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Impacts of ocean warming on the Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*)

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Statement of contribution

This thesis was conducted under the supervision of Prof. Morgan Pratchett[1], Dr. Jennifer Donelson[1], Dr. Ciemon Caballes[1,4], Dr. Sven Uthicke[2] and Prof. Andrew Hoey[1]. Please see the table below for specific contributions by supervisors and other collaborators.

Chapter	**Contributor**	**Nature of Assistance**
Chapter 1: General introduction	Morgan Pratchett[1]	Conceptualisation/chapter preparation
Chapter 2: Impacts of ocean warming on echinoderms: A meta-analysis	Jennifer Donelson[1]	Conceptualisation/data analysis/manuscript preparation
	Kevin Bairos-Novak[1]	Conceptualisation/data analysis/manuscript preparation
	Carolyn Wheeler[1,3]	Conceptualisation/data analysis/manuscript preparation
	Ciemon Caballes[1,4]	Manuscript preparation
	Sven Uthicke[2]	Manuscript preparation
	Morgan Pratchett[1]	Conceptualisation/manuscript preparation
Chapter 3: Impacts of ocean warming on the settlement success and post-settlement survival of Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*)	Ciemon Caballes[1,4]	Conceptualisation/data analysis/manuscript preparation
	Sven Uthicke[2]	Conceptualisation/larval rearing/data analysis/manuscript preparation
	Peter Doll[1]	Conceptualisation/larval rearing/data analysis/manuscript preparation
	Jennifer Donelson[1]	Conceptualisation/data analysis/manuscript preparation
	Morgan Pratchett[1]	Conceptualisation/data analysis/manuscript preparation
Chapter 4: Metabolic responses of Pacific crown-of-thorns starfish	Jennifer Donelson[1]	Conceptualisation/data collection/data analysis/manuscript preparation
	Ciemon Caballes[1,4]	Data analysis/manuscript preparation

(*Acanthaster* cf. *solaris*) to acute warming	Peter Doll[1] Morgan Pratchett[1]	Data collection/data analysis/manuscript preparation Conceptualisation/data collection/data analysis/manuscript preparation
Chapter 5: Effects of elevated temperature on the performance and survival of Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*)	Jennifer Donelson[1] Ciemon Caballes[1,4] Sven Uthicke[2] Peter Doll[1] Morgan Pratchett[1]	Conceptualisation/data collection/data analysis/manuscript preparation Data analysis/manuscript preparation Conceptualisation/data analysis/manuscript preparation Conceptualisation/data collection/data analysis/manuscript preparation Conceptualisation/data collection/data analysis/manuscript preparation
Chapter 6: General discussion	Morgan Pratchett[1] Jennifer Donelson[1]	Conceptualisation/chapter preparation Chapter preparation

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Abstract

Anthropogenic climate change and concomitant ocean warming are threatening marine ecosystems globally. Coral reefs are particularly vulnerable, with the incidence and severity of temperature-induced coral bleaching events rapidly increasing. Coral reefs in the Indo-Pacific region are further threatened by population irruptions of crown-of-thorns starfish (CoTS; *Acanthaster* spp.), which preferentially feed on reef-building corals. While coral bleaching is likely to worsen as the oceans warm, there is currently limited understanding of how elevated temperatures may impact CoTS. This understanding is critical in order to predict the future spatial and temporal dynamics of CoTS population irruptions, and hence the resilience and vulnerability of coral reefs under near-future climate change.

The overarching objective of this thesis was to assess how elevated temperatures will affect the physiological performance and population dynamics of Pacific CoTS (*Acanthaster* cf. *solaris*). To address this objective, four distinct studies (presented as independent chapters) were undertaken to (1) establish broad patterns in responses of echinoderms to elevated temperature, in order to anticipate the vulnerability of CoTS to warming, (2) determine the effect of warming on the processes associated with CoTS larval settlement and successful recruitment into the benthic population, (3) establish the effect of acute warming on the metabolism of the coral-feeding life stages of CoTS, at both the organism and biochemical level, and finally (4) ascertain how prolonged warming impacts metabolism, activity and survival in the coral-feeding life stages of CoTS. All CoTS used in the experiments presented herein were collected from the central Great Barrier Reef (GBR), Australia.

Meta-analyses provide a heuristic statistical tool for quantitatively examining broad patterns in responses to environmental conditions among taxa. In **Chapter 2**, a comprehensive literature search resulted in the amalgamation of 710 individual responses from 85 studies exploring the effects of elevated temperature on 47 species of echinoderm. Patterns in responses to warming were explored based on the following predictors: biological response measured, ontogenetic life stage, taxonomic class, and region. In general ocean warming led to a significantly accelerated metabolic rate, but reduced survival in echinoderms, and those in the tropical and sub-tropical regions had the greatest susceptibility to warming induced declines in performance. Starfish appeared to be the most vulnerable class of echinoderms. These results

indicate that CoTS, which are tropical starfish, may be particularly vulnerable to ocean warming. It was also found that elevated temperatures were especially harmful to echinoderms at the larval stage, indicating that this life stage may be the greatest population bottleneck.

These identified negative impacts of elevated temperature on larvae, tropical echinoderms and starfish, suggest that the CoTS larval stage and associated processes may be particularly vulnerable to warming. Settlement of larvae onto algal substrate, and subsequent metamorphosis into juveniles with radial symmetry is considered a major population bottleneck in marine invertebrates, including CoTS. Thus, understanding how these processes may be affected by ocean warming is important. In **Chapter 3**, the impact of elevated temperature on the settlement success, metamorphic success, and post-settlement survival of CoTS were explored, as well as how warming influenced the quality of the crustose coralline algae (CCA) settlement substrate, and thus settlement success. Larvae were divided into four temperature treatments: 28 °C (ambient control), representing the mean summertime temperature, and the temperature at which CoTS spawn on the GBR; 30 °C, representing the maximum summertime temperature; and 32 °C and 34 °C, representing future climate change scenarios, respectively. Within each temperature treatment, half of the larvae were introduced to pre-temperature treated CCA, while the other half contained CCA that was maintained at the control temperature. Larval settlement was robust to warming, with the majority of larvae settling across the treatments, although at temperatures of ≥ 32 °C, settlement success was 12-14% lower on pre-treated CCA. At temperatures as cool as 30 to 32 °C, metamorphic success and survival were significantly lower than at ambient temperature (5 and 15% lower respectively). Together with prior knowledge regarding the thermal effects on the early life stages of CoTS, these observations indicate that the cumulative effects of warming on physiological processes early in life, may inhibit successful recruitment, and reduce the replenishment of the adult population.

There is little knowledge regarding how adult CoTS will respond to near-future ocean warming. Answering this question is of paramount importance, considering that adults feed on vast quantities of reef-building corals, and are responsible for reseeding the population. Acute thermal ramping experiments are a useful way of visualising thermal performance and limits in marine organisms and may provide an indication of responses to short-term marine heatwaves. In **Chapter 4**, acute physiological responses to warming were assessed both at the organism and biochemical levels in order to establish the thermosensitivity of coral-feeding CoTS. The standard metabolic rate of CoTS was determined using oxygen consumption rate as a proxy, which was measured incrementally over a 24 to 36 °C thermal range, at a warming rate of 1 °C hr^{-1}. Spectrophotometry was used to measure the activity of the enzymes citrate synthase (CS), which is a proxy for aerobic metabolism, and lactate dehydrogenase (LDH) which is a proxy for anaerobic metabolism, again over wide thermal ranges (15 to 40 °C and 20 to 45 °C respectively). In large CoTS, the standard metabolic rate increased with warming up until a certain point, with clear metabolic depression observed > 33 °C, while on the other hand, there was only some evidence of metabolic depression > 35 °C in small CoTS. Taken together these results may indicate that the reproductive maturity of large CoTS reduces the energy reserves available, and increases the vulnerability to warming. Activity of CS declined with warming, with negligible activity occurring at similar temperatures to which the standard metabolic rate was constrained (≥ 35 °C). LDH activity, on the other hand, increased with warming, demonstrating a greater reliance on unsustainable anaerobic metabolism at higher temperatures.

Under near-future climate change, CoTS may experience supra-optimal temperatures for periods of weeks to months. Therefore, understanding how prolonged warming impacts the physiology, behaviour and persistence of CoTS is necessary. In **Chapter 5**, coral-feeding CoTS were exposed to one of four temperature treatments: 26 °C (ambient control), representing the mean annual temperature, 28 and 30 °C, representing the average and maximum summertime temperatures, and 32 °C representing a temperature expected to frequently occur on the reef under near-future ocean warming. The righting time, movement rate, standard metabolic rate and survival of the CoTS from each treatment were measured at various points over approximately 60 days. While starfish righted themselves over the temperature range with little difficulty, movement rate was significantly impacted by temperature, with a peak in activity at 30 °C, followed by a decline at 32 °C. This pattern was mirrored by the observations for standard metabolic rate, which may be expected considering that metabolic depression likely leads to a shortage of energy available for activity. Ultimately, CoTS survival was significantly lower at 32 °C compared to the cooler temperature treatments, indicating that at this temperature their thermal optimum is surpassed.

The findings presented herein, together with prior knowledge of thermal effects on immature CoTS and other tropical echinoderms, indicate that the physiological performance of CoTS may be compromised at temperatures ≥ 30 °C, which are predicted to frequently occur on the reef in the near-future. Large adult and settlement-stage CoTS appear particularly vulnerable, with the latter being further impacted by a reduced food and habitat quality at elevated temperatures. Longer exposure periods may introduce additional constraints to both the CoTS and the benthic algae or coral that they rely on. Considering the increased prevalence and intensity of marine heatwaves in recent years, supra-optimal temperatures may be exceeded far sooner for periods of days to weeks. Thus, reductions in the occurrence and severity of population outbreaks may be expected in the near-future, relieving predation pressure on prey corals. Interactions between CoTS and their thermosensitive coral prey in a warmer ocean may also have profound effects on the persistence of CoTS populations, in addition to possible acclimation, adaptation and range shifts, and are thus important directions of further research.

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Chapter 1: General Introduction

1.1 Climate change and impacts on marine ectotherms

Climate change, caused by anthropogenic greenhouse gas emissions, is the most significant emerging threat facing living organisms (IPCC, 2021). Global sea surface temperature have risen by more than 1 °C since pre-industrial times, and are expected to increase a further ~3 °C by the end of the century (relative to 1986-2005; IPCC, 2019; 2021). Predicted end-of-century ocean temperatures are already being experienced in the marine environment during severe marine heatwaves (*i.e.*, warming above the 90th percentile for at least 5 consecutive days; Hobday et al., 2016; Oliver et al., 2018; GBRMPA, 2019; Bureau of Meteorology, 2020; IPCC, 2021), with significant impacts on marine ecosystems and their inhabitants already evident (Poloczanska et al., 2016; Mellin et al., 2019; Filbee-Dexter et al., 2020; Strydom et al., 2020). These ongoing increases in water temperatures have important implications for most marine organisms as they are ectothermic, meaning their physiological processes are inherently governed by their thermal environment (Lagerspetz & Vainio, 2006; Cheung et al., 2009; Nguyen et al., 2011). When physiological thermal limits (Pejus limits; Pörtner et al., 2017) are exceeded, the condition and performance of these organisms is compromised, which can ultimately lead to changes in the abundance and distribution of species (Poloczanska et al., 2016).

1.2 Physiological responses to warming

The physiological capacity of marine ectotherms is governed by metabolism, which is the sum of all chemical reactions occurring within the body (de Nava & Raja, 2022). Due to increased rates of reactions at higher temperatures, the metabolic rate of ectotherms scales positively with warming until an optimal temperature (Clarke & Fraser, 2004; Schulte et al., 2011; Schulte, 2015). At temperatures above the thermal optimum (supra-optimal temperatures), organisms are unable to respire fast enough to meet the growing oxygen demand, which ultimately leads to declines in metabolic rate and performance (Pörtner & Knust, 2007; Pörtner et al., 2017). With increasing temperatures above the thermal optimum, organisms begin to rely on energy deficient and unsustainable anaerobic metabolism, which can greatly impact on individual performance and condition, possibly even resulting in mortality (Pichaud et al., 2017; Pörtner & Knust, 2007; Pörtner et al., 2017), as documented for a wide diversity of marine invertebrates and fishes (Svendsen et al., 2016; Pereira Santos et al., 2021; Sampaio et al., 2021). Temperature induced mortality may also result from protein denaturation (Pörtner et al., 2017). However, the initiation of a heat shock response, which leads to the repair of damaged proteins, may enable marine ectotherms to endure periods of supra-optimal temperatures (Sconzo et al., 1986; Pörtner et al., 2017; Kühnhold et al., 2019b)

1.3 Patterns in vulnerability to warming

The vulnerability of marine ectotherms to warming (defined throughout this thesis as the relative amount of harm experienced to an organism when their thermal optimum is exceeded; Malone & Engel, 2011), varies both among and within species (Byrne, 2011; Sunday et al., 2011; Hughes et al., 2018a; Dahlke et al., 2020; Sampaio et al., 2021). Owing to the relatively low annual thermal variability at low latitudes, tropical ectotherms are characterised by a narrower thermal breadth (the range of temperatures in which performance remains high, Schulte et al., 2011), in comparison to those from temperate latitudes (Sunday et al., 2011; 2014; Hughes et al., 2018a). Tropical species live particularly close to their thermal limits, have limited acclimation capacity, and are thus highly vulnerable to ocean warming (Nguyen et al., 2011; Vinagre et al., 2016; Hughes et al., 2018a). Accordingly, Sampaio et al. (2021) showed that tropical organisms (including fishes, molluscs and crustaceans) were generally more adversely affected by elevated temperature than their temperate counterparts.

The vulnerability of marine ectotherms to warming may vary throughout different ontogenetic life stages (Pandori & Sorte, 2018). This may in part be due to a change in the habitats occupied, and the corresponding thermal environment, throughout the life cycle. Marine ectotherms often invest a great deal of energy in gamete production, shrinking the reserves available for normal bodily functions and processes (Lawrence, 1984; Dahlke et al., 2020). Thus, large, fecund adult individuals, immediately prior to spawning, may be highly vulnerable to warmer, more physiologically demanding temperatures (Pörtner et al., 2017). For example, spawning adult fishes were shown to have reduced thermal limits that were more than 50% lower than non-spawning fishes (Dahlke et al., 2020). Furthermore, embryos, larvae and early juveniles have limited energy reserves and physiological capacities and may be less able to maintain homeostasis by ion regulation, a heat shock response, or behavioural thermoregulation compared to adults (Munday et al., 2008; Byrne, 2011; Nguyen et al., 2011; Dahlke et al., 2020). Indeed, embryos and larvae of a plethora of marine invertebrates were found to have > 60% more negative responses to warming, in comparison to adults (Pandori & Sorte, 2018).

1.4 Ontogenetic shifts in vulnerability to warming

The early life stages (embryos, larvae and juveniles) of marine ectotherms often represent a major population bottleneck, whereby high rates of mortality during these critical life stages often constrain population replenishment and population size (Doherty et al., 2004; Byrne, 2011; Wilmes et al., 2018). This bottleneck may intensify in a warmer ocean if the early life stages are particularly vulnerable to elevated temperature (Dahlke et al., 2020), and if the timing of marine heatwaves aligns with spawning and early development (Shanks et al., 2019). Gametes and the fertilisation process are often considered the most thermally robust of the early stages, due to maternal provisioning of energy and molecular chaperones including heat shock proteins, which protect eggs from supra-optimal temperatures (Hamdoun & Epel, 2007; Byrne, 2011; Caballes et al., 2017; Lockwood et al., 2017; Gall et al., 2021). A number of studies have reported little effect of warming on gamete quality and fertilisation success in marine ectotherms (Ling et al., 2008; Byrne et al., 2010; Kamya et al., 2014; Suckling et al., 2015). However, less maternal provisioning in the embryonic and larval stages may impede their ability to cope with temperatures above the thermal optimum (Hamdoun & Epel, 2007; Munday et al., 2008; Pankhurst & Munday, 2011; Gall et al., 2021; Sampaio et al., 2021). One study found an ~18% decline in survival of embryos of the Sydney Rock Oyster (*Saccostrea glomarata*) at temperatures 4 °C above those experienced in their natural environment (Parker et al., 2010), while another determined that, with only 2 °C of warming above the control temperature, mortality of larvae of the starfish *Meridiastra calcar* (also from the Sydney area) increased by ~67% (Nguyen et al., 2012). Aside from reduced survival, diminished quality of these early life stages may impede the ability for them to successfully settle and metamorphose into juveniles (Underwood & Fairweather, 1989; Hoey & McCormick, 2004; Przeslawski et al., 2008; Uthicke et al., 2015). Metamorphosis is considered an energetically costly process and may add further constraints on recruitment in a warmer ocean (in this thesis, recruitment is defined as the process by which individuals survive the population bottleneck at the early life stages and are added to the adult population; Shilling et al., 1996; Wendt, 2000). However, most studies examining thermal effects on early development and processes, do not consider this important bottleneck at settlement and metamorphosis (but see Li et al., 2011; Chen & Chen, 1992; Randall & Szmant, 2009), which likely has major impacts on the replenishment of the adult population (Pörtner et al., 2017; Espinel-Velasco et al., 2018; Wilmes et al., 2018; Glockner-Fagetti & Phillips, 2020; Sampaio et al., 2021).

In a warmer ocean, adult marine ectotherms may exhibit reduced growth, movement, and reproductive capacity (Pörtner & Knust, 2007; Byrne, 2011; Johansen et al., 2015; Pereira Santos et al., 2021; Sampaio et al., 2021). When exposed to temperatures 3 °C above the summertime maximum temperature, somatic and reproductive growth in the sea urchin *Echinometra sp.* from the GBR declined by ~29% and ~18%, respectively (Uthicke et al., 2014). Furthermore, temperatures of just 1.5 °C above the average summertime temperature, reduced the reproductive output of the coral reef damselfish *Acanthochromis polyacanthus* by ~26%, and their clutch size and egg area by ~17% and ~11%, respectively (Donelson et al., 2010). When provided with low food, no fish reproduced at this temperature, indicating energetic constraints associated with warming (Donelson et al., 2010). Less energy available at warmer temperatures may lower the capacity for marine ectotherms to move in their environment (Kidawa et al., 2010; Wood et al., 2010; Brothers & McClintock, 2015; Sun et al., 2018). The righting and reburying behaviour in Antarctic molluscs *Laternula elliptica* and *Nacella concinna*, for instance, halved with only 2 to 3 °C of warming above ambient temperature (Peck et al., 2004); while the Antarctic starfish *Odontaster validus* were no longer able to right themselves at 11 °C above ambient, which was linked to the metabolic depression observed at a similar temperature (Peck et al., 2008). The ability to reduce activity levels in order to conserve energy may be beneficial during short-term exposure to supra-optimal temperatures, with this plasticity contributing towards the lower vulnerability of adults to warming, compared to the early life stages (Byrne, 2011; Nguyen et al., 2011; Sun et al., 2018; Dahlke et al., 2020). However, constraints to movement may lead to a reduced ability to obtain nutrition and evade predators, which may increase mortality risk (Peck et al., 2004; 2008; Kidawa et al., 2010; Wood et al., 2010; Brothers & McClintock, 2015; Harianto et al., 2018; Sun et al., 2018).

1.5 Indirect effects of ocean warming

Temperature-induced constraints on movement, feeding and predator evasion, may be further exacerbated by indirect effects, such as climate-induced habitat degradation (De'ath et al., 2012; Ling, 2013; Hughes et al., 2017; Huggett et al., 2018; Filbee-Dexter et al., 2020; Strydom et al., 2020). On coral reefs for example, changing environmental conditions represent the foremost threat to habitat structure, whereby climate-induced coral bleaching has caused extensive mass coral bleaching and coral loss (Hughes et al., 2017). Coral reef ecosystems are also reliant on crustose coralline algae (CCA), which contributes to the structure of coral reefs, via cementation, and provides a substrate for larval settlement of marine invertebrates (Tiechert et al., 2020). However, studies have demonstrated that temperature-induced bleaching of CCA and a change in their microbial community can negatively impact successful settlement and metamorphosis of marine invertebrates at elevated temperatures (Dworjanyn & Pirozzi, 2008; Webster et al., 2011; Huggett et al., 2018). A reduced abundance of coral and CCA in a warmer ocean may result in a phase shift to fleshy macroalgae (Hoegh-Guldberg et al., 2007). Climate-driven phase shifts have also been reported in kelp forests, with impacts on the abundance, composition, and diversity of habitat specialists (Ling, 2008, 2013; Filbee-Dexter et al., 2020). Most notably, the sea urchin *Centrostephanus rodgersii* has instigated the shift of kelp forests to urchin barrens off the coast of Tasmania, culminating in the loss of around 150 taxa that relied on kelp for survival (Ling, 2008; 2013).

1.6 Effect of exposure duration

Supra-optimal temperatures may occur for short periods of time, during marine heatwaves, or may be continuous and accelerating due to sustained and progressive ocean warming (IPCC, 2019; 2021). The length of time that marine ectotherms, and their habitat and prey, are exposed to supra-optimal temperatures has a major bearing on the magnitude and extent of impacts. For example, adult life stages may be robust to short-term exposure to elevated temperatures, due to acclimation via physiological and behavioural plasticity (Kidawa et al., 2010; Kühnhold et al., 2019a; b; Mardones et al., 2022), including increasing metabolic rate to meet energetic demands (Mardones et al., 2022), and reducing activity to conserve energy (Peck et al., 2004; 2008). Prolonged warming, however, may be detrimental, as has been shown in various trout species, where the thermal tolerance limits declined with increasing exposure duration (up to 63 days; Wehrly & Wang, 2007). This may be due to various energetic trade-offs (Mardones et al., 2022), as observed in the gastropod *Ocenebra erinaceus*, which was able to physiologically acclimate after 95 days of exposure to both near-future warming and acidification conditions, but ceased reproduction after ~300 days of exposure (Mardones et al., 2022). If marine ectotherms can continue normal reproduction, positive carryover effects (transgenerational plasticity) may enable offspring to be better equipped to cope with extreme environmental conditions, as has been observed in a number of marine invertebrates and fishes in response to ocean warming and acidification (*e.g.*, Donelson et al., 2011; Parker et al., 2015; Heckwolf et al., 2018). However, a 25-month study on the sea urchins *Echinometra* sp., discovered that instead of transgenerational plasticity, negative carryover effects were observed over successive generations, when exposed to predicted future ocean warming and acidification conditions (Uthicke et al., 2021).

The vulnerability of the habitat and prey of marine ectotherms also has an exposure-time dependence. Because of this, in coral reef studies, degree heating weeks (DHW, the number of weeks that the temperature exceeds the maximum summer sea surface temperature) is commonly used to predict risk of coral bleaching (Kayanne et al., 2017). Four DHWs for instance, are predicted to be the threshold for some bleaching to occur, and eight DHWs are considered to result in widespread bleaching and some mortality (Kayanne et al., 2017). Under near-future climate change, the duration, prevalence, and intensity of marine heatwaves will increase, and the mean ocean temperature will continue to rise, with ecological implications for marine ectotherms, their habitats and the ecosystem (Poloczanska et al., 2016; IPCC, 2019; McWhorter et al., 2022).

1.7 Ecological implications

Changes in abundance, demography, phenology, and distribution in response to warming have already been reported in a plethora of marine organisms, including phytoplankton, fishes, crustaceans, and molluscs (Poloczanska et al., 2016). Poloczanska et al. (2016) reviewed responses of marine organisms to ocean warming that had been reported in the Marine Climate Change Impacts Database (MCID). They found that changes in abundance were the most commonly reported response to warming (41% of the MCID), with a similar number of studies reporting positive and negative changes, and a shift to the latter response is expected in the near-future (Poloczanska et al., 2016). Poloczanska et al. (2016) also found that distributional shifts were common (40% of the MCID), with most reporting the expansion of the leading edges of species distributions and poleward displacements. Poleward range expansions are typically facilitated by larval dispersal and currents. The East Australian Current (EAC), for instance, has shifted southwards by approximately 350 km in less than 60 years, and has created warming hotspots closer to the poles (Ridgway, 2007; Lough, 2008; Ling et al., 2009). Changes to the EAC, as well as ocean warming, is considered to have facilitated the migration of the echinoderm *C. rodgersii* southwards at a rate of about 160 km per decade, and consequently, has caused widespread declines of kelp forests (Ling et al., 2009, 2018; Ling, 2013).

1.8 Echinoderms

Many echinoderms are considered "keystone" or influential species, having disproportionate roles in maintaining the diversity, distribution and abundance of associated species (Paine, 1966). Echinoderms are exclusively marine ectotherms, consisting of around 7000 species of starfish (Asteroidea), sea urchins (Echinoidea), sea cucumbers (Holothuroidea), brittle stars (Ophiuroidea), and sea lilies and feather stars (Crinoidea; Byrne & O'Hara, 2017). This taxon is characterised by cyclical boom-bust population dynamics, where population irruptions are followed by sharp population declines when resources are depleted (Uthicke et al., 2009). Thus, changes in their abundance may have profound impacts on the marine environment (Ling et al., 2009; Uthicke et al., 2009; Menge et al., 2016; De'ath et al., 2019). High densities of echinoderms may have positive or negative impacts, depending on their ecological function. The starfish *Pisaster ochraceus* for instance, the first species to be coined a "keystone" species (Paine, 1966; Wagner, 2010), provides top-down control on mussel populations along the West coast of North America. This species has recently experienced significant population declines due to Sea Star Wasting Disease (SSWD), possibly reducing invertebrate diversity (Paine & Trimble, 2004; Menge et al., 2016; Miner et al., 2018). Population declines due to SSWD have been associated with ocean warming in this species, as well as other starfish including the endangered sunflower starfish *Pycnopodia helianthoides* (Menge et al., 2016; Miner et al., 2018; Harvell et al., 2019). Other echinoderms, most notably, *C. rodgersii* and crown-of-thorns starfish (*Acanthaster* spp.; CoTS), cause substantial damage to the marine environment at high densities, and thus reductions in their abundance would in fact be beneficial (Ling et al., 2009; De'ath et al., 2012; Pratchett et al., 2014).

1.9 Crown-of-thorns starfish

Crown-of-thorns starfish (CoTS) are native to coral reefs throughout the Indo-Pacific (Moran, 1986). Despite previously being considered a single species, *Acanthaster planci* [Linnaeus, 1758], there are at least four distinct species of CoTS (Haszprunar & Spies, 2014; Haszprunar et al., 2017). The northern Indian Ocean species, *A. planci* is morphologically distinct from the Eastern Pacific species, which is nominally regarded as *Acanthaster solaris* (Haszprunar et al., 2017). *Acanthaster solaris* was originally named based on specimens collected near Jakarta (Haszprunar et al., 2017), and while morphologically similar, it is still not certain that CoTS from the Western Pacific, such as on the GBR, are the same species. All CoTS used in the research presented herein were collected from the GBR are therefore referred to as *Acanthaster* cf. *solaris*.

Crown-of-thorns starfish are gonochoric broadcast spawners (Caballes & Pratchett, 2014). Embryos resulting from spawning events hatch into planktonic algal-feeding larvae (Uthicke et al., 2009; Uthicke et al., 2015), with most settling within 22 days in specific reef environments, before metamorphosing into juvenile starfish (Pratchett et al., 2017). Juvenile CoTS feed on CCA initially, before shifting to feeding on corals at > 6 months post-settlement (Birkeland & Lucas, 1990; Johnson et al., 1991). The specific timing of dietary shifts is dependent on the availability of preferred coral prey (Wilmes et al., 2020; Neil et al., 2022). After juveniles commence coral-feeding, growth accelerates, and CoTS reach reproductive maturity at around two years of age (Pratchett et al., 2014). Following maturation, fecundity increases with size, whereby larger (> 40 cm diameter) individuals are capable of producing > 100 million eggs per spawning season (Babcock et al., 2016; Pratchett et al., 2021c).

Crown-of-thorns starfish often undergo population irruptions (or outbreaks; Birkeland, 1982), whereby densities may increase 100- to 1000-fold in a few years (Kayal et al., 2012). Population irruptions have occurred on reefs throughout the Indo-west Pacific (Chesher, 1969; Yamaguchi, 1986; Baird et al., 2013; Babcock et al., 2016). On the GBR, there have been four documented population irruptions since 1962 (Pratchett et al., 2014; Brodie et al., 2017; Mellin et al., 2019). CoTS predominantly feed on reef-building corals (Ormond et al., 1976; Pratchett, 2001). Thus, recurrent population irruptions have contributed significantly to sustained and rapid coral loss and reef degradation over large expanses of the GBR (De'ath et al., 2012; Mellin et al., 2019). Putative causes of CoTS population irruptions remain equivocal (Pratchett et al., 2014). The inherent life-history dynamics of CoTS, especially their exceptional reproductive capacity makes them predisposed to population irruptions (Babcock et al., 2016), which may be facilitated or exacerbated by anthropogenic degradation of coral reef ecosystems, caused by coastal modification and elevated discharge of nutrients (Birkeland, 1982; Brodie, 2005; Fabricius et al., 2010; Brodie et al., 2017), and over-fishing and localised depletion of key predators (Endean, 1969; Cowan et al., 2017). As in many other echinoderms, *e.g.*, *Diadema antillarum*, *Paracentrotus lividus* and *Asterias* spp. (Haley & Solandt, 2001; Weil et al., 2005; Uthicke et al., 2009), population irruptions of CoTS are generally followed by population collapses, likely due to the depletion of resources (Chesher, 1969; Uthicke et al., 2009), or changing environmental conditions (Uthicke et al., 2009). Elevated environmental temperatures have already been linked to collapses in a number of echinoderms populations, including *Pisaster ochraceus* (discussed in 1.8; Menge et al., 2016; Miner et al., 2018), as well as the large sub-tropical predatory starfish *Heliaster kubiniji* in the Northern Pacific (Dungan et al., 1982; Uthicke et al., 2009).

1.10 Impact of ocean warming on crown-of-thorns starfish

Population collapses of CoTS, as well as the occurrence and severity of future population irruptions may also be initiated by ocean warming (Uthicke et al., 2009; Table 1.1). Previous research has determined that reproduction and the early life stages of CoTS are vulnerable to warming (Rupp, 1973; Kamya et al., 2014; 2016; 2018; Lamare et al., 2014; Caballes et al., 2017; Hue et al., 2020). Hue et al. (2020) found that the gonadosomatic index of CoTS from New Caledonia declined by up to 50% at 29 °C (+2 °C above ambient), with potential detrimental impacts on reproductive output. Due to density dependence of fertilisation, a reduced quantity of gametes would likely reduce successful fertilisation and consequently, the density of larvae (Uthicke et al., 2009). The process of fertilisation has been determined to be thermally robust to warming (Rupp, 1973; Kamya et al., 2014; Caballes et al., 2017). However, Hue et al. (2020) revealed a three-fold decline in fertilisation success when parents were exposed to 29 °C for several months prior to spawning, demonstrating the important role of parental exposure. Resultant embryos also appear vulnerable to warming, with less CoTS embryos from the GBR and Guam undergoing cleavage and gastrulation when exposed to 32 °C (+4 °C above ambient) post-fertilisation (Lamare et al., 2014; Caballes et al., 2017). Embryonic abnormality was much more prevalent at temperatures as low as 30 °C (Lamare et al., 2014). The same magnitude of warming, resulted in developmental arrest at the larval stage of CoTS from the GBR, and larvae were smaller at temperatures as low as 28 °C (+2 °C above ambient; Kamya et al., 2014). However, faster development of CoTS larvae at higher temperatures (Uthicke et al., 2015) may mitigate against the negative effect of temperature on growth and survival to some extent, by reducing the time by which larvae are the most vulnerable to predators (Cowan et al., 2017). There is a lack of prior research on how the process of larval settlement is impacted by warming, but resultant juvenile CoTS may be more resilient to elevated temperatures than earlier life stages, once large enough to have made it through the population bottleneck experienced by larvae and early post-settlement juveniles (Cowan et al., 2016; 2017; Wilmes et al., 2018). In the same cohort of starfish as in Kamya et al. (2014), further studies observed that growth rates remained high at 30 °C in algae-feeding and coral-feeding juveniles, which may be explained by their propensity to consume food at a faster rate under higher temperatures (Kamya et al., 2016; 2018). Furthermore, coral-feeding juveniles reared at 30 °C had a greater number of spines, likely improving their capacity to avoid predation (Kamya et al., 2018). Coral-feeding adult CoTS may be more sensitive to warming than juveniles, given their high energy investment in reproduction (Lawrence, 1984; Caballes & Pratchett, 2014; Babcock et al., 2016). However, other than the work by Hue et al. (2020) on the reproductive capacity of adult CoTS, there is little knowledge of how elevated temperature impacts this latter life stage.

Table 1.1 Studies on the impacts of elevated temperature on crown-of-thorns starfish (*Acanthaster* cf. *solaris*) at different ontogenetic stages.

Study	Collection location (latitude)	Measure	Thermal range tested (Ambient temperature, °C)	Temperature at which significant negative (↓) effect occurs (°C)
Gametes				
Caballes et al., 2017	Guam (13 °N)	Sperm speed (μm s^{-1})	20-36 (28)	Nil
		Sperm motility (%)		↓ 36
		Fertilisation (%)		↓ 32
Hue et al., 2020	New Caledonia (22 °S)	Egg volume (mm^3)	27-29 (27)	↓ 29
		Fertilisation success (%)		↓ 29
Kamya et al., 2014	GBR (16 °S)	Fertilisation success (%)	26-30 (26)	Nil
Rupp, 1973	Guam (13 °N)	Fertilisation success (%)	28-36 (28)	↓ 34
Embryos				
Caballes et al., 2017	Guam (13 °N)	Cleavage (%)	20-36 (28)	↓ 32
		Gastrulation (%)		↓ 32
Lamare et al., 2014	GBR (16 °S)	Cleavage (%)	19-37 (27.5)	↓ 32
		Hatching (%)		↓ 32
		Gastrulation (%)		↓ 29
		Abnormality (%)		↓ 30
Rupp, 1973	Guam (13 °N)	Cleavage success (%)	28-36 (28)	↓ 34
Larvae				
Kamya et al., 2014	GBR (16 °S)	Normal development (%)	26-30 (26)	↓30
		Length (mm)		↓ 28
		Width (mm)		↓ 28
Lamare et al., 2014	GBR (16 °S)	Bipinnaria (%)	19.4-37 (27.5)	↓33.2
		Abnormality (%)		↓30.2
		Length (μm)		↓30.2
		Width (μm)		↓31.6
Uthicke et al., 2015	GBR (16 °S)	Mid-late brachiolaria (%)	28-30 (28)	Nil

Juveniles				
Kamya et al., 2016	GBR (16 °S)	Growth (% day^{-1})	26-30 (26)	Nil
		Arm number		Nil
		Spine number		Nil
		Food consumption (mm^{2})		Nil
Kamya et al., 2018	GBR (16 °S)	Growth (mg week^{-1})	26-30 (26)	Nil
		Arm number		Nil
		Spine number		Nil
		Consumption (mm^{2})		Nil
Adults				
Hue et al., 2020	New Caledonia (22 °S)	Gonadosomatic index (%)	27-29 (27)	↓ 29

1.11 Purpose of thesis and outline

The purpose of this thesis was to establish the effect of increasing ocean temperatures on CoTS, focussing on the Pacific species, *Acanthaster* cf. *solaris*. Considering the boom-bust population dynamics exhibited by CoTS and other echinoderms (Uthicke et al., 2009), supra-optimal temperatures may initiate population busts of present population irruptions, and future irruptions may be rarer and less intense. These responses may be a consequence of both direct impacts of warming on CoTS physiology (Kamya et al., 2014; 2016; Pörtner et al., 2017; Hue et al., 2020), as well as reductions in the quality and availability of algal and coral prey for the early and later life stages (Johnson & Sutton, 1994; Webster et al., 2011). Considering that the southern GBR (*e.g.*, the Swains region) may be a thermal refugia for coral reef species, including corals, at least until mid-century (McWhorter et al., 2022), CoTS populations may continue to irrupt in these cooler parts of the GBR, with implications for remaining coral communities. Despite some previous studies exploring the effect of warming on CoTS (Table 1.1), there are obvious knowledge gaps. In particular, it is still unknown how elevated temperatures influence the process of larval settlement in CoTS, and how the coral feeding life stages of CoTS are impacted by warming. A knowledge of how CoTS perform in predicted near-future thermal conditions throughout their life cycle, will improve the ability to predict when these changes in population dynamics may occur, and the consequences for coral communities.

This thesis consists of four data chapters (**Chapters 2** to **5**). **Chapter 2** provides context by formally reviewing the state of knowledge regarding the vulnerability of echinoderms to warming and investigating broad patterns in responses to ocean warming among echinoderms. **Chapters 3**, **4** and **5** explicitly focus on CoTS and aim to address the aforementioned gaps in our understanding, specifically, the effect of warming on the processes associated with larval settlement, as well as on the coral feeding life stages.

Chapter 2 represents a meta-analysis that explores responses to ocean warming among echinoderms. Although there have been a multitude of publications in recent years, focusing on understanding the effects of elevated temperatures on echinoderm species, mostly in laboratory settings (*e.g.*, Peck et al., 2008; Li et al., 2010; 2011; Christensen et al., 2011; 2017; Binet & Doyle, 2013; Kamya et al., 2014; 2016; 2018; Parvez et al., 2018; Balogh & Byrne, 2020), there has yet to be any systematic attempt to analyse this knowledge to explore general patterns on the impacts of warming or the causes of differential responses to elevated temperature within this phylum. This chapter compiled and analysed the results from 85 studies (710 individual responses), which measured effects of warming on the performance of echinoderms, specifically echinoids (sea urchins), holothuroids (sea cucumbers), ophiuroids (brittle stars), and asteroids (starfish). The magnitude and direction of the response to warming was compared among different biological responses, ontogenetic life stages, taxonomic classes, regions, as well as experimental designs. Establishing patterns in vulnerability to elevated temperature between these important predictors, will aid the ability to forecast the population dynamics of ecologically and economically important species, in a warmer ocean. Notably, this meta-analysis revealed that tropical starfish, may be particularly vulnerable to ocean warming, thus highlighting the importance of understanding the effect of elevated temperatures on CoTS, a tropical starfish that has disproportionate impact on coral reef ecosystems.

Settlement of planktonic larvae and subsequent metamorphosis in marine invertebrates is considered one of the greatest population bottlenecks (Balch & Scheibling, 2001; Uthicke et al., 2009). However, the effect of ocean warming on these fundamental processes is rarely studied in marine ectotherms (Espinel-Velasco et al., 2018). In **Chapter 3**, the effect of temperature on settlement success, metamorphosis and post-settlement survival of CoTS was explored, as well as how the quality of the CCA settlement substrate at high temperatures further impacts settlement. The temperature range used represented the summertime average

(28 °C) and maximum (30 °C) temperatures in the collection location, and predicted temperatures under near-future warming scenarios (32 °C and 34 °C; IPCC, 2019; AIMS, 2022). If the processes associated with settlement are negatively impacted by ocean warming, there will be a reduction in recruitment and replenishment of the adult population, that may also be compromised at supra-optimal temperatures, especially considering the vulnerability of their coral prey (Mellin et al., 2019).

The impacts of ocean warming on the coral-feeding stages of CoTS are important to understand given that these are the life stages that cause mass coral destruction and seed the next generation of coral predators (Pratchett et al., 2014). In **Chapter 4**, the physiological responses of sub-adult and adult CoTS to acute warming were explored. Acute responses to elevated temperatures do not allow for compensatory mechanisms, such as acclimation (Schulte et al., 2011; Healy & Schulte, 2012; Pörtner et al., 2017), and so represent how organisms may respond to acute thermal anomalies (Pörtner, 2002; Schulte et al., 2011; Frölicher & Laufkötter, 2018; Gall et al., 2021). Organism-level thermal sensitivity was explored by measuring oxygen consumption rate as a proxy for standard metabolic rate of sub-adults and adults over a 24 to 36 °C temperature range. Thermal sensitivity of both life stages was also assessed at the biochemical level, by measuring the maximal activity of enzymes involved in aerobic metabolism (citrate synthase, CS; 15 °C to 40 °C) and anaerobic metabolism (lactate dehydrogenase, LDH; 20 °C to 45 °C), to determine the causes of organism level responses. The results presented in this chapter provide an indication of the critical thermal limit of CoTS, and a reference for understanding responses to longer-term thermal exposure (Schulte et al., 2011; Kühnhold et al., 2019a; b; Torossian et al., 2020).

Understanding responses of coral-feeding CoTS to prolonged exposure to elevated temperatures is important for predicting how populations of this species will fare under the sustained ocean warming predicted to occur in the near-future, as well as during long-term marine heatwaves (IPCC, 2019). In **Chapter 5,** coral-feeding starfish (predominantly mature adults) were exposed to temperatures between 26 and 32 °C, a temperature range comprising the annual and summertime average temperatures in the collection location, as well as the current and predicted near-future summertime maximum temperatures (AIMS, 2021). The standard metabolic rate, righting time, movement rate, and survival of CoTS was measured at various timepoints over ~60 days, as well as the impacts of starfish weight on these measures. CoTS may have the ability to acclimate to warmer conditions over this duration of time, meaning that the starfish may perform well over the range of temperatures tested (Schulte et al., 2011; Suckling et al., 2015; Morley et al., 2019). In the absence of acclimation, metabolic depression may occur, which would inevitably diminish energy reserves required for CoTS to return to their natural orientation following disturbance and move with haste over the reef, reducing the ability of CoTS to avoid predators, and seek coral prey (Pörtner & Knust, 2007; Peck et al., 2008; Kidawa et al., 2010; Wood et al., 2010; Brothers & McClintock, 2015; Sun et al., 2018). The ultimate response to sustained warming is mortality, which in nature, would significantly impact population dynamics of CoTS on the reef, and their interaction with coral prey (Chen & Chen, 1993; Pörtner, 2001; 2002; Peck et al., 2009; Fang et al., 2015a; b; Christensen et al., 2011, 2017; Pörtner et al., 2017; Harianto et al., 2018).

Chapter 6 discusses the major findings of the aforementioned chapters, the ramifications of the collective findings in both an ecological and management context and explores key avenues for further research that will enhance understanding of this topic.

Chapter 2: Impacts of ocean warming on echinoderms: A meta-analysis[1]

Publication

[1] Lang, B. J., Donelson, J. M., Bairos-Novak, K. R., Wheeler, C. R., Caballes, C. F., Uthicke, S., & Pratchett, M. S. (*In Review*) Impacts of ocean warming on echinoderms: A meta-analysis.

2.1 Abstract

Rising ocean temperatures are threatening marine species and populations worldwide, and ectothermic taxa are particularly vulnerable to warming. Echinoderms are an ecologically important phylum of marine ectotherms and shifts in their population dynamics can have profound impacts on the marine environment. The effects of warming on echinoderms are highly variable across controlled laboratory-based studies. Accordingly, synthesis of these studies will facilitate the better understanding of broad patterns in responses of echinoderms to ocean warming. Herein, a meta-analysis incorporating the results of 85 studies (710 individual responses) is presented, exploring the effects of warming on various performance predictors. The mean responses of echinoderms to all magnitudes of warming were compared across multiple biological responses, ontogenetic life stages, taxonomic classes, and regions, facilitated by multivariate linear mixed effects models. Further models were conducted which only incorporated responses to warming greater than the projected end-of-century mean annual temperatures at the collection sites. This meta-analysis provides evidence that ocean warming will generally accelerate metabolic rate (+ 32%) and reduce survival (–35%) in echinoderms, and echinoderms from sub-tropical (– 9%) and tropical (– 8%) regions will be the most vulnerable. The relatively high vulnerability of echinoderm larvae to warming (– 20%) indicates that this life stage may be a significant developmental bottleneck in the near-future, likely reducing successful recruitment into populations. Furthermore, asteroids appear to be the class of echinoderms that are most negatively affected by elevated temperature (– 30%). When considering only responses to magnitudes of warming representative of end-of-century climate change projections, the negative impacts on asteroids, tropical species and juveniles were exacerbated (– 51%, – 34%, and – 40% respectively). The results of these analyses enable better predictions of how keystone and invasive echinoderm species may perform in a warmer ocean, and the possible consequences for populations, communities, and ecosystems.

2.2 Introduction

Ocean warming, a repercussion of anthropogenic climate change, is one of the principal emerging threats facing marine ecosystems (IPCC, 2019). An increased prevalence and intensity of marine heatwaves (*i.e.*, warming above the 90th percentile for at least five consecutive days; Hobday et al., 2016) in recent years, has already resulted in the degradation of some of the world's most vulnerable marine ecosystems, such as coral reefs, sea grass beds and kelp forests (Mellin et al., 2019; Filbee-Dexter et al., 2020; Strydom et al., 2020). Within these ecosystems, ectotherms are particularly thermosensitive as their body temperature, and therefore their physiology and behaviour, are naturally linked to their thermal environment (Lagerspetz & Vainio, 2006). Accordingly, ocean warming has been implicated in population declines, phenological shifts, and range shifts in an array of marine ectotherms (Poloczanska et al., 2016). Considering the ecological and economic importance of many marine ectotherms (Ling et al., 2009; Li et al., 2011; Munday et al., 2013), it is essential to understand how elevated temperatures will impact these organisms.

Due to the widespread threat of ocean warming, it is important to understand how common biological and ecological aspects shape the responses of marine ectotherms to a warming environment. For example, certain biological responses (*e.g.*, metabolic activity and survival rates) and life stages may have differing sensitivities to elevated temperatures (Dahlke et al., 2020; Sampaio et al., 2021; Pereira Santos et al., 2021). Biological responses associated with physiology are likely to be particularly sensitive to environmental warming in ectotherms, with their magnitude and direction dependent upon where the temperatures experienced fall within the organisms' thermal window (Pörtner et al., 2017; Sampaio et al., 2021). Physiological performance is often assessed non-lethally via oxygen consumption rate measurements, which serves as a proxy for aerobic metabolism, and evidence suggests that this measure can operate as a good proxy for other aspects of performance (Svendsen et al., 2016; Pörtner et al., 2017). In general, aerobic metabolism is only able to increase to the organisms' thermal limit, after which, constraints on oxygen delivery will lead to metabolic depression and declines in energy production (Pörtner et al., 2017; Lang et al., 2021). The additional aerobic metabolic costs of warming may impact growth, development, activity, feeding and reproduction (Schulte, 2015; Pörtner et al., 2017). Reproduction is typically sensitive to elevated temperatures, and resulting embryos and larvae are often highly vulnerable to warming (Byrne, 2011; Dahlke et al., 2020; Collin et al., 2021), and other concurrent stressors, such as ocean acidification (Kroeker et al., 2010; Byrne, 2011; Przeslawski et al., 2015; Pandori & Sorte, 2018). On the other hand, juveniles and adults are usually more thermotolerant, testament to their well-developed physiological capacity and greater ability to modify their behaviour to withstand warmer conditions (Byrne, 2011; Nguyen et al., 2011; Dahlke et al., 2020; Lang et al., 2021; Sampaio et al., 2021). Behavioural plasticity and thermoregulation could allow marine ectotherms to buffer their experience of stressful thermal conditions and potentially reduce the costs of increased metabolic demands in warmer oceans (Wong & Candolin, 2015).

Responses of marine ectotherms to warming may be in part, a reflection of their evolutionary and thermal history. Responses to elevated temperatures in marine ectotherms have been demonstrated to vary taxonomically (*e.g.*, Nguyen et al., 2011; Sampaio et al., 2021). These patterns are due to taxa having differing capacities to adjust their physiology and behaviour in response to warming, which may be associated with their phylogeny and thermal experience throughout evolutionary time (Nguyen et al., 2011; Sunday et al., 2011; Bennett et al., 2021). Tolerance to elevated temperatures is often attributed to the amount of thermal variation experienced (Sunday et al., 2011; Hughes et al., 2018a). Seasonal thermal variability is low at tropical latitudes (Sunday et al., 2011; Madeira et al., 2017; Hughes et al., 2018a). Consequently, marine ectotherms living in these relatively stable thermal environments often have a narrow thermal breadth, and live closer to their upper thermal limits compared to those inhabiting more variable thermal environments (Schulte et al., 2011; Sunday et al., 2011; Sherman, 2015; Woolsey et al., 2015; Poloczanska et al., 2016; Pinsky et al., 2019). Marine ectotherms exposed to greater seasonal thermal variability at temperate latitudes for instance, often have wider thermal windows and have evolved greater capacity to withstand periods of thermal extremes via enhanced physiological and behavioural thermoregulation (Sokolova & Pörtner, 2001; Sunday et al., 2011; Woolsey et al., 2015; Madeira et al., 2017). This geographic pattern in vulnerability to warming may not always hold true however, because vulnerability may also be dependent on other spatial factors, including the breadth of the geographic range, the part of the range in which they occur, their depth and habitat (Pey et al., 2011; O'Connor et al., 2012; Poloczanska et al., 2016; Drake et al., 2017; Zettlemoyer & Peterson, 2021; Sasaki et al., 2022).

The phylum Echinodermata, consisting of ~7,000 extant species, including asteroids, echinoids, holothuroids and ophiuroids are an ecologically and economically important taxon in all oceans (Byrne & O'Hara, 2017). The ecological importance of echinoderms is partly linked to their "boom and bust" population dynamics, and the remarkable beneficial or detrimental effects that these changes can have on marine ecosystems (Lessios, 1988; Uthicke et al., 2009; Ling, 2013; Menge et al., 2016; Byrne & O'Hara, 2017; Mellin et al., 2019). Population irruptions of crown-of-thorns starfish (*Acanthaster* cf. *solaris*), for instance, have resulted in significant coral loss on Australia's Great Barrier Reef (Mellin et al., 2019), while shifts in the distribution of large populations of the echinoid *Centrostephanus rodgersii,* have contributed to the accelerated loss of highly diverse kelp forest ecosystems in Tasmania (Ling, 2013). Ocean warming is considered to be the primary cause of the distributional shift of *C. rodgersii* (Ling et al., 2009). Ocean warming has also been implicated in increasing the incidence and severity of disease outbreaks in a variety of echinoderm species (Dungan et al., 1982; Lester et al., 2007; Clemente et al., 2014; Menge et al., 2016). For instance, the asteroid *Pisaster ochraceus*, the first species to be coined a "keystone" species (Paine, 1966; Wagner, 2010), helps maintain invertebrate diversity by providing top-down control on mussel populations along the Pacific coast of North America, but has recently experienced significant population declines due to Sea Star Wasting Disease, which may be linked to ocean warming (Menge et al., 2016). Considering the numerous ecosystem impacts resulting from a "boom" or "bust" in echinoderm populations (Uthicke et al., 2009; Menge et al., 2016), there is a strong impetus to understand how echinoderm species may be impacted by a changing climate.

Despite an increasing number of controlled laboratory-based studies focusing on understanding the effects of elevated temperatures on echinoderm species, there is little synthesis of the overarching impacts of warming, or explanations for the variation in vulnerability to elevated temperature observed within this phylum. Meta-analyses have provided a useful statistical tool for quantitatively assessing broad patterns in the responses of marine ectotherms such as fish, molluscs, and crustaceans to environmental change, including ocean warming, ocean acidification, hypoxia and reduced salinity (*e.g.,* Kroeker et al., 2010; Przeslawski et al., 2015; Pandori & Sorte, 2018; Pereira Santos et al., 2021; Sampaio et al., 2021; Hu et al., 2022). To the authors' knowledge, there are no comparable meta-analyses to date that have focused on understanding whether echinoderms adhere to general biological, ecological, or evolutionary expectations regarding their vulnerability to ocean warming (Byrne, 2011; Sunday et al., 2011; Poloczanska et al., 2016; Dahlke et al., 2020; Sampaio et al., 2021). Herein, a meta-analysis using a comprehensive empirical dataset is presented, consisting of information from 85 studies that explore the effect of warming on echinoderms. Specifically, the direction and magnitude of the effect of warming (relative to control temperatures) on performance was investigated, and how this varies depending on the (1) biological response tested, (2) ontogenetic life stage, (3) taxonomic class, (4) region, and (5) experimental design. To further explore the potential effect of future ocean warming, a subset of the data was examined in which responses were only included if the experimental temperature was above the predicted end-of-century mean annual temperature (MAT; IPCC, 2019) at the location of collection. The outcomes of this meta-analysis will aid in assessing the vulnerability of key echinoderm species to current and future ocean warming, and the broader impacts on the marine environment.

2.3 Methods

2.3.1 Literature search

Relevant publications on the effect of warming on echinoderms were initially identified using systematic searches within ISI Web of Science (studies published before June 2021). The keywords used were: “temp*”, “thermal”, “thermotolerance”, “warm*” and “climate change” (an asterisk represents the wildcard operator in Web of Science, used to find different endings of keywords). These words were combined (using the “AND” boolean) with both the common and/or Latin names of the five classes of echinoderms *i.e.*, Asteroidea (starfish/sea stars), Echinoidea (sea urchins and sand dollars), Ophiuroidea (brittle stars), Holothuroidea (sea cucumbers) and Crinoidea (feather stars and sea lilies). The latter class was not included in this review due to a lack of relevant studies. The field tag used was “TS = Topic”. To avoid any bias associated with conducting literature searches in a single database (Martín-Martín et al., 2018), systematic searches using ISI Web of Science were supplemented using Google Scholar and by exploring studies referenced in relevant publications. There was no limit on the year of publication for included studies, but field-based studies were excluded, due to limited ability to control for other factors that may influence responses.

2.3.2 Data collection and selection

The papers that met these initial criteria ($n = 143$) were further screened for eligibility (see Appendix 2.1 for a flow diagram of the selection process). Studies were only incorporated if the responses fell under one of the following key biological responses: developmental success, feeding and nutrition, growth, metabolic rate (routine or resting), movement, reproductive success, and survival ($n = 3$ studies that did not fall under the aforementioned biological responses and were therefore excluded). Acute “ramping” (*i.e.*, warming rate > 2 °C hr^{-1})

studies were not included due to the associated cumulative stress likely causing confounding deleterious effects on organism responses to warming (n = 5 studies excluded). Studies and individual responses were excluded if the control temperature was not specified or clearly stated (n = 21 studies excluded), or if the mean response for a control and at least one experimental temperature were not reported (n = 4 studies excluded). Finally, studies were excluded if the standard deviation or sufficient information for it to be calculated/estimated (*i.e.*, standard error, upper and lower 95% confidence limits, interquartile range, minimum and maximum values) and/or the sample sizes were not provided (n = 22 studies excluded). If multiple synonymous responses were included in the publication (*e.g.*, gonad wet weight and gonad dry weight), only one was included in the analyses. The responses at all elevated temperatures were included regardless of how realistic these temperatures were in the context of near-future climate change. If a response was measured at multiple timepoints, in general the response at the first and last timepoint were used in the analyses, to avoid pseudoreplication, and taxonomic biases. If the first timepoint was immediately after environmental conditions were changed, the second and last timepoint were used instead (see Appendix 2.2 for a reference list of the data sources for the analyses).

For each response, the mean, standard deviation, and number of replicates at both the control and experimental (elevated) temperatures were recorded. The control temperature was the temperature at which the individuals were habituated to prior to the experiments, or if there was no laboratory habituation period, then the temperature at the collection location was used. When raw or summary data tables were not published, but were presented in a graphical format, data was mined from the primary literature using the program WebPlotDigitizer (Automeris.io, 2021). The biological response being tested, ontogenetic life stage, the taxonomic class of the test subjects and the region were also recorded (see Appendix 2.3 for a table of these predictors and the groups within them). An attempt was made to gather data on the collection depth, and the latitudinal range of each species (to consider how range size, and the position of the individuals within the range, may impact vulnerability to warming), however, insufficient data was available (Living Australia, 2021). Experimental variables were recorded or calculated for each study, namely the habituation time at ambient temperature prior to experiments (days), the warming rate from the control to the experimental temperature (°C hr^{-1}), the exposure time at experimental temperatures (days), and the natural logarithm of the ratio of the experimental and control temperatures (*LnSR*; Pereira Santos et al., 2021; Sampaio et al., 2021).

The resulting values for *LnSR* were dependent not only on the difference between the temperature values, but also depended upon how hot or cold the control values were themselves; thus, the temperatures were adjusted accordingly. As in Pereira Santos et al. (2021) and Sampaio et al. (2021), all control temperatures were set to 2 °C, and new experimental temperatures were calculated as the original experimental temperature minus the original control temperature, plus the new baseline control temperature. The mean annual temperature (MAT) for each study location was also established by extracting COBE long term mean sea surface temperature estimates from the National Oceanic and Atmospheric Administration (NOAA Physical Sciences Laboratory, 2021) using the coordinates of the collection locations (or approximate coordinates if not available). For each study, the temperature data from the 10 years prior to the year of the experiment was used to establish the MAT.

2.3.3 Effect size and variance calculation

Effect sizes, *i.e.*, the ln-transformed response ratios (*LnRR*) were calculated using the equation outlined in Hedges et al. (1999):

$$LnRR = \ln\left(\frac{\bar{X}_E}{\bar{X}_C}\right)$$

where $\bar{X}_E$ and $\bar{X}_C$ are the mean responses in the experimental and control treatments respectively. The natural logarithm linearises these values, causing the values of both the experimental and control means to be weighted equally, and removes some of the skewness of the sampling distribution (Hedges et al., 1999). A positive *LnRR* indicates that the response is positively impacted by elevated temperature, while a negative *LnRR* means that the response is negatively impacted. Furthermore, if the *LnRR* is zero, then there is no effect of elevated temperature on the response. If for a given response, a higher mean value indicated a more detrimental effect (*i.e.*, percent abnormality, percent mortality, development time, and righting time), the sign of the *LnRR* was reversed for a more intuitive visualisation. The variance (inverse-variance weights) of each response was calculated using the equation of Hedges et al. (1999):

$$v = \frac{(S_E)^2}{n_E \bar{X}_E^2} + \frac{(S_C)^2}{n_C \bar{X}_C^2}$$

where, S is the standard deviation and n is the sample size. The variance enables the precision of the estimate to be established. Observations with a greater sample size and lower standard deviation are more heavily weighted, as they are a more precise estimate of the effect size (Kroeker et al., 2010).

2.3.4 Data analyses

Multivariate multilevel linear mixed effects models were conducted in R v.4.1.2 (R Core Team, 2021) and used to establish the mean effects of various predictor variables (see Appendix 2.3). Separate models were fitted for each predictor (*i.e.,* biological response, life stage, taxonomic class, region, habituation time at the control temperature, warming rate, and exposure time at the experimental temperature), as there was insufficient data available to consider multiple predictors simultaneously. Responses for metabolic rate were only included in the analysis for the "biological response" predictor, due to the uncertainty regarding whether positive or negative changes in the metabolic rate translate to beneficial or detrimental effects on organismal fitness (Schulte, 2015; Pörtner et al., 2017; Hu et al., 2022). All models were carried out using the *rma.mv* function from the "metafor" package in R (Viechtbauer, 2010). These models account for the hierarchical structure of meta-analytic data and considers the variation both within and between studies, while also accounting for any non-independence of effect sizes (Konstantopoulos, 2011; Jackson et al., 2011; Cheung, 2019). The formula for each of the models, adapted from Pereira Santos et al. (2021) and Sampaio et al. (2021), was as follows:

Model = rma.mv(yi = *LnRR*, v = Variance, mods = ~ *LnSR*: Predictor - 1, test="*t*", random = list(~1|Study number/Response number), method = "REML", data = data)

The categorical predictors (*i.e.,* biological response, life stage, taxonomic class, and region), were interacted with *LnSR* ("*LnSR*: Predictor - 1"), which allowed for varying slopes of *LnSR* on *LnRR* for each predictor group, with a fixed intercept of 0 as in previous studies (Sampaio et al., 2021). *LnSR* was not required in the experimental variable models, as all predictors were continuous. The variance (v), was included in all models to weight responses based on their precision, as well as the random effects structure. "Response number" was the number allocated to all responses at the different experimental temperatures for a given measure (*e.g.*, percent fertilisation success, gonad index, wet weight, and locomotion speed) within a single study (see Appendix 2.4 for a full list of measures included within each "biological response" group). The random effect was nested within the study number (~1|Study number/Response number). In most cases there was only a single study per publication, however if the paper included independent experiments on multiple echinoderm species, these were included as multiple studies per publication. Restricted maximum likelihood was used to fit all models, and *t*-statistic methods were implemented, which are more conservative than default *z*-statistic methods (Knapp & Hartung, 2003). To account for any deviations from the assumptions of the model, such as heteroscedasticity, non-normality, as well as non-independence of effect sizes, cluster-robust confidence intervals (clustered by "Study number") were calculated using the *robust* function in the "metafor" package (Viechtbauer, 2010; Cheung, 2019). The Robust Test of Moderators (Q_M) was used to establish whether there was a significant overall difference between the effect sizes for the different groups within each predictor model. Considering that it is not possible to get a test for residual heterogeneity based on cluster robust methods, the Test for Residual Heterogeneity (Q_E) from the original model was utilised.

Sensitivity analyses were conducted on all models; specifically, the presence of influential observations and pseuedoreplication were tested. The influence of the three most represented species: *Acanthaster* spp. (tropical asteroid; *n* = 110 data points), *Apostichopus japonicus* (temperate holothuroid; *n* = 78 data points) and *Strongylocentrotus droebachiensis* (temperate and polar echinoid; *n* = 53 data points) were also tested in the taxonomic class and region models and were found not to substantially drive the results (see Appendix 2.5 for a table detailing the number of data points for each species of echinoderm included in the analyses, and Appendix 2.6.1 for model results). Furthermore, tests for publication bias were conducted, because studies may be more likely to be published if their results are significant (Duval & Tweedie, 2000). Lastly, tests were conducted to see if the relationship between the response (*LnRR*) and the degree of warming (*LnSR*) was linear or not (see Appendix 2.6 for methods and results of the sensitivity, publication bias and linearity tests).

2.3.5 End-of-century scenario

All the predictor models above were re-run using a subset of the data where responses were only included if the experimental temperatures were above the MAT predicted for the end of the century (+2.58 °C, RCP8.5 global mean sea surface temperature projection 2081-2100; IPCC, 2019) at the collection sites. This allowed the assessment of whether there is evidence of an increased negative impact on echinoderms when experiments use magnitudes of warming tantamount to end-of-century predictions, and whether testing temperatures that are already frequently experienced by the organism may underestimate the potential effects of future warming.

2.4 Results

A total of 85 studies met the selection criteria for the "biological response" model. The dataset contained 710 individual responses from 47 species of echinoderm from around the globe (Asteroidea, $n = 13$; Echinoidea, $n = 21$; Holothuroidea, $n = 6$; Ophiuroidea, $n = 7$; Figure 2.1; see Appendix 2.5). The models containing all other predictors excluded five of the 85 studies, which included 52 individual responses measuring the effect of temperature on metabolic rate. The effect sizes (converted to a percentage change) are based on the average magnitude of warming across the dataset (~5 °C).

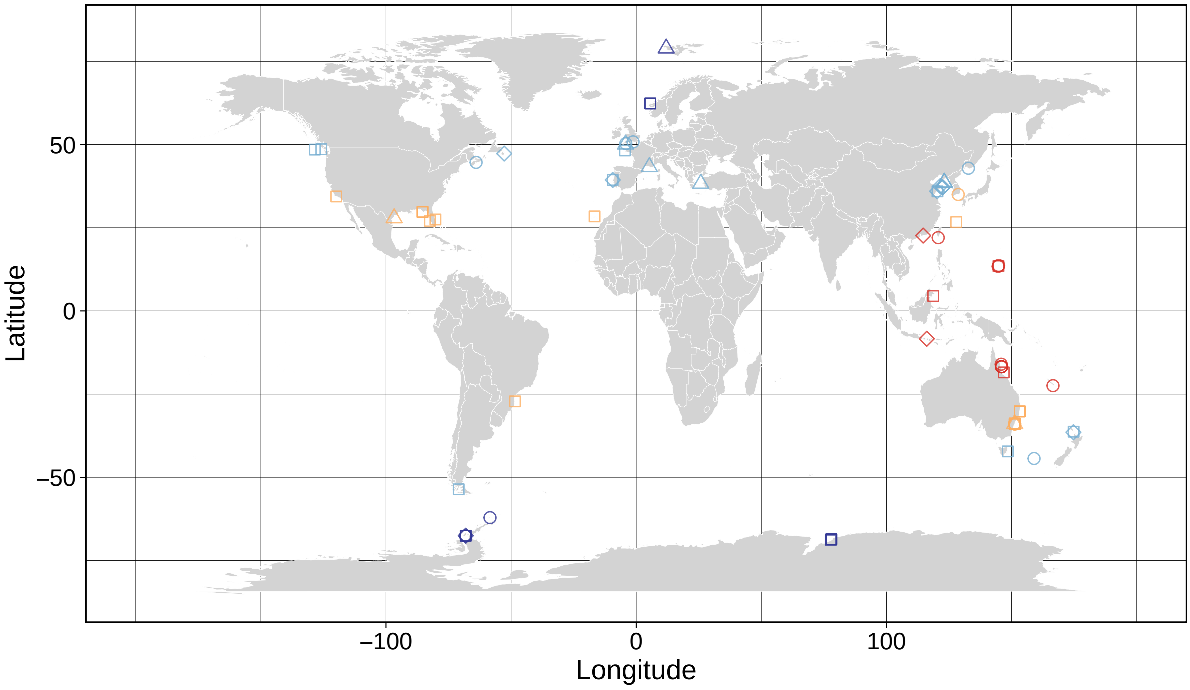

Figure 2.1 World map illustrating the collection locations of the echinoderms included in this meta-analysis. The points are colour coded by whether the organisms were found in tropical (red), sub-tropical (yellow), temperate (light blue) or polar (dark blue) waters. The shapes represent the four classes of echinoderm included in the meta-analysis: Asteroidea (circles), Echinoidea (squares), Holothuroidea (diamonds) and Ophiuroidea (triangles).

The response to warming was found to vary in magnitude and direction among the different biological responses considered (Robust Q_M; $F = 5.00$, $df = 7{,}78$, $P < 0.001$; Figure 2.2A, E). Survival was reduced by 35% on average ($P < 0.001$), while metabolic rate was the only biological response that increased with warming, and was on average 32% higher ($P = 0.013$). There was a negative effect of warming on both development success as well as feeding and nutrition (12% and 10% decline, respectively), however these effects were not significant. In the case of development success there was evidence of non-linearity in the response which could have resulted in the lack of significance (see Appendix 2.6.3). The effects of warming on growth, movement and reproductive success were not distinguishable from zero ($P > 0.050$).

Warming affected echinoderm life stages to differing extents (Robust Q_M; $F = 4.65$, $df = 5{,}75$, $P = 0.001$; Figure 2.2B, F). The larval stage was the most vulnerable to warming, with elevated temperature leading to a 20% decline in performance ($P < 0.001$). To a lesser extent, gametes (including fertilisation) were also significantly negatively affected ($P = 0.039$), however their performance only declined by 3% on average. This contradictory finding may be due to negative outliers and a relatively small sample size ($n = 85$ responses), that biased results towards significance. Despite the effect sizes not being significant, embryos and juveniles were the second and third most negatively affected life stages (based on percentage declines in performance), with a 14% ($P = 0.162$) and 9% ($P = 0.103$) decline in performance, respectively. The average *LnRR* was close to zero for adults, suggesting no overall effects of warming on this life stage ($P = 0.855$).

Vulnerability to warming was significantly different between taxonomic classes of echinoderms (Robust Q_M; $F = 14.74$, $df = 4{,}76$, $P < 0.001$; Figure 2.2C, G). Asteroidea were the most negatively affected, with an average 30% decline in performance ($P < 0.001$). Holothuroidea exhibited a 10% decline in performance ($P < 0.001$). Ophiuroidea appeared to respond positively to thermal challenge, with a 15% increase in performance, but this was not significant ($P = 0.244$). Considering the relatively even spread of data points around zero for this class, the high estimate may be a consequence of low resolution due to the small sample size ($n = 20$ responses). For Echinoidea, the mean *LnRR* was close to zero ($P = 0.638$). Removal of the three species that contributed the most data points changed the model output very little (see Appendix 2.6.1).

The response to warming varied based on the region (Robust Q_M; $F = 2.84$, df = 4,76, $P = 0.030$; Figure 2.2D, H); however, only echinoderms from sub-tropical (26-35°) latitudes were significantly affected by warming ($P = 0.020$), with an average 9% reduction in performance.

Tropical (4-23°) and temperate (36-53°) echinoderms exhibited 8% and 6% declines in performance respectively, while polar (62-78°) echinoderms experienced slight (3%) increases in performance (all $P > 0.050$). Tropical species exhibited non-linearity in their response which may explain the non-significant mean effect (see Appendix 2.6.3). When the tropical *Acanthaster* sp. were removed from the analysis, the vulnerability tropical species became significantly negatively affect by warming ($P = 0.040$), although the percentage change was 5% lower (see Appendix 2.6.1). This discrepancy may be a consequence of the much smaller sample size for tropical echinoderms ($n = 42$ data points) after the removal of this species (which contributed $n = 110$ data points). The removal of *Apostichopus japonicus* ($n = 78$ data points) and *Strongylocentrotus droebachiensis* ($n = 53$ data points) individually from the analysis did not significantly alter the response in the different region groups. However, the removal of *A. japonicus* resulted in the overall effect of the model becoming non-significant ($P = 0.086$; see Appendix 2.6.1).

Differences in the experimental design (*i.e.*, habituation time at the control temperature, warming rate, and exposure time at the experimental temperature) had no significant effect on echinoderm responses ($P > 0.050$). For all predictor models there was significant residual heterogeneity ($P < 0.001$).

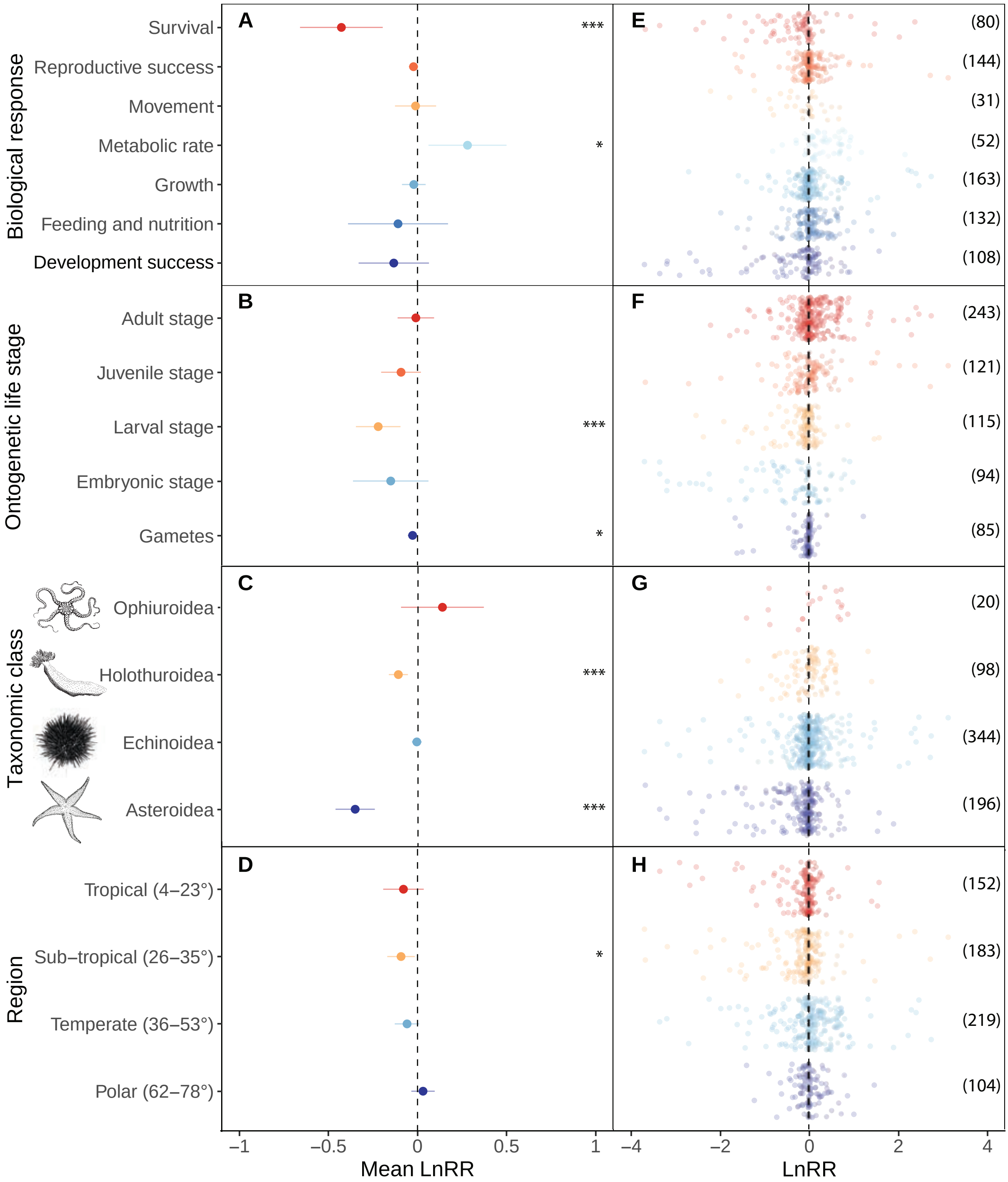

Figure 2.2 The effect of warming on echinoderms from around the globe, a comparison between biological responses (A, E), ontogenetic life stages (B, F), taxonomic classes (C, G) and regions (D, H). The mean effect sizes and the robust 95% confidence intervals are provided (left panels, A-D), as well as the raw data points (right panels, E-H). The number of responses in each group are provided in parentheses in the right panels. Significant mean effect sizes are

indicated by an asterisk (* P < 0.05; ** P < 0.01; *** P < 0.001) in the left panels. Mean effect sizes and 95% confidence intervals have been corrected for differences between the control and experimental temperatures (*LnSR*). Effect sizes above and below zero indicate a positive and negative response to warming, respectively.

2.4.1 End-of-century scenario

When only the responses that applied experimental temperatures relevant to end-of-century projections were tested, it was found that for some of the groups within predictors, the effect size and/or significance had changed (see Appendix 2.7 for a figure illustrating these results). For instance, metabolic rate was no longer significantly positively impacted by warming, and gametes were no longer significantly negatively impacted by warming ($P > 0.050$), yet the negative impact of warming on juveniles increased by 31% ($P < 0.001$). Under this end-of-century scenario, Asteroidea and Holothuroidea were also more negatively affected, with performance declining by a further 21% ($P < 0.001$) and 18% ($P = 0.049$), respectively. There was also a further 26% decline in performance of echinoderms from tropical (4-23°) latitudes, although this group still failed to exceed the threshold for significance ($P > 0.050$). Sub-tropical (26-35°) echinoderms were no longer significantly impacted by warming under the end-of-century scenario ($P = 0.384$), however, the percentage change in performance remained the same.

2.5 Discussion

For echinoderms, finding patterns in vulnerability to warming between biological responses, life stages, taxonomic classes or regions will aid the ability to predict how species and populations will respond to future climate change. The present meta-analysis found a number of interesting patterns. While warming accelerated metabolic rates and reduced survival, several biological responses were robust to thermal challenge, including growth, feeding and nutrition, movement, and reproduction. This may suggest that these biological responses are not good predictors of the vulnerability of echinoderms to ocean warming, as their performance can be maintained up until thermal limits are reached. The findings presented herein also suggest that under greater ocean warming, the larval stage may act as a developmental bottleneck in the life cycle of echinoderms, asteroids may be the most vulnerable group of echinoderms, and that populations from warmer latitudes may be living particularly close to their thermal limit. This information may facilitate predictions of how various ecologically and economically important echinoderms may fare in a warmer ocean, and will aid decision making for marine natural resources managers, and policy makers (Kroeker et al., 2010).

It is widely accepted that for ectotherms, metabolic rate increases up until a critical temperature, after which energy production can no longer keep pace with the energetic demands for the maintenance of homeostasis and normal behaviours (Schulte et al., 2011; Schulte, 2015; Pörtner et al., 2017). As expected, there was a significant (32%) increase in metabolism in echinoderms with warming, testament to the acceleratory effects of warming on cell processes (Schulte, 2015). Under the end-of-century experimental conditions the effect size for metabolic rate was less positive, indicating that echinoderms may live closer to their physiological thermal limits by 2100. Interestingly, while similar positive metabolic responses to warming were found in other meta-analyses on marine invertebrates and fishes, they have generally found far lower metabolic rate increases for similar magnitudes of warming (~7-17% increase with 3-4 °C of warming on average; Pereira Santos et al., 2021; Sampaio et al., 2021; Hu et al., 2022). A comparable response could have been anticipated for development success, as this would also be expected to shift due to an increase in the rate of cell processes (Munday et al., 2008; Pörtner & Peck, 2010; Schulte, 2015; Pörtner et al., 2017). However, there was no evidence that warming increased the speed at which early life stages reached developmental milestones, and more than ~3 °C of warming was particularly detrimental to development success. Consequently, when considering warming consistent with end-of-century predictions, development success declined by 35%, indicating a possible negative effect of temperature on the cell cycle (Schulte, 2015), which may be further exacerbated by other anthropogenic stressors, such as ocean acidification (Kroeker et al., 2010; Byrne, 2011; Przeslawski et al., 2015). Developmental success may be more closely linked with survival, considering that slower development may indicate incompetency, and is associated with a higher risk of predation in the field (Munday et al., 2008; Ross et al., 2011).

Mortality due to supra-optimal thermal exposure will ultimately lead to declines in abundance, population viability, and even extinctions (Byrne, 2011; Eisenlord et al., 2016). A 35% decline in survival was observed across all echinoderms. Consistent with the findings in the present study, Sampaio et al. (2021) observed a ~32% reduction in survival for a variety of marine taxa (including corals, crustaceans, echinoderms, molluscs, and fishes), but for an average 3 °C of warming. In contrast, elasmobranch survival was robust, with a mere ~3% reduction in survival with ~4 °C of warming (Pereira Santos et al., 2021). Surprisingly, although echinoderm survival declined with thermal challenge in the current analysis, limited impacts on other biological responses such as growth, feeding, or movement were found. This is concerning, as previous studies have considered these metrics as indicators of sensitivity to climate change (Pereira Santos et al., 2021; Sampaio et al., 2021). Since these biological responses were not affected by warming in the models presented herein, echinoderms may maintain these behaviours and processes in a warmer ocean, leading to an energy deficit, and subsequent mortality. Behaviour is often expected to be the first response to environmental change as a means for individuals to buffer the negative effects (Wong & Candolin, 2015). However, since echinoderms are relatively limited in their mobility, they may have reduced capacity to alter their behaviour in response to warming. Given the relatively short experimental durations in most *ex situ* studies, it must be considered that behavioural responses to temperature may occur after lengthier exposure durations. Nevertheless, if echinoderm metabolic rate and hence energetic demands increase greatly with warming, while feeding and nutrition and therefore energy intake do not increase proportionally, an overall energy shortfall is expected during projected warming periods (Lawrence, 1984; Munday et al., 2008; Pörtner & Peck, 2010; Schulte, 2015). This may result in widespread mortality and population contractions (Munday et al., 2008; Eisenlord et al., 2016; Poloczanska et al., 2016).

In the life cycle of marine ectotherms, highly fecund adults immediately prior to and after spawning, as well as the planktonic early life stages are often considered the most vulnerable to ocean warming, and these stages will likely determine population persistence into the future (Dahlke et al., 2020). The present meta-analysis found only partial support for this expectation, with reproduction and gametes being relatively robust to warming (3% decline in performance with warming), even when exploring only end-of-century relevant responses. It appears the negative impact of warming may increase as development progresses from gametes (including the process of fertilisation) to embryos and finally to larvae, respectively. A 20% decline in performance was observed at this latter planktonic life stage, and the greatest declines in larval performance were observed when warming exceeded ~3 °C. These observations may be explained by protective maternal factors (*e.g.,* heat shock proteins), which shield offspring from extreme environmental conditions, but have more of an effect in gametes and embryos than larvae (Sconzo et al., 1986; Hamdoun & Epel, 2007; Byrne, 2011; Foo et al., 2012; Lockwood et al., 2017). The findings presented herein are corroborated by those from previous studies, including meta-analyses, which indicate a greater relative vulnerability of the larval stage to extreme environmental conditions, including ocean warming, ocean acidification, reduced salinity and hypoxia (Kroeker et al., 2010; Byrne, 2011; Harvey et al., 2013; Przeslawski et al., 2015; Pandori & Sorte, 2018; Dahlke et al., 2020; Sampaio et al., 2021). Notably, ocean acidification appears to significantly reduce the calcification rate of marine larvae (Kroeker et al., 2010; Byrne, 2011; Harvey et al., 2013). Reduced larval performance under further climate change is concerning, as this will likely interfere with settlement success and recruitment into the population (Ross et al., 2011; Pörtner et al., 2017). Recruitment success may be further hindered by declines in performance of juvenile echinoderms, when exposed to magnitudes of warming consistent with future predicted temperatures. Juveniles may have a reduced physiological capacity and ability to exhibit adaptive strategies (*e.g.*, aestivation and burrowing) to buffer the effects of supra-optimal temperatures, compared to adults, which were found to be thermally robust even under end-of-century conditions (Nguyen et al. 2011; Zamora & Jeffs, 2012; Marshall et al., 2013; Christensen et al., 2017; Pandori & Sorte, 2018; Dahlke et al., 2020; Sampaio et al., 2021). Contrary to expectations, adult reproduction showed no measurable decline in response to thermal challenge. However, the thermosensitivity of reproduction likely requires further investigation into echinoderms with different modes of reproduction (*e.g.*, brooding versus broadcasting), which could not be explored here due to the lack of studies on brooding species (Menge, 1975; Pechenik, 1999). Despite the relative consistency of patterns of vulnerability to warming between life stages in prior research (Byrne, 2011; Przeslawski et al., 2015; Pandori & Sorte, 2018; Dahlke et al., 2020; Sampaio et al., 2021), comparisons warrant some caution, considering that the measures used to assess responses to warming often differ between ontogenetic life stages, particularly between the planktonic and benthic stages (Pottier et al., 2022). Furthermore, some life stages have significantly fewer applied measures of performance than others, for instance to measure the vulnerability of gametes to warming, most studies use fertilisation success.

Variation in the response to warming in marine ectotherms may be attributable to phylogenetic or evolutionary differences, leading to variabilities in thermal tolerance windows and adaptive strategies (Zamora & Jeffs, 2012; Marshall et al., 2013; Christensen et al., 2017; Bennett et al., 2021; Pereira Santos et al., 2021; Sampaio et al., 2021). It was determined that both the classes Asteroidea and Holothuroidea were negatively impacted by warming, while Ophiuroidea and Echinoidea were thermally robust. However, this pattern was not clearly related to phylogeny as Asteroidea and Ophiuroidea cluster in the subphylum Asterozoa while Holothuroidea and Echinoidea are in the subphylum Echinozoa (Reich et al., 2015). The pattern may, however, be linked to variations in metabolic processes and the relative allocation of resulting energy to various body components, behaviours (*e.g.*, movement) and processes (*e.g.*, reproduction) within the body, which differs between taxonomic classes (Lawrence, 1984; Whitehill & Moran, 2012). A comparative study on echinoid (*Arbacia punctulata*) and ophiuroid (*Ophiocoma alexandri*) larvae, found that metabolic rates were lower in ophiuroids, suggested to be a consequence of their lower feeding rates which cannot sustain greater energy requirements (Whitehill & Moran, 2012). A lower metabolic rate in the ophiuroid larvae compared to the echinoid larvae may be advantageous when food is limited, but a concomitant slower development rate may offset this benefit (Whitehill & Moran, 2012). In the present analysis, asteroids were deemed the most negatively affected by warming (30% decline in performance) and their performance declined by an even greater extent when only considering responses where warming magnitudes were in line with end-of-century predictions. In contrast to asteroid performance, ophiuroids showed trends of enhanced performance with warming. However, due to the paucity of studies on this class, this analysis may not have had the resolution to detect any negative effects. An ability to make generalisations of thermal performance in different taxonomic groups may be helpful when estimating the responses of species, particularly those that have the ability to alter the structure and function of an ecosystem, and further research on under-represented taxa are warranted (Ling, 2013; Menge et al., 2016).

The findings in this meta-analysis, to some extent, supports the general trend that the vulnerability of marine ectotherms to elevated temperatures increases with latitude from the poles to the equator, likely due to the increase in thermal stability (Sunday et al., 2011; Woolsey et al., 2015; Poloczanska et al., 2016; Hughes et al., 2018a; Pinsky et al., 2019). When all data were included, echinoderms from sub-tropical latitudes, were the most negatively impacted by warming (9% decline in performance), and tropical echinoderms only exhibited declines in performance with ~4 °C of warming. Although, removal of *Acanthaster* spp. led to tropical echinoderms becoming significantly negatively affected by warming. Furthermore, the negative impact was 25% greater for tropical compared to sub-tropical echinoderms in the end-of-century scenario, due to non-linearity in the thermal response. While temperate echinoderms were more tolerant to warming, there was still an overall negative effect on performance, which more than doubled in the end-of-century scenario. In contrast, there was an overall positive impact of warming on polar echinoderms, which became three times more positive in the aforementioned scenario. It is necessary to consider, that since environmental conditions not only vary on a global scale, but also on a local scale, this may have had some influence on the patterns observed across regions. For instance, shallow water species in tidal environments experience greater diurnal temperature variation in comparison to deeper water species and may also be living closer to their thermal limits (Pey et al., 2011; Sunday et al., 2011; Drake et al., 2017).

2.5.1 Data gaps, limitations, and future directions

The present meta-analysis identified a number of data gaps and limitations. Firstly, the authors were unable to gather sufficient information from studies in order to establish the effect of local scale thermal variability on the vulnerability to warming. Additionally, sufficient data on the spatial distribution of echinoderm species is lacking, and it is possible that responses to ocean warming may differ depending on the breadth of the species thermal range, and whether the individuals were collected from the leading or trailing edges, or the central part of the range (Sunday et al., 2011; O'Connor et al., 2012; Poloczanska et al., 2016; Collin et al., 2018; Zettlemoyer & Peterson, 2021; Sasaki et al., 2022). Some classes of echinoderms were underrepresented in the dataset, specifically there were only 20 data points for ophiuroids, and astonishingly, the authors did not find relevant studies on crinoids during the literature search. There were also relatively few studies that covered multiple life stages or generations, which are important to understand the plastic and adaptive capacity of echinoderms (Wernberg et al., 2012; Przeslawski et al., 2015; Suckling et al., 2015; Pandori & Sorte, 2018; Byrne et al., 2020; Uthicke et al., 2021). Moreover, many studies were excluded because not all data required for the models were provided in the papers (*i.e.*, means, $n = 4$, error values or sample sizes, $n = 22$, temperatures, $n = 21$; see Appendix 2.1). A consequence of the fact that many relevant studies could not be included, there was insufficient data available to include multiple predictors in a single model. The detection of publication bias in the dataset suggests that even more studies relevant for this meta-analysis were not included, because they were simply not published. This bias may have led to the over-estimation of effects on taxa (Duval & Tweedie, 2000; see Appendix 2.6.2).

These data gaps and limitations gave rise to the suggestion of several key considerations for future experimental studies on the impacts of ocean warming on echinoderms:

- An increased effort should be made to record, not only co-ordinates of the collection location, but information regarding the habitat and the geographic range of the species.

- There should be a greater focus on studying groups of echinoderms that are poorly represented in the literature, as well as those that are of high ecological importance.
- There should be an accelerated effort in publishing studies that span multiple life stages and generations.
- All relevant data required for meta-analyses should be incorporated into the papers, including temperatures, means, standard deviations and sample sizes.
- All results should be published, even if they show non-significant effects.

A greater number of studies overall, would provide the resolution to detect patterns in thermal responses with greater reliability. Moreover, this would allow for the assessment of interactions between multiple predictors in meta-analytic models, to better forecast the vulnerability of echinoderm species into the future. To provide a more realistic assessment however, it is necessary to consider the myriad of other environmental and ecological factors (*e.g.*, ocean acidification, food availability and predation) that may exacerbate, or even alleviate, echinoderm vulnerability to a warming ocean (Kroeker et al., 2010; Uthicke et al., 2015; Aziz, 2017; Lucey et al., 2020; Melzner et al., 2020; Sampaio et al., 2021). An important next step would be to incorporate multiple stressors associated with climate change, in a meta-analysis on echinoderm performance, that may have synergistic, additive, or antagonistic effects when combined with warming (Harvey et al., 2013; Przeslawski et al., 2015; Pandori & Sorte, 2018; Sampaio et al., 2021).

2.6 Conclusions

This meta-analysis provides a critical first step in understanding patterns of thermal responses among echinoderms and adds to the ever-growing body of literature predicting the negative impacts of climate change on marine species (Byrne, 2011; Sunday et al., 2011; Poloczanska et al., 2016; Dahlke et al., 2020; Pereira Santos et al., 2021; Sampaio et al., 2021). This taxon is clearly vulnerable to ocean warming, and many of the patterns observed in this meta-analysis fitted with broad expectations for marine ectotherms. For instance, proportionally greater negative responses to warming were observed in sub-tropical and tropical echinoderms and at larval ontogenetic life stages. However, there were some interesting patterns that did not fit initial expectations, including a lack of thermal vulnerability for many biological responses (*i.e.*, growth, feeding and nutrition, movement, and reproduction).

The impacts of ocean warming and accompanying marine heatwaves are already evident in echinoderm populations, with disease-induced die-offs in numerous species being linked to elevate temperatures (Dungan et al., 1982; Lester et al., 2007; Clemente et al., 2014; Menge et al., 2016). Despite this growing threat, echinoderms may be able to persist in warmer oceans through range shifts and/or acclimation and adaptation (Munday et al., 2008; Ling et al., 2009; Poloczanska et al., 2016). However, there is a growing consensus that future ocean warming may be too rapid and marine heatwaves may be too abrupt for these mitigation strategies to keep pace with climate change (Radchuk et al., 2019). Considering the disproportionately large role that echinoderms play in structuring communities and ecosystems, understanding how members of this phylum will fare in under further warming is of paramount importance (Byrne & O'Hara, 2017; Ling, 2013; Menge et al., 2016).

Chapter 3: Impacts of ocean warming on the settlement success and post-settlement survival of Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*)[2]

Publication

[2] Lang, B. J., Caballes, C. F., Uthicke, S., Doll, P. D., Donelson, J. M., & Pratchett, M. S. (2022) Impacts of ocean warming on the settlement success and post-settlement survival of Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*). *Coral Reefs*, 42, 143-155. https://doi.org/10.1007/s00338-022-02314-y

3.1 Abstract

Ocean warming and population irruptions of crown-of-thorns starfish (CoTS; *Acanthaster* cf. *solaris*) are two of the greatest threats to coral reefs. As such, there is significant interest in understanding how CoTS may be directly impacted by rising ocean temperatures. Settlement of planktonic larvae and subsequent metamorphosis is purported to be a major population bottleneck in marine invertebrates, yet it is unknown how ocean warming will impact these processes in CoTS. Herein, the effect of temperature (28 °C ambient, 30 °C, 32 °C, 34 °C), on the settlement success, metamorphic success, and post-settlement survival of this corallivore was explored. While larval settlement was robust to elevated temperature, with at least 94% of larvae settling after 48 h across all temperatures, it was observed that settlement success was lower on substrate that had been pre-treated ≥ 32 °C. Metamorphic success was also significantly constrained at temperatures ≥ 32 °C. At 32 °C and 34 °C metamorphic success was 16% and 63% lower than at ambient temperature, respectively. Significant adverse effects of warming on post-settlement survival were observed at even cooler temperatures, with 10% lower survival at 30 °C compared to at ambient temperature, and at 34 °C, survival was 34% lower. Substantial reductions in metamorphic success and early post-settlement survival at elevated temperatures, as well as negative impacts of warming on the settlement substrate and its capacity to induce settlement, may present a bottleneck for recruitment in a warmer ocean.

3.2 Introduction

Climate change is a growing threat to marine ecosystems, and coral reefs are particularly vulnerable (Hoegh-Guldberg et al., 2017; Hughes et al., 2017; IPCC, 2019; Mellin et al., 2019; Pratchett et al., 2021b). Ocean warming and the higher prevalence and intensity of marine heatwaves are causing increased severity, frequency, and extent of mass coral bleaching events on reefs (Bahr et al., 2017; Hoegh-Guldberg et al., 2017; Hughes et al., 2017; Mellin et al., 2019; Pratchett et al., 2021b). Moreover, many coral reef ecosystems, such as the Great Barrier Reef (GBR), are facing a multitude of other threats (Vercelloni et al., 2017; Suchley & Alvarez-Filip, 2018; Bellwood et al., 2019; Mellin et al., 2019). Crown-of-thorns starfish (*Acanthaster* spp.*;* CoTS), are causing extensive damage throughout the Indo-Pacific region, due to their tendency to feed on reef-building corals, and their propensity to occur at high densities (Pratchett et al., 2014; Vercelloni et al., 2017; Yasuda 2018; Pratchett et al., 2021a). The future threat of this destructive starfish depends largely upon their tolerance to ocean warming, as well as the state of their habitat and prey (Kamya et al., 2016; 2018; Lang et al., 2021; 2022).

Throughout their life cycle, CoTS remain vulnerable to warming (Kamya et al., 2014; 2016; 2018; Lamare et al., 2014; Uthicke et al., 2015; Caballes et al., 2017; Hue et al., 2020; Lang et al., 2021; 2022). Like most benthic marine invertebrates, CoTS are broadcast spawners, meaning gametes are released into the water column, where fertilisation occurs (McEdward & Miner, 2001; Uthicke et al., 2009; Caballes & Pratchett, 2014). Resulting embryos develop into swimming planktotrophic larvae, which eventually settle on the benthos (after ~12-14 days in optimal conditions; Lamare et al., 2014) and develop into juvenile and adult starfish with radial symmetry (Caballes & Pratchett, 2014). Prior research has determined that the thermal limit of fertilisation, and the embryonic and larval stages of CoTS, is generally exceeded between 30 to 34 °C, temperatures which are expected to be regularly surpassed on the GBR in the near-future (IPCC, 2019; AIMS, 2022a). Temperatures of 30 °C have been shown to have little effect on the performance of algal and coral feeding juvenile CoTS (Kamya et al., 2016; 2018). At 32 °C however, the thermal limit is surpassed, with coral-feeding juvenile and adult CoTS exhibiting significant reductions in metabolic performance and survival over ~60 days of thermal exposure (see **Chapter 5**; Lang et al., 2022). Coral-feeding CoTS may be able to tolerate short-term marine heatwaves (by definition, when the temperature surpasses the 90th percentile for a minimum of five consecutive days; Hobday et al., 2016) but may be unable to endure prolonged and sustained warming expected to occur as the climate changes (Lang et al., 2021; 2022). Prolonged warming may impact reproductive capacity and the quality of progeny, and the early life stages of CoTS may be further impaired by marine heatwaves that coincide with spawning, and subsequent early development and settlement (Rupp, 1973; Kamya et al., 2014; Lamare et al., 2014; Uthicke et al., 2015; Caballes et al., 2017; Hue et al., 2020).

Larval settlement and metamorphosis are essential processes in the life cycle of CoTS and other marine invertebrates (Rodríguez et al., 1993; Hadfield & Paul, 2001), and typically involve the planktonic larvae attaining metamorphic competence, active searches for suitable substrate, initial substrate contact, exploration, selection, fixation (*i.e.*, permanent attachment), and subsequent metamorphosis into their juvenile form (Pawlik, 1992; Doll et al., 2022). These processes are considered major bottlenecks in the life cycle of marine organisms with planktonic larvae, and the success of settlement and metamorphosis is likely to have a pronounced influence on recruitment and population dynamics (Balch & Scheibling, 2001; Uthicke et al., 2009; Wilmes et al., 2018). Few studies have assessed the effect of temperature on settlement and metamorphosis in marine invertebrates (relative to other life stages and processes; Espinel-Velasco et al., 2018), with the majority of such research focussing on corals (*e.g.*, Nozawa & Harrison, 2007; Putnam et al., 2008; Randall & Szmant, 2009). Nevertheless, a field study found that in warmer years, settlement success was higher in the sea urchin *Diadema* aff. *Antillarum* from the Canary Islands (Hernandez et al., 2010). It was anticipated that the additive effect of elevated temperature and over-fishing of sea urchin predators would increase their abundance in a warmer ocean, with negative downstream impacts on marine biodiversity (Hernandez et al., 2010; Mos et al., 2011). Conversely, *ex-situ* studies on the sea cucumber *Apostichopus japonicus* and the sea urchin *Tripneustes gratilla* found that at certain critical temperatures, settlement was negatively impacted (Li et al., 2010; Mos et al., 2011). This may be largely a consequence of an increasing energetic demand at higher temperatures, meaning less energy is available for maintaining normal physiological processes and behaviours (Pörtner & Peck, 2010; Ross et al., 2011; Pörtner et al., 2017; Espinel-Velasco et al., 2018).

Even if settlement rates remain high in a warmer ocean, this may not necessarily result in successful recruitment (Rowley, 1989; Keesing & Halford, 1992; Nozawa & Harrison, 2007; Hernandez et al., 2010; Vermeij et al., 2010; Mos et al., 2011; Glockner-Fagetti & Phillips, 2020). Newly metamorphosed juveniles may have reduced energy reserves in a warmer ocean, interfering with the maintenance of homeostasis, and the growth and development of protective structures, such as spines (Wolfe et al., 2013; Wilmes et al., 2018; 2019). These newly settled juveniles may therefore experience high mortality rates (Nozawa & Harrison, 2007; Ross et al., 2011; Pörtner et al., 2017; Espinel-Velasco et al., 2018), particularly on the reef where they are highly vulnerable to predation (Cowan et al., 2017; Keesing et al., 2018; Wilmes et al., 2018; 2019).

Settlement success and early post-settlement survival in marine benthic invertebrates, such as CoTS, may also be hindered by the indirect effect of warming on the settlement substrate (Dworjanyn & Pirozzi, 2008; Webster et al., 2011; Mos et al., 2011; Huggett et al., 2018). At a local scale, many marine invertebrate larvae respond to chemical stimuli associated with particular biogenic substrates (mostly marine algae) indicating a suitable environment for their benthic life-history stage (Hadfield & Paul, 2001; Huggett et al., 2006; Doll et al., 2022). When tested in isolation from the biogenic substrate, marine biofilms (and associated bacteria or diatoms) are often identified as the harbour of specific chemical components that induce larval settlement in CoTS (Johnson & Sutton, 1994), other echinoderms (reviewed by Doll et al., 2022), and a wide range of benthic marine invertebrates (reviewed by Hadfield & Paul, 2001; Hadfield, 2011). Elevated temperatures can cause shifts in the microbial community structure on the algal surface (Webster et al., 2011; Whalan & Webster 2014; Huggett et al., 2018), but also directly bleach the algae (Webster et al., 2011; Vásquez-Elizondo & Enríquez, 2016; Huggett et al., 2018). In a warmer ocean, settlement success and early post-settlement processes in CoTS may thus be indirectly inhibited by the effects of thermal stress on settlement substrates, and their capacity to induce settlement (Hadfield & Paul, 2001; Huggett et al., 2018).

The aims of this study were to establish the effect of warming on (1) settlement success (2) metamorphic success, and (3) post-settlement survival of CoTS. This study further aimed to (4) unravel the effect of elevated temperature on the crustose coralline algae (CCA) settlement substrate, and hence settlement and metamorphic success. Based on previous research on the early life-history stages of this species (Table 1.1; Rupp, 1973; Kamya et al., 2014; 2016; Lamare et al., 2014; Uthicke et al., 2015; Caballes et al., 2017), it was hypothesised that temperatures ≥ 30 °C would reduce the percentage of larvae that successfully settle and metamorphose, and would increase mortality rates during the early post-settlement stage. It was further hypothesised that settlement success would be constrained by negative effects of warming on settlement substrates (Diaz-Pulido et al., 2011; Webster et al., 2011; Vásquez-

Elizondo & Enríquez, 2016; Huggett et al., 2018; Muñoz et al., 2018). An inadvertent reduction in recruitment would unequivocally constrain the replenishment of adult CoTS populations, that may themselves be directly affected by warming (Lang et al., 2021; 2022).

3.3 Methods

3.3.1 Collection and rearing

Adult Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*) were collected from John Brewer Reef (18°38'S, 147°02'E) and Wheeler Reef (18°47'S, 147°31'E) in the Central Great Barrier Reef in late-October and early-November 2021. At the time that CoTS were collected, the water temperature was ~26-27 °C. CoTS were acclimated at the Australian Institute of Marine Science National SeaSimulator in Townsville, Australia, in filtered seawater (FSW) at 28 ± 0.5 °C. Eggs and sperm were obtained from six females and six males on the 17th November 2021. Ovary lobes were removed from females and placed in a beaker with 200 ml FSW at 28 °C. These ovary lobes were then washed over a 500 µm mesh to remove loose eggs. Ovary lobes were placed in a beaker containing 200 ml FSW with 10^{-5} M 1-methyladenine for 60 min to induce maturation of eggs. Approximately 20 min before the aforementioned step was complete, sperm was removed from males. Dry sperm from each male (2 µL) was mixed with 15 ml FSW at 28 °C, and then 1 ml of the sperm stock solution was added to the egg stock solution. Fertilisation was immediately assessed under a microscope, indicated by the presence of a fertilisation envelope. Fertilisation was determined to be at least 80%. Embryos were then transferred to 70 L vats on low bubbling at a density of approximately 10 to 15 embryos ml^{-1} (See Figure 3.1). Twenty-four hours after fertilisation, 100% water exchanges were conducted to remove undeveloped embryos and dead larvae (which sank to the bottom of the vats when air lines were removed). At 48 h post-fertilisation, 16 L flow through cones (14 L working volume) were stocked with healthy larvae at a density of approximately 1 larvae ml^{-1} and fed a continuous supply of *Dunaliella* sp. (70%) and *T-Isocrysis* sp. (30%) via an automated feeding system (Uthicke et al., 2018). Larval development was microscopically examined three times per week, and water changes were carried out concurrently.

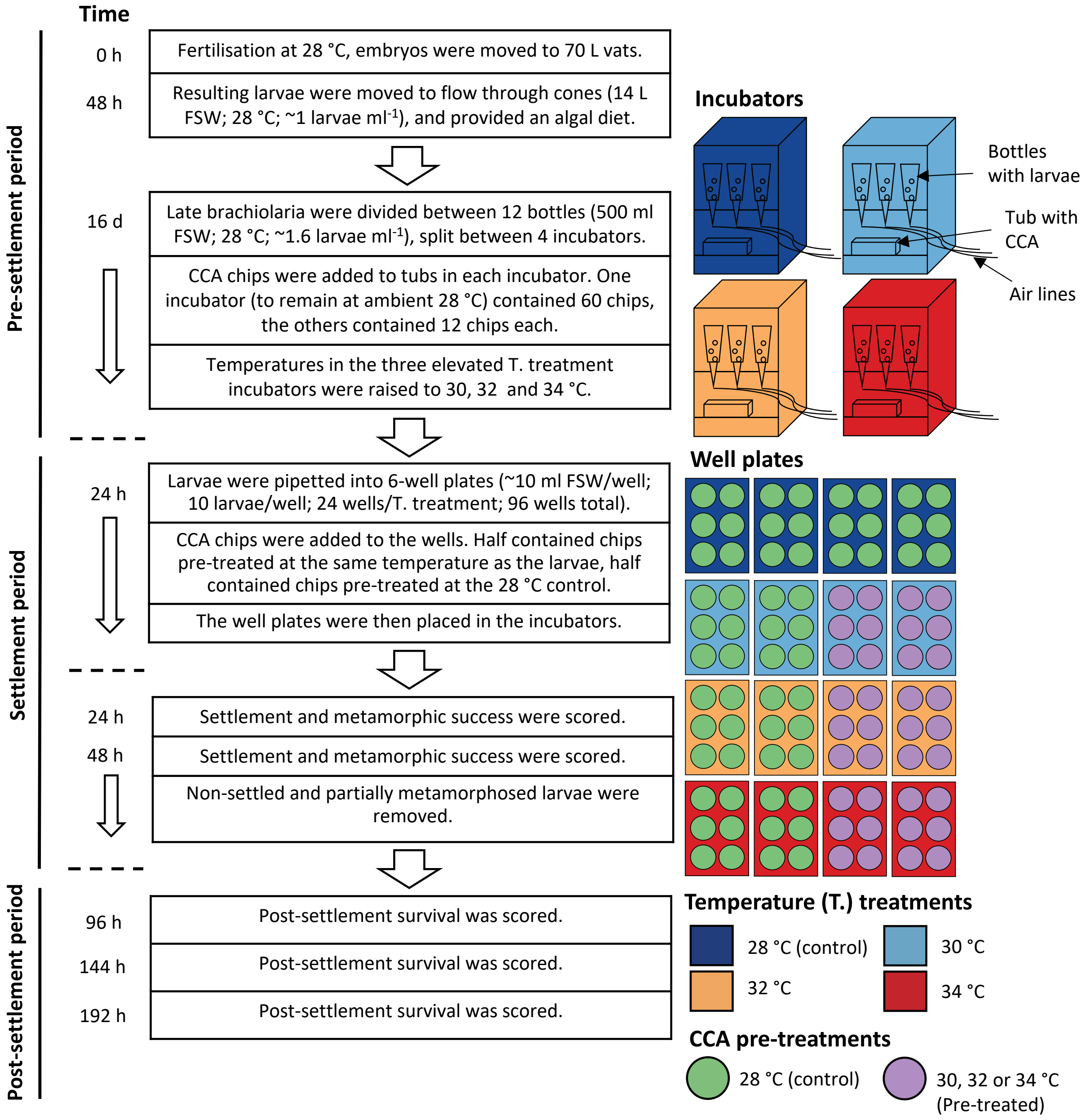

Figure 3.1 A flow diagram summarising the methodology and accompanying diagrams illustrating the set-up of the incubators for the four temperature treatments (28 °C: dark blue; 30 °C: light blue; 32 °C: orange; 34 °C: red), and the well plates containing crown-of-thorns starfish (*Acanthaster* cf. *solaris*) larvae and crustose coralline algae (CCA, *Lithothamnion* sp.; control: green; pre-treated: purple) incubated within them. Dashed lines on the flow diagram indicate time beginning again from zero hours, and arrows indicate a continuation of the methodology at the same point in time as the previous steps.

3.3.2 Experimental setup

At 16 days post-fertilisation, larvae reached the late brachiolaria stage with a well-developed rudiment, indicating that they were competent to settle. Larvae were transported to James Cook University in Townsville and kept in one of twelve plastic bottles containing ~500 ml FSW at a density of ~1.6 larvae ml^{-1}. These were held within one of four Sanyo MIR-553 incubators (12:12 h light:dark cycle) and maintained at ambient 28 °C (three bottles per incubator; Figure 3.1). Air lines connected to the base of each bottle ensured that the water remained oxygenated and prevented the larvae from settling prematurely. A Perspex tub was placed in each incubator, containing crustose coralline algae (*Lithothamnion* sp., CCA) chips with a mean surface area of 0.87 cm^2 (SE ± 0.03 cm^2). This CCA was collected from various northern and central reefs on the GBR. The tub in one incubator, which was to become the 28 °C control treatment, contained at least 60 chips of CCA. The tub in the other incubators (which were to become the 30, 32, and 34 °C treatments) contained at least 12 chips of CCA each. The temperatures in the latter incubators were then set to the desired temperatures, and the temperatures increased at a rate of 1-2 °C h^{-1}. The larvae and CCA were left to acclimate at these temperatures for 24 h. The temperature range used in the present study represents the approximate current mean (28 °C) and maximum (30 °C) summertime sea surface temperature in the collection vicinity, as well as temperatures similar to those projected to occur under the most optimistic (32 °C) and pessimistic (34 °C) end-of-century (2081-2100) ocean warming scenarios (IPCC, 2019; AIMS, 2022a).

3.3.3 Settlement and metamorphic success

Competent larvae were pipetted into 6-well plates. Four plates were used in each of the four larval temperature treatments (24 replicate wells per temperature, 96 wells total; Figure 3.1). Approximately 10 larvae were added to ~10 ml FSW in each well, followed by a CCA fragment. Fragments that had acclimated to the three elevated temperatures for 24 h (pre-treated CCA) were added to 12 wells of the corresponding larval temperature treatment, and fragments acclimated at the 28 °C control temperature (control CCA) were placed in the remaining 12 wells. All wells of the control temperature treatment contained CCA acclimated at the 28 °C control temperature (Figure 3.1). Testing settlement using pre-treated and control CCA permitted the observation of whether the quality of CCA, and therefore its relative inductive capacity, may have an impact on settlement success, and whether this impact is greater at higher magnitudes of warming. The four 6-well plates containing larvae for each temperature treatment were placed in the respective incubators and maintained at these temperatures.

Settlement and metamorphic success were scored under a microscope after 24 and 48 h, during which 50% water changes were carried out. Specifically, a single observer (B.J.L.) scored the number of larvae in each well, which had (1) successfully attached to the substrate or bottom of the well and commenced metamorphic transformation (settlement success; Figure 3.2A), (2) completed metamorphosis into juveniles with a radial symmetry (metamorphic success; Figure 3.2B). After scoring was complete, non-settled larvae and partially metamorphosed individuals that had not settled on CCA were removed. All other individuals remained in the wells to assess post-settlement survival.

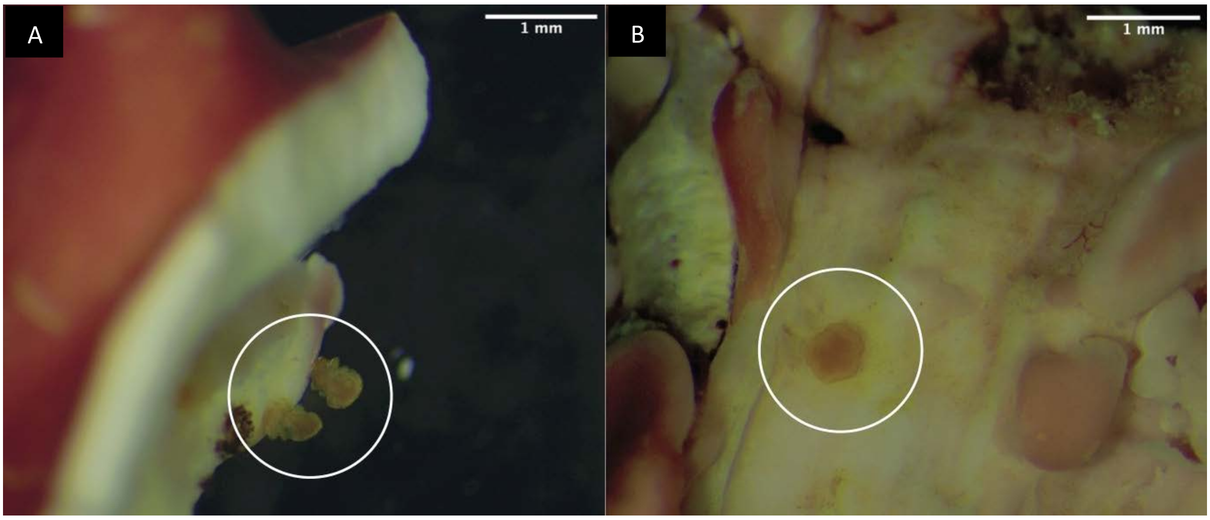

Figure 3.2 Images showing A. metamorphosing larval crown-of-thorns starfish (*Acanthaster* cf. *solaris*) absorbing the larval body and B. post-metamorphic juveniles with radial symmetry. Both have settled on crustose coralline algae (CCA, *Lithothamnion* sp.).

3.3.4 Post-settlement survival

At 96, 144 and 192 h after the commencement of the experiments, the number of post-metamorphic juveniles remaining was counted under a microscope to determine percentage survival in the four temperature treatments. Missing CoTS were presumed to be dead, and thus survival may be underestimated to some degree as a result of detectability limitations. Water changes were carried out daily over this time period.

3.3.5 Statistical analyses

Statistical analyses were carried out in R v.4.1.2 (R Core Team, 2021). Generalised linear models were used to establish the effect of temperature, time (24 and 48 h), and their interaction, on the percentage of larvae that settled (settlement success, %) and the percentage of settled individuals that completed metamorphosis (metamorphic success, %). The observations from the control and pre-treated CCA wells were pooled for this analysis. Further generalised linear models were conducted to establish the effect of the CCA treatment on settlement and metamorphic success. For these models, an interaction between CCA treatment and time and CCA treatment and temperature were included. The observations at the 28 °C control temperature was removed from these latter models, because all of the CCA was treated the same for this temperature treatment. All models were conducted using the *glm* function in the "stats" package, with the quasibinomial family (link: logit), which accounted for the overdispersion of the data. The surface area of the CCA was included in initial models as a covariate but registered no significant effect. Model diagnostics (using the *check_model* function) were conducted in order to check that the model assumptions were met and to choose the models with superior fit, as such, the surface area of the CCA was excluded from all models. The estimated marginal means for the responses in each of the temperature and CCA treatments were established, and post-hoc analyses using the Tukey method were conducted in order to establish significant pairwise differences between treatments and timepoints.

A log-rank test was conducted, using the *survdiff* function in the "survival" package, to compare survival of metamorphosed juveniles in the four temperature treatments. Confidence intervals were established using the *survfit* function ("survival" package), and a Kaplan-Meier survival curve was fitted using the *ggsurvplot* function in the "survminer" package, in order to visualise the data.

3.4 Results

3.4.1 Settlement and metamorphic success

Settlement success of CoTS was high at all tested temperatures, with ≥ 83% of larvae settled at 24 h across all temperatures, which increased to ≥ 94% at 48 h (Figure 3.3A). Despite high rates of settlement, success did vary significantly among temperature treatments (F = 6.87, df = 188, P < 0.001) and times (F = 23.36, df = 187, P < 0.001), although there was no significant interaction between these variables (*F* = 1.67, *df* = 184, *P* = 0.175). Settlement success was highest at 30 °C (24 h: 97%, 48 h: 100%). At 24 h, settlement success at both the 28 °C control temperature and at 32 °C was 86%, significantly lower than at 30 °C (*P* = 0.034 and *P* = 0.041 respectively), and at 34 °C, settlement success was 83%, again significantly lower than at 30 °C (*P* = 0.013). At 48 h, there was little difference in settlement success between the temperatures tested (28 °C: 95%; 30 °C: 100%; 32 °C: 94%; 34 °C: 99%).

Despite the majority of larvae settling throughout the range of temperatures tested, fewer larvae completed metamorphosis when exposed to warmer temperatures (Figure 3.3B). Temperature significantly impacted metamorphic success (*F* = 39.51, *df* = 188, *P* < 0.001), but there was no significant effect of time (*F* = 0.07, *df* = 187, *P* = 0.790) or an interaction between temperature and time (*F* = 0.76, *df* = 184, *P* = 0.519). The percentage of the total number of settled larvae that completed metamorphosis was 96%, 91%, 81% and 36% for the 28 °C, 30 °C, 32 °C and 34 °C treatments respectively. Metamorphic success was significantly lower at 32 °C than at the 28 °C control temperature (*P* = 0.013), and at 34 °C compared to the three cooler temperatures (*P* < 0.001 for all).

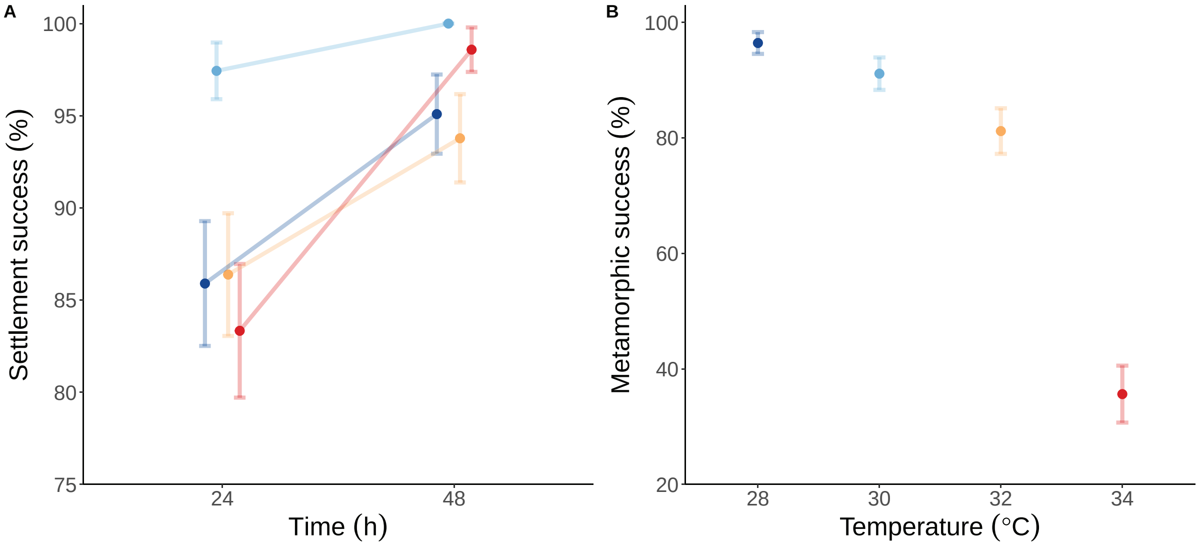

Figure 3.3 The effect of temperature (28 °C: dark blue; 30 °C: light blue; 32 °C: orange; 34 °C: red) on crown-of-thorns starfish (*Acanthaster* cf. *solaris*) A. settlement success at 24 and 48 h (*n* = 24 replicates per temperature treatment at each timepoint), and B. metamorphic success (*n* = 48 replicates per temperature treatment, replicates for the two timepoints are pooled). The bars are the mean values ± SE.

3.4.2 Post-settlement survival

Post-settlement survival remained high (≥ 62%) in all treatments (Figure 3.4), however a significant effect of temperature on post-settlement survival was found (Log-rank test, $\chi^2 = 56.5$, $df = 3$, $P < 0.001$). At 192 h, survival of early juveniles that remained following the 48 h scoring, was 94% (95%CI: 91-97%) at 28 °C, 85% (95%CI: 80-90%) at 30 °C, 86% (95%CI: 81-91%) at 32 °C, and 62% (95%CI: 52-72%) at 34 °C. Survival was significantly lower at 30 °C ($P = 0.003$), 32 °C ($P = 0.006$) and 34 °C ($P < 0.001$), compared to the 28 °C control temperature, and was also significantly lower at 34 °C compared to 30 °C and 32 °C ($P < 0.001$ for both).

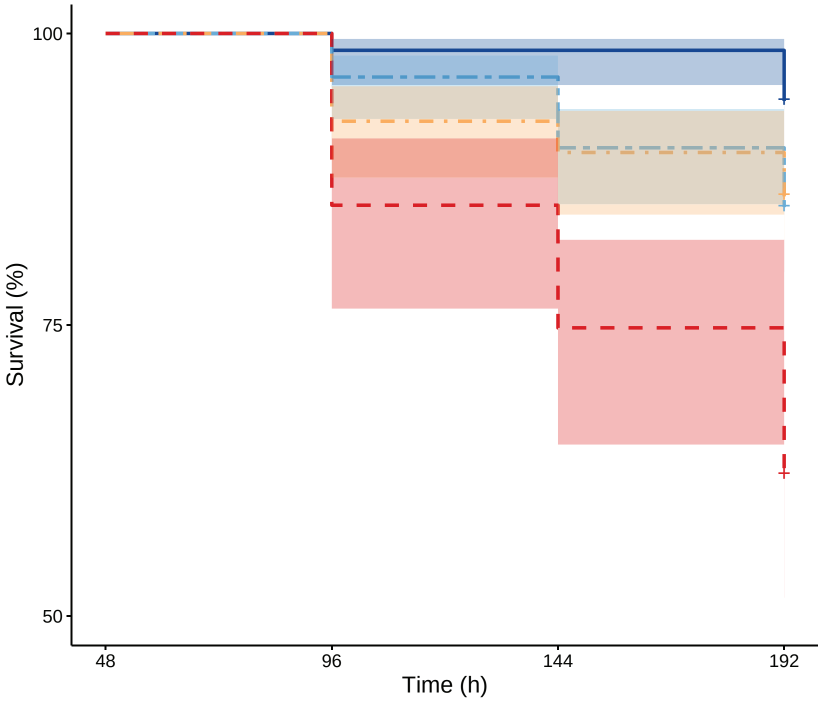

Figure 3.4 Kaplan-Meier survival curve illustrating the effect of temperature (28 °C: dark blue solid line; 30 °C: light blue twodash line; 32 °C: orange dotdash line; 34 °C: red dashed line) on the probability of crown-of-thorns starfish (*Acanthaster* cf. *solaris*) surviving post-settlement (%) from 48 to 192 h (*n* = 24 replicates per temperature treatment at each timepoint). The ribbons represent the 95% confidence intervals.

Ultimately, it was determined that the percentage of the number of individuals that remained at the conclusion of the experiments (192 h), compared to the number of individuals present at the start of experiments (24 h), declined to an even greater extent with temperature.

Specifically, at 28 °C, 30 °C, 32 °C and 34 °C there were 83%, 78%, 67% and 27% of all starting individuals remaining at 192 h, respectively.

3.4.3 Settlement substrate

Exposure of CCA to elevated temperature for an additional 24 h prior to the commencement of settlement experiments impacted settlement success. Settlement success was significantly lower in the pre-treated CCA wells, compared to the control CCA ($F = 41.14$, $df = 142$, $P < 0.001$). At 24 h, settlement success was 89% on pre-treated CCA and 96% on control CCA ($P = 0.007$). At 48 h, settlement success was high (98-100%) in both CCA treatments ($P = 0.080$). It was found that there was no significant interaction between CCA pre-treatment and time ($F = 0.73$, $df = 138$, $P = 0.396$; Figure 3.5A), but there was a significant interaction between CCA pre-treatment and temperature ($F = 3.95$, $df = 136$, $P = 0.021$; Figure 3.5B). At 30 °C there was little difference in settlement success between CCA pre-treatments, given the high (99%) settlement success at this optimal temperature ($P = 0.783$). However, at 32 °C and 34 °C settlement success was 85-87% on pre-treated CCA, but 99% on control CCA ($P < 0.001$ at both temperatures).

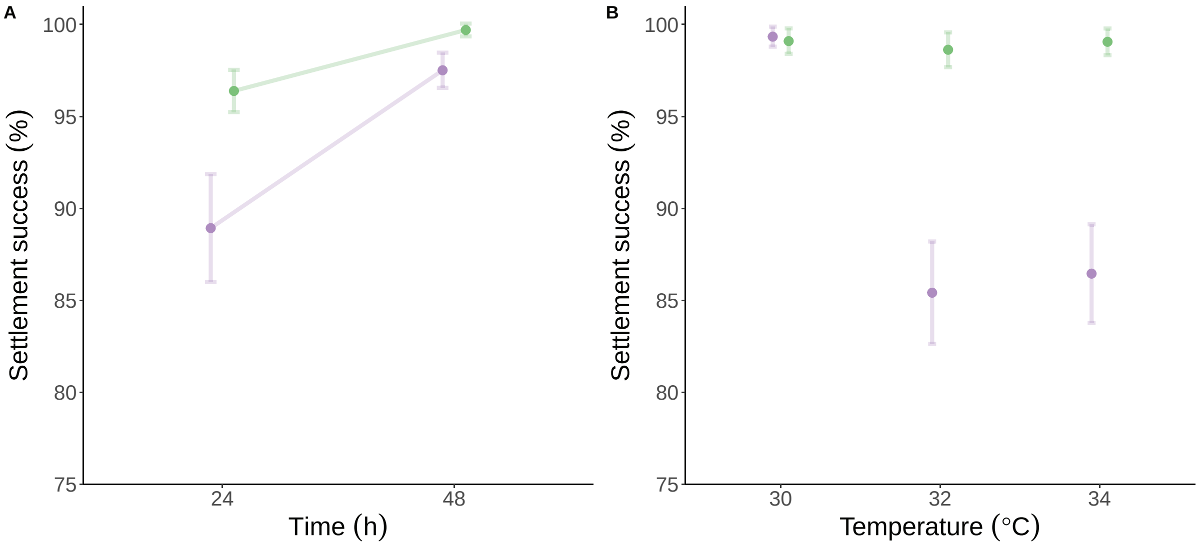

Figure 3.5 The effect of crustose coralline algae (CCA; *Lithothamnion* sp.) treatment (control: green; pre-treated: purple) on crown-of-thorns starfish (*Acanthaster* cf. *solaris*) settlement success over A. time (30, 32 and 34 °C treatments pooled; $n = 36$ replicates per CCA treatment at each timepoint) and across B. the temperature (timepoints pooled; $n = 24$ replicates per CCA treatment at each temperature treatment). The bars are the mean values ± SE.

There was no impact of CCA pre-treatment on metamorphic success ($F = 0.34$, $df = 142$, $P = 0.562$), and no significant interaction between CCA pre-treatment and time ($F = 0.62$, $df = 138$, $P = 0.433$) or temperature ($F = 1.39$, $df = 136$, $P = 0.253$). Some loss of pigmentation (bleaching) on the CCA was observed at 32 °C, and significant bleaching was observed at 34 °C (192 h, Figure 3.6).

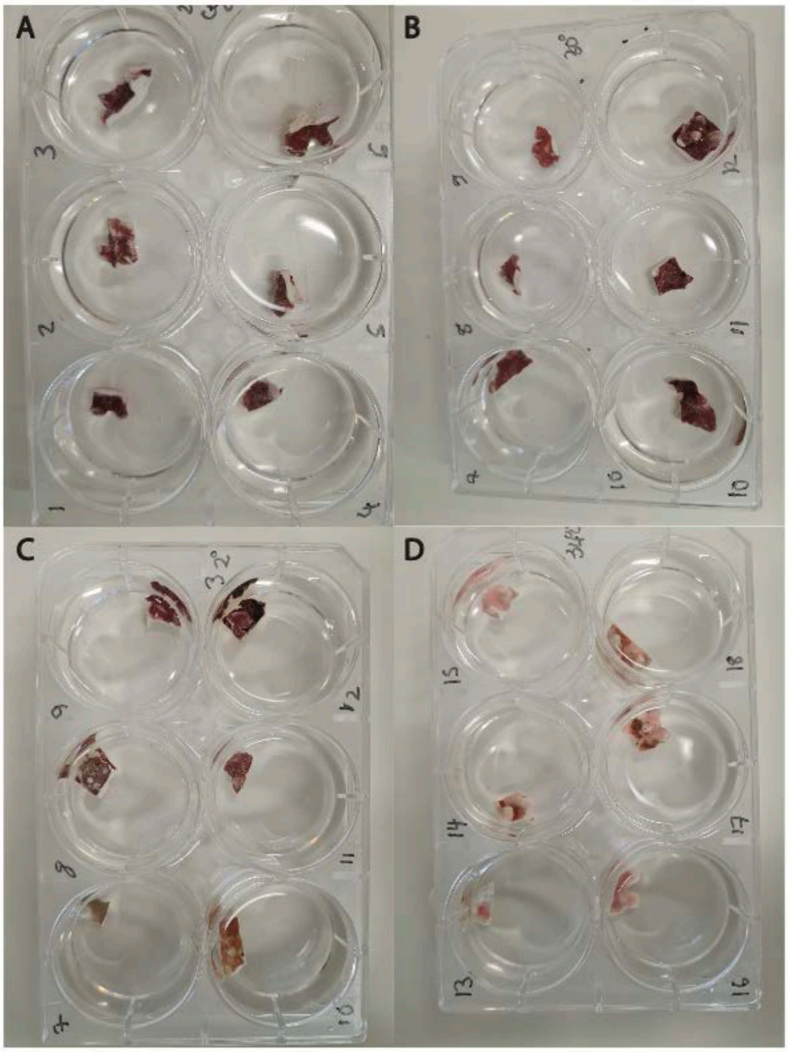

Figure 3.6 Photographs of the crustose coralline algae (CCA; *Lithothamnion* sp.) in six-well plates from the A. 28 °C, B. 30 °C, C. 32 °C and D. 34 °C temperature treatments at 192 h, indicating loss of pigment (bleaching). The well plates contain the pre-treated rather than the control CCA.

3.5 Discussion

This study provides evidence that larval settlement rates of *Acanthaster* cf. *solaris* will be largely insensitive to ocean warming. However, the cumulative effects of temperatures ≥ 30

°C will limit metamorphic success, and survival of early juveniles. Such effects may be further exacerbated by negative effects of warming on the settlement substrate (*i.e.*, CCA) and its capacity to induce settlement. These results indicate that recruitment and thus population replenishment of this corallivorous starfish may be constrained at elevated temperatures. Further, these findings add to the growing body of evidence suggesting that CoTS will be vulnerable to near-future ocean warming and may experience population declines (Table 1.1).

The robustness of CoTS settlement to elevated temperatures (up to at least 34 °C) is surprising, especially considering that pre-settlement embryonic and larval development appear very vulnerable to elevated temperatures (Table 1.1). However, there is similar evidence from tropical scleractinian corals that larval settlement may be unaffected by or even benefit from acute exposure to elevated temperatures (Edmunds et al., 2001; Nozawa & Harrison, 2007). For instance, larval settlement in the coral *Favites chinensis* was found to be ~62% higher at 34 °C (ca. ambient + 7 °C) when exposed to the temperature treatments for 1 h prior to the introduction of settlement substrate, and 24 h after (Nozawa & Harrison, 2007). However, the short exposure time in these studies may not be sufficient to instigate negative effects on settlement (Nozawa & Harrison, 2007; Randall & Szmant, 2009; Conaco & Cabaitan, 2020). Li and colleagues (2010) assessed the settlement success of the sea cucumber *Apostichopus japonicus* after 5 d of exposure to the temperature regimes and settlement substrate, and observed a 22% decline at 24 °C (ca. ambient + 6 °C). However, exposure of larvae to warmer conditions prior to larval competence in experimental studies, as in Li and colleagues (2010), may lead to difficulties in disentangling direct effects of temperature on settlement capacity from the indirect effects of warming induced constraints on normal larval development, as has been observed in CoTS (Table 1.1). As a corollary, persistent warming during the larval stage of CoTS in nature may exacerbate effects of warming on settlement success, and lead to detrimental effects at temperatures lower than observed in the present study. Yet, if the species can take advantage of natural thermal heterogeneity on the reef, then CoTS may not be as dramatically affected as these numbers suggest (Johnson et al., 1991; Nozawa & Harrison, 2007).

Even if larvae are able to settle effectively at higher temperatures, they may have reduced capacity to complete metamorphosis (Nozawa & Harrison, 2007; Mos et al., 2020). A significant decline in metamorphic success at elevated temperatures was recorded from 32 °C (ca. ambient + 4 °C) in the present study. Likewise, a study on the tropical sand dollar

Arachnoides placenta found that although larval development rate increased with temperature from ambient 28 °C to 34 °C, the incidence of metamorphosis declined by almost 40% over this range (Chen & Chen, 1992). The resulting post-metamorphic juveniles were also smaller at higher temperatures (Chen & Chen, 1992). These observations may be largely due to the greater oxygen demands and metabolic rates of larvae and juveniles at elevated temperatures, whereby energy reserves may be insufficient for successful metamorphosis, which has been determined to be a particularly energetically costly process in other marine invertebrate taxa, such as Bugula and Abalone (Shilling et al., 1996; Wendt, 2000; Randall & Szmant, 2009; Pörtner et al., 2017; Espinel-Velasco et al., 2018).

Diminished energy reserves may lead to increased mortality of post-metamorphic juveniles (Randall & Szmant, 2009; Pörtner et al., 2017). The present study revealed that for the most part, survival of post-metamorphic juveniles declined with warming. The vulnerability to near-future predicted temperatures observed in our study is similar to that observed in the coral *Porites astreoides*, where the survival of coral recruits exposed to temperatures of 30 °C was 1%, compared to 10% at ambient 27 °C (Ross et al., 2013). However, survival of post-metamorphic juveniles of the sea urchin *Heliocidaris erythrogramma* appeared unchanged by 4 °C of warming above ambient temperature (Wolfe et al., 2013). In this latter study, organisms were not exposed prior to the juvenile stage being reached, and it therefore did not account for potential carry-over effects from thermal effects on settlement-stage larvae (Ross et al., 2011; Wangensteen et al., 2013; Pörtner et al., 2017; Espinel-Velasco et al., 2018). Yet, a study on the sea urchin *Arbacia lixula* found no carry-over effects of temperature from the larval to the juvenile stages (Wangensteen et al., 2013). In nature, mortality directly resulting from elevated temperature may be secondary to high mortality resulting from predation (Wolfe et al., 2013). Constraints on growth and development of protective structures (*e.g.*, spines) for instance, may increase the vulnerability of juvenile CoTS to benthic predators (Cowan et al., 2017).

Elevated ocean temperatures may also reduce the availability of settlement substrate (*i.e.*, CCA) and inhibit their capacity to induce settlement (Webster et al., 2011; Vásquez-Elizondo & Enríquez, 2016; Huggett et al., 2018), with subsequent downstream impacts on settlement and metamorphic success and early post-settlement survival. In the present study a reduction in settlement success in response to the presence of CCA that had been exposed to ≥ 32 °C for an additional 24 h was observed. There was clear evidence of CCA bleaching from 32 °C.

Bleaching likely reduces the nutritional quality of the algal food, with impacts on juvenile survival (Webster et al., 2011; Huggett et al., 2018). In addition to bleaching, high temperatures can modify the bacterial communities of biofilms on the algal surface, alter the production of inductive metabolites and chemicals, promote pathogenic bacteria which may be harmful to the larvae, and increase the abundance of bacteria, diminishing oxygen levels (Li et al., 2010; Huggett et al., 2018).

On the reef, there may be further biotic and abiotic factors that may exacerbate (or alleviate) the negative effects of warming on the processes of settlement and metamorphosis, and ultimately survival of CoTS. Potential changes to larval food availability in a warmer ocean may affect the condition of the larvae at competence, impacting settlement and subsequent processes (Uthicke et al., 2013; 2018; Pörtner et al., 2017). Uthicke and colleagues (2018) for instance, showed that settlement rates of CoTS were greater when larvae were provisioned with higher concentrations of algae, thus nourished larvae may be better able to settle at supra-optimal temperatures than malnourished larvae. Ocean acidification, a further consequence of climate change (IPCC, 2019), may also have significant impacts on these processes (Kamya et al., 2016; 2017). Kamya and colleagues (2016) demonstrated that growth and feeding rates of post-metamorphic juvenile CoTS on CCA were in fact higher under acidified conditions. A later study found that the carbon to nitrogen ratio and carbonate levels were lower under ocean acidification, likely increasing the palatability of the CCA (Kamya et al., 2017). Considering the significant effects of these factors, exploring the additive and interactive effects of these stressors combined with temperature on the processes discussed herein, is an important next step (Uthicke et al., 2013; Wangensteen et al., 2013; Kamya et al., 2016).

3.6 Conclusions

While larval settlement appears relatively robust to near-future ocean warming, metamorphic success and post-settlement survival of CoTS is lower at ≥ 32 and ≥ 30 °C respectively, and at ≥ 32 °C the quality and inductive capacity of CCA is reduced. These observations suggest that the cumulative effects of warming, which may be more pronounced under parental and early larval exposure, will seemingly limit recruitment in the near future (Ross et al., 2011; IPCC, 2019; AIMS, 2022a). Sea surface temperatures of ~30 °C are already experienced during marine heatwaves in the central GBR (AIMS, 2022a), and by 2081-2100 sea surface temperatures are expected to increase by 1.64-3.51 °C (5-95% model range, relative to 1986-

2005, RCP8.5; IPCC 2019). The observations presented herein add to the growing body of evidence indicating that near-future ocean warming will ultimately lead to an abatement in the incidence or severity of population irruptions of CoTS (Table 1.1), which could alleviate predation pressure on important reef-building corals (Pratchett et al., 2021a; Song et al., 2021). Adaptation or acclimation of CoTS to warmer conditions may be possible however, *e.g.*, via transgenerational plasticity that may improve the hardiness of the early life stages, yet there is a growing consensus that the oceans may be warming too fast, and marine heatwaves may be too intense for these responses to keep pace with climate change (Munday et al., 2008; Morley et al., 2019; Radchuk et al., 2019). Furthermore, other direct and indirect stressors, both relating to and independent of climate change, may add additional pressures on CoTS populations at different stages of their life cycle (Kamya et al., 2016, 2017; Cowan et al., 2017; Uthicke et al., 2018). Notably, the increasing frequency and intensity of coral bleaching and mortality events, and the corresponding reduction in the availability of coral prey, may further undermine the viability of CoTS populations (Caballes et al., 2016; Hoegh-Guldberg et al., 2017; Hughes et al., 2017; Mellin et al., 2019; Pratchett et al., 2021a; b).

Chapter 4: Metabolic responses of Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*) to acute warming[3]

Publication

[3] Lang, B. J., Donelson, J. M., Caballes, C. F., Doll P. C., Pratchett, M. S. (2021) Metabolic responses of pacific crown-of-thorns sea stars (*Acanthaster* sp.) to acute warming. *Biological Bulletin*, 241. https://doi.org/10.1086/717049

4.1 Abstract

Climate change and population irruptions of crown-of-thorns starfish (*Acanthaster* sp.) are two of the most pervasive threats to coral reefs. Yet there has been little consideration regarding the synergies between ocean warming and the coral-feeding sub-adult and adult stages of this asteroid. Here the thermosensitivity of the aforementioned life stages were explored by assessing physiological responses to acute warming. Thermal sensitivity was assessed based on the maximal activity of enzymes involved in aerobic (citrate synthase) and anaerobic (lactate dehydrogenase) metabolic pathways, as well as the standard metabolic rate of sub-adult and adult starfish. In both life stages, citrate synthase activity declined with increasing temperature from 15 °C to 40 °C, with negligible activity occurring >35 °C. On the other hand, lactate dehydrogenase activity increased with temperature from 20 °C to 45 °C, indicating a greater reliance on anaerobic metabolism in a warmer environment. The standard metabolic rate of sub-adult starfish increased with temperature throughout the testing range (24 °C to 36 °C). Adult starfish exhibited evidence of thermal stress, with metabolic depression occurring from 33 °C. Here, it was demonstrated that crown-of-thorns starfish are sensitive to warming but that adults, and especially sub-adults, may have some resilience to short-term marine heatwaves in the near-future.

4.2 Introduction

Environmental temperature is one of the most important abiotic factors influencing marine ectotherms (Munday et al., 2008; 2009; Nguyen et al., 2011; Schulte 2015; Hoey et al., 2016; Pinsky et al., 2019). In these animals, which include starfish and other echinoderms, the rate of cellular processes and, consequently, their physiology are inherently linked to the ambient thermal environment (Lawrence, 1984; Lagerspetz & Vainio, 2006; Nilsson et al., 2009; Donelson et al., 2010; Hofmann & Todgham, 2010; Byrne, 2011; Uthicke et al., 2014; Caballes et al., 2017). Because of the important role that temperature plays in the function and performance of these animals, elevated temperatures may have a major influence on their abundance and distribution (Lawrence, 1984; Schulte et al., 2011; Ling, 2013). Critically, sustained ocean warming is expected in the coming decades as a result of climate change (Helmuth et al., 2014; Hoegh-Guldberg et al., 2018; IPCC, 2021), which will be further compounded by the increasing incidence and severity of marine heatwaves (Lynch et al., 2014; Frölicher & Laufkötter, 2018; Hoegh-Guldberg et al., 2018; IPCC, 2021).

In general, rates of cellular processes accelerate as temperature rises, following the Arrhenius law (Clarke & Fraser, 2004; Schulte et al., 2011; Schulte, 2015). Consequently, thermal performance is largely determined by flux through metabolic pathways, influenced predominantly by the activity of metabolic enzymes (Pörtner, 2001; 2002; Kühnhold et al., 2017; 2019a; Pörtner et al., 2017; Illing et al., 2020). Enzymes are catalysts in almost all cellular processes and are thermally sensitive (Cooper, 2000; Seebacher et al., 2004; Tullis & Baillie, 2005; Kühnhold et al., 2017; 2019a). Enzymatic performance is influenced by the temperature dependence of the activation energy required for the conversion from substrate to product (Schulte, 2015), the state of the enzymes involved (*i.e.*, the proportion active, inactive, and denatured), and the trade-off with thermodynamically induced increases in reaction rates (Clarke & Fraser, 2004; Schulte et al., 2011; Schulte, 2015). Measuring the tissue-specific activity of enzymes involved in aerobic and anaerobic metabolic pathways is a commonly used proxy for understanding the capacity of aerobic and anaerobic metabolism at various temperatures (Lemos et al., 2003; Blank et al., 2004; Seebacher et al., 2004; Tullis & Baillie, 2005; Fields et al., 2008; Ekström et al., 2017). There is a lack of such studies on starfish; but combining metabolic enzyme activity and metabolic rate measurements, as in studies on other marine ectotherms (*e.g.*, Lemos et al., 2003; Blank et al., 2004; Tullis & Baillie, 2005), will be useful for understanding the mechanistic complexity behind the observed physiological responses of starfish in a warmer environment.

Increasing enzymatic activity and rates of cellular processes at higher temperatures tend to result in enhanced function and performance at the level of the organism and a greater demand for oxygen (Pörtner, 2001; 2002). In order to meet this demand, organisms increase their metabolic rate, as has been observed in a number of starfish species (Propp et al., 1983; Murphy & Jones, 1987; Peck et al., 2008; Fly et al., 2012; McElroy et al., 2012; McGaw et al., 2015). There are, however, inherent constraints on oxygen delivery and corresponding limits on metabolic rate (Pörtner, 2001; 2002; Nilsson et al., 2009; Pörtner et al., 2017; Kühnhold et al., 2019b), such that animals exposed to temperatures above their optimal thermal range may experience compromised performance, which may be compounded by protein denaturation (Pörtner et al., 2017). Limits to aerobic metabolism are further exacerbated by the associated accelerated rate of proton leakage (Schulte et al., 2011) and lower oxygen solubility in seawater at supra-optimal temperatures (Weiss, 1970; Pörtner, 2001; 2002; Pörtner, et al., 2017).

Measuring the physiological performance of organisms exposed to relatively rapid changes in temperature (*e.g.*, 1-2 °C h^{-1}) is a common way to visualise thermal performance and limits (Schulte et al., 2011; Kühnhold et al., 2019b; Torossian et al., 2020), although such studies on asteroids are limited (but see Fly et al., 2012; McElroy et al., 2012). Such tests of organismal responses to quick changes in temperature do not allow for compensatory mechanisms like acclimation (Pörtner, 2001; 2002; Schulte et al., 2011; Healy & Schulte, 2012; Pörtner et al., 2017; Torossian et al., 2020) and may be more representative of how organisms will respond to acute temperature stress associated with severe heatwaves (Pörtner, 2002; Schulte et al., 2011; Lynch et al., 2014; Frölicher & Laufkötter, 2018; Balogh & Byrne, 2020; Campbell et al., 2020; Gall et al., 2021). Measuring physiological responses to acute thermal exposure is the first step to understanding thermal sensitivity in marine ectotherms such as starfish and may also provide a reference for understanding responses to longer-term exposure (Schulte et al., 2011; Kühnhold et al., 2019a; b; Torossian et al., 2020).

Crown-of-thorns starfish (CoTS; *Acanthaster* cf. *solaris*) are highly fecund, large-bodied, coral-feeding asteroids (Keesing et al., 2021; Pratchett et al., 2021a; c) that have contributed greatly to sustained coral loss, particularly across the Indo-West Pacific, as a result of their propensity to undergo major population irruptions (De'ath et al., 2012; Mellin et al., 2019). Tropical marine ectotherms, in general, are considered to be particularly sensitive to increasing temperatures and are often living especially close to their thermal limits (Nguyen et al., 2011; Stuart-Smith et al., 2015; Comte & Olden, 2017; Pinsky et al., 2019; Negri et al., 2020). Studies have thus far demonstrated that early life stages of CoTS are thermosensitive, whereby fertilisation success, development, and larval size are compromised at 2 °C to 4 °C above ambient temperature (Rupp, 1973; Kamya et al., 2014; Caballes, 2017; Caballes et al., 2017; Sparks et al., 2017; Hue et al., 2020). Conversely, increasing temperature has been linked to more rapid larval development and higher larval survival (Uthicke et al., 2015) and also enhanced growth of algae-feeding juvenile CoTS (Kamya et al., 2016). Little is known, however, about the thermal sensitivity of coral-feeding sub-adult and adult CoTS and how they may respond to projected ocean warming and increasing incidence and severity of marine heatwaves (but see Hue et al., 2020). Crown-of-thorns starfish reach adulthood when their energy investment in growth declines, but the allocation of energy to reproduction increases markedly (Lawrence, 1984; Pratchett et al., 2014; 2021b; Babcock et al., 2016; Caballes et al., 2017). As a result of the high energetic cost of reproduction and the high reproductive potential of mature asteroids, it is considered that adult starfish may be more sensitive to warming than sub-adults (Lawrence, 1984; Caballes & Pratchett, 2014; Babcock et al., 2016).

The purpose of this study was to explore the thermal performance and sensitivity of sub-adult and adult CoTS. Thermal performance was assessed by measuring changes in the maximal cellular enzymatic performance (proxies for both aerobic and anaerobic metabolism) and the standard metabolic rate (SMR; proxy for aerobic metabolism) across a range of temperatures. Based on previous research on weight-specific fecundity in CoTS, starfish were deemed to be adults if their wet weight exceeded 700 g (Babcock et al., 2016; Pratchett et al., 2021c). Cellular performance was determined by measuring the maximal activity of the enzymes citrate synthase (CS) and lactate dehydrogenase (LDH) throughout the temperature ranges of 15 °C to 40 °C and 20 °C to 45 °C, respectively. The enzyme CS was selected as a proxy of aerobic capacity because of its role in the first step of the citric acid cycle, which takes place in the mitochondria (Blank et al., 2004; Savoie et al., 2008; Pichaud et al., 2017; 2019). The enzyme LDH was chosen as a proxy for anaerobic glycolysis (Savoie et al., 2008), being involved in the reduction of pyruvate to lactate in the cytoplasm (Ekström et al., 2017; Illing et al., 2020). Considering that a common expectation of ectotherm physiology is a greater reliance on unsustainable anaerobic metabolism at supra-optimal temperatures, due to the depression of aerobic metabolism (Pörtner 2001; 2002; Pörtner et al., 2017), it was expected that the activity of LDH would increase with temperature while the activity of CS would decline. The SMR of a separate set of CoTS was measured using intermittent-flow respirometry across a wide range of temperatures (24 °C to 36 °C). It was hypothesised that the SMR of CoTS would increase up until some critical level, which would be followed by abrupt metabolic depression; and it was suggested that this may occur at cooler temperatures in adult CoTS (Schulte et al., 2011; Schulte 2015). The metabolic responses to acute warming measured in this study provide an insight into the temperature sensitivity of CoTS physiology and its relationship with life stage, and aid in understanding how these starfish may respond to projected increases in ocean temperatures and the frequency of marine heatwaves.

4.3 Materials and methods

4.3.1 Collection and maintenance

Pacific CoTS (*Acanthaster* cf. *solaris*) used in this study were collected from shallow reef environments (2-8 m) on mid-shelf reefs in the central Great Barrier Reef (GBR), Australia, specifically Big Broadhurst (18°58'S, 147°43'E), Keeper (18°45'S, 147°17'E), Kelso (18°26'S, 146°59'E), Little Broadhurst (18°56'S, 147°41'E), Little Kelso (18°28'S, 146°59'E), Lodestone (18°42'S, 147°06'E), and Rib (18°28'S, 146°53'E) reefs. Seawater temperatures tend to range from 24 °C in July to 29 °C in February (AIMS, 2021a) at these locations and depths, and the water was 28 °C to 29 °C at the time of sampling (January 2020). Some CoTS (n = 29; 63-3868 g, 10-49.5 cm diameter) were used for immediate tissue sampling, whereby ~0.3-1.4 g of tube feet were removed, which was stored in liquid nitrogen for transport and then kept at 280 °C prior to processing for enzyme analyses. Other CoTS (n = 25; 24-1800 g wet weight, 8.1-35.5 cm diameter) were maintained in flow-through aquaria while being transported to the Marine and Aquaculture Research Facilities Unit at James Cook University, Townsville, Queensland, Australia. These CoTS were then distributed between 4 380-L aquaria ($n < 10$ starfish per aquaria) attached to semi-closed recirculating systems in a temperature-controlled room with a 12h:12h light:dark cycle. CoTS were habituated to ambient conditions (26 °C, salinity 37.5 ppt, pH 8.24) for 15 to 22 days before the commencement of the SMR experiments. The CoTS were not fed throughout the habituation period and SMR experiments.

4.3.2 Maximal enzyme activity

The maximal enzymatic activity of both CS and LDH from tube feet samples were measured. Tube feet, along with papulae, are essential for the uptake of oxygen into the water vascular (respiratory) system in asteroids (Nichols, 1966). They are also important for locomotion, featuring terminal suckers that provide adhesion to substrate and complex muscles and connective tissue fibres that allow the tube feet to contract and relax, enabling movement along a surface (Nichols, 1966).

The enzyme analysis protocol was adapted from Thibault et al. (1997), Seebacher et al. (2003), and McClelland et al. (2005). Samples from *n* = 29 CoTS were defrosted on ice and homogenised using a microtube homogenizer (BeadBug 6, Benchmark Scientific, Edison NJ) in 1:5 proportions with a buffer consisting of 50 mmol L^{-1} 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 1 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA), 0.01% Triton X-100, and 99.99% Milli-Q water (Millipore Sigma, Burlington, MA) and adjusted to pH 7.4, using sodium hydroxide (NaOH). At least 250 mL of the homogenate was taken for CS analysis and frozen in a -80 °C freezer for later use. The remaining homogenate was centrifuged (Eppendorf Centrifuge 5430, Hamburg, Germany) at 500 rpm for 1 min, and at least 625 mL of supernatant removed for LDH analysis was also stored in a -80 ºC freezer. In order to retain the mitochondria in the supernatant, CS homogenates were not centrifuged. The samples in 1-mL cuvettes were analysed using a spectrophotometer (UV5, MettlerToledo, Columbus, OH); the associated program LabX digitised the absorbance readings. The cuvettes were heated using a Loop L100 circulation thermostat (Lauda, Lauda-Königshofen, Germany).

Citrate synthase was assayed in 2 mmol L^{-1} 5,5'-dithobis-(2-nitrobenzoic acid) (DTNB)-ethanol solution, 12 mmol L^{-1} acetyl coenzyme A-lithium salt-Milli-Q water solution, and 50 mmol L^{-1} Tris-HCl-Milli-Q water solution (pH 8.0); 20 mmol L^{-1} of oxaloacetic acid-Tris solution (pH 8.0) was added to commence the reaction in all cuvettes except the background control cuvette. Here the reduction of DTNB and the associated change in absorbance were measured at 412 nm (Seebacher et al., 2003; Blank et al., 2004). The maximal CS activity was measured in triplicate at 15, 20, 25, 30, 35 and 40 °C (2-s readings, with 20 readings over 13 min). Lactate dehydrogenate was assayed in 0.5 mmol L^{-1} of *b*-nicotinamide adenine dinucleotide reduced disodium salt hydrate (NADH)-Tris solution (pH 7.4) and 50 mmol L^{-1} of sodium-pyruvate-NADH-Tris solution (pH 7.4). The background control cuvette included all assay components except the sample. Here the absorbance of NADH at 340 nm was measured (Seebacher et al., 2003). The maximal LDH activity was measured in triplicate at 20, 25, 30, 35, 40 and 45 °C (2-s readings, with 20 readings over 13 min). The temperature ranges for both of these enzymes were decided based on observations from pilot studies. In the case of LDH, negligible activity (*i.e.*, enzyme slopes were indistinguishable from blank controls) was recorded at 15 °C; in the case of CS, negligible activity was recorded at 45 °C. All readings across the temperature range for a sample were completed within 2.5 h, and the starting temperature was alternated to avoid time-related bias.

The average of the three absorbance slopes was used to establish the maximal enzyme activity. The first reading from all slopes, including the background slopes, was removed for consistency. The slopes for background activity were subtracted from the enzyme activity slopes if the background was more than 5% of the average slope value. Background activity was removed from only 6.4% and 1.3% of slopes for CS and LDH, respectively. The maximal enzyme activity was calculated in units per milligram tissue (U mg^{-1} tissue), where U is equivalent to micromoles per minute (mmol min^{-1}) (Thibault et al., 1997; Seebacher et al., 2003; McClelland et al., 2005).

4.3.3 Standard metabolic rate

The SMR (mg O_2 g^{-1} h^{-1}) of CoTS was measured by using intermittent-flow respirometry (Clark et al., 2013; Svendsen et al., 2016; Kühnhold et al., 2019b). Acute temperature ramping experiments were conducted by exposing individuals to temperatures ranging from 24 °C to 36 °C in 1 °C increments. The SMR was quantified within sealed acrylic respirometry chambers (2 x 4.12 L, 2 x 9.99 L, and 2 x 21.77 L). All chambers were placed within a 1000-L capacity water bath. Recirculating pumps allowed the water to constantly mix within the chambers at a flow rate of 2 to 4 L min^{-1}. Oxygen sensor spots and aligned Firesting oxygen probes (PyroScience, Aachen, Germany) attached to the recirculatory tubing allowed continuous measurements of percentage oxygen. The data were fed into the PyroScience program via an oxygen meter, which enabled the creation of slopes exhibiting the decline in oxygen concentration over time. Flush pumps (flow rate of 2 to 4 L min^{-1}) delivered oxygenated water from the water bath into the chambers between measurement periods, and snorkels attached to the top of the chambers allowed oxygen-depleted water to escape.

At least 12 h prior to the experiments, five starfish of various sizes were placed individually in appropriately sized respirometry chambers to acclimate overnight, and one chamber was left empty as a control for background respiration. A chiller allowed the gradual cooling of the water from ambient temperature (26 °C) to 24 °C at a rate of no more than 0.5 °C h^{-1}. Experiments began between 07:00 and 09:00 the following day. During each 12-h run, the decrease in percentage oxygen in the chambers was measured, with the starfish and the control chamber for every degree temperature increment between 24 °C and 36 °C. Each testing cycle consisted of a 30-minute measurement period (stable temperature), which was considered a suitable period of time to ensure that oxygen levels did not fall below ~75%. The measurement period was followed by a 30-minute flush period, during which the water in the chambers was returned to ~100% oxygen and was heated a further 1 °C. After the first day of testing, it was decided that above 31 °C the cycle would consist of a 20-minute measurement period, followed by a 40-minute flush period, to mitigate the faster rate of oxygen depletion and the slower rate of temperature elevation from this temperature point. In total, five runs were required in order for all 25 starfish to be tested. Control runs on a separate cohort of starfish (*n* = 6; 68-739 g wet weight, 10.2-27.1 cm diameter) were also conducted. The same 30:30 measurement:flush cycle was used, but the temperature remained at 26 °C for the entire 12-h run in order to control for the effect of time in the chambers. However, there was no correlation between time in the chambers and SMR in these control starfish ($R^2 = -0.160$, $P = 0.180$), indicating that the order of temperatures unlikely impacted measurements to a significant degree.

For consistency, the first 8 min from all oxygen depletion slopes were removed, due to a lack of linearity common at the start of measurement periods explained by the delay in the time it took for the water in the chambers to become completely mixed following the flush cycle. The SMR of the CoTS was calculated in milligrams of oxygen consumption per gram per hour, as per Svendsen et al. (2016), with the rates calculated from the background chamber slopes for each respective run subtracted.

4.3.4 Statistical analysis

All statistical analyses were carried out in R (v.3.6.3, R Core Team, 2020). Third-order polynomial linear mixed-effects models were conducted in order to establish the effect of temperature on the maximal activity of CS and LDH. The models examined changes in the maximal enzyme activity relative to temperature and its interaction with life stage. The two life stages included in the models were sub-adults (*n* = 10; 126-565 g wet weight) and adults (*n* = 19; 900-3868 g wet weight). The sample for one sub-adult weighing 170 g was only large enough to test CS activity; therefore, *n* = 9 sub-adults were included in the analyses for LDH. Starfish ID was included as a random effect in the models to account for repeated measures for each sample. The estimated marginal means for enzyme activity over the temperature range were extracted and used for all further data investigation, such as establishing temperature (Q_{10})

coefficients, using the equation by Prosser (1991) and the LDH:CS ratio at each temperature increment between 20 °C and 40 °C.

The SMR at the various temperatures was analysed using an additional third-order polynomial linear mixed-effects model. The model observed the effect of temperature on the SMR, which was log transformed in order to meet the assumptions of the statistical model. The model included an interaction between temperature and life stage, whereby starfish were again separated into sub-adults (n = 13; 24-282 g wet weight) and adults (n = 12; 713-1800 g wet weight); Starfish ID was included as a random effect. The estimated marginal means (on the response scale) were again extracted from the model and utilised for all further investigation of the data, including the calculation of Q_{10} coefficients. The metabolic scaling exponent (b), which is the slope value of log SMR (mg O_2 h^{-1}) and log weight (g), was established for each temperature between 24 °C and 36 °C, using linear ordinary least squares regression analyses.

4.4 Results

4.4.1 Maximal enzyme activity

There was a significant interaction between temperature and life stage in influencing the maximal activity of CS ($F_{3,143}$ = 3.21, P = 0.025, model conditional R^2 = 81.5%). Temperature alone also had a significant effect on the response ($F_{3,143}$ = 205.42, $P < 0.0001$), but life stage did not ($F_{1,29}$ = 3.75, P = 0.063). For sub-adult CoTS, CS activity was highest at 15 °C, with a mean activity of 0.516 U mg^{-1} tissue (95% confidence interval [CI], 0.454-0.577), which then declined with increasing temperature, with negligible activity at 40 °C (0.040 U mg^{-1} tissue; 95% CI -0.021-0.102; Table 4.1; Figure 4.1A). The average rate of decline in CS activity with warming was -0.019 U mg^{-1} tissue °C^{-1}, and the Q_{10} coefficient was 0.36 for the temperature range 15 °C to 40 °C. For adult CoTS, CS activity ranged from 0.388 U mg^{-1} tissue (95% CI, 0.343-0.433) at 15 °C, down to 0.031 U mg^{-1} tissue (95% CI -0.014-0.076) at 40 °C (Table 4.1; Figure 4.1B). The average rate of decline in CS activity was -0.014, and the Q_{10} coefficient was identical to that of sub-adults.

There was a significant effect of temperature on the activity of LDH ($F_{3,130}$ = 132.37, $P <$ 0.0001, model conditional R^2 = 79.1%) but no effect of life stage ($F_{1,28}$ = 2.36, P = 0.136) or interaction between these variables ($F_{3,130}$ = 2.18, P = 0.093). For sub-adult CoTS, LDH activity was lowest at 20 °C, with a mean activity of 0.508 U mg^{-1} tissue (95% CI, 0.075-0.941). The activity then increased up to 3.197 U mg^{-1} tissue (95% CI, 2.747-3.646) at 45 °C (Table 4.1; Figure 4.1C). Here the average rate of LDH activity elevation was 0.108 U mg^{-1} tissue $°C^{-1}$, and the Q_{10} coefficient was 2.09 for the temperature range 20 °C to 45 °C. For adults, the activity of LDH ranged from 0.437 U mg^{-1} tissue (95% CI, 0.145-0.728) at 20 °C to 2.460 (95% CI, 2.168-2.752) at 45 °C (Table 4.1; Figure 4.1D). For adults, the average rate of LDH activity elevation was 0.081 U mg^{-1} tissue $°C^{-1}$, and the Q_{10} coefficient was 2.00 for the temperature range 20 °C to 45 °C.

Table 4.1 Estimated marginal means for the effect of temperature on the maximal activity of citrate synthase (CS, U mg^{-1} tissue) and lactate dehydrogenase (LDH, U mg^{-1} tissue) in tube feet of *Acanthaster* cf. *solaris* (CS sub-adults, *n* = 10, adults *n* = 19; LDH sub-adults *n* = 9, adults *n* = 19). Upper and lower confidence intervals are in brackets.

Temperature (°C)	CS activity Sub-adult CoTS	CS activity Adult CoTS	LDH activity Sub-adult CoTS	LDH activity Adult CoTS
15	0.516	0.388	NA	NA
	(0.454-0.577)	(0.343-0.433)		
20	0.425	0.360	0.508	0.437
	(0.372-0.478)	(0.321-0.399)	(0.075-0.941)	(0.145-0.728)
25	0.305	0.267	0.756	0.566
	(0.254-0.355)	(0.230-0.304)	(0.371-1.141)	(0.306-0.826)
30	0.183	0.152	1.117	0.929
	(0.132-0.234)	(0.116-0.189)	(0.752-1.481)	(0.682-1.177)
35	0.086	0.060	1.627	1.432
	(0.032-0.139)	(0.021-0.098)	(1.268-1.987)	(1.183-1.681)
40	0.040	0.031	2.326	1.981
	(-0.021-0.102)	(-0.014-0.076)	(1.953-2.698)	(1.722-2.241)
45	NA	NA	3.197	2.460
			(2.747-3.646)	(2.168-2.752)

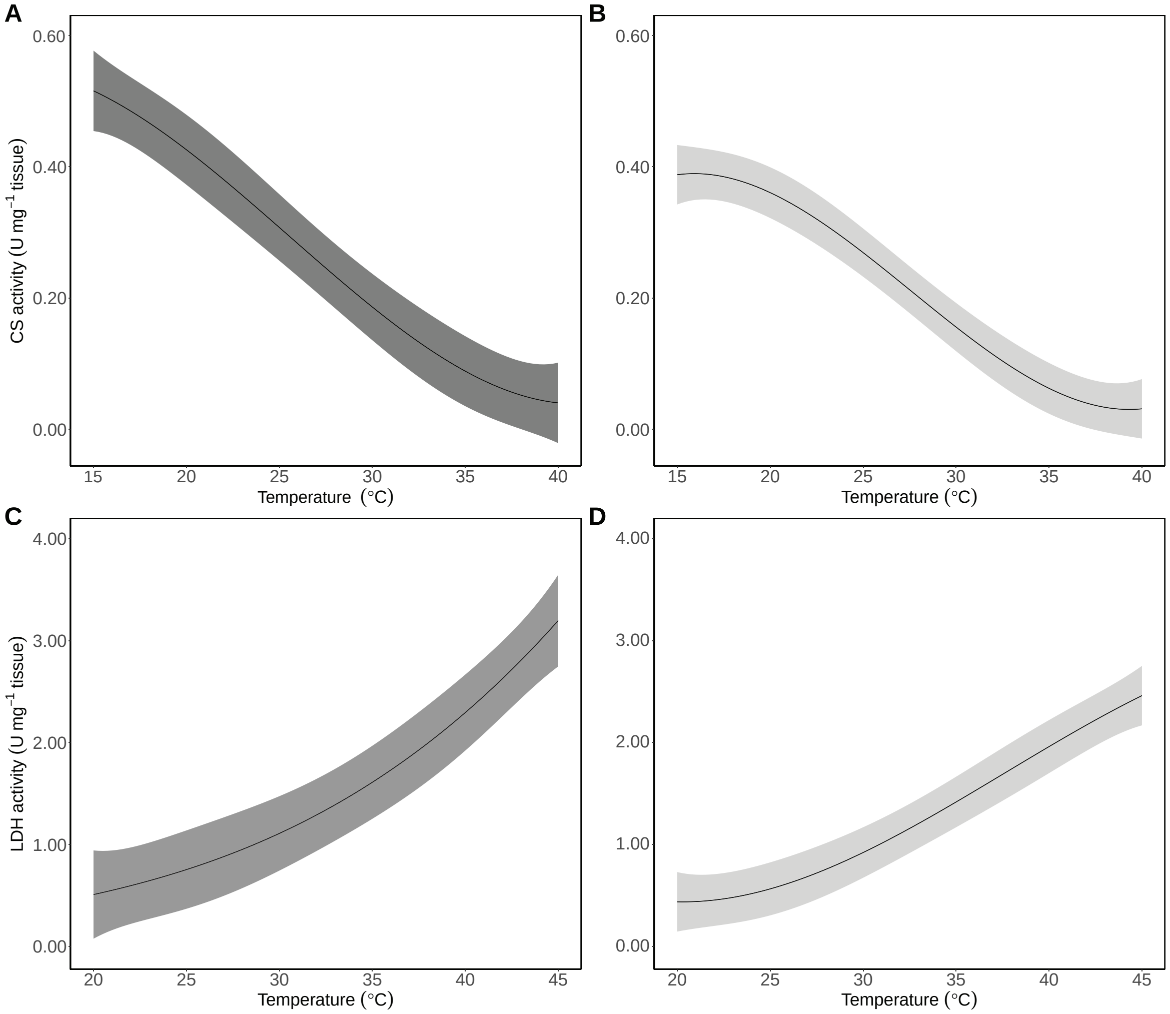

Figure 4.1 Estimated marginal means for the effect of temperature on the maximal activity of citrate synthase activity (CS, U mg^{-1} tissue) for A. sub-adult (*n* = 10, dark grey) and B. adult (*n* = 19, light grey) *Acanthaster* cf. *solaris.*; and the maximal activity of lactate dehydrogenase (LDH, U mg^{-1} tissue) for C. sub-adult (*n* = 9, dark grey) and D. adult (*n* = 19, light grey) *Acanthaster* cf. *solaris*. The ribbons surrounding the lines represents the upper and lower 95% confidence intervals.

The LDH:CS ratio increased exponentially with increasing temperature, from 1.195 at 20 °C to 58.150 at 40 °C for sub-adults and from 1.214 at 20 °C to 63.903 at 40 °C for adults (Table 4.2). The ratios are similar in sub-adults and adults between 20 °C and 30 °C but from 35 °C, a lower activity of CS relative to LDH in adults resulted in higher LDH:CS ratios at these warmer temperatures.

Table 4.2 The ratio of the estimated marginal means for the effect of temperature (between 20 and 40 °C) on the maximal activity of lactate dehydrogenase (LDH, U mg^{-1} tissue) and citrate synthase (CS, U mg^{-1} tissue) in tube feet of *Acanthaster* cf. *solaris*. Upper and lower confidence intervals are in brackets.

Temperature (°C)	LDH:CS ratio Sub-adult CoTS	LDH:CS ratio Adult CoTS
20	1.195	1.214
25	2.479	2.120
30	6.104	6.112
35	18.919	23.867
40	58.150	63.903

4.4.2 Standard metabolic rate

When assessing changes in the SMR of CoTS, a significant interaction between temperature and life stage was found ($F_{3,\ 275}$ = 93.12, P < 0.0001, model conditional R^2 = 90.2%). The individual effects for both temperature ($F_{3,275}$ = 220.41, P < 0.0001) and life stage ($F_{1,25}$ = 23.49, P < 0.0001) were also significant. In sub-adult CoTS, the SMR increased throughout the whole temperature range from 24 °C to 36 °C. The SMR ranged from 0.013 mg O_2 g^{-1} h^{-1} (95% CI, 0.010-0.016) at 24 °C to 0.063 mg O_2 g^{-1} h^{-1} (95% CI, 0.049-0.081) at 36 °C, with a rate of increase of 0.004 mg O_2 g^{-1} h^{-1} °C^{-1} and a Q_{10} value of 3.73 over the full temperature range (Table 4.3; Figure 4.2A). In adult CoTS, the SMR increased from a mean of 0.011 mg O_2 g^{-1} h^{-1} (95% CI, 0.008-0.014) at 24 °C to a peak of 0.019 mg O_2 g^{-1} h^{-1} (95% CI, 0.015-0.025) at 32 °C, with a rate of increase of 0.001 mg O_2 g^{-1} h^{-1} °C^{-1} and a Q_{10} value of 1.98 over the 24 °C to 32 °C temperature range. Beyond this temperature, metabolic depression was observed, with a -0.002 mg Q_{10} g^{-1} h^{-1} °C^{-1} decrease (Q_{10} = 0.20) in the SMR to 0.010 mg O_2 g^{-1} h^{-1} (95% CI, 0.008-0.014) between 32 °C and 36 °C (Table 4.3; Figure 4.2B). At 35 °C and 36 °C, many of the adult starfish were characterised by swelling, loss of turgor, twisting of arms, and inability to return to their natural orientation.

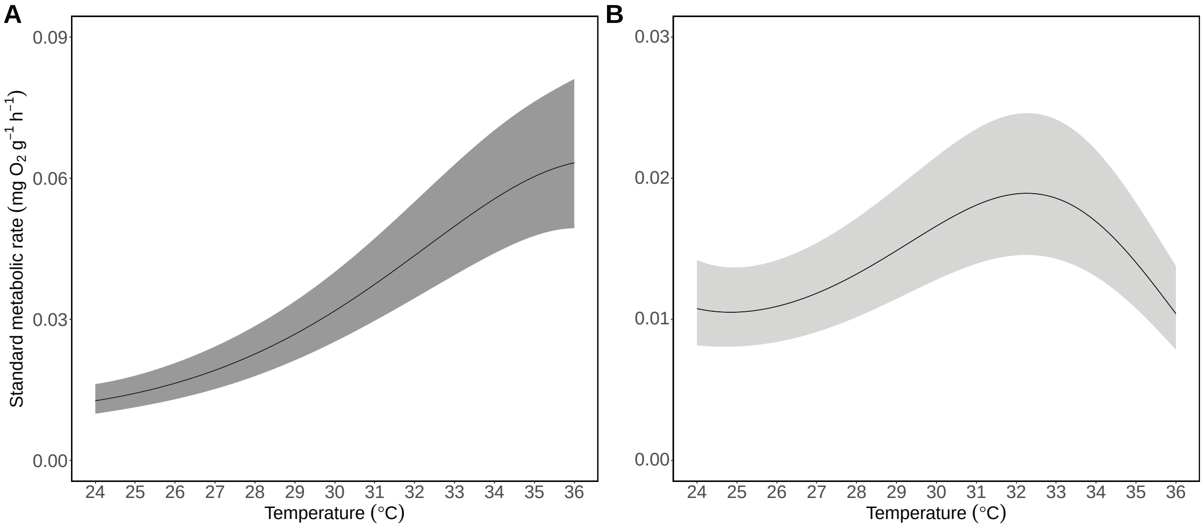

Figure 4.2 Estimated marginal means for the effect of temperature on the standard metabolic rate (SMR, mg O_2 g^{-1} h^{-1}) in A. sub-adult (*n* = 13, dark grey) and B. adult (*n* = 12, light grey) *Acanthaster* cf. *solaris*. The ribbons surrounding the lines represent the upper and lower 95% confidence intervals.

It was also found that the metabolic scaling exponent (*b*) declined linearly with temperature from 0.95 at 24 °C to 0.22 at 36 °C (77% decline; Table 4.3). This indicates that at higher temperatures, the positive correlation between weight and SMR is weaker, again highlighting that the metabolic rate of larger adult CoTS declined at warmer temperatures.

Table 4.3 Estimated marginal means for the effect of temperature on the standard metabolic rate (SMR, mg O_2 g^{-1} hr^{-1}) of *Acanthaster* cf. *solaris* (sub-adults, *n* = 13, adults, *n* = 12). Upper and lower confidence intervals are in brackets. The metabolic scaling exponent (*b*) for each temperature is also provided, which is the slope of log SMR (mg O_2 h^{-1}) vs. log weight (g)

Temperature (°C)	SMR Sub-adult CoTS	SMR Adult CoTS	Metabolic scaling exponent (*b*)
24	0.013 (0.010-0.016)	0.011 (0.008-0.014)	0.95
25	0.014 (0.011-0.018)	0.011 (0.008-0.014)	0.86
26	0.017 (0.013-0.021)	0.011 (0.008-0.014)	0.80
27	0.019 (0.015-0.024)	0.012 (0.009-0.015)	0.82
28	0.023 (0.018-0.029)	0.013 (0.010-0.017)	0.80
29	0.027 (0.022-0.034)	0.015 (0.012-0.020)	0.74
30	0.032 (0.025-0.040)	0.017 (0.013-0.022)	0.72
31	0.038 (0.030-0.047)	0.018 (0.014-0.024)	0.66
32	0.044 (0.034-0.055)	0.019 (0.015-0.025)	0.61
33	0.050 (0.040-0.064)	0.018 (0.014-0.024)	0.59
34	0.056 (0.044-0.071)	0.017 (0.013-0.022)	0.54
35	0.060 (0.048-0.076)	0.014 (0.011-0.018)	0.42
36	0.063 (0.049-0.081)	0.010 (0.008-0.014)	0.22

4.5 Discussion

This study provides the first insight into the acute thermal sensitivity of the sub-adult and adult life stages of the coral-feeding Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*) from the GBR, with clear responses in maximal cellular enzyme activity as well as the SMR. In both life stages, CS activity, which is a proxy for aerobic capacity (Blank et al., 2004; Savoie et al., 2008; Pichaud et al., 2017; 2019), was highest at 15 °C and decreased markedly with increasing temperature. The activity of CS was negligible at 35 °C to 40 °C, which may reflect the thermal limit of aerobic metabolism in these starfish. In contrast, the activity of LDH in sub-adults and adults was positively correlated with temperature. This resulted in higher LDH:CS ratios at the warmest testing temperatures; from 30 °C to 40 °C, every 5 °C increase in temperature resulted in a > three-fold increase in the LDH:CS ratio. This indicates a greater reliance on anaerobic metabolism, compared to aerobic metabolism at higher temperatures, particularly > 30 °C

(Pörtner 2001; 2002; Seebacher et al., 2004; Tullis & Baillie, 2005; Pörtner et al., 2017). The SMR of sub-adult CoTS increased throughout the 24 °C to 36 °C temperature range. In adult CoTS, however, the SMR increased up to an aerobic thermal ceiling at 32 °C and then declined moderately until 34 °C, with greater metabolic depression occurring thereafter. These results suggest that adult and, particularly, sub-adult CoTS from the central GBR may be fairly resilient to short-term marine heatwaves in the near-future (AIMS, 2021a; b).

The thermal limit of CS function was reached only at 35 °C to 40 °C, although it appears that CoTS exposed to warmer conditions will have increasingly constrained aerobic capacity and a greater reliance on anaerobic metabolism than at cooler temperatures. This appears to be the first known study to assess the influence of temperature on the relative activity of aerobic versus anaerobic metabolic enzymes in an asteroid. However, a similar study on another tropical echinoderm, *Holothuria scabra*, found that the LDH:isocitrate dehydrogenase ratio (another proxy for aerobic metabolism) was greater at 6 °C above the ambient control temperature, indicating that this may be a common physiological response to acute warming (Kühnhold et al., 2017). The fact that the maximal activity of LDH was higher than CS, in general (*i.e.*, the ratio is always greater than 1), may be expected, considering that sedentary marine animals, such as CoTS, use anaerobic metabolic pathways more than active species (*e.g.*, most fishes), even at ambient temperatures (Pörtner & Knust, 2007; Kühnhold et al., 2019a; b). This life-history strategy may enable CoTS to withstand short-term exposure to very high temperatures and thereby survive marine heatwaves (Li et al., 2015). Because this study examined the effect of temperature on tissue samples taken directly from CoTS in the field (at ~29 °C), only the effect of temperature on enzymes that were present at the time of sampling could be established. In nature, CoTS may also be able to increase or decrease enzyme production (Vetter, 1995) or produce more thermally tolerant isozymes of important metabolic enzymes (Lin & Somero, 1995), which may further improve the ability for CoTS and other asteroids to endure marine heatwaves.

In the present study, the SMR of CoTS increased over a wide thermal range, from 24 °C to at least 32 °C. An increase in metabolic rate with acute warming aligns with observations on other asteroid species (Peck et al., 2008; Fly et al., 2012; McElroy et al., 2012; McGaw et al., 2015). Fly et al. (2012), for instance, found that over a 10 °C range, the metabolic rate of the temperate intertidal starfish *Pisaster ochraceus* more than doubled. Most studies on asteroids and other echinoderms, including Fly et al. (2012), did not test individuals at temperatures that exceed their aerobic thermal ceiling (*e.g.*, Siikavuopio et al., 2008; Wood et al., 2010; Christensen et al., 2011; 2017; Fly et al., 2012; McElroy et al., 2012; Uthicke et al., 2014; Fang et al., 2015a; b; Carey et al., 2016; Yu et al., 2018). Due to our wide range of testing temperatures, metabolic depression at 33 °C and warmer in adult CoTS was observed, although the aerobic thermal ceiling of sub-adults was not reached in this study. The temperature of 33 °C is several degrees above maximum summer temperatures (~29 °C) in the central GBR; even during severe heatwaves, temperatures have not been recorded to exceed 32 °C (AIMS, 2021a; b), corroborating the view that CoTS may be resilient to short-term marine heatwaves in the near-future. Similarly high physiological thermal limits have been observed in comparable studies on the Antarctic starfish *Odontaster validus* and the tropical sea cucumber *Holothuria scabra*, where metabolic depression was observed 10 °C to 15 °C above the ambient control temperatures, indicating that the physiology of asteroids and other echinoderms may be robust in relation to acute warming (Peck et al., 2008; Kühnhold et al., 2019b). Marine ectotherms are, however, generally capable of coping with higher levels of acute environmental change, compared with sustained warming; consequently, this study may underestimate the vulnerability of CoTS to global warming (Lawrence, 1984; Peck et al., 2008; Brothers & McClintock, 2015; Pörtner et al., 2017).

This study has further revealed marked differences in the physiological responses of sub-adult versus adult CoTS to acute thermal exposure; this is consistent with studies across a broad range of species, including starfish and other echinoderms, that have shown that temperature sensitivity varies based on individual size, age, or life stage (*e.g.*, Seebacher et al., 2004; Peck et al., 2013; Dahlke et al., 2020; Illing et al., 2020). While the SMR was similar at 24 °C in both life stages (~0.01 mg O_2 g^{-1} h^{-1}), in sub-adults metabolic rate increased throughout the entire temperature range to ~0.06 mg O_2 g^{-1} h^{-1} at 36 °C. However, in adults the SMR reached a maximum of only ~0.02 mg O_2 g^{-1} h^{-1} at 32 °C, after which metabolic depression was observed. Although adult CoTS appear to have a lower thermal limit than sub-adults, it was found that the thermal sensitivity of sub-adult CoTS (SMR $Q_{10} = 3.73$) was far higher than that of adults (SMR $Q_{10} = 1.98$). This suggests that they may be better able to compensate for the heightened energetic demands in a warmer ocean (Schulte, 2015; Pörtner et al., 2017). This observation contrasts those of other asteroids (Murphy & Jones, 1987; Fly et al., 2012), such as the starfish *P. ochraceus*, for instance, where individuals < 300 g had an SMR Q_{10} coefficient of 1.95 compared to 3.79 in individuals > 500 g over a 10 °C temperature range (Fly et al., 2012). This difference may be a consequence of differential natural thermal environments experienced by the two species. Hailing from an intertidal environment, *P. ochraceus* adults are likely better adapted to cope with acute warming than adult CoTS, which inhabit a much more stable thermal environment (Fly et al., 2012; AIMS, 2021a; b). The activity of the metabolic enzymes tested provides some insight into the observed differences seen in the thermosensitivity and physiological thermal limits (Pejus limits; Pörtner et al., 2017) of sub-adult and adult CoTS. It was found that the activity of CS was lower and the LDH:CS ratios were higher in adults compared to sub-adults at the warmest testing temperatures. This indicates that in adult CoTS, aerobic constraints at the biochemical level may contribute to the lower aerobic capacity at the level of the organism.

The metabolic scaling exponent (*b*) declined from 0.95 to 0.22 with increasing temperature (24 °C to 36 °C), with *b* falling below 0.5 from 35 °C. In asteroids and other echinoderms, *b* generally ranges from 0.5 to 1.0, where 1.0 indicates an isometric relationship between the metabolic rate and the organism weight, while a value of 0.5 indicates a negatively allometric relationship (Lawrence & Lane, 1982; Murphy & Jones, 1987; Glazier, 2005; Carey et al., 2014). The exceeding of physiological thermal limits is evidenced by *b* decreasing with temperature, which has been observed in other marine invertebrate taxa (Doyle et al., 2012) and fishes (Ohlberger et al., 2012; Silva-Garay & Lowe, 2021). Conversely, Carey et al. (2014) found that *b* was not impacted by temperature (10 °C to 20 °C) in the temperate starfish *Asterias rubens*, although, again, the variable natural thermal environment experienced by this starfish may explain its thermotolerance. In the present study, a decrease in *b* with temperature further highlights limits to oxygen supply and energy production at more extreme temperatures in heavier (adult) individuals, which is not observed in lighter (sub-adult) conspecifics (Lawrence, 1984; Pörtner, 2001; 2002; Doyle et al., 2012; Pörtner et al., 2017). There are a number of possible explanations for the lower thermal limit of adult or larger CoTS. It is considered that oxygen supply may be less efficient in larger individuals and that mitochondrial function declines with age (Peck et al., 2013). Furthermore, life stage and size-based differences in thermal sensitivity may also be attributed to differences in the energetic investment in growth and reproduction (Lawrence, 1984). Given the very high investment in reproduction by large female CoTS, particularly in the warmer summer months (Caballes & Pratchett, 2014; Babcock et al., 2016; Pratchett et al., 2021c), it is likely that reproductive adult CoTS will be less tolerant of elevated temperatures during marine heatwaves and sustained warming compared to nonreproductive sub-adults, especially immediately prior to spawning (Uthicke et al., 2019; Caballes et al., 2021). As a result of the physiological constraints of heavier starfish at higher temperatures, a decrease in the maximum weight of this species may be observed as the oceans continue to warm (Pörtner & Knust, 2007), which may have consequences for reproductive success (Pratchett et al., 2021c) and vulnerability to predation (Rivera-Posada et al., 2014; Deaker et al., 2021).

It is axiomatic that thermally induced physiological incompetencies will have consequences for many asteroid species and other marine taxa as they are increasingly exposed to higher temperatures (Pörtner & Knust, 2007; Munday et al., 2008; Ling, 2013; Eisenlord et al., 2016; Hoey et al., 2016). Higher energetic demands with elevated temperature will impose inevitable constraints on movement (Pörtner 2002; Pörtner & Knust, 2007; Peck et al., 2008; Yuan et al., 2009; Kidawa et al., 2010; Günay et al., 2015; Sun et al., 2018), growth and reproduction (Lawrence, 1984; Pörtner, 2002; Pörtner & Knust, 2007), and, ultimately, survivorship (Peck et al., 2009; Christensen et al., 2011; 2017; Fang et al., 2015a; b; Harianto et al., 2018). It is unknown, however, when (rather than whether) different species of tropical marine organisms will begin to be impacted by sustained increases in ocean temperatures and/or severe heatwaves if, indeed, they are not already being affected. Given the threat posed by high densities of CoTS to coral reef ecosystems, including the GBR (Mellin et al., 2019), the extent to which CoTS are potential winners or losers under projected environmental change will be highly pertinent for tropical marine conservation (Przeslawski et al., 2008; De'ath et al., 2012; Mellin et al., 2019; Pratchett et al., 2021a). Yet any gains associated with a reduced abundance and impact of CoTS at higher temperatures may be offset, to some extent, by direct effects of ocean warming and heatwaves on corals and reef ecosystems (Hughes et al., 2018b; Pratchett et al., 2021a). It is suggested, however, that CoTS, particularly sub-adult CoTS, may be resilient to short-term marine heatwaves in the near-future; thus, CoTS predation and marine heatwaves may have cumulative or synergistic negative effects on coral reefs (Vercelloni et al., 2017). While this study provides a step to understanding the thermosensitivity of coral-eating sub-adult and adult CoTS, explicitly testing the effects of prolonged exposure to elevated temperatures by using CoTS from a wide range of thermal environments (*i.e.*, latitudes) is warranted in order to better understand how this asteroid will fare under further climate change (see **Chapter 5**).

Chapter 5: Effects of elevated temperature on the performance and survival of Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*)[4]

Publication

[4] Lang, B. J., Donelson, J. M., Caballes, C. F., Uthicke, S., Doll, P. C., Pratchett, M. S. (2022) Effects of elevated temperature on the performance and survival of pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*). *Marine Biology*, 169. https://doi.org/10.1007/s00227-022-04027-w

5.1 Abstract

Population irruptions of Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*) have caused substantial damage to coral reefs, but it is largely unknown how this asteroid will fare in a warmer ocean. These starfish were exposed to one of four thermal treatments, with final temperatures of 26 °C (control, annual average), 28 °C (summer average), 30 °C (summer maximum) and 32 °C (predicted summer maximum by 2100). The righting time, movement rate, standard metabolic rate and probability of survival of the crown-of-thorns starfish were measured at various timepoints over ~ 60 days. It was found that while temperature did not affect righting time, it did significantly affect movement rate. The movement rate of starfish increased across the 26 to 30 °C range, with those at 28 °C and 30 °C moving 18 and 27% faster than those at the control temperature. Similarly, the standard metabolic rate of starfish increased from 26 to 30 °C, with metabolism 100% and 260% faster at 28 °C and 30 °C compared to those at the 26 °C control. At 32 °C, individual starfish exhibited a 14% slower movement rate, a 33% slower metabolic rate, and also exhibited a fivefold lower probability of survival than those at 30 °C. These results indicate that 32 °C is above the thermal optimum of crown-of-thorns starfish, suggesting that prolonged exposure to temperatures that are expected to be regularly exceeded under near-future climate change may be detrimental to this species.

5.2 Introduction

Population irruptions of the coral-feeding Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*; CoTS) and climate change are considered two of the greatest contributors to coral loss, especially in the western Pacific (De'ath et al., 2012; Baird et al., 2013; van Hooidonk et al., 2016; Mellin et al., 2019; Castro-Sanguino et al., 2021). The tropical and subtropical waters surrounding coral reefs have warmed approximately ~ 1 °C since pre-industrial times (Lough et al., 2018; Negri et al., 2020), and climate change projections indicate that they will likely heat further over the coming decades (IPCC, 2013; 2021). Climate change is already having significant impacts on coral reef ecosystems in the form of a higher incidence and severity of marine heatwaves in recent years (Bureau of Meteorology, 2020; IPCC, 2021). These short-term periods of abnormally high ocean temperatures (by definition, when the temperature exceeds the 90th percentile for at least five consecutive days; Hobday et al., 2016), have resulted in mass coral bleaching events (van Hooidonk et al., 2016; Bureau of Meteorology, 2020). Extreme temperatures and CoTS predation are considered to have cumulative or synergistic negative effects on corals, reducing the resilience of coral reef ecosystems (Vercelloni et al., 2017; Kamya et al., 2018; Haywood et al., 2019; Keesing et al., 2019; Castro-Sanguino et al., 2021). However, in order to predict the future resilience of the reef, the direct impact of ocean warming on this coral predator must be considered (Lang et al., 2021).

For ectothermic species, such as asteroids, environmental temperature is a strong determinant of the rate of physiological processes, which affects the ability of these individuals to move in their natural environment (Kidawa et al., 2010; Nguyen et al., 2011; Brothers & McClintock, 2015; Illing et al., 2020). Measuring the time it takes for an individual to return to its natural orientation following inversion (righting time) is a useful measure of neuromuscular coordination (Kleitman, 1941; Reese, 1966; Binyon, 1972) and has been used as a proxy for overall performance in response to temperature in echinoderms (Peck et al., 2008; Kidawa et al., 2010; Wood et al., 2010; Brothers & McClintock, 2015). In the starfish *Odontaster validus,* while temperatures of 2 °C above ambient had little effect on righting time, starfish righted more slowly when heated by a further degree (Kidawa et al., 2010). Likewise, the sea urchin *Heliocidaris erythrogramma* righted at similar speeds at ambient temperature and with a 2 °C temperature increase, but a further degree of warming more than doubled the righting time (Minuti et al., 2021). In contrast, several studies have found that the righting time of echinoderms, such as the sea urchin *Strongylocentrotus intermedius* (Zhang et al., 2017) and the brittle star *Ophiura ophiura* (Wood et al., 2010), changed very little with 3 °C and 4.5 °C of warming (above ambient), respectively. Rates of movement along a surface serves as another useful proxy for performance in a warmer environment, but is rarely studied in echinoderms (but see Kidawa et al., 2010). This behaviour may be even more thermosensitive than righting, with the movement rate of *O. validus* increasing only until 1 °C above ambient temperature, but declining with an additional degree of warming (Kidawa et al., 2010). Activity rate declines in marine organisms at high temperatures may diminish the ability to effectively escape predators and seek out suitable prey, lessening the capacity to meet the heightened energetic demands in a warmer ocean (Pörtner & Knust, 2007; Peck et al., 2008; Kidawa et al., 2010; Wood et al., 2010; Brothers & McClintock, 2015; Sun et al., 2018).

It is a general physiological rule that the standard metabolic rate (SMR) increases with temperature, in an attempt to meet the greater energy requirement for maintenance of homeostasis (Peck & Prothero-Thomas, 2002; Clarke & Fraser, 2004). However, there are limits to the capacity to supply sufficient oxygen to sustain metabolism, produce enough energy to support normal cellular processes, and maintain organism performance and fitness (Lawrence, 1984; Pörtner, 2001; 2002; Pörtner et al., 2017). Harianto et al. (2018), for instance, observed significant declines in SMR and survival at 26 °C (~ 4 °C above the average summer temperature, AST) in the temperate sea urchin *H. erythrogramma*. Observed metabolic depression at elevated temperatures is largely a consequence of the increased reliance on anaerobic metabolism, which is much less efficient at producing energy than aerobic metabolism (Pörtner, 2001; 2002; Pörtner et al., 2017; Lang et al., 2021). Evidence for this has been observed in CoTS at the biochemical level, with reduced activity of citrate synthase (a proxy for aerobic capacity) and increased activity of lactate dehydrogenase (a proxy for anaerobic capacity) at warmer temperatures (see **Chapter 4**; Lang et al., 2021). The consequent hypoxia contributes towards the disruption of cellular oxidative phosphorylation, protein denaturation, mitochondrial failure and ultimately mortality, which often occur after extended exposure to elevated temperatures (Chen & Chen, 1993; Pörtner, 2001; 2002; Peck et al., 2009; Fang et al., 2015a; b; Christensen et al., 2011; 2017; Pörtner et al., 2017; Harianto et al., 2018).

In many marine ectotherms, including CoTS, thermal sensitivity is not uniform across life stages, and sensitivity to elevated temperature often varies depending on the measures assessed (*e.g.*, Rupp, 1973; Kashenko, 2006; Rahman et al., 2009; Byrne et al., 2013; Uthicke et al., 2015; Caballes et al., 2017). Laboratory-based investigations have demonstrated that gametogenesis and the early life stages of CoTS are thermosensitive, whereby 2 to 4 °C warming (above AST) results in smaller eggs and a lower gonadosomatic index (Hue et al., 2020), as well as reduced fertilisation success (Caballes et al., 2017; Hue et al., 2020), and proportion of embryos and larvae undergoing normal development (Kamya et al., 2014; Caballes et al., 2017; Sparks et al., 2017). In contrast, the same degree of warming either enhances or does not affect the development rate (Lamare et al., 2014; Uthicke et al., 2015), survival (Lamare et al., 2014; Uthicke et al., 2015) and growth (Lamare et al., 2014) of larvae, as well as the growth of algae-feeding (Kamya et al., 2016) and coral-feeding juveniles (< 1.5 cm; Kamya et al., 2018). Larger coral-feeding CoTS may be more thermally robust, as shown in a recent study where the metabolic rate of sub-adults was found to increase with temperature to at least 36 °C, and metabolic depression was only observed in adult CoTS from 33 °C (> 5 °C above AST; see **Chapter 4**; Lang et al., 2021). However, these individuals were exposed to acute ramping trials (rate of increase, 1 °C h^{-1}; Lang et al., 2021) and thus may not experience additional physiological costs that are associated with the longer durations of heat exposure that naturally occur on the reef (Pörtner, 2001; 2002; Pörtner et al., 2017). Whether coral-feeding juvenile (> 1.5 cm), sub-adult and adult CoTS experience compromised performance at cooler temperatures with prolonged exposure, remains to be tested.

Understanding how the coral-feeding life stages of CoTS perform in a warmer environment is essential for predicting the future impact of this predator on the reef. Herein, the effect of prolonged (~ 60 days) exposure to various thermal treatments were explored in Pacific CoTS from Australia's Great Barrier Reef (GBR). The size range tested incorporates large coral-feeding (> 5.5 cm) juveniles, as well as sub-adults and adults (Pratchett et al., 2014; 2021a; Caballes, 2017). The measures used to assess the effect of temperature on this species were: righting time, average movement rate, SMR (weight-specific) and probability of survival. Because it is considered that species that inhabit coral reefs are living particularly close to their thermal limits (Stuart-Smith et al., 2015; Negri et al., 2020), it was hypothesised that temperatures of only a few degrees above the AST will compromise performance during prolonged exposure to elevated temperatures (Lang et al., 2021). Compromised performance and increased mortality of CoTS in a warmer ocean will unequivocally lead to population contractions (Eisenlord et al., 2016), and possibly re-distributions (Ling, 2013; Sill & Dawson, 2021), with variable impacts on the corals that they interact with.

5.3 Materials and methods

5.3.1 Collection and maintenance

Pacific crown-of-thorns starfish (*n* = 48, 10-1556 g, 5.5-38 cm diameter) were collected from mid-shelf reefs in the central GBR between 28th and 30th January 2020, at depths of 2-8 m. These reefs were Big Broadhurst (18°58'S, 147°43'E), Keeper (18°45'S, 147°17'E), Kelso (18°26'S, 146°59'E), Little Broadhurst (18°56'S, 147°41'E), Little Kelso (18°28'S, 146°59'E), Lodestone (18°42'S, 147°06'E), and Rib (18°28'S, 146°53'E). Sea surface temperatures at the collection locations generally range from 24 °C to 29 °C annually (AIMS, 2021a), and at the time of collection the temperature of the water was 28-29 °C. These CoTS were transported to the Marine and Aquaculture Research facilities Unit (MARFU), James Cook University, Townsville, in flow-through aquaria. Upon arrival, the starfish were placed individually in 42 L tanks attached to one of four semi-closed recirculating systems (which would eventually become the four temperature treatments). The water temperature was set to 26 °C, which is the annual average temperature in the collection vicinity (AIMS, 2021a). The seawater was naturally at pH 8.24 and had a salinity of 37.5 ppt. The systems were housed within a temperature-controlled room with a 12 h:12 h light:dark cycle. The CoTS were acclimated to laboratory conditions in these tanks for 25 days. The CoTS were not fed throughout the acclimation period and the duration of experiments, to avoid any potential effect of differential feeding on individual performance (*sensu* Caballes et al., 2016). This starvation unlikely impacted energy reserves to a significant extent, due to the low levels of activity of CoTS in the laboratory, and the fact that they are known to survive for many months without feeding (Moran, 1988).

5.3.2 Experimental design

The experiments to assess righting time and average movement rate commenced after the acclimation period on 24th February 2020. Starfish were distributed evenly among four treatments (26, 28, 30 and 32 °C, *n* = 12 per treatment). The treatment names represent the final temperatures experienced by the starfish rather than the temperatures at the beginning of the experiments (26 °C in all treatments, Figure 5.1). The mean weights of these starfish were 313 g ± 90 (s.e.), 309 g ± 120 (s.e.), 294 g ± 131 (s.e.), and 193 g ± 50 (s.e.) for the 26, 28, 30 and 32 °C treatment, respectively. The weights of the starfish were not significantly different among treatment groups (LM, $F_3 = 0.30$, $P = 0.819$). The starfish were initially tested in each of the temperature treatments at the control temperature of 26 °C (day 1), after which the temperatures in the 28, 30 and 32 °C treatments were raised to 28 °C, before repeating the experiments on all 48 starfish (week 2, day 8). Upon completion, the temperatures of the 30 °C and 32 °C treatment systems were further raised to 30 °C, and again repeated the experiments (week 3, day 15). Finally, the temperatures of the 32 °C treatment were further raised to 32 °C, and the last round of righting and movement experiments were completed, with all starfish being at their final temperatures (week 4, day 22, Figure 5.1). In between each week, the temperatures in the relevant treatments were raised at rates of 0.5 °C day^{-1}, which allowed for a three-day acclimation period at the testing temperature before experiments commenced. The treatments were warmed gradually rather than immediately exposing starfish to their final temperatures, in order for the environmental conditions to be more representative of those that may be experienced by CoTS in nature, during a warming event (AIMS, 2021a; b). The temperature of the seawater in the systems was on average ± 0.25 °C around the set-point level throughout the experiments.

The SMR of each starfish was tested twice unless mortality occurred before testing. One run (same week, SW) involved testing the SMR of all surviving starfish between days 32-35 for the 26 (n = 12), 28 (n = 12), 30 (n = 11) and 32 °C (n = 8) temperature treatments (Figure 5.1). The CoTS were tested over the same four days in order to control for an effect of time in the laboratory. To further control for an effect of time, the starfish from all four treatments were tested each day. The other run (consecutive weeks, CW) involved testing the SMR of the same starfish exactly 4 weeks after first exposure to the final temperatures for each treatment (except in the case of the 26 °C control). Specifically, testing occurred on days 29-30 for the 26 °C treatment (n = 12), day 36 for the 28 °C treatment (n = 11), day 43 for the 30 °C treatment (n = 11) and day 50 for the 32 °C treatment (n = 3; Figure 5.1). On each of the warmer testing days (36, 43 and 50), the same six starfish from the 26 °C control treatment were re-tested in order to control for any effect of time, however there was no significant effect of this variable (P = 0.529). The CW run was conducted to account for an effect of differing lengths of time at the final temperatures among temperature treatments, and to allow for better assessment of the exposure-time dependence of thermal effects on physiological performance.

The CoTS were checked once or twice per day for mortality from day 1 until day 59 (Figure 5.1). Starfish were considered deceased when they exhibited all or a number of typical indicators of mortality, such as flattened or detached spines, lesion formation, exposure of internal organs and loss of turgor. Upon death, the starfish were immediately removed in order to avoid system contamination, and they were not replaced. The time of death was recorded for later analysis.

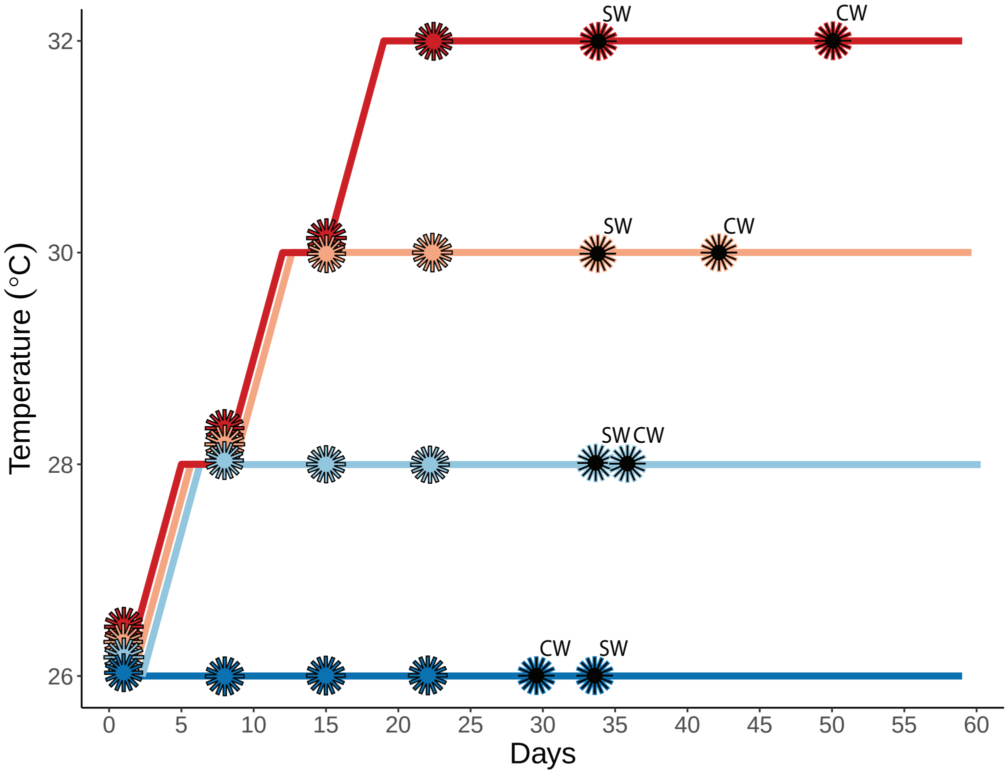

Figure 5.1 Experimental design timeline. The figure indicates the temperature profiles for the 26 °C (dark blue), 28 °C (light blue), 30 °C (orange) and 32 °C (red) treatments, from day 1 of the study until day 59. Experiments to assess righting time and average movement rate (symbols with coloured fill) were carried out on days 1, 8, 15 and 22 for all *Acanthaster* cf. *solaris* during the temperature ramping period. Experiments to measure the standard metabolic rate of the starfish (symbols with black fill) took place after endpoint temperatures were reached. The same week 'SW' run was carried on days 32 to 35 on all starfish, while the consecutive weeks 'CW' run was conducted on days 29-30, 36, 43 and 50 for the 26, 28, 30 and 32 °C treatments, respectively. The overlapping lines of the four treatments were offset horizontally, so that their complete temperature profiles could be visualised. The overlapping symbols were offset vertically. Experiments began with n = 12 starfish per treatment, and any mortality was recorded over the 59 days.

5.3.3 Righting time and movement rate

The experiments to measure the righting time and average movement rate for each CoTS was conducted in one of four 1.1 m diameter round aquaria, connected to their respective recirculation systems to ensure consistency of environmental conditions. To measure righting time (s), the starfish were inverted and placed in the centre of the aquarium and permitted to return to their natural orientation. Once righted, the starfish were allowed to move from the centre to the edge of the aquarium. All responses were video recorded using GoPro Hero 7 cameras (GoPro Inc., California, USA) for later analysis. The righting time and mode of righting (the style of movement used to return to the correct orientation) were determined from the videos. Three modes of righting were utilised by the CoTS. Mode 1 involved the starfish raising all arms upwards so that they were almost touching, causing them to tip to the side. The top arms finally moved over the bottom arms until they reached the surface. Starfish utilising mode 2 raised some arms upwards so that they were almost touching, while the other arms bent backwards in order for them to flip themselves over. Mode 3 involved the starfish bending their arms backwards and flipping themselves over without raising their arms up first. Adobe After Effects software (Adobe Systems Inc., California, USA) was used to establish the average rates of movement (cm min^{-1}) over the total distance travelled (the sum of the distance travelled in all directions).

5.3.4 Standard metabolic rate

To assess the SMR of the starfish in the four temperature treatments, intermittent-flow respirometry was used (Clark et al., 2013; Svendsen et al., 2016; Kühnhold et al., 2019b; Lang et al., 2021). Four acrylic respirometry chambers of various sizes (4.12, 9.99 and 21.77 L) were placed within a 1000 L capacity water bath. Flush pumps (flow rate; 400 to 500 L h^{-1}) delivered oxygenated water from the water bath into the chambers between measurement periods, and snorkels attached to the top of the chambers expelled oxygen depleted water. Recirculatory pumps (flow rate; 400 to 500 L h^{-1}) enabled the water within the chambers to constantly mix. Oxygen sensor spots were glued to the recirculatory tubing with silicone gel, and aligned with Firesting oxygen probes (PyroScience, Aachen, Germany). These probes delivered oxygen depletion data via a Pyro oxygen meter into the PyroScience program installed on a computer via fibre-optic cables. A chiller and two heaters (1 kW) cooled/heated the water to the final temperature for the 26, 28, 30 and 32 °C treatments prior to the introduction of the starfish. Four chambers were run at once (three containing starfish, with one left empty to control for background respiration). The starfish were allowed to settle in the chambers for 45 min with the flush pumps switched on, after which the chambers were sealed, and oxygen depletion was measured in each chamber over 30 min. After the measurement period the flush pumps were again switched on, enabling oxygen levels in the chambers to return to ~ 100%. Four to six cohorts of starfish were tested each day between 09:00 and 20:30.

For consistency the first eight minutes from all oxygen depletion slopes were removed, as a consequence of a delay in the water within the sealed chambers becoming completely mixed. The slopes for background respiration from each run were subtracted from the CoTS respiration slopes. The weight-specific SMR in mg O_2 g^{-1} h^{-1} was calculated following Svendsen et al. (2016).

5.3.5 Statistical analysis

All statistical analyses were carried out in R v.4.1.2 (R Core Team, 2021). The effect of temperature (at the time of testing) on righting time (s) was analysed using a linear mixed effects model. The response was log-transformed in order to meet the normality assumption. The experimental week, the log-transformed starfish wet weight (g) and mode of righting were also included as covariates, to control for differences in these variables among temperature treatments. Starfish ID was included as a random effect to account for the repeated measures design. In addition, a Fisher's exact test was conducted in order to establish whether there was an association between temperature and the mode of righting utilised by the starfish. An additional linear mixed effects model was used to establish the effect of temperature on the average movement rate (cm min^{-1}). Again, the experimental week, the log-transformed wet weight (g) and starfish ID were included in the model, in addition to the log-transformed distance travelled (cm). Observations were excluded from the analysis if the starfish did not move within 20 min of righting (n = 3). As a result of the significance of wet weight in influencing the average movement rate, this relationship was explored further by establishing the scaling exponent (b), which is the slope value of this factor and the response. This value was calculated for each temperature using linear ordinary least squares regression analyses. The estimated marginal means for the righting time (on the response scale) and average movement rate of the starfish were established for all temperatures. These are the means that have been adjusted for covariates in the model *i.e.*, weight, mode of righting and distance travelled. These estimated marginal means were used for further data investigation including post-hoc comparisons using the Tukey method.

The SMR (log-transformed, mg O_2 g h^{-1}) was analysed using a further linear mixed effects model. Temperature treatment and run were included as interacting fixed effects, and starfish ID as a random effect. Observations were removed from the analysis that were so low that they could not be distinguished from background respiration ($n = 3$). The estimated marginal means (on the response scale) of SMR in the four treatments were used for post-hoc analysis, including comparisons using the Tukey method. As a result of there being little effect of the run in the model, only the compiled estimated marginal means for the four treatments were reported. However, these estimated marginal means adjust for other factors in the model. The temperature (Q_{10}) coefficient between the estimated marginal means of the four treatments was calculated to establish thermal sensitivity, using the equation by Prosser (1991). For each treatment the scaling exponents (*b*) for the relationship between the natural logarithms of SMR at the organism level (mg O_2 h^{-1}) and weight (g) was also established. These were calculated using linear ordinary least squares regression analyses.

The survival probability (%) for the starfish in the four temperature treatments was explored using a Kaplan–Meier survival plot and an accompanying log-rank test. Pairwise comparisons were conducted using the Benjamini–Hochberg method. A Cox proportional hazards model was also conducted to determine the hazard ratios associated with the three elevated treatments. These models are unable to include groups with no "events", and therefore the 26 °C control treatment (no mortality, see results) was eliminated from this analysis. A Cox proportional hazards model was also conducted to observe the hazard ratios for the effect of wet weight (g) on the probability of survival.

5.4 Results

5.4.1 Righting time and movement rate

No significant impact of temperature on righting time (Figure 5.2A, Table 5.1, see Appendix 5.1), and no significant effect of the experimental week or the wet weight of the starfish was found (Table 5.1). Likewise, temperature did not significantly influence the mode of righting utilised (Fisher's exact test, $P = 0.328$), although there was a significant effect of mode of righting on righting time (Table 5.1). Starfish utilising mode 3 righted the fastest, with an average righting time of 158 ± 35 s. This was also the rarest mode of righting ($n = 4$). Mode 1 was the second fastest mode of righting (194 ± 13 s) and was the most commonly used by the CoTS (*n* = 161). Starfish utilising mode 2 of righting were the slowest (*n* = 26, 258 ± 27 s).

A significant effect of temperature on the average movement rate of the starfish was found (Table 5.1). There was an 18 and 27% faster movement rate of starfish at 28 °C and 30 °C, respectively, compared to those at 26 °C. However, starfish at 32 °C were 14% slower than those at 30 °C (Figure 5.2B, see Appendix 5.1). Rates of movement did not vary significantly among experimental weeks, although a significant effect of distance travelled and wet weight on average movement rate was found. Starfish that had faster rates of movement travelled further to reach the edge of the aquaria and were heavier (Table 5.1). The positive relationship between wet weight and movement rate was similar between 26 and 30 °C ($b = 3.58$-3.93), but was lower at 32 °C ($b = 1.80$, see Appendix 5.2).

Table 5.1 Output of the linear mixed effects models on the effect of temperature on righting time (s) and average (Av.) movement rate (cm min^{-1}) in *Acanthaster* cf. *solaris* (*n* = 48 CoTS, *n* = 191 righting time observations, *n* = 188 average movement rate observations). The influence of the covariates: experimental week, wet weight (g), mode of righting and distance travelled (cm) are also included. Responses and covariates with an asterisk (*) were log-transformed in the models to meet the normality assumption. Significant *P* values are in bold.

Response	Factor	Num *df*, Den *df*	*F*	*P* value
Righting time (s)*	Temperature	3, 179	0.24	0.871
	Experimental week	3, 164	1.20	0.312
	Wet weight*	1, 47	0.17	0.686
	Mode of righting	2, 181	5.94	**0.003**
Av. Movement rate (cm min^{-1})	Temperature	3, 173	3.42	**0.019**
	Experimental week	3, 162	1.26	0.290
	Wet weight*	1, 55	73.88	**<0.001**
	Distance travelled*	1, 184	9.24	**0.003**

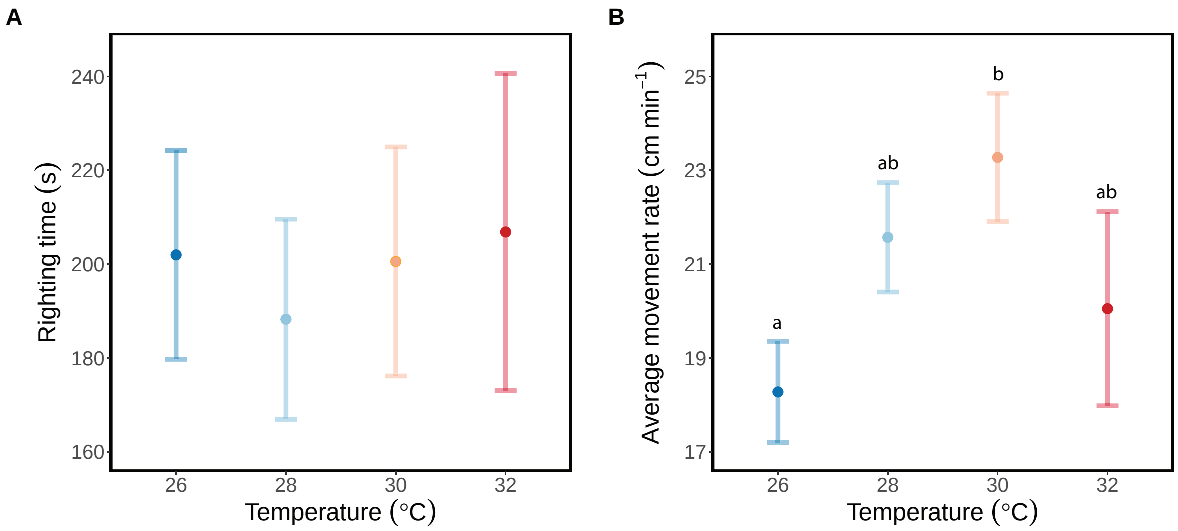

Figure 5.2 The effect of temperature on A. righting time (s) and B. average movement rate (cm min^{-1}) in *Acanthaster* cf. *solaris* (n = 48 CoTS, n = 191 righting time observations, n = 188 average movement rate observations). Estimated marginal means are provided (±SE). Letters represent significant differences between temperatures (Tukey method, $P < 0.05$).

5.4.2 Standard metabolic rate

It was found that temperature treatment significantly influenced the SMR (Table 5.2). The SMR was 100% (Q_{10}: 32.0) and 260% (Q_{10}: 24.6) higher in the 28 °C and 30 °C treatments compared to the 26 °C treatment, respectively. However, the SMR of starfish in the 32 °C treatment was 33% (Q_{10}: 0.1) lower than those in the 30 °C treatment (Figure 5.3, see Appendix 5.3). The run did not significantly impact the SMR, and there was no interaction between temperature treatment and run (Table 5.2). The positive correlation between the weight of the starfish and the SMR was similar in the majority of the treatments (b = 0.96, 1.02 and 0.97 for the 26, 28 and 32 °C treatments, respectively), but was lower in the 30 °C treatment (b = 0.70, see Appendix 5.4).

Table 5.2 Output of the linear mixed effects model on the effect of temperature (T.) treatment, run, and their interaction on the standard metabolic rate (mg O_2 g^{-1} h^{-1}) of *Acanthaster* cf. *solaris* (*n* = 42 CoTS, *n* = 76 observations). The response was log-transformed in the model to meet the normality assumption. Significant *P* values are in bold.

Response	Factor	Num *df*, Den *df*	*F*	*P* value
Standard metabolic rate	T. treatment	3, 38	11.76	**< 0.001**
(mg O_2 g^{-1} h^{-1})	Run	1, 30	0.30	0.587
	T. Treatment x Run	3, 30	0.34	0.798

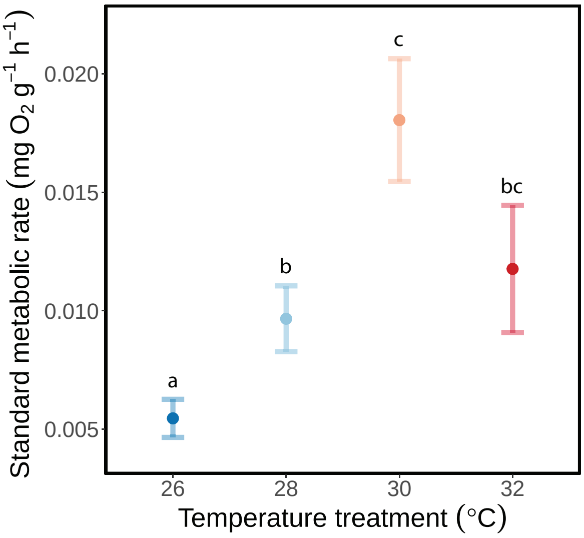

Figure 5.3 The estimated marginal means (±SE) for the effect of temperature treatment on the standard metabolic rate (mg O_2 g^{-1} h^{-1}) of *Acanthaster* cf. *solaris* (*n* = 42 CoTS, *n* = 76 observations). Letters represent significant differences between treatments (Tukey method, $P < 0.05$).

5.4.3 Survival

An overall significant effect of temperature treatment on the probability of survival was found (Log-rank, $\chi^2_3 = 25.30$, $P < 0.001$). The difference in survival between treatment pairs was only significant for the 32 °C (75% mortality) treatment compared to the 26 °C ($P < 0.001$, 0% mortality), 28 °C ($P = 0.008$, 17% mortality) and 30 °C ($P = 0.012$, 25% mortality) treatments, respectively (Figure 5.4). The Cox proportional hazards model further supports this result (Likelihood ratio test = 11.66, *df* = 2, $P = 0.003$), with hazard ratios revealing an eightfold ($P = 0.007$) and fivefold ($P = 0.012$) lower probability of survival in the 32 °C compared to the

28 °C and 30 °C treatments, respectively (Table 5.3). The first mortality event was observed on day 17. The deceased starfish was from the highest temperature treatment when the water temperature was approximately 31 °C. All remaining deaths occurred after the final temperatures were reached, with the two subsequent deaths occurring three days after 32 °C was reached in the applicable temperature treatment. The first deaths in the 30 °C and 28 °C treatments occurred on days 28 and 36, respectively, 13 and 28 days after these final temperatures were reached. No significant effect of starfish wet weight (g) on the probability of survival was found (Cox proportional hazards model, Likelihood ratio test = 1.45, *df* = 1, *P* = 0.228).

Table 5.3 Results of the Cox proportional hazards model indicating the probability of survival in *Acanthaster* cf. *solaris* (*n* = 48 CoTS) between temperature treatment pairs. The 26 °C control treatment could not be included due to the lack of "events". The hazard ratios, lower and upper 95% confidence intervals and *P* values (significant values in bold) are provided.

Treatment comparison	Hazard ratio	Lower 95% CI	Upper 95% CI	*P* value
28-30 °C	1.51	0.25	9.05	0.651
28-32 °C	8.30	1.76	39.07	**0.007**
30-32 °C	5.49	1.46	20.70	**0.012**

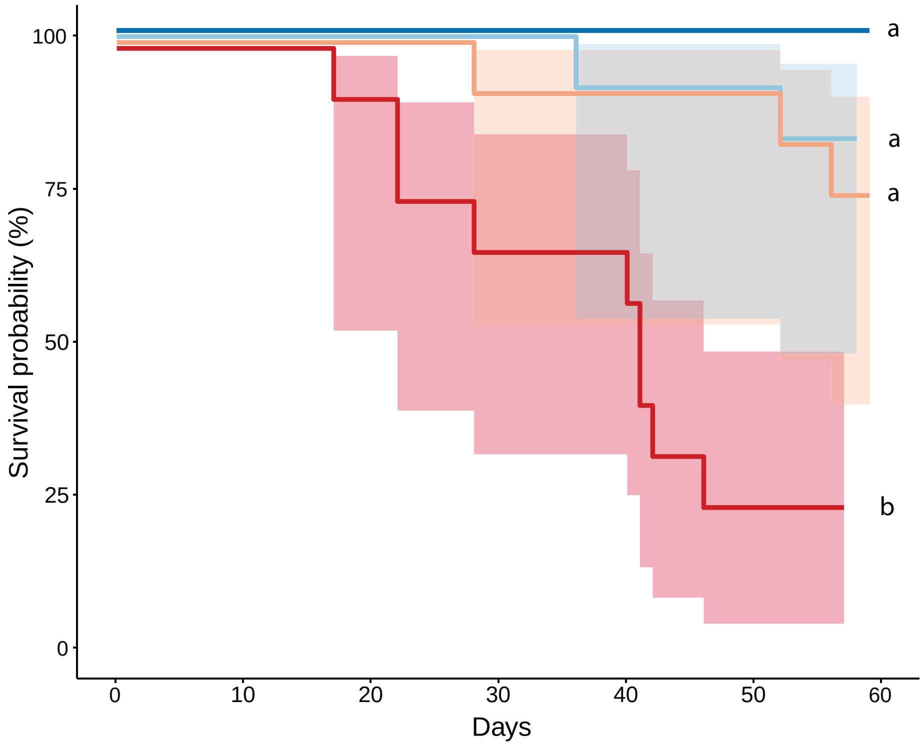

Figure 5.4 Kaplan–Meier survival curve for the effect of the 26 °C (dark blue), 28 °C (light blue), 30 °C (orange) and 32 °C (red) treatments on survival probability (%) in *Acanthaster* cf. *solaris* (*n* = 48) over 59 days. The shaded bands represent the 95% confidence intervals. The survival curve for each temperature treatment began at 100%. The overlapping lines of the four treatments were offset horizontally, in order for their complete survival curve to be visualised. Final temperatures were reached for the 28, 30 and 32 °C treatments on days 5, 12, and 19, respectively. The righting and movement experiments took place on days 1, 8, 15 and 22. Letters represent significant differences between temperature treatments (Benjamini–Hochberg method, $P < 0.05$).

5.5 Discussion

Establishing the thermal sensitivity of the corallivorous Pacific crown-of-thorns starfish is an essential step forward in understanding how future shifts in environmental temperature may alter the impact of this asteroid on the reef. In the present study, it was found that seawater temperatures of 32 °C had negative effects on the performance and survival of CoTS. Average movement rates and SMR increased from 26 °C to 30 °C, and over this thermal range survival was, for the most part, maintained. However, starfish at 32 °C had a lower SMR, reduced movement rate and a lower probability of survival, than those at 30 °C. This temperature is approximately 4 °C above the average, and 2 °C above the maximal summer temperatures commonly experienced on the central mid-shelf of the GBR (AIMS, 2021a; b), but it is predicted that temperatures of 32 °C will be frequently exceeded under near-future climate change (IPCC, 2013; 2021; Hoegh-Guldberg et al., 2018).

In nature it is expected that activity rates of organisms will decline when thermally stressed (Peck et al., 2008; Kidawa et al., 2010; Pörtner et al., 2017; Sun et al., 2018; Lang et al., 2021). In the present study, it was observed that the average movement rate increased with temperature until 30 °C, but slowed at 32 °C (14% decrease compared to 30 °C), indicating that starfish may experience thermal stress at this degree of ocean warming. In contrast, water temperature had little effect on righting time. This lack of thermal sensitivity is not uncommon (Wood et al., 2010; Zhang et al., 2017) and could be a consequence of the importance of this behaviour to avoid injury and predation, such that CoTS have evolved to invest a great deal of energy in maintaining their natural orientation (Lawrence, 1984; Wood et al., 2010; Deaker et al., 2021). The differing responses between the two activities may, however, be a product of the methodological design. Because movement rate was assessed directly after righting time, the latter response may be impacted by the energy investment in the prior activity (Lawrence, 1984). Whatever the exact cause of these observations, the additional costs of activity seem to be limited at 32 °C, due to reduced aerobic metabolism, and therefore energy production of CoTS at this temperature, which may have been further constrained by starvation. Constraints in energy production at high temperatures appears particularly relevant to larger (adult) individuals, highlighted by the lower value of b for average movement rate at 32 °C, compared to cooler temperatures (Lawrence, 1984; Pörtner, 2001; 2002; Doyle et al., 2012; Pörtner et al., 2017). It is currently unknown how the activity of CoTS will be influenced by warmer temperatures in nature. Environmental signals from predators, prey and conspecifics have a substantial impact on this asteroid, and provide a strong impetus to move away or towards such signals (Hall et al., 2017; Pratchett et al., 2017). This may result in the starfish maintaining high activity levels during marine heatwaves, potentially at the expense of other biological processes and behaviours (Lawrence, 1984).

Reductions in aerobic capacity and associated energy reserves is expected to be a major contributor to compromised performance (including activity levels) in a warmer ocean (Pörtner et al., 2017; Lang et al., 2021). In this study, starfish in the 32 °C temperature treatment had a 33% slower SMR, compared to those in the 30 °C treatment, similar to the decline in movement rate observed between the same temperatures. The lower SMR in the highest temperature treatment is likely a result of the depression of aerobic metabolism, and an increased reliance on energy deficient, and unsustainable anaerobic metabolism (Pörtner, 2001; 2002; Pörtner et al., 2017; Lang et al., 2021). In the present study, aerobic metabolic decline occurred at temperatures which were at least 1 °C cooler than observed in acute ramping trials (24-36 °C over 12 h) in CoTS that were collected from the same location and at the same time of the year. Although for smaller CoTS, metabolic depression was not observed even at temperatures as high as 36 °C when acutely exposed (Lang et al., 2021). The differing observations between these two studies is likely a result of factors that generally lead to compromised performance only after prolonged exposure to sub-optimal temperatures, such as mitochondrial failure (Pörtner, 2002; Schulte et al., 2011), protein denaturation (Pörtner, 2001; 2002; Pörtner et al., 2017) and oxidative damage (Pörtner, 2002; Pörtner et al., 2017)..

Aside from the lower SMR of CoTS at 32 °C, a 260% higher SMR of individuals in the 30 °C compared to the 26 °C treatment (Q_{10}: 24.6) was observed. This is likely a result of the inherent increased rate of chemical reactions and cellular processes at higher temperatures (Peck & Prothero-Thomas, 2002; Clarke & Fraser, 2004; Schulte, 2015; Harianto et al., 2018). The high Q_{10} coefficient indicates that CoTS have a very high physiological thermal sensitivity across this testing range, which is far greater than observed in temperate echinoderms (Q_{10}: ~1.0-3.0; Christensen et al., 2011; Weber et al., 2013; Harianto et al., 2018). It is not surprising that tropical species, such as CoTS may be more thermosensitive than temperate species, considering that they evolved in a stable thermal environment, and are therefore less equipped to cope with extreme temperature fluctuations (Peck et al., 2010; Nguyen et al., 2011). Such a substantial increase in SMR with temperature is concerning because this would mean that CoTS would need to consume more coral prey to meet increased energetic demands, potentially resulting in greater impacts on coral communities (Barbeau & Scheibling, 1994; McBride et al., 1997; Siikavuopio et al., 2008; Kamya et al., 2018; Haywood et al., 2019; Keesing et al., 2019; Kühnhold et al., 2019a; b). Although, a smaller *b* in the 30 °C treatment (that has the highest mean SMR), highlights lower relative upper limits to SMR in the larger individuals, which naturally consume more coral, compared to smaller conspecifics (Lawrence, 1984; Pörtner, 2001; 2002; Doyle et al., 2012; Pörtner et al., 2017).

While CoTS are able to withstand temperatures exceeding 36 °C acutely (Lang et al., 2021), it was observed that the physiological thermal limits of this asteroid are likely between 30 and 32 °C, which appears conserved between starfish across the tested weight distribution (10-1556 g). Here, starfish in the 32 °C temperature treatment exhibited a five- and eightfold lower probability of survival than those in the 30 °C and 28 °C treatments, respectively. Mortality occurred within a couple of days of the water temperature reaching ~ 31-32 °C, indicating that even brief marine heatwaves that reach these temperatures may induce mortality in CoTS. Yet, 25% of starfish were able to survive extended exposure to 32 °C, suggesting that thermally tolerant individuals may persist in a warmer ocean (Binyon, 1972; Johnson & Babcock, 1994; Pörtner, 2002). The present aquarium study may, however, overestimate survival that would occur in nature, due to a higher risk of disease at higher temperatures, either due to the greater pathogen virulence or lower host defences (Bates et al., 2009; Kohl et al., 2016; Hewson et al., 2018; Uthicke et al., 2021). Even if some CoTS are able to survive future extreme heating events, a greater amount of energy required for maintaining homeostasis may result in a lower energy investment in reproduction (Brockington & Clarke, 2001; Siikavuopio et al., 2006; 2008; Uthicke et al., 2014; Delorme & Sewell, 2016; Hue et al., 2020). CoTS from New Caledonia, for instance, produced significantly smaller eggs, had poorer fertilisation success and a reduced gonadosomatic index with only 2 °C of warming above the ambient summertime seawater temperature (Hue et al., 2020). Furthermore, a recent cross-generational study on the sea urchin *Echinometra* sp. A from the GBR found that there were negative carry-over effects from parents to offspring reared in predicted near-future ocean conditions (+ 1 °C, 685 ppm; + 2 °C, 940 ppm), providing uncertainty regarding the adaptive capacity of echinoderms (Uthicke et al., 2021).

In the current study, evidence for thermal effects on the performance and survival of CoTS is presented. An increased performance of CoTS when exposed to current maximal summertime temperatures (~ 30 °C), which may become more commonly experienced prior to the end of the century (AIMS, 2021a; b; IPCC, 2021; Hoegh-Guldberg et al., 2018), could result in a higher incidence and severity of population irruptions. Considering the faster metabolic rates (260%), movement rates (27%) and probably feeding rates of CoTS at this optimal temperature, these irruptions may result in increased coral loss on the GBR (De'ath et al., 2012; Mellin et al., 2019). Elevated coral predation will reduce the likelihood of recovery for corals already suffering from cumulative disturbance effects caused by temperature-induced bleaching and cyclones, among other stressors (De'ath et al., 2012; Vercelloni et al., 2017; Mellin et al., 2019).

While moderate increases in temperature (up to 30 °C) may increase the incidence and impacts of population irruptions of CoTS, the performance and survival of coral-feeding CoTS is expected to be greatly compromised at 32 °C, corresponding with projected end-of-century temperatures (IPCC, 2013; 2021; Kamya et al., 2014; Hoegh-Guldberg et al., 2018; Lough et al., 2018; AIMS, 2021a; b). However, at these temperatures, increased coral bleaching and mortality will more than offset benefits accrued from a reduced incidence of population irruptions of CoTS (Eisenlord et al., 2016; van Hooidonk et al., 2016; Castro-Sanguino et al., 2021). Moreover, CoTS may have some capacity to acclimate or adapt to a warmer environment (Schulte et al., 2011; Schulte, 2015; Pörtner et al., 2017; but see Uthicke et al., 2021). If CoTS are able to adjust their metabolism and oxygen requirements, and activity levels at supra-optimal temperatures, they may be able to conserve more energy, reducing their nutritional requirements (Pörtner et al., 2017). As a consequence, CoTS may switch their feeding preferences to feed more readily on partially bleached corals, which have lower nutritional value, but will be more common in a warmer ocean (MacArthur & Pianka, 1966; Glynn, 1985; Rodrigues & Grottoli, 2007; Keesing et al., 2019). If unable to acclimate or adapt to warmer conditions, CoTS populations may persist by redistributing to cooler, more southerly reefs of the GBR, as well as more distant reefs such as the fringing reefs of Lord Howe Island, facilitated by larval dispersal (Dight et al., 1990; Benzie, 1992; Sill & Dawson, 2021). However, population redistributions may be constrained by the dispersal capacity of CoTS larvae in a warmer ocean (Dight et al., 1990) and the availability of suitable settlement substrate (Britton et al., 2021). Acclimation and adaptation capacity, as well as range shifts may result in CoTS continuing to be a significant threat on the reef for years to come (De'ath et al., 2012; Mellin et al., 2019). For this reason, it is axiomatic that these possibilities are investigated.

Chapter 6: General Discussion

Understanding responses to elevated temperature is necessary for predicting future population dynamics of ectothermic taxa (Poloczanska et al., 2016). Accordingly, this thesis has greatly advanced understanding of the effects of acute and chronic warming on Pacific crown-of-thorns starfish (CoTS; *Acanthaster* cf. *solaris*). Notably, CoTS are one of the greatest threats (alongside climate change) to tropical coral reefs throughout the Indo-Pacific region, because of their tendency to undergo population irruptions and propensity to feed on reef-building corals (De'ath et al., 2012; Mellin et al., 2019). Establishing how CoTS may be affected by ocean warming was therefore the main aim of the present thesis.

Marine ectotherms that occupy relatively stable thermal environments closer to the equator generally have a narrower thermal breadth and are living closer to their thermal limit than those from more variable thermal environments at higher latitudes (Sunday et al., 2011; 2014; Hughes et al., 2018a). While yet to be established for echinoderms, tropical marine species are predicted to be much more vulnerable to changing environmental conditions than their temperate counterparts (Sunday et al., 2011; 2014). **Chapter 2** showed that sub-tropical and tropical echinoderms experience a greater decline in performance than temperate echinoderms, when exposed to ~5 °C of warming. Considering only data where the experimental temperature was above the predicted near-future mean annual temperature (IPCC, 2019), tropical echinoderms appeared to be by far the most vulnerable latitudinal group, exhibiting an average 34% decline in performance with warming across studies. This result, as well as the large 51% reduction in performance with warming observed for asteroids, indicates that tropical starfish such as CoTS, may be highly vulnerable to warming. This, combined with the differential vulnerability of echinoderms to warming throughout their life cycles (**Chapter 2**), stresses the importance of better understanding the optimal and supra-optimal temperatures of CoTS, throughout ontogeny.

6.1 Thermal tolerance of coral-feeding CoTS

Understanding the physiological thermal optima and limits of CoTS is essential for predicting the threat that this species will pose to the reef in the future, on various spatial and temporal scales. Metabolism is required for the provision of energy for cellular processes and the maintenance of homeostasis (de Nava & Raja, 2022). Thus, a slowing of the metabolic rate at supra-optimal temperatures, may lead to an increased risk of mortality (Pörtner et al., 2017; Harianto et al., 2018; **Chapter 5**). The research presented in the meta-analysis showed that metabolic rate increased in response to warming in echinoderms (**Chapter 2**); although there was some evidence of supra-optimal temperatures being reached, with metabolic rates no longer being significantly positively impacted by warming under the end-of-century scenario. The findings in **Chapters 4** and **5** support those in **Chapter 2**. In the acute thermal ramping trials (**Chapter 4**), the metabolic rate of adult CoTS increased until at least 32 °C, with metabolic depression (*i.e.*, reduced oxygen consumption rate) observed after this temperature was exceeded. When exposed to prolonged and sustained warming (**Chapter 5**), metabolic rate increased up to 30 °C in adult CoTS, while metabolic depression was observed at 32 °C (6 °C above the control temperature and ~2 °C above the summertime maximum temperature).

When physiological limits are reached at supra-optimal temperatures, a mismatch between oxygen supply and demand leads to a decline in aerobic metabolism, and an increased reliance on energy-deficient anaerobic metabolism (Pörtner et al., 2017). This was evidenced by the activity of citrate synthase (the proxy for aerobic metabolism) in CoTS decreasing with warming, and negligible activity occurring at the temperature in which metabolic depression was observed at the organism level (**Chapter 4**). This change was accompanied by a concomitant increase in the activity of lactate dehydrogenase (the proxy for anaerobic metabolism). Anaerobic metabolism, being markedly less energy efficient than aerobic metabolism, cannot be sustained for long periods of time (Pörtner, 2002; Kühnhold et al., 2019b). Loss of essential biological functions, as a consequence of ATP deficiency, will inevitably lead to mortality if optimal conditions are not restored (Peck et al., 2004; Pörtner & Knust, 2007).

The lower temperature at which metabolic depression of mature CoTS was observed following chronic exposure to elevated temperature (**Chapter 5**) compared to acute exposure to elevated temperature (**Chapter 4**), indicates that physiological thermal limits may be lower if exposed for longer periods of time (*e.g.*, during long-term marine heatwaves and sustained and progressive ocean warming). Passive plasticity (thermosensitivity), and possibly active plasticity (acclimation), may be observed during brief spikes in temperature, which may enable CoTS to withstand relatively high temperatures in the short term (Schulte et al., 2011). On the other hand, associated energetic trade-offs of acclimation under prolonged exposure, may lead to an energy deficit for movement, reproduction, growth, and survival (Sandersfeld et al., 2015; Mardones et al., 2022). For instance, negative energetic trade-offs were linked to an 84% reduction in growth of the Antarctic fish *Trematomus bernacchii* (Sandersfeld et al., 2015), and a cessation of reproduction in the temperate gastropod *Ophiocoma erinaceus* (Mardones et al., 2022), after extended thermal exposure.

Energetic trade-offs may explain the reduced activity of CoTS at 32 °C observed in **Chapter 5**, as well as the thermal robustness of righting. The ability of the starfish to right effectively across the tested thermal range may, in part, be a consequence of righting being a relatively quick behaviour in CoTS, and may require little energy (Peck et al., 2004). Alternatively, the result could be due to this behaviour being evolutionarily conserved at the expense of other aspects of performance, because of its importance for avoiding predation (Lawrence, 1984; Wood et al., 2010; Deaker et al., 2021). An interesting future avenue of research would be to establish whether other evolutionarily conserved predator defensive behaviours, such as the formation of "spine balls", would be similarly robust to ocean warming (Deaker et al., 2021). The finding that the activity of righting was not impacted by warming, is supported by the fact that there was little overall impact of warming on echinoderm movement (which includes righting behaviour) in the meta-analysis presented in **Chapter 2**. The slowing of CoTS movement rate at higher temperatures in **Chapter 5**, however, contradicts this finding, and indicates that CoTS mobility may be more vulnerable to warming than many echinoderm species. This may in part be a consequence of the relatively stable thermal environment CoTS experience, living in the tropics (Sunday et al., 2011; 2014; Hughes et al., 2018a). Reductions in rates of movement suggests that CoTS will be less able to find sufficient food (particularly in areas where suitable prey is scarce) and avoid predators in a warmer ocean, leading to increased risk of mortality (Peck et al., 2004; 2008; Kidawa et al., 2010; Wood et al., 2010; Brothers & McClintock, 2015; Harianto et al., 2018; Sun et al., 2018).

This thesis found direct impacts of warming on mortality in coral-feeding CoTS. **Chapter 2** showed that reduced survival is a common response among echinoderms in controlled laboratory-based studies, with survival being on average 35% lower when exposed to 5 °C of warming. Likewise, C**hapter 5** demonstrates that the risk of mortality in coral-feeding CoTS was eight-fold higher at 32 °C compared to 28 °C. Mortality occurred within a couple of days of the water temperatures reaching ~31-32 °C (~5-6 °C above the control temperature; ~1-2 °C above the summertime maximum temperature), indicating that, in fact, brief marine heatwaves that reach these temperatures may induce mortality in the coral-feeding life stages. However, as previously discussed, in nature CoTS may exhibit some degree of acclimation in order to maintain survival in the short-term. This may involve adjusting their positioning on the reef, or increasing food intake (Spirlet et al., 2000; Kamya et al., 2018; Minuti et al., 2021), if this possibility is not constrained by a reduced availability of prey (Hughes et al., 2017), or ability to seek it out (**Chapter 5**, Harianto et al., 2018; Sun et al., 2018). Furthermore, the increased virulence of disease-causing bacteria and viruses at higher temperatures may increase mortality risk in CoTS (Sutton et al., 1988). Echinoderms are highly susceptible to disease, and diseases such as Seastar Wasting Disease has been linked to warmer than average ocean temperatures on the West coast of North America (Menge et al., 2016). If CoTS experience population declines in a warmer ocean, a possible reduction in genetic diversity may further increase vulnerability to disease-induced mortality (Uthicke et al., 2009).

Risk of mortality in mature echinoderms may be dependent on their age and size, with possible implications on the demographics of their populations (Seebacher et al., 2004; Peck et al., 2013; Poloczanska et al., 2016; Dahlke et al., 2020; Illing et al., 2020). **Chapter 4** demonstrated that heavier starfish (> 700 g) had lower thermal limits than lighter starfish (< 700 g), which did not experience metabolic depression throughout the 24 °C to 36 °C thermal range. The exceeding of physiological thermal limits was evidenced by the metabolic scaling exponent (*b*, the slope value of log metabolic rate and log weight) declining with increasing temperature, indicating greater limits to oxygen supply and energy production at higher temperatures in heavier individuals (Lawrence, 1984; Pörtner, 2001; 2002; Doyle et al., 2012; Pörtner et al., 2017). In **Chapter 5**, *b* was also low ($b = 0.70$) at 30 °C, highlighting the lower aerobic capacity of heavier CoTS. Despite heavier, and probably older CoTS having lower thermal limits, **Chapter 4** showed that lighter CoTS are more thermosensitive, indicated by higher Q_{10} values. This implies that lighter, and likely younger CoTS may be better able to compensate in a warmer environment by increasing their metabolic rates (Schulte, 2015; Pörtner et al., 2017). These observations at the organism level are supported by those at the biochemical level. In **Chapter 4**, CS activity was lower and LDH:CS ratios were higher at the warmest temperatures in adult individuals than in sub-adults, explaining the lower aerobic capacity of larger individuals at this level of organisation (Doyle et al., 2012). The lower thermal limit of heavier individuals may in part be a consequence of their higher investment in the energetically costly process of reproduction than their lighter conspecifics (Lawrence, 1984; Pratchett et al., 2021c). The experiments for both **Chapters 4** and **5** were conducted in the months following the spawning season (which takes place November-December) on the GBR, and therefore more fecund starfish may have had reduced energy levels following gamete release (Uthicke et al., 2019; Caballes et al., 2021; Pratchett et al., 2021c). If heavier CoTS are more vulnerable to mortality, the average size of CoTS within populations on the reef may decrease in size (Pörtner & Knust, 2007). Considering that larger CoTS are more fecund, and have longer spines, a concomitant reduction in reproductive output and increase in predation risk may exacerbate population declines (Stump & Lucas, 1990; Rivera-Posada et al., 2014; Deaker et al., 2021; Pratchett et al., 2021c).

6.2 Thermal tolerance of CoTS early life stages

Diminished survival rates of CoTS early life stages may exacerbate the effects of a decline in reproductive output on starfish populations in a warmer ocean. Studies have observed increased abnormality and reduced size of CoTS larvae at temperatures as low as 30 °C (Table 1.1; Kamya et al., 2014; Lamare et al., 2014). Although other studies have found increased development rates of larvae at this temperature (Uthicke et al., 2015). Faster development reduces the time spent as vulnerable larvae and possibly increases the probability of settlement on suitable habitat (Cowan et al., 2017). Interestingly, this thesis demonstrates that CoTS larval settlement was not constrained by temperatures in experimental studies where larvae were exposed to temperatures up to 34 °C (**Chapter 3**). The resilience of settlement to warming may reflect the limited energetic investment required, as per righting behaviour discussed previously (Lawrence, 1984). Larval metamorphosis on the other hand, is deemed an energetically costly process (Shilling et al., 1996; Wendt, 2000; Randall & Szmant, 2009). Fewer CoTS larvae metamorphosed at temperatures ≥ 32 °C, and survival of newly metamorphosed juveniles declined at temperatures as low as 30 °C (**Chapter 3**). In these experiments, CoTS were only exposed when the larvae were at the late brachiolaria stage, and measurements of metamorphosis and survival occurred only after 2-3 days and 5-9 days of thermal exposure, respectively. It is possible that after extended exposure beginning at an earlier life stage, cumulative impacts and carry-over effects of warming may lead to even greater negative effects of elevated temperature on these processes (Espinel-Velasco et al., 2018). Specifically, constrained larval physiology and development may reduce settlement success and growth and survival of early post-settlement juveniles (Espinel-Velasco et al., 2018). Li and colleagues (2010) for instance, observed a 22% reduction in the settlement success of the temperate sea cucumber *Apostichopus japonicus* with 6 °C of warming after 5 days of larval exposure, purported to be a consequence of larval energetic constraints. These results together indicate that upon settlement, CoTS may experience a substantial population bottleneck when environmental temperatures reach ≥ 30 °C, which may be exacerbated by the relatively high rate of predation at these early stages, with only an estimated 10% of newly settled juvenile CoTS surviving until one-month post-settlement (Cowan et al., 2016; 2017; Wilmes et al., 2018).

The findings from **Chapter 3** are supported by those from the meta-analysis presented in **Chapter 2**, which demonstrated that larvae are the most vulnerable life stage across a range of echinoderms, with a mean 20% decline in performance with approximately 5 °C of warming. The high vulnerability of larvae may be a consequence of physiological and behavioural constraints (Byrne, 2011; Nguyen et al., 2011; Dahlke et al., 2020; Sampaio et al., 2021). Although CoTS larvae exhibit some swimming ability, their positioning in the water column is highly dependent on oceanographic conditions, and thus they may have limited capacity to escape supra-optimal environmental temperatures and find more suitable thermal environments (Metaxas, 2001). The findings that early juveniles are highly sensitive to warming also aligns with those from **Chapter 2** showing that when exposed to end-of-century conditions, juvenile echinoderms experienced a significant (40%) decline in performance with an average ~5 °C of warming. Yet, Kamya et al. (2016) determined that rates of growth and feeding of algal and coral-feeding juvenile CoTS (that had been exposed to elevated temperatures at these life stages for 6-8 weeks) remained high at 30 °C. As a corollary, CoTS that are able to avoid mortality during metamorphosis and immediately after settlement, may be relatively robust to warming (Kamya et al., 2016; 2018; Wilmes et al., 2018). Nevertheless, this robustness may be trivial if very few CoTS reach this life stage in a warmer ocean (**Chapter 3**, Kamya et al., 2014; Lamare et al., 2014).

6.3 Indirect effects of ocean warming on CoTS

Direct negative effects of elevated temperature on both the early and later life stages of CoTS may be further exacerbated by reduced habitat quality and/or limited food in a warmer ocean. CoTS settle on CCA (which is also their food source) before transitioning to a coral diet (Johnson & Sutton, 1994; Kamya et al., 2016; 2018). **Chapter 3** found that settlement success was about 15% lower on CCA that had been pre-treated for 24 h at temperatures of 32 °C to 34 °C compared to 28 °C. This is likely in part due to the bleaching of the CCA that occurred at these temperatures. However, studies have indicated that coralline algae, including *Lithothamnion* sp., experience shifts in their microbial communities at higher temperatures (Webster et al., 2011; Whalan & Webster, 2014; Huggett et al., 2018), that may affect the inductive capacity of the substrate for settlement. Ocean acidification, which often accompanies ocean warming, may further impact the settlement substrate. Kamya and colleagues (2017) for instance found that lower pH decreased the C:N ratio and carbonate levels in the algae. However, interestingly, this appears to improve its palatability, resulting in higher feeding rates by CoTS juveniles (Kamya et al., 2016; 2017).

Not only will climate change impact the food for the early life stages, it will also impact the coral prey of the latter life stages. Already, the effects of climate change are evident on the GBR with devastating mass coral bleaching events occurring in 2016, 2017, 2020, and 2022, despite the latter year being characterised by La Niña (cooler summer) conditions (AIMS, 2022b). It remains unclear how CoTS feeding preferences change during bleaching events (see *6.4.3 Further research* section below). Despite this uncertainty, it is clear that extensive coral loss will inevitably lead to CoTS population declines.

6.4 Conclusions

This thesis adds to current knowledge of the effects of warming on echinoderms, particularly CoTS. Importantly, tropical asteroids (such as CoTS) may be particularly vulnerable to ocean warming (**Chapter 2**). Moreover, results from experimental studies that explicitly assessed the thermal sensitivity of *A.* cf. *solaris* **(Chapters 3** to **5)** demonstrated that this highly influential species is likely to be impacted (both directly and indirectly) by ongoing ocean warming. Critically, exposure to temperatures of ≥ 30 °C are likely to have negative impacts on the physiology, activity and survival of *A.* cf. *solaris*, at least in the northern and central GBR, and any such effects will likely be compounded by indirect effects of environmental change on habitat structure and prey availability.

6.4.1 Ecological implications

Without extreme climate change mitigation, by 2050 the mean sea surface temperature may rise by +1 °C (RCP8.5; IPCC, 2019). Further, the number of degree heating weeks are expected to increase from ~5 (in 2020) to ~15 weeks (in 2050; RCP8.5), indicating an increased prevalence and duration of marine heatwaves (McWhorter et al., 2022). Although, coral-feeding CoTS may continue to perform well with only a degree increase in their environmental temperature, regular marine heatwaves may lead to physiological constraints, reduced reproductive capacity, and periodic mortality events (**Chapters 4** and **5**). Lower densities of adult CoTS will inevitably affect fertilisation rates, which will further constrain population replenishment (Caballes, 2017). If marine heatwaves occur during spawning and early development, successful fertilisation, development success and recruitment may be further constrained (**Chapters 2** and **3;** Rupp, 1973; Kamya et al., 2014; Caballes et al., 2017; Hue et al., 2020), which may be exacerbated by a reduced quality of crustose coralline algae (**Chapter 3**). Diminished quality and availability of coral prey may further reduce the proportion of early CoTS that become highly fecund coral-feeding adults (Caballes et al., 2016; Caballes et al., 2017; Hughes et al., 2017). This negative feedback loop, characteristic of echinoderms exhibiting boom-bust population dynamics (Uthicke et al., 2009), indicates that by mid-century, thermally-induced population busts may occur, as well as fewer and less severe future outbreaks. The impact of warming on CoTS population dynamics may also vary spatially. For instance, there may be fewer outbreaks in the warmer Northern GBR by mid-century, yet intense CoTS outbreaks may continue in the cooler Southern GBR, *e.g.*, the Swains, which is considered a climate refugia for corals (McWhorter et al., 2022).

By 2100, under "business as usual" scenarios, the mean sea surface temperature is predicted to increase by around +3 °C (RCP8.5; IPCC, 2019), and the number of degree heating weeks on the GBR may increase by 44 weeks per year (relative to 2020; RCP8.5; McWhorter et al., 2022). This extreme heat stress will likely prevent future outbreaks from occurring, aided by reduced food availability. McWhorter and colleagues (2022) predicted that by the end of the century, there will no longer be coral refugia in the southern portion of the GBR under the most extreme projected climate change scenario. If the oceans do not warm by such a considerable degree, or if CoTS and their coral prey have a strong ability to acclimate (*i.e.*, non-genetic heritable changes) or adapt (*i.e.*, genetic heritable changes) to warming, than CoTS outbreaks may continue to be a problem on the reef for centuries to come (Schulte et al., 2011; Humanes et al., 2022; McWhorter et al., 2022).

6.4.2 Management implications

The research presented in this thesis, as well as prior knowledge on thermal impacts on CoTS early life stages (Table 1.1), can aid predictions of spatial and temporal changes in the risk and severity of population irruptions. This information is critical for informing and improving management of CoTS populations (Plagányi et al., 2020). The CoTS Control Program facilitated by the Great Barrier Reef Marine Park Authority utilises an integrated pest management strategy to plan the control of CoTS on the GBR (Westcott et al., 2021). The Program currently includes a reef prioritisation framework for culling efforts, which incorporates both economic (*i.e.*, importance for tourism) and ecological factors (*e.g.*, coral condition and connectivity to other reefs; Westcott et al., 2021). Reef prioritisation would benefit from information on the suitability of the thermal environment for the establishment of CoTS population irruptions (**Chapters 3** to **5**), and projected shifts in thermal envelopes (Lough, 2008; Lough et al., 2018; McWhorter et al., 2022). Latitudinal thermal bands have moved 63 to 313 km southwards along the North-East coast of Australia between 1950-1969 and 1987-2006 and will likely shift further in the coming decades (Lough, 2008). These shifts will not only impact CoTS, but also their habitat and prey (Hughes et al., 2017; McWhorter et al., 2022).

6.4.3 Further research

For the purpose of management, an important conundrum to consider, is whether a reduced coral availability due to thermal bleaching and subsequent mortality will constrain CoTS population irruptions before they are constrained by the direct impacts of warming. The preferred coral prey of CoTS, *Acropora*, is considered one of the most thermally sensitive genera of coral, with a relatively high susceptibility to bleaching (Marshall & Baird, 2000; Baird & Marshall, 2002; Pratchett, 2007; Keesing et al., 2019). One study observed a 90-95% decline in survivorship of two GBR *Acropora* species commonly preyed upon by CoTS in response to 5 weeks of exposure to temperatures of 31 °C (relative to the 26 °C control; Anderson et al., 2019). Another found that fertilisation success of *Acropora*, and embryo settlement from the same region was reduced by 14% as low 30 °C, and at 32 °C, larval settlement was 23% lower (compared to the 27 °C control; Humanes et al., 2017), indicating possible constraints to population replenishment. Together, these findings suggests that the abundance of high value prey may be diminished in the near future. CoTS are however, known to exhibit diet shifts in response to a reduced availability of preferred food (De'ath & Moran, 1998; Keesing et al., 2019; Keesing, 2021). For example, in a study by Keesing (2021), only Acroporidae was significantly selected for by CoTS, when coral cover was above 50%. However, when coral cover was less than 10%, all four major families of coral (Acroporidae, Pocilloporidae, Faviidae, and Poritidae) were significantly selected for. A number of coral taxa on the GBR have a low susceptibility to bleaching, such as *Leptastrea* and *Cyphastrea* in the family Faviidae, and *Goniopora* in the family Poritidae (Marshall & Baird, 2000). Furthermore, novel reef restoration techniques *e.g.*, assisted evolution through selective breeding, pre-conditioning and microbiome manipulation may mitigate against large scale coral loss (McLeod et al., 2022). Therefore, if CoTS were able to persist in a warmer ocean, they may feed on remaining thermally robust corals, and other marine organisms *e.g.*, soft corals, clams and other molluscs, possibly threatening ecologically important species that were not previously targeted by CoTS (Moran, 1988).

A critical next step is to better understand how this relationship between CoTS and their coral prey will change in a warmer ocean. One pertinent question is: in an era of regular coral bleaching events, will CoTS prefer to feed on healthy or bleached coral? (Hughes et al., 2017; McWhorter et al., 2022). CoTS may prefer to feed on healthy coral that have greater energy reserves, provided by the algal symbionts (Rodrigues & Grottoli, 2007). Alternatively, they may prefer to feed on bleached coral, which despite being of lower nutritional value due to decreased *Symbiodinium* spp. densities (Rodrigues & Grottoli, 2007), they have diminished host defences, *e.g.*, nematocysts and crustacean symbionts (Glynn, 1985; McIlwain & Jones, 1997; Tsuchiya, 1999; Pratchett, 2001; 2007; Stella et al., 2011). Bleached coral may also be more detectable by CoTS due to the chemicals they release when stressed (McIlwain & Jones, 1997). Research on the feeding behaviour of CoTS on healthy and bleached coral at predicted near-future temperatures, will enable the better understanding of whether CoTS may prosper during bleaching events, or whether they will starve if the corals are unable to quickly recover. This information, along with current knowledge regarding the feeding selectivity of CoTS, and the abundance and vulnerability of preferred coral genera/species to bleaching, will facilitate projections of the future spatial and temporal dynamics of CoTS population irruptions (De'ath & Moran, 1998; Pratchett, 2007; Keesing, 2021).

Projections of the future threat CoTS will pose to the reef will also benefit from better knowledge on the effects of sustained and progressive warming on the species. Although the CoTS in **Chapter 5** were exposed to prolonged warming (60 days), even longer exposure durations would allow the assessment of whether CoTS may acclimate to temperatures at which reduced performance was observed, or whether even greater performance declines may be observed. At the highest magnitude of warming, 75% of CoTS died after 60 days of exposure (**Chapter 5**). Thus, in order to retain enough living starfish for such trials, a greater sample size or the provision of food may facilitate sufficient survival rates. Further, cross-generational studies on CoTS may enable the detection of transgenerational plasticity. Transgenerational plasticity occurs when the exposure of parents to supra-optimal conditions results in subsequent generations possessing advantageous phenotypes for performing well in these conditions. This has been observed at elevated temperatures in the tropical damselfish *Acanthochromis* *polyacanthus* (Donelson et al., 2011) and the temperate marine stickleback *Gasterosteus aculeatus* (Heckwolf et al., 2018), for instance. This plasticity may facilitate the slower process of genetic adaptation (Lucassen et al., 2006). If CoTS are able to exhibit transgenerational plasticity, then their populations may fare better in a warmer ocean than the results presented herein suggest. However, akin with findings from long-term (~15-25 month) studies on the sea urchins *Echinometra* sp. (Uthicke et al., 2021) and *Strongylocentrotus intermedius* (Zhao et al., 2018), Hue et al. (2022) found negative carry over effects on development success of CoTS larvae, after 20 weeks of adult exposure to + 2 °C of warming. This study suggests that fewer and less severe population irruptions of CoTS may be expected in the coming decades. Nevertheless, further research where the F_1 generation are followed through settlement, the juvenile stages, and ultimately to spawning adults at predicted near-future temperatures, are warranted, facilitating the testing of transgenerational effects on the F_2 generation (Donelson et al., 2011; Parker et al., 2015; Heckwolf et al., 2018). Such studies, using treatments which encompass the range of temperatures that may be expected under various RCP scenarios will unequivocally enhance understanding on the future threat posed by CoTS on the reef (Zhao et al., 2018; IPCC, 2019; Uthicke et al., 2021; Hue et al., 2022).

Additional stressors associated with climate change may further constrain CoTS populations in the near-future and should also be considered in further studies. Prior studies have shown variable impacts of ocean acidification on the early life stages, both as a single stressor, and in conjunction with ocean warming. For instance, Caballes et al. (2017) and Uthicke et al. (2013) observed negative effects of ocean acidification (pH 7.9-7.6) on sperm activity and fertilisation success. Yet, Kamya et al. (2014) and Hue et al. (2020) found no effects of similar magnitudes of ocean acidification on successful fertilisation, both individually, and in combination with ocean warming (+ 2 or 4 °C). The former study discovered that the percentage of normal larvae was lowest in the warmest, most acidified treatment. A later study by Hue et al. (2022) corroborates this observation, finding that after parental exposure, there were more abnormal larvae in the combined warming (+ 2 °C) and acidification (pH 7.75) treatments, compared to the those with sole stressors, or the control treatment. Despite this, larval survival appeared unaffected. It is unclear, whether there may be potential additive, synergistic or possibly antagonistic effects of combined stressors (including seldom studied stressors, such as hypoxia, reduced salinity, and sedimentation), on mature CoTS. This knowledge will further strengthen predictions regarding the future population dynamics of this troublesome echinoderm, and the outlook for coral reefs of the Indo-Pacific.

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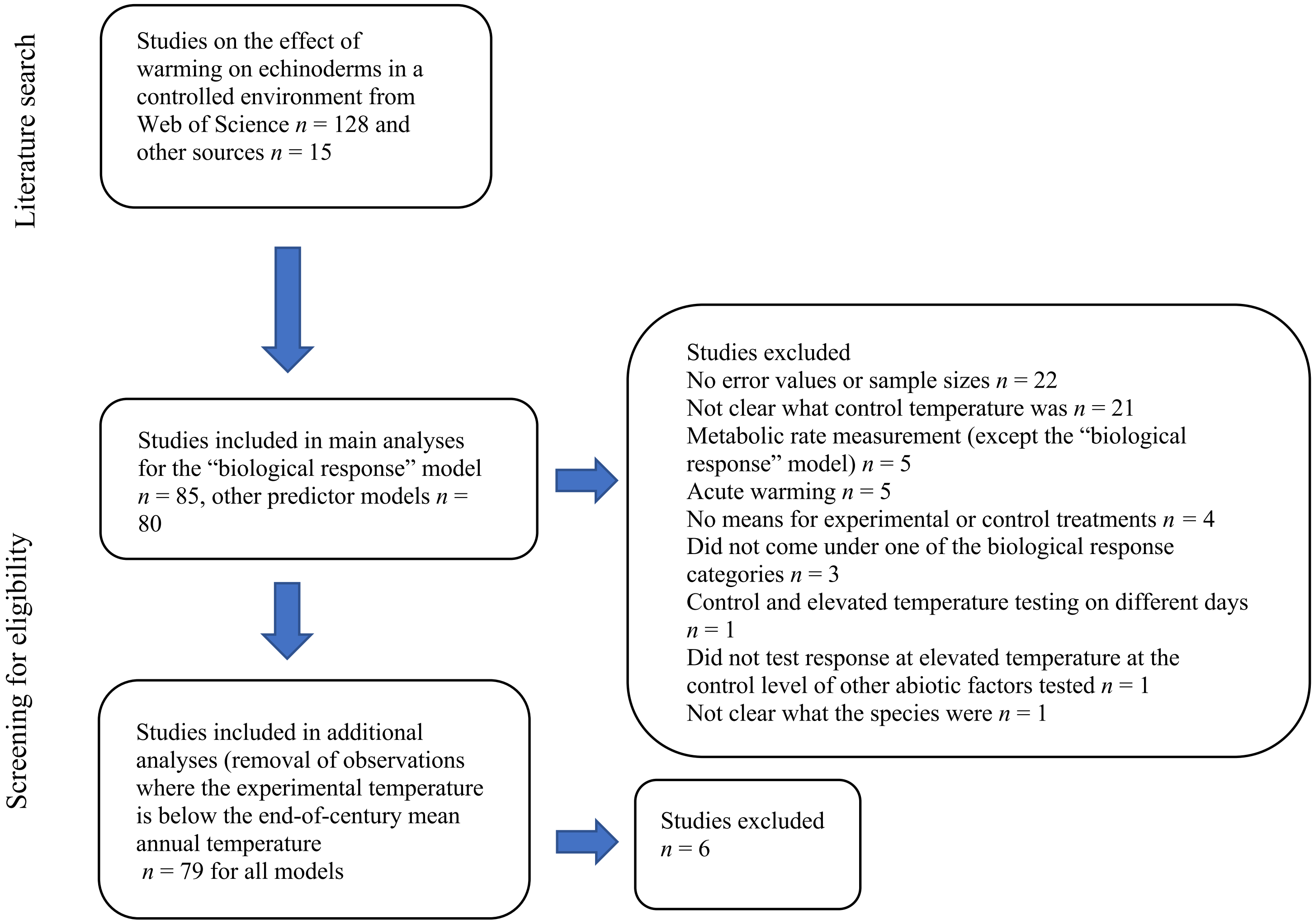
Appendices

Appendix 2.1 Flow diagram illustrating how the literature was searched and screened for eligibility for inclusion in the meta-analysis. The numbers of studies included and excluded (for various reasons) are detailed.



Appendix 2.2 Data sources included in the meta-analysis. * indicates papers measuring metabolic rate that were removed from the main analyses for all moderators except "biological response". ** indicates papers where the experimental temperature was below the near-future mean annual temperature projection, and were therefore removed from the associated subset of data.

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Appendix 2.3 Predictors of variation in echinoderm vulnerability to warming, tested using separate multivariate linear mixed effects models. The predictor name and groups within them are provided.

Model predictor	Groups
Biological response	Development success, feeding and nutrition, growth, metabolic rate, movement, reproductive success, survival
Life stage	Gamete (including fertilisation), embryonic stage, larval stage, juvenile stage, adult stage
Class	Asteroidea, Echinoidea, Holothuroidea, Ophiuroidea
Region	Tropical (4-23°), sub-tropical (26-35°), temperate (36-53°), polar (62-78°)
Habituation time at the control temperature (days)	Continuous
Warming rate (°C hr^{-1})	Continuous
Exposure time at experimental temperature (days)	Continuous

Appendix 2.4 List of measures tested within each "biological response" group

Biological response group	*Measures*
Development success	Percentage undergoing cleavage/gastrulation/hatching Percentage reaching various larval stages Development rate/time Larval arm asymmetry Percentage settled Percentage metamorphosing into juveniles
Feeding and nutrition	Feeding rate/Total food consumed Proportion of individuals feeding Absorption efficiency Feed conversion efficiency Energy consumed Food encounter rate/capture probability Protein/lipid/carbohydrate content Protein/lipid/carbohydrate absorption efficiency
Growth	Body length/width/height Body area/radius/diameter/volume Growth rate Wet weight/Ash free dry weight Number of arms/spines Ciliated band length Mg/Ca ratio Scope for growth Net growth efficiency Percentage intact individuals/regeneration rate/functional recovery/percentage regained arms Muscle density
Metabolic rate	Larval oxygen consumption rate Standard/resting/routine oxygen consumption rate
Movement	Righting time/number of successful righting attempts Locomotion speed Percentage individuals moving Foraging time Percentage exhibiting covering behaviour Tentacle locomotion time
Reproductive success	Sperm speed/motility Percentage of successful fertilisations Sperm mitochondrial activity Egg diameter/volume/sphericity Gonadosomatic index/Gonad growth/Gonad maturity index
Survival	Percentage mortality/survival of embryos/larvae/juveniles Percentage abnormal embryos/larvae/juveniles Percentage of blastulae released from egg membrane Density of larvae Percentage settlement and post-settlement survival

Appendix 2.5 Table of species of echinoderm included in the analysis, their taxonomic class and region groupings and the number of data points for each species.

Species	**Class**	**Region**	**Number of points**
Acanthaster sp.	Asteroidea	Tropical	110
Apostichopus japonicus	Holothuroidea	Temperate	78
Arbacia lixula	Echinoidea	Sub-tropical	3
Arbacia punctulata	Echinoidea	Sub-tropical	22
Asterias amurensis	Asteroidea	Sub-tropical	6
Asterias rubens	Asteroidea	Temperate	5
Asterias vulgaris	Asteroidea	Temperate	9
Asterina pectinifera	Asteroidea	Temperate	3
Australostichopus mollis	Holothuroidea	Temperate	12
Centrostephanus rodgersii	Echinoidea	Temperate	22
Cucumaria frondosa	Holothuroidea	Temperate	10
Culcita novaeguineae	Asteroidea	Tropical	3
Dendraster excentricus	Echinoidea	Temperate	8
Diadema africanum	Echinoidea	Sub-tropical	3
Diadema savignyi	Echinoidea	Tropical	3
Echinometra lucunter	Echinoidea	Sub-tropical	10
Echinometra mathaei	Echinoidea	Tropical	12
Echinometra sp. A	Echinoidea	Tropical	1
Evechinus chloroticus	Echinoidea	Temperate	5
Heliocidaris erythrogramma	Echinoidea	Sub-tropical	39
Heliocidaris tuberculata	Echinoidea	Sub-tropical	6
Holothuria forskali	Holothuroidea	Temperate	8
Holothuria moebii	Holothuroidea	Tropical	2
Holothuria scabra	Holothuroidea	Tropical	8
Linckia laevigata	Asteroidea	Tropical	5
Loxechinus albus	Echinoidea	Temperate	4
Lytechinus variegatus	Echinoidea	Sub-tropical	49
Marthasterias glacialis	Asteroidea	Temperate	3
Meridiastra calcar	Asteroidea	Sub-tropical	9
Microphiopholis gracillima	Ophiuroidea	Sub-tropical	1
Odontaster validus	Asteroidea	Polar	21
Ophiocten sericeum	Ophiuroidea	Polar	5
Ophioderma longicauda	Ophiuroidea	Temperate	10
Ophionereis schayeri	Ophiuroidea	Sub-tropical	1
Ophiopholis mirabilis	Ophiuroidea	Temperate	2
Ophiopholis sarsii	Ophiuroidea	Temperate	2

Ophiura ophiura	Ophiuroidea	Temperate	6
Paracentrotus lividus	Echinoidea	Sub-tropical/Temperate	45
Parvulastra exigua	Asteroidea	Sub-tropical	12
Patiriella pseudoexigua	Asteroidea	Tropical	4
Patiriella regularis	Asteroidea	Temperate	8
Pseudechinus magellanicus	Echinoidea	Temperate	4
Sphaerechinus granularis	Echinoidea	Sub-tropical	6
Sterechinus neumayeri	Echinoidea	Polar	37
Strongylocentrotus droebachi	Echinoidea	Temperate/Polar	53
Strongylocentrotus purpuratu	Echinoidea	Sub-tropical	2
Tripneustes gratilla	Echinoidea	Tropical/Sub-tropical	33

Appendix 2.6 Sensitivity, publication bias and non-linearity tests methods and results.

2.6.1 Sensitivity analyses

Methods The presence of influential observations was tested by ranking the data by the effect size and removing the observations with the largest 10 effect sizes (in either direction) in a stepwise fashion and re-running all categorical models (*i.e.*, biological response, ontogenetic life stage, taxonomic class, and region), to see if the overall significance of Q_M, and the significance of individual groups of a predictor were affected. The effect of pseudoreplication was then explored by removing publications one at a time that contributed two or more "studies" due to measuring responses of multiple species and observing any changes in significance. Again, this was conducted for each of the categorical predictors. Lastly, the ontogenetic life stage and taxonomic class models were re-run, removing the three species that contributed the most data points: *Acanthaster* spp. (tropical asteroid; n = 110 data points), *Apostichopus japonicus* (temperate holothuroid; n = 78 data points) and *Strongylocentrotus droebachiensis* (temperate and polar echinoid; n = 53 data points).

Results These models were robust to the removal of the 10 responses with the highest effect sizes (in either direction). Removal of papers that contribute two or more studies, in most cases did not change the model outputs to a significant extent. However, the responses reported in Rupp (1973) which contributed six studies to the analysis appears to be particularly influential in two models. In the ontogenetic life stage model, gametes (and fertilisation) were no longer being significantly impacted by warming when responses from this paper were removed ($P = 0.661$). Furthermore, in the region model, echinoderms from tropical (4-22°) latitudes became significantly negatively affected by warming ($P = 0.025$), when Rupp (1973) was removed. Moreover, removing Garcia et al. (2018) that contributed four studies to the meta-analysis, resulted in sub-tropical (26-35°) echinoderms being no longer significantly impacted by warming ($P = 0.079$), along with the overall Robust Q_M ($P = 0.077$). Removal of *Acanthaster* spp. resulted in tropical echinoderms becoming significantly affected by warming ($P = 0.040$), and removal of *Apostichopus japonicus* resulted in the overall effect of the model becoming non-significant ($P = 0.086$). In all other instances the significances and direction of the response remained the same for the overall predictor models, and the individual groups.

In the tables, the groups in which the significance changed from the original models are in bold.

Life stage model output after removal of Rupp (1973).

Life stage	estimate	se	tval	df	pval	ci.lb	ci.ub
Gametes	**-0.0127**	**0.0288**	**-0.4407**	**69**	**0.6608**	**-0.07**	**0.0447**
Embryonic stage	-0.1981	0.1499	-1.3218	69	0.1906	-0.497	0.1009
Larval stage	-0.222	0.0633	-3.5059	69	0.0008	-0.3483	-0.0957
Juvenile stage	-0.0929	0.0564	-1.648	69	0.1039	-0.2054	0.0196
Adult stage	-0.0096	0.0516	-0.1857	69	0.8532	-0.1126	0.0934

QM; F = 3.21, df = 5,69, P = 0.012, *n* = 630

Region model output after removal of Rupp (1973).

Region	estimate	se	tval	df	pval	ci.lb	ci.ub
Tropical (4-23°)	**-0.2496**	**0.1087**	**-2.2972**	**70**	**0.0246**	**-0.4664**	**-0.2496**
Sub-tropical (26-35°)	-0.0928	0.0391	-2.3701	70	0.0205	-0.1709	-0.0928
Temperate (36-53°)	-0.059	0.0348	-1.6961	70	0.0943	-0.1284	-0.059
Polar (62-78°)	0.0308	0.033	0.9312	70	0.3549	-0.0351	0.0308

QM; F = 3.66, df = 4,70, P = 0.009, *n* = 630

Region model output after removal of Garcia et al. (2018).

Region	estimate	se	tval	df	pval	ci.lb	ci.ub
Tropical (4-23°)	-0.079	0.0572	-1.3807	72	0.1716	-0.193	-0.079
Sub-tropical (26-35°)	**-0.1209**	**0.0679**	**-1.7808**	**72**	**0.0792**	**-0.2562**	**-0.1209**
Temperate (36-53°)	-0.0589	0.0348	-1.6946	72	0.0945	-0.1282	-0.0589
Polar (62-78°)	0.0308	0.033	0.9337	72	0.3536	-0.035	0.0308

QM; F = 2.21, df = 4,72, P = 0.077, *n* = 637

Class model output after removal of *Acanthaster* spp.

Class	estimate	se	tval	df	pval	ci.lb	ci.ub
Asteroidea	-0.3668	0.1609	-2.2794	68	0.0258	-0.6880	-0.0457
Echinoidea	-0.0066	0.0141	-0.4705	68	0.6395	-0.0348	0.0215
Holothuroidea	-0.1101	0.0269	-4.0968	68	0.0001	-0.1637	-0.0565
Ophiuroidea	0.1360	0.1179	1.1537	68	0.2527	-0.0993	0.3713

QM; F = 5.88, df = 4,68, P < 0.001, *n* = 548

Region model output after removal of *Acanthaster* spp.

Region	estimate	se	tval	df	pval	ci.lb	ci.ub
Tropical (4-23°)	**-0.0280**	**0.0133**	**-2.0974**	**68**	**0.0397**	**-0.0546**	**-0.0014**
Sub-tropical (26-35°)	-0.0927	0.0391	-2.3725	68	0.0205	-0.1707	-0.0147
Temperate (36-53°)	-0.0589	0.0348	-1.6926	68	0.0951	-0.1284	0.0105
Polar (62-78°)	0.0308	0.0331	0.9312	68	0.3550	-0.0352	0.0967

QM; F = 3.44, df = 4,68, P = 0.013, *n* = 548

Class model output after removal of *Apostichopus japonicus*

Class	estimate	se	tval	df	pval	ci.lb	ci.ub
Asteroidea	-0.3549	0.0557	-6.3776	71	<.0001	-0.4659	-0.2440
Echinoidea	-0.0067	0.0141	-0.4758	71	0.6357	-0.0348	0.0214
Holothuroidea	-1.0628	0.5004	-2.1241	71	0.0371	-2.0605	-0.0651
Ophiuroidea	0.1331	0.1156	1.1512	71	0.2535	-0.0974	0.3636

QM; F = 11.68, df = 4,71, P < 0.001, *n* = 592

Region model output after removal of *Apostichopus japonicus*

Region	estimate	se	tval	df	pval	ci.lb	ci.ub
Tropical (4-23°)	-0.0790	0.0572	-1.3803	71	0.1718	-0.1930	0.0351
Sub-tropical (26-35°)	-0.0926	0.0389	-2.3798	71	0.0200	-0.1703	-0.0150
Temperate (36-53°)	-0.0012	0.0442	-0.0267	71	0.9788	-0.0893	0.0869
Polar (62-78°)	0.0308	0.0330	0.9334	71	0.3538	-0.0350	0.0966

QM; F = 2.11, df = 4,71, P = 0.086, *n* = 592

Class model output after removal of *Strongylocentrotus droebachiensis*

Class	estimate	se	tval	df	pval	ci.lb	ci.ub
Asteroidea	-0.3522	0.0554	-6.3601	73	<.0001	-0.4625	-0.2418
Echinoidea	-0.0169	0.0105	-1.6190	73	0.1098	-0.0378	0.0039
Holothuroidea	-0.1101	0.0268	-4.1072	73	0.0001	-0.1636	-0.0567
Ophiuroidea	0.1361	0.1170	1.1630	73	0.2486	-0.0971	0.3693

QM; F = 15.32, df = 4,73, P < 0.001, *n* = 605

Region model output after removal of *Strongylocentrotus droebachiensis*

Region	estimate	se	tval	df	pval	ci.lb	ci.ub
Tropical (4-23°)	-0.0790	0.0572	-1.3812	73	0.1714	-0.1929	-0.0790
Sub-tropical (26-35°)	-0.0927	0.0389	-2.3800	73	0.0199	-0.1703	-0.0927
Temperate (36-53°)	-0.0588	0.0349	-1.6857	73	0.0961	-0.1283	-0.0588
Polar (62-78°)	-0.0310	0.0520	-0.5964	73	0.5528	-0.1346	-0.0310

QM; F = 2.69, df = 4,73, P = 0.038, *n* = 605

2.6.2 Publication bias

Methods Publication bias commonly occurs in meta-analyses because studies are more likely to be published if results are significant (Duval & Tweedie, 2000; Rosenberg, 2005; Shi & Lin, 2019). To test for publication bias, Duval and Tweedie's trim-and-fill method (Duval & Tweedie, 2000) was used, which supresses the most extreme data points on either side of a funnel plot, and by doing so, estimates the number of missing responses due to publication bias in the dataset (Duval & Tweedie, 2000; Shi & Lin, 2019; Sampaio et al., 2021). This method provides conservative estimates, as it does not take into account the random effects (Duval & Tweedie, 2000).

Results Evidence of publication bias was detected, with an estimated 168 responses missing on the left (*i.e.*, negative *LnRR*'s) side. An estimated 150 responses were missing on the left side, when metabolic rate responses were removed. It was estimated that there were no responses missing on the right (*i.e.*, positive *LnRR*'s) side.

2.6.3 Linearity

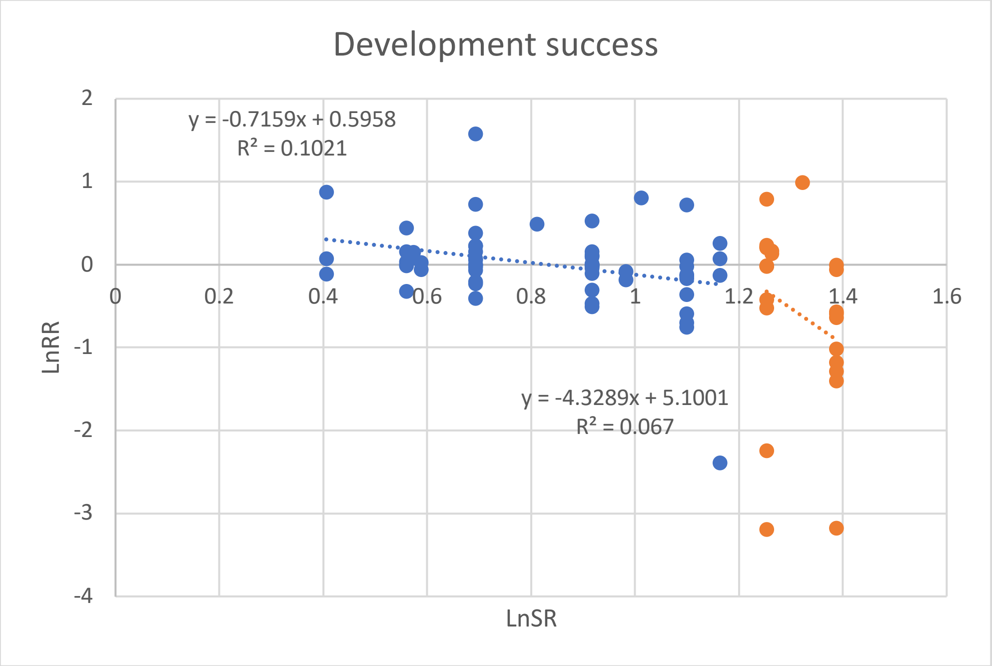
Methods It is a possibility that the response (*LnRR*) may be dependent on the degree of warming (*LnSR*), leading to non-linearity (*i.e.*, thermal performance curves). Any non-linearity in thermal effects on performance was tested for each group of a predictor using polynomial linear models. For those groups where a 2^{nd} or 3^{rd} order polynomial was detected, where relevant, the degree of warming in which *LnRR* became non-linear in a negative direction was recorded.

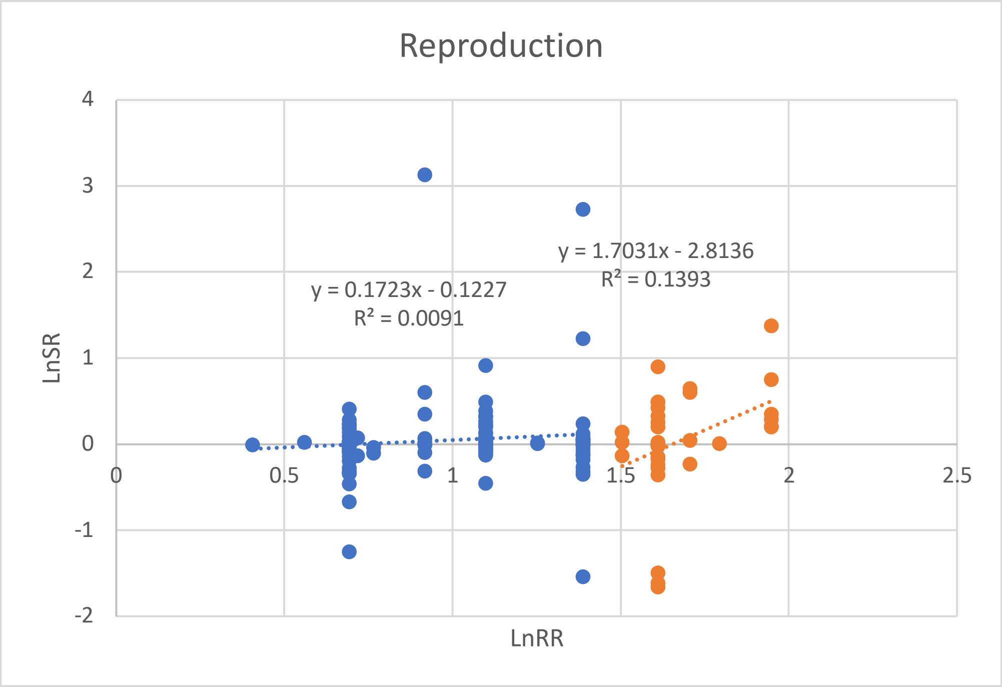
Results Non-linearity in the correlation between *LnSR* and *LnRR* was observed for some groups for the biological responses, life stages and regions. Most notably, the relationship between *LnSR* and *LnRR* for both development success and larvae is a 2nd order polynomial, with deviation from linearity in the negative direction observed at a *LnSR* of 1.2 (equivalent to 3.3 °C of warming) in both instances. 2nd order polynomials are also detected for embryos and the tropical (4-23°) latitude, with negative deviations from linearity detected at *LnSR*'s of 1.1 (equivalent to 3 °C of warming) and 1.4 (equivalent to 3.9 °C of warming), respectively.

In the tables, *LnSR* (the natural logarithm of the degree of warming) is the *LnSR* at which a clear negative deviation from linearity is observed for the relationship between this variable and the effect size (*LnRR*). If the relationship is linear "NA" is given, and if a 2nd or 3rd order polynomial is detected, but there is no clear point at which there is a deviation from linearity "NA*" is given. Exp-Ctl is the degree of warming at which the non-linearity is observed, *i.e.*, the back transformation of *LnSR*.

Detection of non-linearity in the biological response groups.

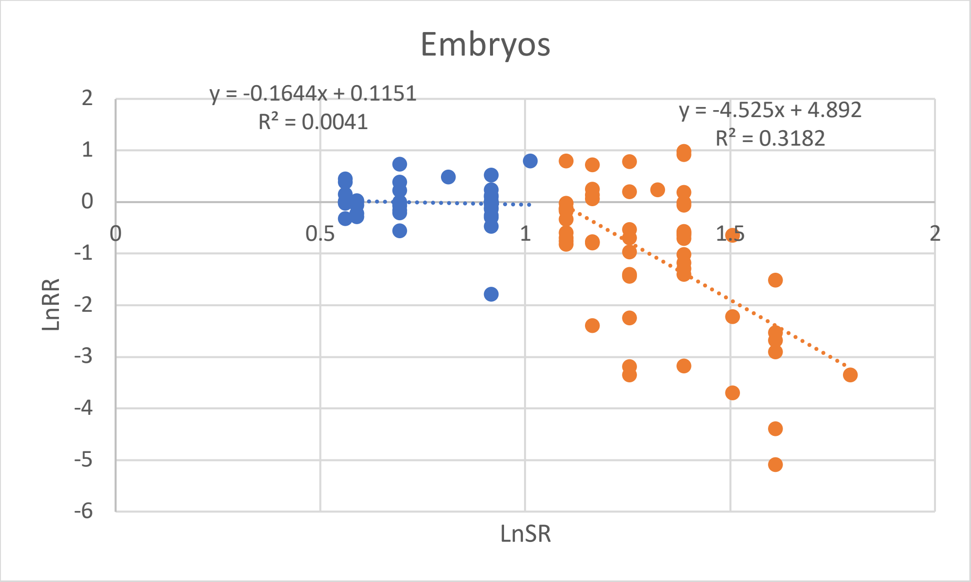
Biological response	Relationship	*LnSR*	Exp-Ctl (°C)
Development	**2nd order polynomial**	**1.17**	**3.25**
Feeding	Linear	NA	NA
Growth	Linear	NA	NA
Metabolism	Linear	NA	NA
Movement	Linear	NA	NA
Reproduction	3rd order polynomial	NA*	NA
Survival	Linear	NA	NA

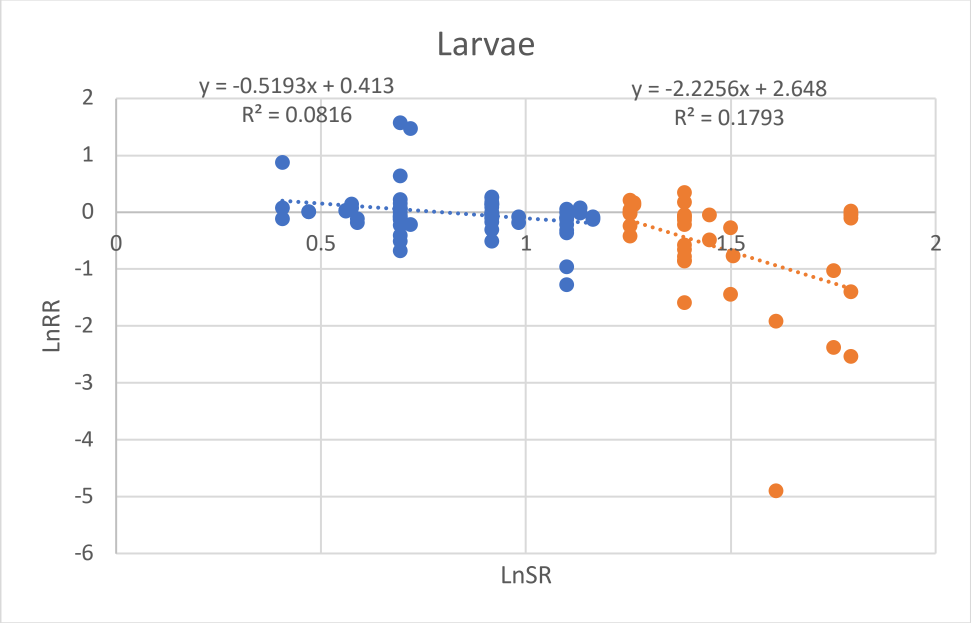

Development success
y = -0.7159x + 0.5958
R² = 0.1021
y = -4.3289x + 5.1001
R² = 0.067
LnRR
LnSR
2
1
0
-1
-2
-3
-4
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6


Reproduction
y = 1.7031x - 2.8136
R² = 0.1393
y = 0.1723x - 0.1227
R² = 0.0091
LnSR
LnRR
4
3
2
1
0
-1
-2
0
0.5
1
1.5
2
2.5

Detection of non-linearity in the life stage groups.

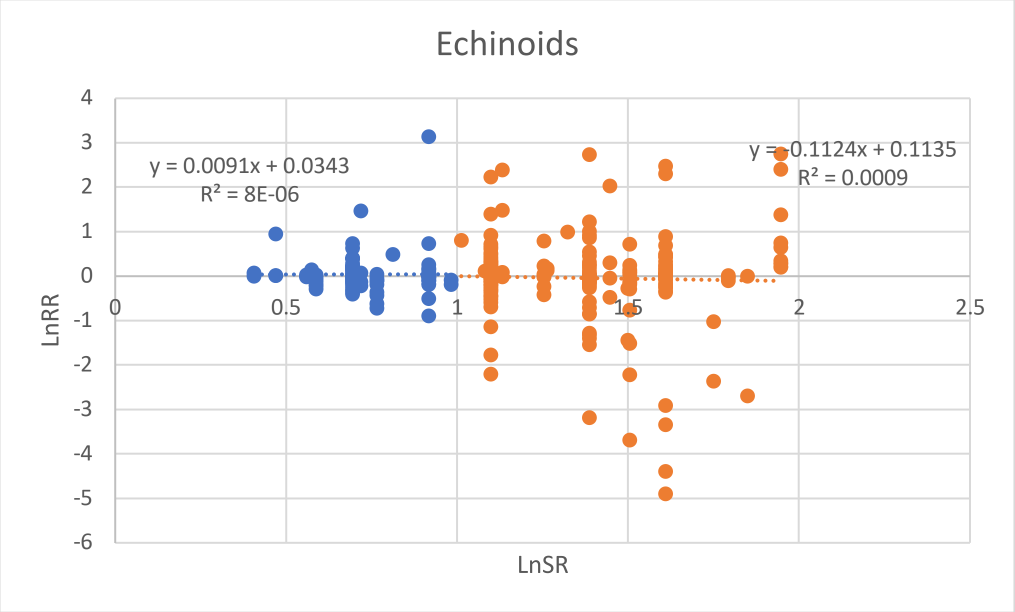
Life stage	Relationship	*LnSR*	Exp-Ctl
Gametes	Linear	NA	NA
Embryos	**2nd order polynomial**	**1.08**	**2.95**
Larvae	**2nd order polynomial**	**1.19**	**3.29**
Juveniles	Linear	NA	NA
Adults	Linear	NA	NA





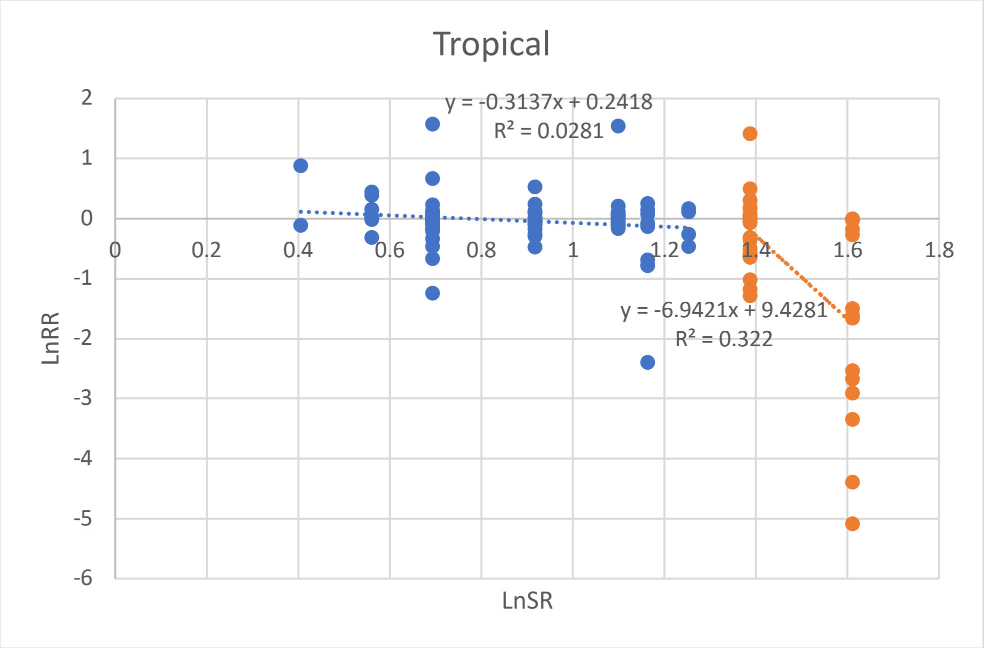
Detection of non-linearity in the class groups.

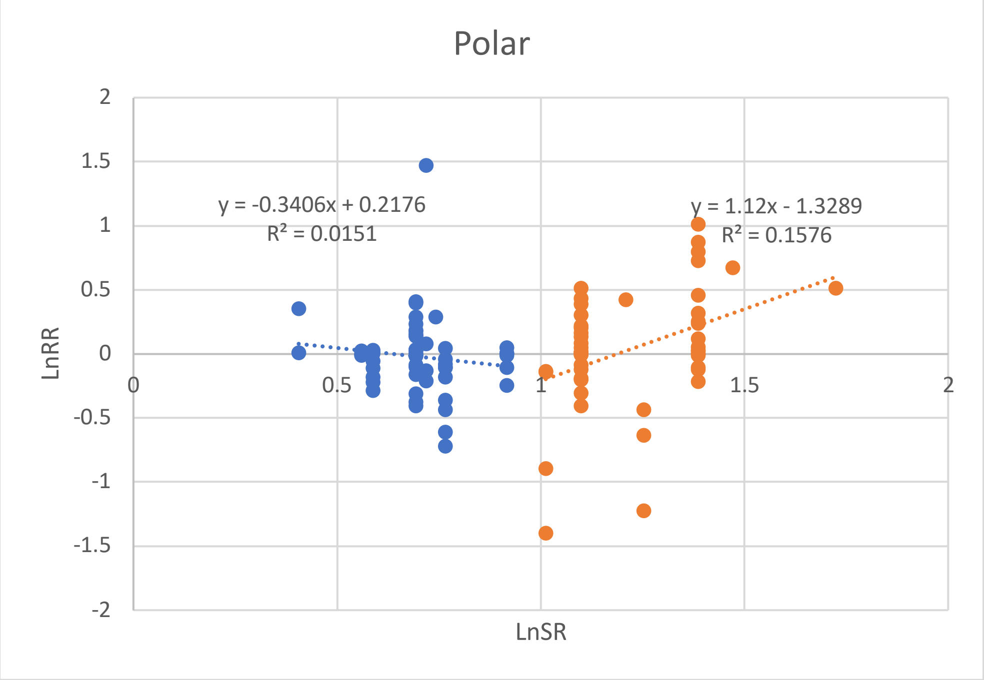
Class	Relationship	LnSR	Exp-Ctl
Asteroids	Linear	NA	NA
Echinoids	3rd order polynomial	NA*	NA
Holothuroids	Linear	NA	NA
Ophiuroids	Linear	NA	NA



Detection of non-linearity in the region groups.

Region	Relationship	*LnSR*	Exp-Ctl
Tropical (4-23°)	**2nd order polynomial**	**1.36**	**3.89**
Sub-tropical (26-35°)	Linear	NA	NA
Temperate (36-53°)	Linear	NA	NA
Polar (62-78°)	2nd order polynomial	NA*	NA*





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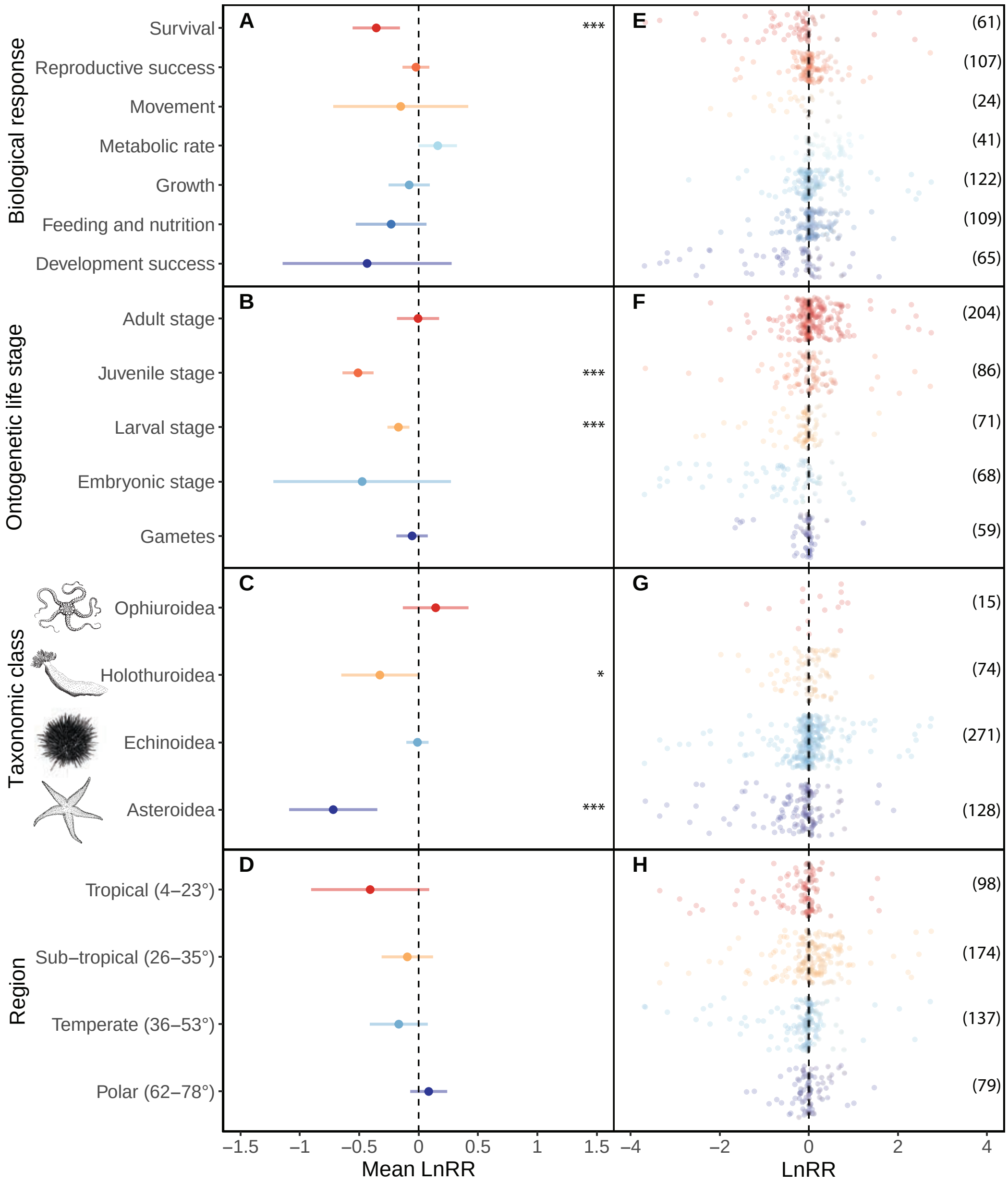
Sampaio, E., Santos, C., Rosa, I. C., Ferreira, V., Pörtner, H. O., Duarte, C. M., . . .Rosa, R. (2021). Impacts of hypoxic events surpass those of future ocean warming and acidification. *Nature Ecology & Evolution*, 5, 311-321. https://doi.org/10.1038/s41559-020-01370-3

Shi, L., & Lin, L. (2019). The trim-and-fill method for publication bias: practical guidelines and recommendations based on a large database of meta-analyses. *Medicine (Baltimore)*, 98, e15987. https://doi.org/10.1097/MD.0000000000015987

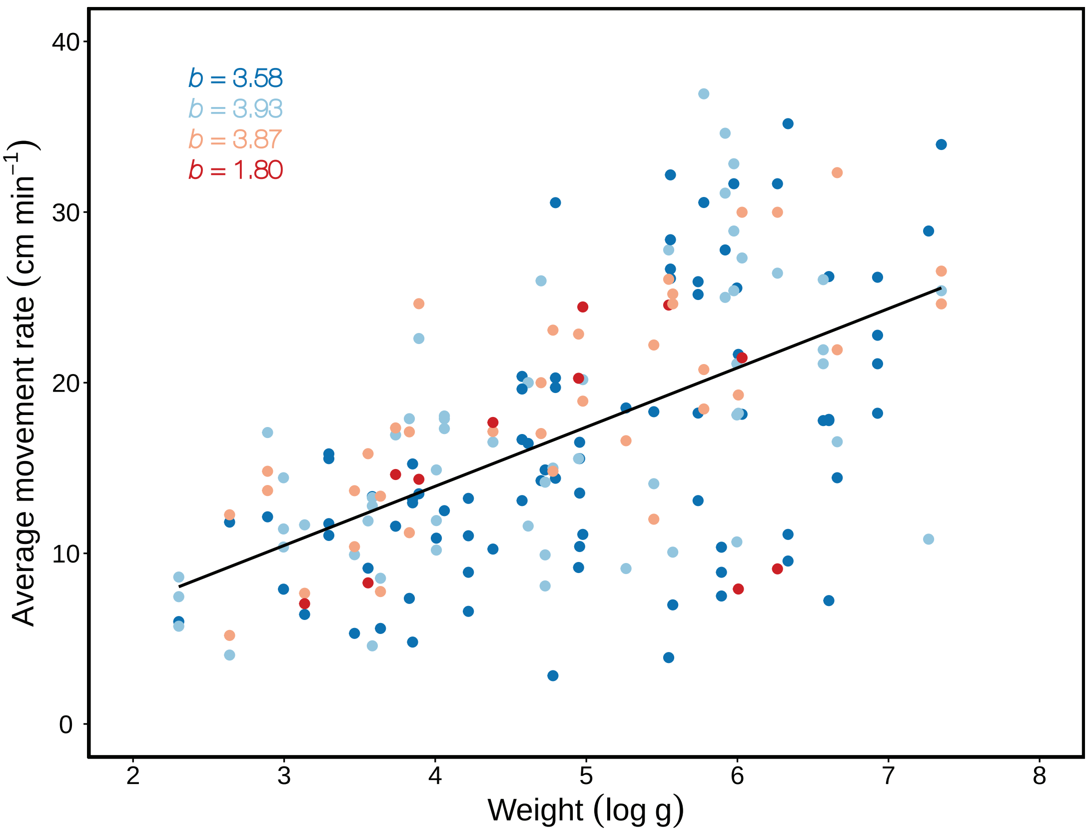
Appendix 2.7 The effect of warming above the predicted mean annual temperature (MAT) for the end of the century (+2.58 °C, RCP8.5, 2081-2100; IPCC, 2019) on echinoderms, a comparison between biological responses (A, E), ontogenetic life stages (B, F), taxonomic classes (C, G) and regions (D, H). The mean effect sizes and the robust 95% confidence intervals are provided (left panels, A-D), as well as the raw data points (right panels, E-H). The number of responses in each group are provided in parentheses in the right panels. Significant mean effect sizes are indicated by an asterisk (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) in the left panels. Mean effect sizes and 95% confidence intervals have been corrected for differences between the control and experimental temperatures (*LnSR*). Effect sizes above and below zero indicate a positive and negative response to warming, respectively.



Appendix 5.1 The estimated marginal means for the effect of temperature (°C) on righting time (s, ± SE) and average movement rate (cm min^{-1}, ± SE) in *Acanthaster* cf. *solaris* (n = 48 CoTS, n = 191 righting time observations, n = 188 average movement rate observations).

Temperature (°C)	Righting time (s)	Movement rate (cm min^{-1})
26	202 ± 22.3	18.3 ± 1.1
28	188 ± 21.3	21.6 ± 1.2
30	201 ± 24.4	23.3 ± 1.4
32	207 ± 33.8	20.0 ± 2.1

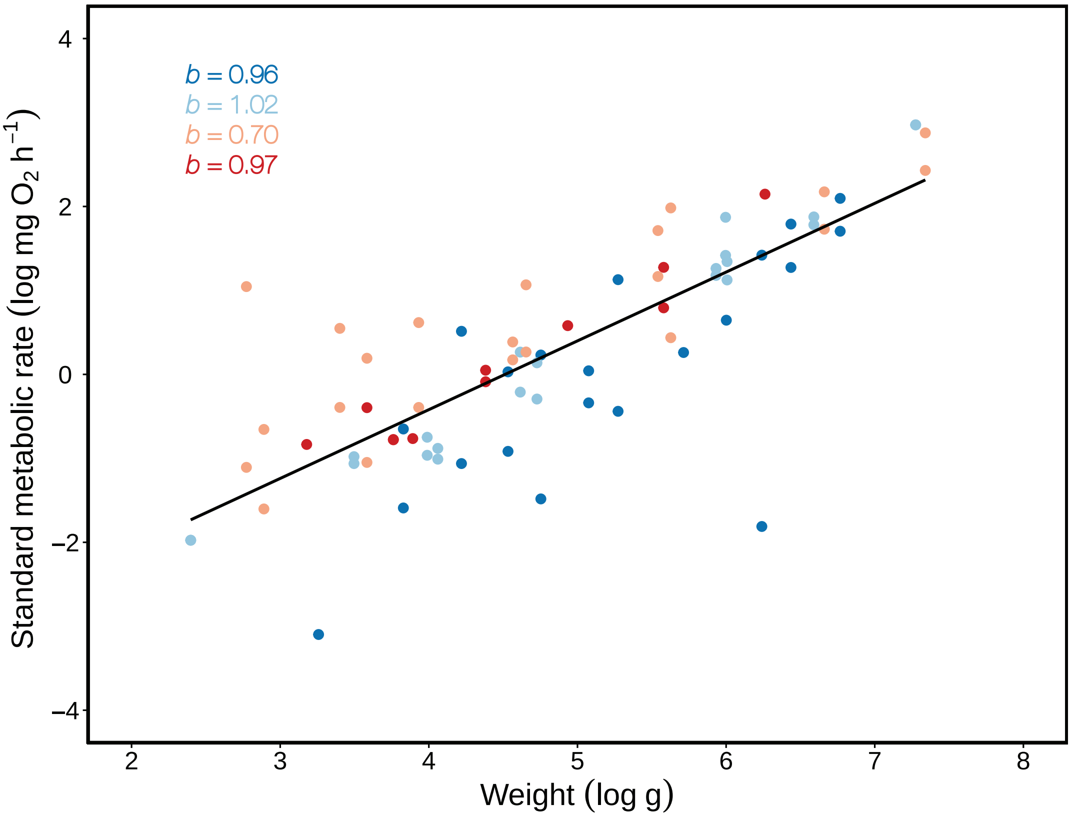
Appendix 5.2 The relationship between the natural logarithm of wet weight (g) and average movement rate (cm min $^{-1}$) in *Acanthaster* cf. *solaris* ($n = 48$ CoTS, $n = 188$ observations). The data points for 26, 28, 30 and 32 °C are coloured dark blue, light blue, orange and red respectively. The regression line was plotted using geom_smooth(method = lm). Scaling exponents (*b*) are also provided for each temperature.



Appendix 5.3 The estimated marginal means for the effect of temperature (°C) on the standard metabolic rate (mg O_2 g^{-1} h^{-1}, ± SE) of *Acanthaster* cf. *solaris* (*n* = 42 CoTS, *n* = 76 observations) in the four experimental treatments. Responses are provided for the overall effect of the temperature treatments on the standard metabolic rate, as well as the effect of the treatment on the response for runs 1 and 2 separately.

Treatment (°C)	Run	Standard metabolic rate (mg O_2 g^{-1} h^{-1})
26	Overall	0.005 ± 0.001
28	Overall	0.010 ± 0.001
30	Overall	0.018 ± 0.003
32	Overall	0.012 ± 0.003
26	1	0.005 ± 0.001
28	1	0.009 ± 0.002
30	1	0.016 ± 0.003
32	1	0.014 ± 0.003
26	2	0.006 ± 0.001
28	2	0.010 ± 0.002
30	2	0.020 ± 0.004
32	2	0.010 ± 0.004

Appendix 5.4 The relationship between the natural logarithm of wet weight (g) and the natural logarithm of the standard metabolic rate (mg O_2 h^{-1}) in *Acanthaster* cf. *solaris* (*n* = 42 CoTS, *n* = 76 observations). The data points for the 26, 28, 30 and 32 °C temperature treatments are coloured dark blue, light blue, orange and red respectively. The regression line was plotted using geom_smooth(method = lm). Scaling exponents (*b*) are also provided for each treatment.



Appendix 6.1 Other publications contributed to during candidature.

Pratchett, M. S., Caballes, C. F., Cvitanovic, C., Raymundo, M. L., Babcock, R. C., Bonin, M. C., . . .**Lang, B. J.**, . . .Wilson, S. K. (2021) Knowledge gaps in the biology, ecology, and management of the pacific crown-of-thorns sea star *Acanthaster* sp. on Australia's Great Barrier Reef. *Biological Bull*etin, 241, 330-346. https://doi.org/10.1086/717026

Pratchett, M. S., Nadler, L. E., Burn, D., **Lang, B. J.**, Messmer, V., & Caballes, C. F. (2021). Reproductive investment and fecundity of Pacific crown-of-thorns starfish (*Acanthaster* cf. *solaris*) on the Great Barrier Reef. *Marine Biology*, 168. https://doi.org/10.1007/s00227-021-03897-w

Wheeler, C. R., **Lang, B. J.**, Mandelman, J. W., & Rummer, J. L. (2022). The upper thermal limit of epaulette sharks (*Hemiscyllium ocellatum*) is conserved across three life history stages, sex and body size. *Conservation Physiology*, 10. https://doi.org/10.1093/conphys/coac074

Doll, P. C., Uthicke, S., Caballes, C. F., Diaz-Pulido, G., Wahab, M. A. A., **Lang, B. J**., . . .Pratchett, M. S. Settlement cue selectivity by larvae of the destructive crown-of-thorns starfish. *Biology Letters*, 19. https://doi.org/10.1098/rsbl.2022.0399