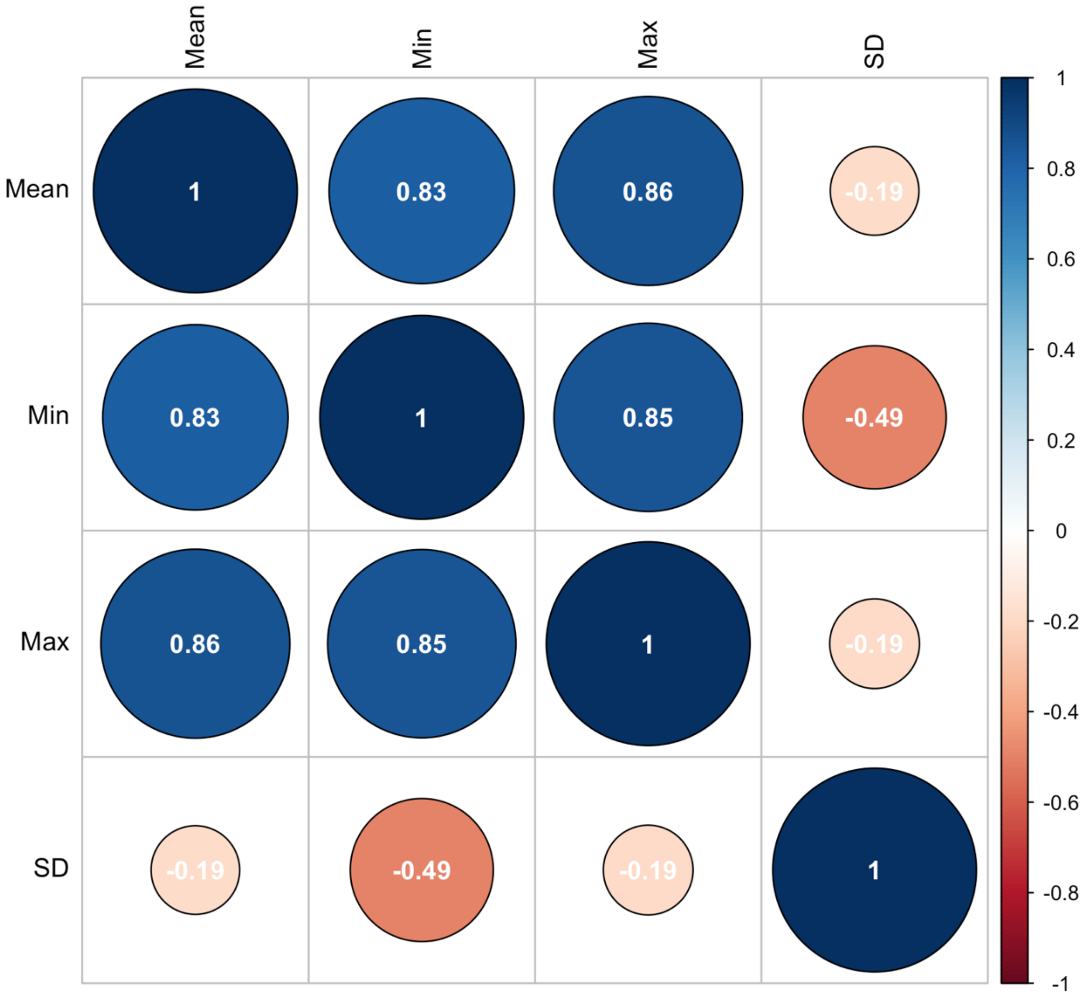

Figure B3. Correlation matrix of temperature parameters extracted for all sites from the Modis Aqua satellite (Parkinson 2003) at 1km spatial resolution for 2010-2019.

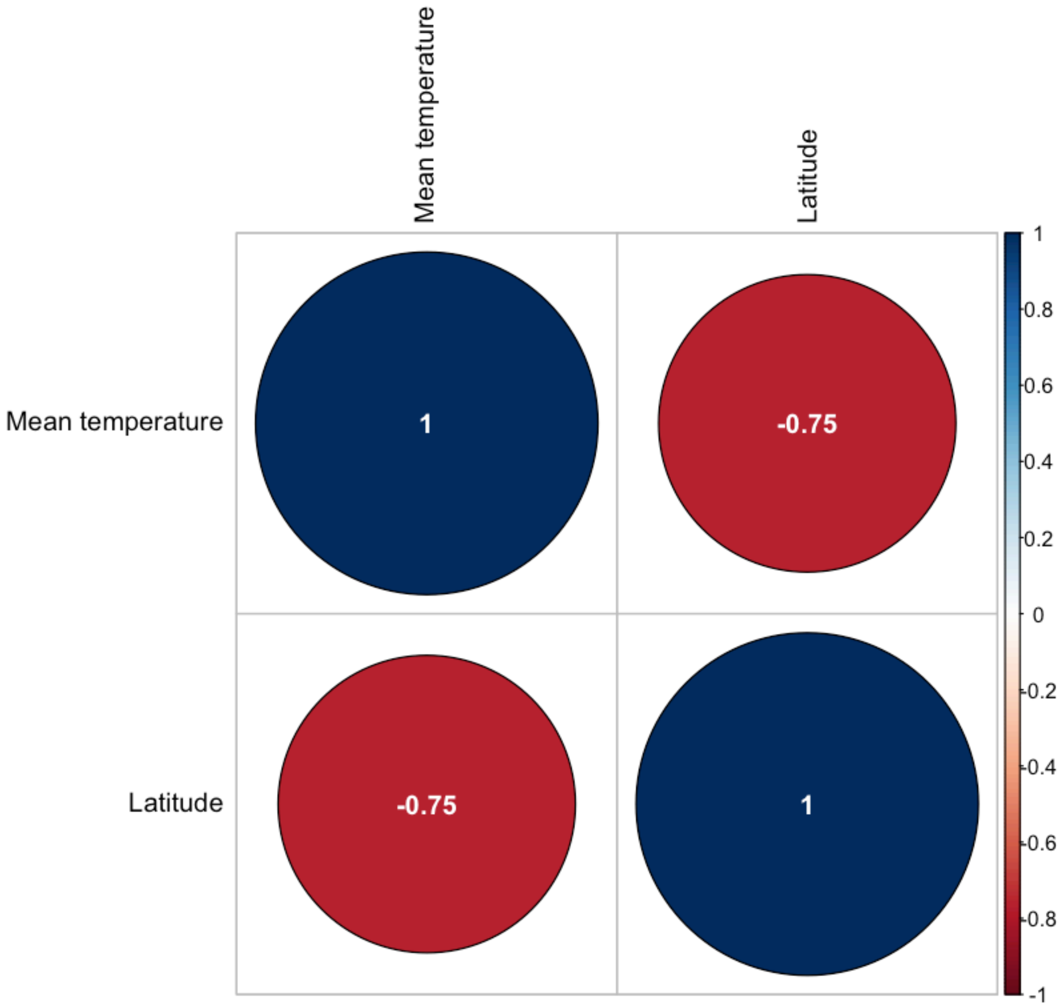

Figure B4. Correlation matrix of mean annual temperature and latitude for all sites from the Modis Aqua satellite (Parkinson 2003) at 1km spatial resolution for 2010-2019.

Table B5. Table of GLMM results for the models examining the relationship between mean temperature and sea urchin densities, and year and sea urchin densities.

Model		χ^2	df	p
Mean temperature ~ species density (m^{-2})				
	C. rodgersii	0.7978	1	0.37
	Diadema spp.	0.8039	1	0.3699
	T. australiae	130.19	1	<0.001*
	P. parvispinus	130.19	1	0.01*
Year ~ species density (m^{-2})				
	C. rodgersii	51.5114	3	<0.001*
	Diadema spp.	35.2437	3	<0.001*
	T. australiae	304.9966	3	<0.001*
	P. parvispinus	7.0481	3	0.07

Appendix C | Chapter 4 supplementary material A

Table C1. Primary morphological traits of *Tripneustes g. gratilla* and *T. australiae* (from McLaren et al. 2023) used to identify specimens at the Australian Museum (AM), Museums Victoria (MV), Sea Urchin Science Centre and Gallery (SUSCG) and the Natural History Museum (NHM).

Trait	*T. gratilla gratilla*	*T. australiae*
Test shape	high (TH > 63%TD)	depressed (mean TH 51%TD)
adapical ambulacral primary tubercles	every second plate	every second plate
ambital ambulacral primary tubercles	every second plate	every fourth plate
adoral ambulacral primary tubercles	every third plate	every second plate
ambulacral plates occluded from perradial suture	none	numerous (2 in 3 at ambitus)
size of interambulacral tubercles	half of plate height	full plate height
mean peristome size	28.1%TD	25.2%TD

Table C2. Latitudinal ranges of T. *australiae* and *T. g. gratilla* in the northwest Indo-Pacific, eastern Australia, the Tasman Sea from the AM, SUSCG, MV and NHM

Species	Range (Latitude)	Northernmost observation	Southernmost observation
Tripneustes g. gratilla	5° 13' 30" S → 35° 7' 12" S	Papua New Guinea	Bowen Island, Jervis Bay, NSW
Tripneustes australiae	28° 37' 0.12" S → 36° 15' 0" S	Byron Bay, Julian Rocks, NSW	Narooma, Montague Island, NSW

Table C3. Longitudinal ranges of *T. australiae* and *T. g. gratilla* in the Australian region and the Tasman Sea from the AM, MV, SUSCG and NHM

Species	Range (Longitude)	Westernmost observation	Easternmost observation	Modal longitude
Tripneustes g. gratilla	96° 52' 1.2" E → 159° 19' 1.2" E	Cocos (Keeling) Island	Lord Howe Island	159° 4' 58.8" E
Tripneustes australiae	150° 13' 48" E → 178° 34' 1.2" E	Narooma, Montague Island, NSW	Kermadec Islands	159° 4' 58.8" E

Table C4. Southern-most collections of *T. australiae* and *T. g. gratilla* in eastern Australia and the Tasman Sea from the AM, MV, SUSCG, and NHM

Species	Southernmost observations	Year of observation	No. of specimens	Institution
Tripneustes g. gratilla	Manly (33° 48' " S)	1979	1	AM
	Camp Cove, Port Jackson (33° 50' 30" S)	1968	1	AM
	Camp Cove, Port Jackson (33° 50' 30" S)	1966	1	AM
	Camp Cove, Port Jackson (33° 50' 30" S)	1999	1	AM
	Camp Cove, Port Jackson (33° 50' 30" S)	1999	1	AM
	Yarra Bay, Botany Bay (33° 59' 03" S)	2007	1	AM
	Jervis Bay (35° 03' " S)	1981	1	AM
Tripneustes australiae				
	Shellharbour (34° 35' " S)	1923	1	AM
	Jervis Bay (35° 03' " S)	1979	1	AM
				AM
	Jervis Bay (35° 03' " S)	1981	1	AM
	Narooma, Montague Island (36° 15' " S)	1978	2	AM
	Narooma, Montague Island (36° 15' " S)	1981	7	AM

Table C5. Eastern-most collections of *T. australiae and T. g. gratilla* in eastern Australia and the Tasman Sea

Species	Eastern-most observations	Longitude	Year collected	No. of specimens	Institution
T. australiae					
	Kermadec Islands	178° 54' 46.8" W	2016	1	NIWA
	Kermadec Islands	178° 49' 54.12" W	1977	1	NIWA
					NIWA
	Kermadec Islands	178° 49' 48.0072" W	1974	2	NIWA
T. g. gratilla					
	Lord Howe Rise	159.317	-	1	MV
	Lord Howe Island	-31.55	-	22	AM

Table C6. Post-hoc pairwise comparisons between ecoregions and respective groupings and co-efficient. Letters indicate Tukeys post-hoc groups. Groups that do not share a letter are significantly different (p <0.05). If two or more means share the same grouping symbol, then we cannot show them to be different based on the sample size. Confidence level is 0.95.

Ecoregion	Response	SE	df	Lower CL	Upper CL	Group
Cape Howe	0.010	0.0047	245	0.003	0.035	a
Central and Southern Great Barrier Reef	0.0119	0.005	245	0.0037	0.038	ab
Torres Strait Northern Great Barrier Reef	0.008	0.01	245	0.0003	0.2	ab
Coral Sea	0.01	0.0062	245	0.0023	0.051	ab
Manning-Hawkesbury	0.015	0.0036	245	0.0076	0.028	a
Tweed-Moreton	0.027	0.006	245	0.015	0.049	ab
Lord Howe and Norfolk Islands	0.046	0.0096	245	0.03	0.08	b

Table C7. Models and model coefficients used in the model selection process for habitat suitability modelling for *T. australiae* and *T. g. gratilla*. Models are presented with AIC values that were used for model selection.

	Model	AIC	Coefficient	Estimate	Standard error	z-value	p-value
T. australiae							
	presence ~ Mean_SST + Minimum_Depth + Mean_Surface_Salinity + Seawater_Velocity_at_Surface + ph	146.76	(Intercept)	-797.8	673.0	-1.185	0.236
			Mean_SST	0.761	0.199	3.83	<0.001 ***
			Minimum_depth	0.00718	0.002799	2.565	0.0103 *
			Mean_Surface_Salinity	-2.302	4.432	-0.519	0.603
			Seawater_Velocity_at_surface	-9.32	2.498	-3.731	<0.001 ***
			pH	-797.8	673.0	-1.185	0.236
	presence ~ Mean_SST + Minimum_Depth + Seawater_Velocity_at_Surface + Mean_Surface_Salinity	141.6	(Intercept)	355.15	77.59	4.577	<0.001 ***
			Mean_SST	0.754	0.180	4.177	<0.001 ***
			Minimum_Depth	0.004	0.001729	2.313	0.0207 *
			Seawater_Velocity_at_Surface	-12.0	2.295	-5.229	<0.001 ***
			Mean_Surface_Salinity	-10.36	2.233	-4.638	<0.001 ***
	presence ~ Mean_SST + Minimum_Depth + Seawater_Velocity_at_Surface + ph	141.49	(Intercept)	-1180.0	265.3	-4.447	<0.001 ***
			Mean_SST	0.8194	0.1963	4.175	<0.001 ***
			Minimum_Depth	0.008302	0.002474	3.356	0.00079 ***
			Seawater_Velocity_at_Surface	-8.772	1.811	-4.845	<0.001 ***
			ph	144.0	32.47	4.434	<0.001 ***
	presence ~ Mean_SST + Minimum_Depth + Mean_Surface_Salinity + ph	149.96	(Intercept)	-2307.0	605.5	-3.810	<0.001 ***

			Mean_SST	0.4629	0.1738	2.664	0.0077 **
			Minimum_Depth	0.01059	0.002851	3.716	0.0002 ***
			Mean_Surface_Salinity	10.24	3.221	3.180	0.0015 **
			ph	239.0	61.45	3.889	<0.001 ***
T. g. gratilla							
	presence ~ Mean_SST + Minimum_Depth + Mean_Surface_Salinity + Seawater_Velocity_at_Surface	106.38	(Intercept)	27.87	21.38	1.303	0.192
			Mean_SST	-0.2479	0.1024	-2.422	0.0154 *
			Minimum_Depth	0.0008394	0.0004239	1.980	0.0477 *
			Mean_Surface_Salinity	-0.6043	0.5538	-1.091	0.275
			Seawater_Velocity_at_Surface	-1.737	1.662	-1.045	0.296
	presence ~ Minimum_Depth + Seawater_Velocity_at_Surface + Mean_Surface_Salinity	107.1	(Intercept)	-1.808	11.84	-0.153	0.879
			Minimum_Depth	0.000937	0.0004304	2.177	0.0295 *
			Seawater_Velocity_at_Surface	-1.667	1.653	-1.009	0.313
			Mean_Surface_Salinity	0.0628	0.3389	0.185	0.853
	presence ~ Mean_SST + Minimum_Depth + Mean_Surface_Salinity	104.07	(Intercept)	28.04	20.02	1.401	0.161
			Mean_SST	-0.1857	0.09922	-1.871	0.061
			Minimum_Depth	0.001248	0.0005606	2.226	0.026 *
			Mean_Surface_Salinity	-0.6599	0.5164	-1.278	0.201
	presence ~ Mean_SST + Minimum_Depth	103.08	(Intercept)	2.424	1.750	1.385	0.166
			Mean_SST	-0.08983	0.06932	-1.296	0.195
			Minimum_Depth	0.001297	0.0005676	2.285	0.022 *

Appendix D | Chapter 4 supplementary material B

Methods

Kermadec Island material from museums in New Zealand

Specimens from the National Institute of Water and Atmospheric Research (NIWA, New Zealand) (n = 60) were not directly examined. As a result, they were not included in the distribution analysis but were considered to contribute information on the distribution of *T. australiae* in New Zealand. Specimens from NIWA that were identified as *T. kermadecensis*, were assumed to represent *T. australiae*, and were all collected from the Kermadec Islands. The collection of *T. australiae* from the Kermadec Islands is not numerous, and as such we have used collection data reported in the description of *T. kermadecensis* (Bronstein et al. 2017) in addition to the specimens from NIWA and the AM. These specimens are housed in the Natural History Museum Vienna.

Habitat suitability modelling

Environmental predictors

Distribution data for *Tripneustes australiae was* derived from the museum collections data outlined above. Mean sea surface temperate (SST), sea water surface velocity (SWV), mean pH (pH), mean sea surface salinity (SSS) and minimum depth (MD) were extracted for the period from 2010-2020 from Bio-Oracle for the area of interest (***T. australiae:*** - 40 to - 20 S and 179.5 to 165.5 W).

Model and model validation

Habitat suitability modelling was undertaken using a Maximum Entropy Model (Maxent) implemented through the R package *dismo* (Hijmans et al. 2010). Maxent model are machine learning models that model species distributions using presence only data (Phillips 2006). We performed model selection using the *glm* function in ENMTools (Warren et al. 2021) to determine the Akaike Information Criterion (AIC) of different predictor combinations (Warren & Seifert 2011). The predictor variables were checked for co-linearity ($p < 0.7$), pH and SST were colinear, and as such pH was removed from the model. Initially, a full model was constructed for both species using all environmental variables (SST, SWV, SSS and MD) and presence. Model selection was then performed by eliminating non-significant variables and selecting the model with the lowest Akaike Information Criterion (AIC). Then the model with the lowest AIC value was used to create a Maxent model for analysis and predictions. These were MD and SSS (AIC = 10.75, Table D1). To account for any sampling bias across the museum institutions in the modelling process, a 'bias layer' was constructed for both species using the kernel density estimation in *MASS* (Ripley and Venables 2009) and used in the final models. All predictor layers were projected using GDA94 using the R package *Raster* (Hijmans et al. 2010). The MaxENT models were trained on 75% of the presence data set with the final model variables as predictors and the remaining 25% were used for model validation. The models were 5-fold cross validated, resulting in 5 replicate models. The area under the curve (AUC) from a receiver operating characteristic (ROC) plot was averaged over the replicate runs to give a global AUC to determine the predictive accuracy of the models.

Results

The final habitat suitability model (variables: MD and SSS) for *T. australiae* in the Kermadec Island region had a high predictive capacity (AUC = 0.982± 0.013 SD), indicating the model prediction was reliable. Depth was the only predictor of *T. australiae* habitat suitability (100%). As depth was the only accurate driver of habitat suitability for *T. australiae* in the Kermadec Islands, and because depth is not an accurate measure of species physiology and should be avoided when performing future projections (Anderson et al. 2011) no predictions for the 2090-2100 period were included.

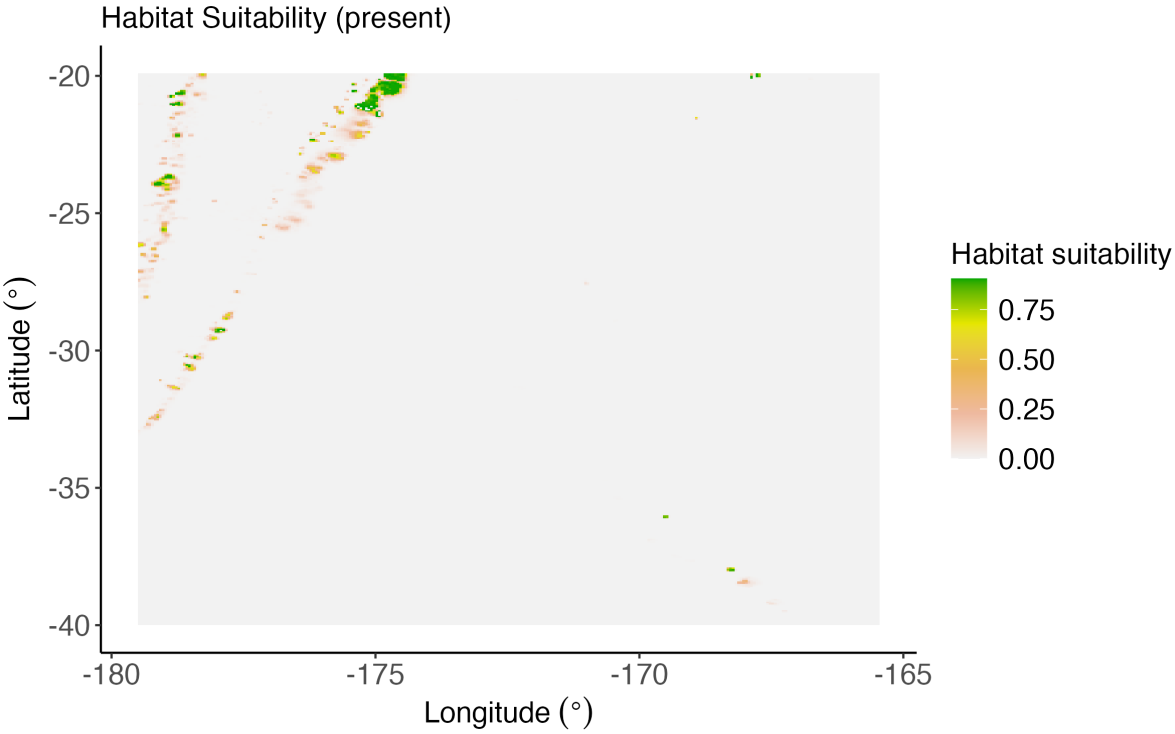

Figure D1. Habitat suitability of *T. australiae* in the Kermadec Islands on model predictions using minimum depth and current sea surface salinity.

Appendix E | Chapter 5 supplementary material

Table E1. Post-hoc pairwise comparisons for survival between day post fertilisation and rearing temperatures and their respective groupings and co-efficient. Letters indicate Tukeys post-hoc groups. Groups that do not share a letter are significantly different (p <0.05). If two or more means share the same grouping symbol, then we cannot show them to be different based on the sample size. Confidence level is 0.95.

Day	Temp	Response	SE	df	Group
1	14	0.947	0.045	78	a
	17	0.998	0.010	78	a
	20	0.992	0.021	78	a
	23	0.962	0.043	78	a
	26	1.000	0.000	78	a
2	14	0.977	0.033	78	a
	17	0.996	0.015	78	a
	20	0.931	0.057	78	a
	23	0.918	0.062	78	a
	26	0.971	0.038	78	a
3	14	0.948	0.050	78	a
	17	0.988	0.025	78	a
	20	0.989	0.023	78	a
	23	0.992	0.018	78	a
	26	0.992	0.018	78	a
5	14	0.440	0.091	78	a
	17	1.000	0.000	78	b
	20	0.998	0.010	78	b
	23	0.947	0.050	78	b
	26	0.992	0.021	78	b
7	14	0.000	0.000	78	a
	17	0.971	0.038	78	c
	20	0.907	0.065	78	bc
	23	0.939	0.054	78	bc
	26	0.644	0.108	78	b

Table E2. Post-hoc pairwise comparisons for normality between day post fertilisation and rearing temperatures and their respective groupings and co-efficient. Letters indicate Tukeys post-hoc groups. Groups that do not share a letter are significantly different (p <0.05). If two or more means share the same grouping symbol, then we cannot show them to be different based on the sample size. Confidence level is 0.95.

Day	Temperature	Response	SE	df	Group
1	14	0.999	0.005	78	a
	17	0.992	0.015	78	a
	20	0.952	0.036	78	a
	23	0.969	0.029	78	a
	26	0.988	0.018	78	a
2	14	0.988	0.018	78	b
	17	0.996	0.011	78	b
	20	0.979	0.024	78	b
	23	0.994	0.013	78	b
	26	0.705	0.076	78	a
3	14	0.954	0.035	78	b
	17	0.998	0.008	78	b
	20	0.947	0.038	78	b
	23	0.964	0.028	78	b
	26	0.716	0.067	78	a
5	14	0.999	0.004	78	b
	17	0.976	0.026	78	ab
	20	0.956	0.034	78	ab
	23	0.984	0.021	78	b
	26	0.784	0.069	78	a
7	14	1.000	0.000	78	b
	17	0.942	0.039	78	b
	20	0.923	0.044	78	b
	23	0.901	0.050	78	ab
	26	0.629	0.081	78	a

Table E3. The mean number of *Tripneustes australiae* larvae across replicates (± SEM, n = 4) at different developmental stages for each day post-fertilization (n = 6) and rearing temperature (n = 5).

Day	Temp	Developmental Stage	Mean	SEM
1	14	Cleavage	100	0
	17	Blastula	100	0.00
	20	Blastula	100	0.00
	23	Blastula	100	0.00
	26	Blastula	100	0.00
2	14	Abnormal/Dead	6.67	3.33
	14	Blastula	95	2.89
	17	Blastula	100	0.00
	20	Abnormal/Dead	15.00	8.33
	20	Blastula	58.33	9.08
	20	Gastrula	34.17	12.72
	23	Abnormal/Dead	13.33	NA
	23	Blastula	15.83	5.51
	23	Gastrula	80.00	5.93
	23	Prism	3.33	NA
	26	Blastula	6.79	3.21
	26	Gastrula	8.89	5.56
	26	Pluteus	14.29	NA
	26	Prism	86.37	3.70
3	14	Abnormal/Dead	3.33	NA
	14	Blastula	99.17	0.83
	17	Abnormal/Dead	3.33	NA
	17	Blastula	12.22	1.11
	17	Gastrula	89.17	2.5
	17	Prism	3.33	NA

	20	Abnormal/Dead	6.67	3.33
	20	Blastula	22.22	8.68
	20	Gastrula	49.17	7.12
	20	Prism	38.89	19.37
	23	Abnormal/Dead	3.33	0.00
	23	Blastula	21.67	1.67
	23	Gastrula	13.33	6.67
	23	Pluteus	100	0
	23	Prism	61.67	5
	26	Abnormal/Dead	13.33	NA
	26	Blastula	35	11.67
	26	Gastrula	12.5	5.34
	26	Pluteus	61.7	20.21
	26	Prism	10	6.67
5	14	Abnormal/Dead	46.67	43.33
	14	Blastula	25.56	20.58
	14	Gastrula	57.5	24.20
	17	Abnormal/Dead	6.67	NA
	17	Gastrula	8.56	5.23
	17	Pluteus	76.21	12.63
	17	Prism	23.79	11.08
	20	Abnormal/Dead	26.67	NA
	20	Gastrula	10	NA
	20	Pluteus	73.33	12.69
	20	Prism	23.33	7.70
	23	Blastula	3.33	NA
	23	Gastrula	3.33	NA
	23	Pluteus	97.5	1.60
	23	Prism	3.33	NA
	26	Abnormal/Dead	23.33	NA

	26	Gastrula	6.67	NA
	26	Pluteus	92.5	5.51
7	14	Abnormal/Dead	100	0.00
	17	Abnormal/Dead	17.78	4.84
	17	Pluteus	65.83	15.18
	17	Prism	41.67	21.67
	20	Abnormal/Dead	22.22	8.89
	20	Pluteus	83.33	8.39
	23	Abnormal/Dead	25.83	5.99
	23	Pluteus	74.17	5.99
	26	Abnormal/Dead	88.89	5.88
	26	Blastula	3.33	NA
	26	Pluteus	43.33	28.35
9	17	Abnormal/Dead	35.83	14.81
	17	Pluteus	61.67	17.29
	17	Prism	10	NA
	20	Abnormal/Dead	25	6.45
	20	Pluteus	74.17	6.44
	20	Prism	3.33	NA
	23	Abnormal/Dead	35.56	9.69
	23	Pluteus	73.33	11.22
	26	Abnormal/Dead	96.67	3.33
	26	Gastrula	6.67	NA

Fin