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The Thermal Performance and Tolerance of Tropical cleaner shrimp (Lysmata amboinensis)

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The Thermal Performance and Tolerance of Tropical cleaner shrimp (*Lysmata amboinensis*)

By

SANCHA CONWAY HOLROYD

A thesis submitted to the University of Plymouth
in partial fulfilment for the degree of

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AUTHORS DECLARATION

At no time during the registration for the degree of Research Masters (ResM) in Marine Science has the author been registered for any other University award without prior agreement of the Doctoral College Quality Sub-Committee.

Work submitted for this research degree at the University of Plymouth has not formed part of any other degree either at the University of Plymouth or at another establishment.

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Abstract

This thesis explores the theoretical and experimental applications of ecophysiological research for the management of aquatic organismal health within the ornamental trade. Ecophysiology investigates the interactions between an organism's physiology and its environment, and offers a promising approach to improving the management of health outcomes for traded species. Chapter One presents a comprehensive and critical literature review that explores the potential of ecophysiology to redefine our understanding of health, by integrating ecophysiological perspectives into practice. Using Hatch's (1962) theoretical framework as a foundation to define health, the review discusses the importance of understanding species-specific responses to key physio-chemical parameters for traded ornamental shrimp. It concludes, stating that ecophysiological knowledge can support more systematic, evidence-based approaches to health, ultimately, reducing stress and mortality in traded organisms. Chapter two investigates the thermal performance and tolerance of the tropical cleaner shrimp (*Lysmata amboinensis)* under thermal warming and cooling scenarios, providing insight into how a tropical species of shrimp responds to thermal change. Overall, this species showed a broad thermal tolerance similar to other tropical shrimp studied. Cardiorespiratory system failure coincided with the upper thermal limit (CT_{max} = 35.25°C), but it failed to explain the complete collapse of ventilation at critically low limits (CT_{min} = 7.5°C), and anaerobic metabolism was not exclusive to temperatures near CT_{max} or CT_{min}. Chapter Three is a call for collaborative research initiatives that align scientific inquiry with industry goals to produce pragmatic outcomes, based on the results of the first two chapters. It recommends the development of standardised health indicators, and species-specific guidelines to bolster the management of traded species. While this thesis contributes to a relatively understudied area of the thermal biology of tropical species, it serves as a test case for integrating scientific study with ornamental industry-driven agendas, and the development of evidence-based strategies to optimise the health of traded shrimp.

List of Content

Chapter One:

How can ecophysiology inform the health of ornamental shrimp during trade and transportation and the aims and objectives of this thesis?

1. Abstract

Ecophysiology investigates the interactions between an organism's physiology and its environment. It seeks to understand how an organism functions and adapts under changing conditions. Therefore, it presents exciting applications in the management of health and wellbeing of organisms involved in live trade industries, including the marine and tropical freshwater ornamental industries, where organisms experience a wide range of environmental conditions/multi-stressors that can threaten their health and survival. Specifically, this review explores the use of ecophysiology and its applications for managing health of a group of key ornamental shrimp, using Hatch's (1962) theoretical approach to disease and dysfunction as a tool to define and distinguish health. We review the evidence for the proposition that ecophysiology has applications to managing the health of ornamental shrimp during their trade and transportation. We do so by 1) showing how an understanding of ecophysiology can be used to inform an operational definition of health, using Hatch's (1962) scheme as originally applied to occupational health, 2) consider the notion that taking an ecophysiological approach, and applying ecophysiological understanding, to managing transport could improve the process to the benefit of the animals and those involved in the retail of this resource, 3) cover points 1-2 with particular reference to tropical ornamental shrimp, and 4) review existing ecophysiological knowledge on tropical species of shrimp to emphasise the benefits and opportunities ecophysiological principles offer in promoting organismal health. We conclude that a

greater integration of ecophysiological knowledge into the industry could support and improve the management of organismal health and reduce cases of associated mortality, overall, optimising the processes, ethics, and profit of this key sector.

1.2. Introduction

1.2.1 What is the Aquarium Trade?

The marine and tropical ornamental industries describe the international trade of tropical saltwater and freshwater aquatic species for home and public aquariums. Although exclusive industries, they are often adopted under the umbrella term of the 'aquarium trade' (Palmtag, 2017; Borgess, 2022). Small-to-medium-scale fisheries operating across the Indo-Pacific, Neotropical, Oceanic and Afrotropical regions drive the global supply of aquarium livestock (Wabnitz *et al.,* 2003; Rhyne *et al*., 2012; Palmtag, 2017; Evers *et al.,* 2019). While accurately quantifying the scale and value of this global trade is difficult, due to insufficient catch data and a lack of comprehensive tracking of export and import activities (Wabnitz *et al.,* 2003; Smith *et al.,* 2008), it is evidently of huge economic value. Estimations place the global aquarium trade value between $15-30 billion USD (Penning *et al.,* 2009) with freshwater dominating the market in both the number of species and individuals traded (Evers *et al.,* 2019). Despite the overarching freshwater dominance in numbers of individuals, marine species hold a greater individual retail value, acquiring around an additional 15% of the market value depending on the species (Dey, 2016). Of the >5300 freshwater species sold globally, more than 90% are captive bred individuals (Raghaven *et al.,* 2013). For marine species, the situation is reversed with, approximately, 90% of the market consisting of wild caught specimens (Chapman *et al.,* 1997; Dominguez and Botella, 2014). That said the landscape appears to be shifting with increasing success in marine captive breeding efforts (Olivotto *et al.,* 2011). For the marine ornamental trade, best guesses place its value in the hundreds of millions of dollars, with the number of traded fish, invertebrates and coral ranging between 46-150 million a year across 2000+ species globally (Wabnitz *et al.,* 2003; Livengood and Chapman, 2007; Palmtag, 2017; Rhyne *et al.,* 2017; Raja *et al.,* 2019). Growing demand and a shift in hobbyist interests, from fish-only tanks to emulating micro reef environments (Rhyne *et al.,* 2009) has increased the numbers of species traded.

1.2.2 Ornamental shrimp

Within the aquarium trade ornamental shrimp make up a highly popular category of traded organisms. Estimations place the number of tropical freshwater shrimp traded between 12-17 species, varying between countries, all within the generas *Atyopsis, Atya*, *Arachnochium, Caridina* and *Neocaridina* (Liptak and Vitazkova, 2015). Within the marine ornamental industry, the trade of invertebrates (shrimp, hermit crabs, large polyp stony (LPS) corals, snails and sea slugs) dominates the market, with 2.6x more invertebrates sold than fish (Watson *et al.,* 2023). In the marine market, a handful of species within the genus *Lysmata* and *Stenopus* make up the majority of shrimp traded (Beeza and Behringer, 2017; Calado *et al.,* 2017). The most iconic include *L.amboinensis* (cleaner shrimp), *L. debelius* (fire shrimp), *S. hispidus* (boxing shrimp) and *L. boggessi* (peppermint shrimp) (Baeza and Behringer, 2017; Calado *et al.,* 2017), as well as *Hymenocera picta* (Harlequin shrimp) and *Thor amboinensis* (sexy shrimp). (Calado *et al.,* 2020). Although, realised numbers of species may be significantly higher as many species are traded under the same common names, e.g. *L. bogessi* and *L. vittate* are both traded as ‘peppermint shrimp’. Whilst this is not an issue when scientific names are used, the adoption of common nomenclature can lead to misidentification, and so, misapplication of information for consumers. Furthermore, the lack of biological and ecophysiological studies addressing reproductive biology and larval development, coupled with the difficulties of achieving a successful and sustained brood stock, has hindered the progress of marine ornamental shrimp culture, in contrast to that of their freshwater counterparts (Calado, 2008), leading to a greater reliance on wild collection.

1.2.3 Transportation of Tropical Marine and Freshwater Ornamental Species-Current Practice

International transport is the engine required to support the global aquarium trade. This requires careful packaging for the live exportation and importation of species. Shipping costs can be substantial (Lim *et al.,* 2003; Paterson *et al.,* 2003), hence, it is in the best interest of importers and exporters to transport animals in ways which minimise shipping costs, while maximising survival (Lim *et al.,* 2003; Harmon, 2009). All aspects of ornamental transportation aim to provide the necessary elements for survival, while reducing the metabolic cost of the animal (Paterson *et al.,* 2003; Harmon, 2009). Standard methods for packaging ornamental aquatic species for international transport typically involve a closed system comprising two layers of polyethylene bags filled with water, inflated with air or oxygen (O_2) and sealed (Correia and Rodrigues, 2017). Marine fish and invertebrates are most commonly bagged individually (Chaun *et al.,* 2010), packaging practice for tropical freshwater species often involve high loading densities (Lim *et al*., 2003; Mandal *et al.,* 2010), although exceptions are commonly made for species considered to be aggressive or those with delicate fins (Chaun *et al.,* 2010). Organisms are then, most commonly, shipped internationally by air freight (Watson *et al*., 2010; Correia and Rodrigues, 2017).

The successful shipment of livestock relies on animals arriving in good health, with minimal mortalities. These outcomes are influenced by seemingly minor variations in shipping methods, changes in water quality, and the physiological tolerance of the animals being transported (Chow *et al.,* 1994; Paterson *et al.,* 2003; Harmon, 2009). Throughout the process of collection, acclimation and international transport animals may encounter significant stress, discomfort and abrasion, which can compromise their health and lead to mortality. The water quality condition can undergo changes during transport, exposing organisms to elevated levels of ammonia, carbon dioxide, pH and temperature instability, together with reduced dissolved O_2 (DO) (Berka, 1986; Holmes *et al.,* 1999; Golombieski *et al.,* 2003; Harrison, 2016). The duration of transport governs the factors that impose the greatest mortality risk. For fish, pH reduction is the primary concern during short journeys (≤8 hours), whereas ammonia excretion becomes the predominant risk factor during long journeys (>8 hours) (Sampaio and Freire, 2016: Cerreta and Harrison, 2020). Similar definitive information on the key environmental factors governing survival during transport have yet to be determined for species of ornamental invertebrates. Nonetheless, minimising mortality associated with the trade and transportation of ornamental species is a major challenge in the industry, including invertebrates, a challenge compounded by the accessibility of resources and capital restrictions of exporters (Harmon, 2009). Mortality rates vary widely, ranging from minimal levels (<5%) to as high as 80% in a single shipment, albeit such high levels are very rarely seen (Johannes and Riepen, 1995; Sadovy and Vincent, 2002). Nevertheless, there is little knowledge on the precise changes in water quality during commercial transport that drive poor health and mortality, and limited understanding of the cumulative effects of repeated transport, or exposure to water of varying quality, on the subsequent health and survival of many ornamental species (Vanderzwalmen *et al.,* 2021). Many of the practices employed in the shipping of marine ornamentals have arisen organically, with various mitigation practices employed to mange different physio-chemical challenges aquatic ornamentals face. Here we consider each of the key physico-chemical parameters that may vary during transport and describe the different mitigation strategies employed and discuss their likely benefit or impact.

1.2.3.1 Temperature

Drawing upon empirical practice, it has long been believed that different ornamental species have a preferred temperature range of 24-27°C for tropical marine species (Hunziker, 2005; Kioke, 2013; Correia and Rodrigues, 2017; Velasco-Blanco et al., 2019) and 22-27°C for tropical freshwater species (Ramshorst, 1980). Therefore, it is widely considered that a constant, ‘optimal’, temperature should be maintained during transport; however, this is not entirely feasible during the international transportation of livestock. Instead, it is common to find temperatures gradually decline over the period of transportation (Mandal *et al.,* 2010; Vanderzwalmen *et al.,* 2021). In particular, during air freight temperature profiles can significantly change (Lorenzon *et al.,* 2007; Watson *et al.,* 2010). This is most pronounced when tropical species are transported to temperate climates (Ramachandran, 2002). In most cases, rapid changes in temperature are ameliorated through the adequate insulation of the container box with material such as plastic foam or polystyrene, together with reflective material (Ramachandran, 2002). Thermal heat packs may also be placed within the container, and individuals may be acclimated to lower temperatures prior to shipment (Correia and Rodrigues, 2017). High temperatures, exceeding an organisms tolerance threshold, can also be fatal to organisms, so avoiding water temperatures that rise above initial shipment temperatures is advised

(Ramachandran, 2002; Mandal *et al.,* 2010). It is widely advised that any changes to temperature are kept to a minimal (<5°C) (Mandal *et al.,* 2010), however, there is a dearth of literature providing precise descriptions of safe maximal and minimal values, or safe rates of change. On arrival at a more permanent holding facility, any proposed effects of exposure to changing temperatures are usually remedied through gradually raising or lowering of temperatures back to normal maintenance temperatures (Ramachandran, 2002). Exactly how optimal these current practices are, however, has not received any systematic appraisal.

1.2.3.2 Dissolved O_2

Most sellers would agree that ensuring sufficient O_2 availability during transport is essential to the survival of any animal (Mandal *et al.,* 2010). While well-maintained aquariums seldom experience hypoxic challenges, O_2 decline remains a significant risk during the transportation of livestock (Lim *et al*., 2003; Mandal *et al.,* 2010; Sampaio *et al.,* 2019; Vanderzwalmen *et al.,* 2021). Rates of O_2 consumption during transport are typically elevated, due to increased stress (Mandal *et al.,* 2010; Zarantoniello *et al.,* 2021; Fang *et al.,* 2023), leading to more rapid rates of decline in dissolved O_2 levels. To compensate for reductions in dissolved O_2 during transport, either air or pure O_2 is introduced into the bags before sealing (Lim *et al.,* 2003; Correia and Rodrigues, 2017), with water to oxygen volume ratios usually kept around 20-35% water to 65-80% oxygen, depending on the size of the organism and bag volume (Cole *et al.,* 1999). Organism can also be starved for 12-24 hours prior to shipment to reduce metabolic processes, and thus oxygen depletion (Mandal *et al.,* 2010). Yet, the efficacy of these methods in preserving the health and survival of different ornamental species during transport remains uncertain.

1.2.3.3. Carbonate Chemistry (Carbon Dioxide (CO_2) and pH)

It is known that carbon dioxide (CO_2) accumulates in closed bags during transport, particularly when stocking density is high (Mandal *et al.,* 2010). Increasing CO_2 (hypercapnia) results in chemical changes in the carbonate chemistry of seawater, resulting in amongst other things changes in bicarbonate, carbonate and reduced pH, all of which affect the health and survival of organisms during transport (Melzner *et al.,* 2009; Thomas *et al.,* 2022). CO_2 itself eventually becomes toxic and decreases the ability of organisms to extract O_2 from the water (Ramachandran, 2002). When organisms are transported in absence of continuous aeration, the risk from hypercapnia increases (Ramachandran, 2002). Concentrations in excess of 40mg.L^{-1l} can be fatal (Mandal *et al.,* 2010), however little data exists quantifying this for different species. On arrival, indications of CO_2 toxicity, including a loss of righting, can be addressed through aeration of the water (Ramachandran, 2002), although may not always result in the successful recovery of individuals. Therefore, further investigations are required to elucidate the possible impacts of altered carbonate chemistry, and how it affects health, as well as effective mitigation of adverse conditions, during transport,

1.2.3.4. Salinity

Many in the trade think and argue that, maintaining an ionic balance (osmoregulation) between the extracellular fluids (hemolymph or blood) of an organism and its surrounding environment is required for good health (Mantel and Farmer, 1983; Freire *et al.,* 2008; Rahi *et al.,* 2020). In tropical marine ornamental aquariums, salinity is recommended to be kept between S= 34-35 (Spotte, 1993; Skomal, 2006; Carter, 2023), whereas trace amounts (S=0-0.1) are advised for freshwater species (Ramshorst, 1980; Gay, 2005). When managed correctly, salinity should not be an issue in well maintained aquariums (Hunziker, 2005; Kioke, 2013). However, in saltwater aquariums, the reality of maintaining a constant salinity can be a challenge, due to the constant evaporation of water, necessitating freshwater top-ups (Spotte, 1993; Skomal, 2006; Carter, 2023) and resulting in minor fluctuations in salinity. In many cases, efforts are made to ensure the salinity of the transportation medium is kept identical to the medium in which they are maintained in (Mandal *et al.,* 2010). Whether this state is well maintained during transportation is largely unknown, as there is little quantitative evidence documenting how salinity changes over time during the international exportation and importation of livestock.

1.2.3.5 Ammonia and Nitrogenous Waste

Nitrogenous waste is produced *via* protein catabolism, the decomposition of excreted faecal waste, and unconsumed feed (Randall and Tsui, 2002; Chang *et al.,* 2015). It is mainly in the form of dissolved inorganic nitrogen ions ammonium (NH_4^+), nitrite ($N0_2^-$) and nitrate ($N0_3^-$) (Romano and Zeng, 2013). During transport metabolic waste can accumulate rapidly, especially with high loading densities (Lim *et al.,* 2003). Safe levels of ammonia are considered to be 0ppm, as when toxic unionised ammonia surpasses tolerable levels (<0.25) mortality is known to occur (Lin *et al.,* 2003; Cobo *et al.*, 2014), nonetheless this ascertain seems based on practice and not empirical work. Elevated ammonia during transportation is cited as the key driver of fish mortality during long journeys (>8 hours) (Sampaio and Freire, 2016), with mortality commonly reported when elevated levels reach 1ppm (Mandal *et al.,* 2010). Conversely, toxic concentrations of ammonia remain undetermined for most species of ornamental shrimp. Changes in oxygen concentrations, temperatures and pH affects ammonia toxicity. Higher pH, reduced temperature and lower dissolved O_2 levels all result in greater ionisation, and thus, increasing toxicity of ammonia (Ramachandran, 2002; Mandal *et al.,* 2010). For example, a shift from pH 7-8 produces a ten-fold increase in the quantity of unionised ammonia (Ramachandran, 2002). To mitigate any deterioration in water quality, resulting from an accumulation of metabolic waste, various techniques are regularly employed in the industry including the starvation of organisms prior to packaging (Phillips and Brockway, 1954; Nemato, 1957), lowering temperatures of transport water (Phillips and Brockway, 1954; Nemato, 1957; Teo and Chen, 1993), buffers (McFarland and Norris 1958; Teo *et al.* 1989), anaesthetics (Teo *et al.,* 1989), and for freshwater organisms, even the addition of drugs in the transport water (Ling *et al.,* 2000). What remains unclear is the suitability of each of these techniques in mitigating toxicity effects on ornamental shrimp, from the build-up of ammonia during transportation.

1.2.4 Current Practice- Where do we go from here?

Overall, the success of ornamental trades lies within the execution of effective practices to ensure sufficient oxygenation, reduce the build-up and impact of metabolic waste products, and, generally, avoid the development of unhealthy environments during the transportation of tropical marine and freshwater species that would impact the visual appeal (attractiveness) and underlying health of an organism. This practice has evolved organically, over many years, but has infrequently been informed by scientific research. The extent to which current practice, and thereby the health of the transported livestock may be improved by integrating scientific, specifically ecophysiological knowledge of the responses of aquatic animals, is as yet unknown and untried. Considering the immense popularity of ornamental shrimp, coupled with the fact that ecophysiological approaches are not yet widely integrated in the management of this group during their trade, the shrimp stand out as important and exciting group in which to trail the application of an ecophysiological approach with. Therefore, the aim of this paper is to propose ways in which an ecophysiological approach can benefit the ornamental aquarium trade in terms of improving health during the trade and transportation of livestock. We will do this by 1) demonstrating how an understanding of ecophysiology can be used to inform an operational definition of health, using Hatch's (1962) scheme as originally applied to occupational health, 2) using tropical ornamental shrimp, given their importance and popularity in the marine ornamental trade, and 3) review existing ecophysiological knowledge on tropical species of shrimp to emphasise the benefits and opportunities of such integration in promoting organismal health during their trade.

1.3. Health

1.3.1 What is Health? Physiological Approaches to Health

Although 'health' is a commonly used term, few explicit and consistent definitions exists for what it constitutes, particularly with respect to animal management. Traditional definitions of health are based on the absence of disease or injury, implying that it is a passive state. More contemporary perspectives view health as complete wellbeing (WHO, 1948) and current consensus acknowledges that health extends beyond the absence of disease and cannot be viewed as a dichotomous state (Arah, 2009; Nordenflelt, 2011; Ayres, 2020). Instead, the notion of health is characterised by an individual's ability to capitalise on its full functional ability, meet its needs and adapt or cope in changing environments (Eriksson and Lindström, 2008; Wittrock *et al.,* 2019).

Within the domain of veterinary science, health historically focused on disease-centric approaches, pathophysiology and biological functioning (Nordenfelt, 2011; Wittrock*, et al.,* 2019). As our approaches to health have expanded beyond these confines of contemporary veterinary science has adopted a more holistic perspective, embracing a broader array of factors to assess the health and condition of an animal including 1) normal appearance and behaviour (normality); 2) biological function; 3) physical and psychological well-being; 4) productivity (reproduction); and finally, 5) physiological homeostasis, such as heart rate (Gunnarsson, 2006). Central to this definition is the recognition that physiological homeostasis is a cornerstone of health. Physiological homeostasis, defined as an organism's ability to maintain internal stability while adjusting to changing external conditions (Cannon, 1929), reflects a state of dynamic equilibrium that allows a degree of tolerance to changing environmental conditions (Billman, 2013; Billman, 2020; Libretti and Puckett, 2023). Homeostatic control mechanisms facilitate physiological adjustments to maintain internal equilibrium, operating at various hierarchical levels within the body including molecular, cellular, tissue, organ and physiological (Ayres, 2020). On a cellular level, homeostasis can be observed through biochemical reactions. Each cell benefits from homeostatic control, providing continuous automaticity to the body and supporting functioning (Libretti and Puckett, 2023). The regulation of temperature, O_2, pH, ion and blood glucose concentrations is crucial for enzymes to function optimally, and the generation of metabolic waste must be kept to a minimal to not disrupt the internal environment of the cells. Cells remain alive as long as the internal environment is favourable (Baptista, 2006). The health of an organism is said to be the end product of homeostatic regulation, a culmination of dynamic regulatory processes (Billman, 2020). Thus, a true assessment of health is not possible without appreciation of this concept.

A healthy organism, as described by Hatch (1962) (Figure 1.1) demonstrates the capacity for physiological compensation in response to environmental stimuli. This idea of maintaining physiological homeostasis (vigor) or returning to a ‘normal’ state, where the plasticity of an organism’s physiology enables it to adapt to changes in its environment, is the approach to health we promote within this paper. Defined as such, the notion of health is represented as a continuum, with physiological homeostasis serving as the centre upon which the spectrum of health pivots. We suggest that the ability of an organism to continually adaptively respond to stress through physiological adjustment, minimising the cost of such adjustment and so,

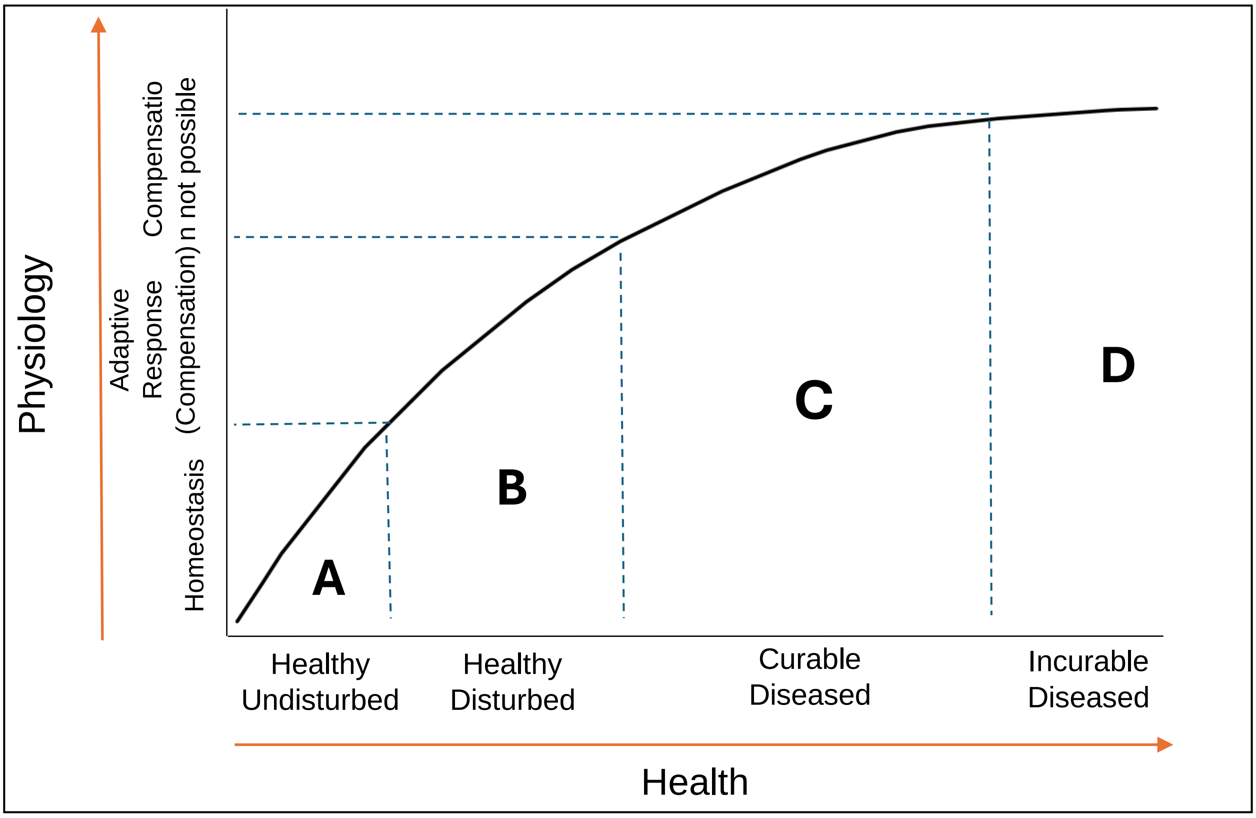

Figure 1.1 The dynamic relationship between health and physiology. Shows an organism’s capacity for physiological compensation in response to environmental stimuli (disturbance) and state of health. Adapted from Hatch (1962).

minimising adverse or chronic implications on future responses, underscores the essence of health.

Despite the importance of integrating physiology in assessment of animal health, challenges persist in making the subject operational. Variability in responses of individuals' (Cockrem, 2022), and ethical considerations of research brings challenges in growing our understanding of animal physiology (Ayres, 2020; Kiani *et al.,* 2022), and in providing accurate guidance to industry. Currently, the literature falls short when it comes to exploring the relationship between physiological homeostasis and environmental stressors. Instead, many studies infer potential implications on health or, more commonly, the eventuality of death when exposure to the stimulus is chronic, repetitive or severe. Despite efforts to advocate for the use of integrative approaches to assess animal health, disease-centric approaches persist as the predominant means of evaluating animal health, (Lerner and Berg, 2017; Ayres, 2020; Stentiford *et al.,* 2020; Piccard, 2022), a trend that is more apparent for aquatic organisms. Moreover, there is no established framework guiding the wider adoption of physiological approaches to health assessments, particularly for the assessment of aquatic livestock. This highlights the need for producing standardised protocols and best practices that incorporates physiological perspectives to form comprehensive health assessments.

1.3.2 Current Assessments of Health in the Aquatic Ornamental Industry

Within the ornamental industry, many sellers diligently strive to acquire and uphold livestock in optimal health. As a result, the regular assessment of livestock health becomes an integral part of daily routine. Health assessments typically involve visual morphological and behavioural evaluations of livestock (Stevens *et al.,* 2017).

Any deviations from established ‘homeostasis’, such as changes to feeding patterns (lethargy), increased ventilation, ‘flashing’ against objects, or to colouration (hyper pigmentation) are interpreted as indicators of underlying stress. Subsequently, appropriate measures are often taken to identify and address the cause, to improve the organism’s health (Hargreaves, 2002; Pasnik *et al.,* 2010). For instance, for the arrival of new livestock, quarantining often helps to prevent the proliferation of pathogenic organisms (Monticini, 2010). For invertebrates, coral, sea anemones, and live rock, a 30-60 day quarantine is recommended to sufficiently interrupt the life cycle of parasites and prevent the spread of pathogens (Wildgoose, 2001). But in practice, quarantining periods vary due to the adoption of divergent monitoring methods by exporters, importers and retail establishments. Morphological and behaviour assessments, for the experienced aquarist, allow for swift diagnosis and treatment. However, these measures utilised within industry can be challenging to interpret and are subject to the interpretation of the observer. Many behaviours remain poorly characterised in scientific literature, and current knowledge is subjectively biased towards vertebrates, given their more distinct physical and behavioural characteristics (Stevens *et al.,* 2017).

For ornamental shrimp, few established health determinants are applied in practice or described in literature. Those that do, appear to adopt a disease and pathogenic centric approach. For example, Maciaszek *et al.,* (2018) sheds light on the criteria used to assess the health and condition of tropical freshwater shrimp in international breeding competitions, focussing on the genera of *Caridina* and *Neocaridina*. Within such breeding competitions, adopted techniques aim to determine the presence of epibionts and genetic defects. Similar disease and pathogenic centric approaches are also, predominately, adopted for the consignment of live aquatic animal including ornamental shrimp. Importations of organisms from non-EU countries are subjected to the requirement of a health certificate under the conditions that animals have 1) been inspected within 72 hours of loading and demonstrated no clinical signs of disease; 2) are not subject to any prohibitions due to unresolved increased mortality; and 3) are not intended for destruction or slaughter for the eradication of diseases (Centre for Science Fisheries & Aquaculture Science, 2023).

Despite the recognition that health constitutes more than the absence of disease (Eriksson and Lindström, 2008; Arah, 2009; Nordenflelt, 2011; Eriksson and Lindström, 2008; Wittrock *et al.,* 2019), industry and regulatory mandates still predominantly rely on disease-centric methodologies. This reliance overlooks the opportunity to stablish health determinants and practical guidelines rooted in broader ecophysiological frameworks. Consequently, there is a missed opportunity to comprehensively understand physiological drivers of health, and to optimise the health and wellbeing of traded ornamentals. Further research, development, and application of relevant ecophysiological knowledge could provide great benefits to the management of organismal health within tropical marine and freshwater industries. Through the diversification of health assessments, digressing from disease and pathogenic-centric approaches, more systematic and comprehensive assessments of health can be undertaken. Therefore, we now turn to what an ecophysiological approach to the health and management of traded ornamentals could look like?

1.4. An Ecophysiological Approach to Ornamental Management

1.4.1 What is Ecophysiology?

Ecophysiology originated from a shared desire to observe and understand how animals work in the wild (Bartholemew, 1987; McNab, 2002). Work by early researchers such as Albert Bennett, George Bartholemew, Laurence Irving, Charlotte Mangum, Per Fredrik Scholander, and Knut Schmidt-Nielsen, considered an organism's physiology and evolutionary history firmly in the context of its natural history and environmental context, with the aim of ultimately understanding how an organism functions and adapts (Bennett, 1987; McNab, 2002). Whilst there is still much discussion around the term, here we define ecophysiology as the study of species' physiological responses to abiotic stimuli and its influence on function, survival, and wider community structuring and processes (Lambers *et al.,* 2008).

To tackle the questions of 'how does this organism work?' and 'how is its physiology modulated by the environment?' multidisciplinary approaches are required, with an interaction of field, laboratory, and natural experiments (Spicer, 2014). Such fundamental questions lay a pathway through which a synthesis between subfields of physiology occurs. Ecophysiological techniques lie in that of comparative animal physiology (CAP), defined as the comparative study of animal physiology to catalogue diversity, reconstruct phylogenetic relations and to determine the role of physiology on organismal and environmental interactions (Withers, 1992; Garland, 2019; Basile *et al.,* 2020). Elements of evolutionary physiology may also be incorporated when cross generational examinations of functional characteristics, influenced by the environment, are undertaken (Garland, 2019). Whilst traditional definitions were built on the absence of molecular biological approaches (Speakman, 2002), the integration of molecular techniques, such as omics approaches, into physiological fields has provided new strategies under which to examine physiological processes from the viewpoint of fundamental biomolecules (Norwood and Gomez, 1994) and mechanistic integration at the whole organism level (Portner *et al.,* 2006; Kuster, *et al.,* 2011). Subsequently, blurring the lines between subfields of research and, nonetheless, the numerous disciplines which ecophysiology draws upon. Having defined and explored what we mean by ‘ecophysiology’, we now try to visualise what this approach might look like when applied to the management of livestock in the ornamental trade.

1.4.2. Ecophysiological Techniques

Techniques used within ecophysiology to determine an organism’s sensitivity to changing environmental conditions often attempt to mimic natural environmental variation or predicted environmental changes over time within the laboratory, incorporating multiple environmental factors, to heighten ecological validity (Schmuckler, 2001; Andrade, 2018; Orr *et al*., 2020). To determine an organism's response to environmental variation, ideally, fitness is measured directly. However, due to the difficult nature of assessing fitness through cross-generational approaches, correlated indirect measures of performance are often used (Miller and Stillman, 2012). For shrimp, this includes the use of heart rate, ventilation, and haemolymph and tissue metabolite profiles as proxies of fitness (Taylor and Spicer, 1987; 1989; 2009; Frederich and Pörtner, 2000; Ern *et al.,* 2014). When physiological mechanisms are measured along an environmental gradient, a ‘physiological performance curve’ is produced, that graphically illustrates the changes in physiological rate(s) in response to an environmental gradient or condition and scopes for areas of unstable metabolic responses (Figure 1.2) (Molnar *et al.,* 2017; Kellermann *et al.*, 2019; Little and Seebacher, 2021). Through examining the shifts in physiological rate(s), these curves offer valuable insights into how an organism may navigate and compensate for changes in their environment, by adjusting its underlying physiology (Little and Seebacher, 2021). Thus, can be a useful tool for discerning the tolerances and sensitivities of specific physiological mechanisms through identifying thresholds where the response becomes compromised or unstable (Killen *et al.,* 2021). However, interpreting physiological proxies for fitness in a species is complicated. How an organism responds to their environment is a complex interaction between autecology, physiology, behaviour and stimuli (Crespi *et al.,* 2012; Johnstone *et al.,* 2015) and metrics alone may not always prove meaningful, particularly when in the absence of broader biological context for interpretation (Peig and Green, 2010). Furthermore, consensus on the measurement of tolerance traits such as critical thermal minima (CT_{min}) and maxima (CT_{max}) remains limited, as determined endpoints traits are largely governed by the physiological mechanism utilised, such as heart rate or scathognathite beats, and the perceived measurement of 'end', such as death or loss of ventilation (Lutterschmidt and Hutchison, 1997; Ørsted *et al.,* 2022).

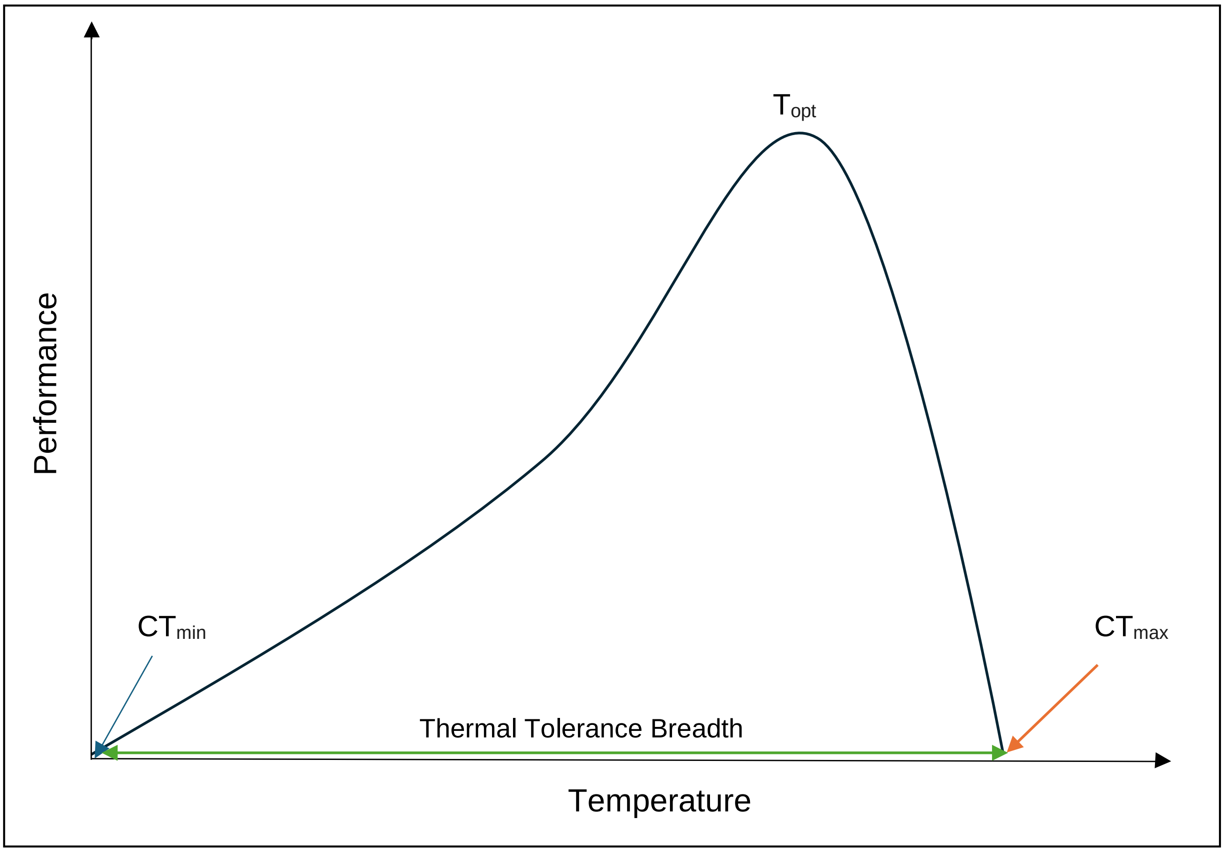

Figure 1.2: General standard thermal performance curve (TPC). Relationship between environmental temperature and a physiological rate of an ectotherm expressed as a thermal performance curve. TPC’s are typically characterised by an initial slow increase in performance with increasing temperatures, reaching maximum rate at the optimal temperature (T_{opt}), followed by a rapid decrease until the point of complete cessation (CT_{max}). CT_{max} and CT_{min} represent the T_A above and below which performance is at a minimum, and T_{opt} represents the T_A at which performance is maximum.

Interestingly, the integration of ecophysiological approaches in the conservation and management of species is notable across various sectors, where it has demonstrated efficacy in addressing key conservation challenges (Stevenson *et al.,* 2005). In the context of climate change, such techniques have been used predict a species' physiological response to current or future environmental perturbation, tolerance to change and likelihood of mortality (Pörtner and Farrell, 2008; Bozinovic and Pörtner, 2015; Penn and Deutsch, 2022). Within sectors of aquaculture for sustainable food production, the application of ecophysiology has supported the optimisation of systems and environment to yield the maximum biomass output (Parker, 2011). Within the aquarium trade, as for all live market industries, the health and management of aquatic livestock is fundamental to success, therefore, efforts are continuously made to minimise poor health and mortality (Johnstone *et al.,* 2015). Yet to date, practice within the aquarium trade has infrequently been informed by scientific research. The physio-chemical determinants underlying poor health and mortality of many species within the aquarium trade remains inadequately understood. The primary advantage of this integration lies in the emergence of a systematic and evidence-based approach to husbandry management strategies, allowing for tailored strategies that optimise health and mitigate risks. Developing a greater understanding of the physiological responses of these organisms to key physico-chemical parameters, will help to identify physiological stress indicators enabling earlier detection for timely intervention and treatment, identify tolerances and sensitivities to optimise husbandry and shipping conditions, and more easily distinguish characteristics displayed by healthy and unhealthy individuals. For example, our understanding of the physiological responses and tolerances of species of ornamental shrimp to hyperoxic, hypoxic and decreasing levels of O_2 is limited. Therefore, optimal conditions for transport remain ambiguous, and the implications of exposure to such conditions during transport on health and survival of shrimp are unclear. One potential solution to this challenge is to identify rates of O_2 consumption (MO_2) and hypoxia tolerance, using indices such as critical O_2 partial pressure (P_{crit}) for a given species. This knowledge can then be applied in practice to optimise dissolved O_2 levels in bags prior to transportation in ways which meet the requirement of the organism, and avoids exposure to suboptimal conditions, while optimising cost-efficiency. Additionally, many mitigation techniques employed by industry to avoid exposure to unsuitable physio-chemical parameters during transport including the use of buffers, heat packs, thermal acclimation, and starvation have no empirical basis on which to assess the significance of these actions on the health and survival of many species, further emphasising the need for scientific contributions.

Having explored what this approach may look like, it now becomes imperative to review how our current ecophysiological understanding, or perhaps lack thereof, could inform the practical management of ornamental livestock. For this, we utilise ornamental shrimp as a model species under which we assess our current ecophysiological knowledge against key physico-chemical parameters (Section 2.3), identify gaps in understanding, and discuss its applications to industry. This is crucial to emphasis the benefits and opportunities of integrating ecophysiological approaches to inform practice, ultimately, to foster optimal health during their trade.

1.5. Physiological Responses of Marine and Freshwater Ornamental Shrimp to Environmental 'Stress'

Many organisms can physiologically adjust to predictable or regular environmental changes, where a state of homeostasis can be maintained or returned to, and no chronic effect on physiology, function or fitness is sustained (Wingfield, 2013). The physiological and behavioural changes that occur in response to an environmental stimulus can be construed as the stress response and are mediated by different levels of endocrine, nervous and immune system mechanisms (Chu *et al.,* 2022). Baseline levels of these signalling and effector molecules maintain homeostatic energy balance and regulate aspects of 'normal' physiological state (McEwen and

Wingfield, 2003; Schultner *et al.,* 2013). In response to a stressful stimulus, it is argued that an adaptive response can initially occur. Albeit not characterised in invertebrates, when fish are confronted with a stress stimulus, HPA activation can causes a sharp increase in the glucocorticoid stress hormones, beyond baseline levels, which mobilises energy reserves and 'switch' the organism into a state in which its physiology and behaviour are geared to maximising its chances of acute survival (Romero, 2004; Wingfield, 2008). However, during chronic, repetitive or severe exposure to environmental stress a gradual deterioration in health can occur as the organism attempts to compensate through adaptation of its physiology (Rapport, 1989; Li *et al.,* 2018). The response can become maladaptive and chronically detrimental to both its physiology and, overall health (Ketchesin *et al.,* 2018).

Addressing shrimp physiology specifically, a general consensus exists amongst biologists that environmental stress can exert multifaceted influences on an organism's physiological state (Chu *et al.,* 2022). Longstanding empirical evidence has consistently demonstrated the effects of environmental stress on physiological function including heart rate, gill ventilation, haemolymph and metabolites used as proxies for fitness (Herreid, 1980; Taylor and Spicer (1987,1989,2009; Ern *et al.,* 2014; Rosa *et al.,* 2014) which change as the organism attempts to compensate and adapt (Rapport, 1989; Li *et al.,* 2018). Overall, ecophysiological approaches have successfully characterised the physiological responses of shrimp to a range of ecologically relevant environmental parameters, but information is less clear for species of ornamental shrimp. Although few studies explicitly align their aims to relevance in the ornamental industry, much of the literature is haphazardly relevant, as, arguably, some similarities exist between environmental stimulus tested upon in labs or experienced in natural marine habitats to those encountered during the trade of ornamental species. Those conditions brought into discussion within this review hold both ecological and industry relevance, though do not encompass the full breadth of environmental factors organisms can experience. Crucially, to discern the independent effects of an environmental factor on physiology, each has been addressed in isolation. In natural settings, however, organisms are likely subjected to a range of multi-stressors, rather than experiencing them in isolation (Crain *et al.,* 2008; Jackson *et al.,* 2016). While understanding the role of multi-stressor interactions is crucial for accurately predicting organismal responses to environmental changes (Orr *et al.,* 2020), many of the interactions are not fully understood for species of shrimp. Therefore, whilst we acknowledge the importance of multi-stressor studies in developing a comprehensive understanding of the effect of stressors on organismal physiology, it is not within the scope of this review to address this literature. First, we begin by conducting a comprehensive review of the effects of key physico-chemical parameters factors on shrimp physiology in isolation to consolidate existing knowledge and identify gaps in our current understanding. In absence of specific research on ornamental shrimp, we broaden our scope by examining a diverse range of shrimp species. In doing so, we aim to demonstrate how the development of similar knowledge could be applied to the management of ornamental shrimp.

1.5.1. Temperature

Temperature stands as a fundamental environmental variable, yielding substantial influence over aquatic ecosystems. Its role in shaping the physiology, ecology and evolution of ectotherms is widely recognised (Nguyen *et al.,* 2011). Many ectothermic organisms, including some species of shrimp, have a constrained capacity to regulate their body temperature autonomously, necessitating a reliance on ambient conditions for thermal regulation (Angilletta, 2009). Consequently, the role of temperature in shaping the survival of shrimp is heavily debated (Somero, 2002; Hoffmann and Todgman, 2010; Whiteley and Mackenzie, 2016).

Temperature can influence the rate at which biochemical reactions occur and the structural and functional properties of macromolecules (Hochacka and Somero, 2002). The temperature coefficient (Q_{10}) provides a valuable way of measuring the extent of an increase in rates of biological processes with a rise in temperature, every 10 °C, quantified through the van't Hoff Equation (Ito *et al.,* 2015; Mundim *et al.,* 2020) and is often used to predict how an organism's total metabolic rate will change with temperature (Halsey *et al.,* 2015). Physiological functions, such as ventilation rate, are composed of intricate interactions involving various physical and biochemical processes, each with slightly different thermal sensitivities and less predictable Q_{10} values (Whiteley and Mackenzie, 2016). Therefore, to mitigate inconsistencies arising from temperature-dependant changes in Q_{10}, changes in physiological rate(s) can be graphed against a thermodynamic temperature gradient, producing a physiological performance curve (Schulte *et al.,* 2011; Molnar *et al.,* 2017; Kellermann *et al.*, 2019; Little and Seebacher, 2021). The Arrhenius equation can then be applied to determine the activation energy of the reaction and identification of breakpoints (Ito *et al.,* 2015; Whiteley and Mackenzie, 2016; Crapse *et al.,* 2021). Through examining the shifts in physiological rate(s), these curves offer valuable insight into how an organism may compensate for changes in their thermal environment, by adjusting underlying physiology (Little and Seebacher, 2021). Identification of discontinuities in the pattern, through the application of models including the Arrhenius break point and critical thermal maximum, minimum and optima (CT_{max} , CT_{min}, T_{opt}), can offer valuable insight into the critical thermal limits and tolerances of organisms (Killen *et al.,* 2021) for set physiological mechanisms, shedding light on their capacity to cope with fluctuating environmental conditions.

For various species of shrimp, such as cleaner shrimp (*Lysmata amboinensis*), peppermint shrimp (*Lysmata seticaudata*), white shrimp (Litopenaeus vannamei), and the common UK species of *Palaemon serratus* and *Palaemon elegans*, studies have examined several physiological mechanisms including scaphognathite beats (ventilation) (Nakanishi and Nishiyama, 1932; Mika, 2013), heart rate (Chung *et al.,* 2012), cessation of pleopod movement (Korhonen and Lagerspetz, 1996; Verberk *et al*., 2018) and cessation of the sensory antennules (Rosa *et al.,* 2014). For example, heart rate and ventilation rate have been shown to vary significantly with changes in temperature. Typically, having an optimum temperature where a peak rate in performance in reached, and declining thereafter (Whitely and Fraser, 2009; Schulte *et al.,* 2011). How an organism's physiology responds to a change in temperature, is driven by several factors including the period of exposure (acute vs chronic), the degree and rate of temperature change, and thermal histories (Sinclair *et al.,* 2006; Peck *et al.,* 2014). All of which gives rise to a broad array of thermal sensitivities and tolerances between populations situated across environmental gradients, as well as those with limited connectivity (Osovitz and Hofmann, 2007; Whiteley and Mackenzie, 2016). Acute exposure to elevated temperatures can trigger rapid but reversible physiological adjustments, dependent on degree of thermal change, where organisms may adjust their underlying physiology in response to acute thermal stress. In contrast, chronic exposure to elevated temperatures can induce more profound and sustained physiological changes leading to acclimation or even adaptation in response (Sinclair *et al.,* 2006; Peck *et al.,* 2014). The degree of thermal change, largely, dictates the severity of physiological disruption. Chronic exposure to temperatures that match or exceed optimal temperatures can decrease phenotypic plasticity, interfering with reproduction and growth (Portner 2002; 2010).

Questions then arise concerning the role of cardiorespiratory performance driving thermal tolerance of ectotherms, particularly where insufficient O_2 supply for basal maintenance drives CT_{max} and CT_{min}, as explained by the Oxygen and Capacity Limited Thermal Tolerance (OCLTT) hypothesis (Frederich and Pörtner, 2000; Pörtner, 2002; 2010), or the alternative, where tissue O_2 supply does not drive the collapse of vital functions (Pörtner, 2010; Ern *et al.,* 2016; Pörtner *et al.,* 2017). This debate is fuelled by observed difference in physiological responses of species towards thermal extremes (Ern *et al.,* 2020). Physiological and biochemical responses to temperature are thought to drive thermal tolerances in temperate shrimp (Rosa *et al.,* 2014; Wang *et al.,* 2019; Ren *et al.,* 2021; Hussain *et al.,* 2023). Albeit studies on the relations between temperature and physiology in species of tropical shrimp are few and far between. An organism's capacity to maintain aerobic scope and supply O_2 to tissues, under the OCLTT hypothesis, is limited by the functional capacity of the ventilatory and circulatory systems (Pörtner, 2002; Hochachka and Somero, 2002; Pörtner, 2010). This hypothesis predicts that as optimal temperatures are reached, aerobic scope is maximal, and with continued warming heart rate and ventilation rate eventually become unable to match the increasing O_2 demand. This temperature marks the reduction in O_2 supply to the tissues, indicating early limitations to thermal tolerance. As temperatures continues to rise above optimum (T_{opt}) organisms experience a loss in aerobic scope, as ventilatory and circulatory systems begin to fail (Frederich and Portner, 2000). Once critical thermal limits are reached, the decrease in systemic O_2 levels (hypoxaemia) causes a discrepancy between the supply and demand of energy associated with elevated temperatures and performance rapidly declines until point of death (Pörtner, 2001; 2002; Melzner *et al.,* 2006). In the absence of a sufficient supply of O_2, organisms may recruit alternative means of acutely supplying energy to tissues through anaerobic respiration, resulting in an increase in the production of anaerobic metabolites such as glucose and lactate, and a decrease in ATP (*Müller, et al., 2012*) however, this response does not postpone succumbing to death indefinitely (Pörtner, 2001; 2002; 2010). Alternative theories suggest insufficient O_2 delivery in relation to metabolic demand may not be the key driver in jeopardising performance at thermal limits, but one of several contributing factors, for example exposure to thermal extremes may cause the loss of structural integrity of metabolic proteins, leading to their denaturation and loss of functionality, prior to the onset of tissue hypoxia (Clark *et al.,* 2013; Ern *et al.,* 2015; Schulte, 2015).

The efficacy of the OCLTT hypothesis in predicting cardiorespiratory performance that drives thermal tolerances of shrimp is much debated, and evidence of its applicability to tropical marine and freshwater ornamental shrimp species is sparse and equivocal. Within literature some species of marine and freshwater shrimp exposed to increasing temperatures, such as giant freshwater shrimp (*Macrobrachium rosenbergii*), have shown evidence of an ability of maintaining cardiorespiratory performance towards thermal extremes with the recruitment of anaerobic respiration upon reaching their CT_{max} values (Ern *et al.,* 2012; Ern *et al.,* 2014). Of the little research conducted on species ornamental of shrimp including that of tropical marine cleaner shrimp (*L. amboinensis*) and peppermint shrimp *(L. seticaudata*), respective CTmax values were calculated as 35.0 °C (*L. seticaudata*) and 35.4 °C (*L. amboinensis*) for a warming scenario that started at 24 °C, and ramped upwards at a rate of 2°C. $hour^{-1}$. The cessation of sensory antennules movement was used to determine endpoint. Experimental groups acclimated to different temperatures (L. *seticaudata*: 18, 21, 24°C and *L. amboinensis:* 24, 27, 30°C) showed HSP70/HSC70 levels in muscle tissue increased with warming for both species (*L. seticaudata*: 266–294 ng mg^{-1}, *L. amboinensis*: 134–161 ng mg^{-1} total protein), respectively ($P > 0.05$) where upregulation of chaperone proteins occurred to compensate the changes in temperature. There was an increase in L-lactate concentrations within the muscle tissue of both species (*L. seticaudata*: 6.2–7.23 μmol g^{-1} dw, *L. amboinensis*: 3.4–4.4 μmol g^{-1} dw). Malondialdehyde (MDA) levels in *L. amboinensis* also increased dramatically, indicating extreme cellular lipid peroxidation occurred. Currently, there is insufficient research to either support or refute the applicability of the predictions made by the OCLTT hypothesis in setting upper thermal tolerances and critical limits of tropical shrimp. Even fewer studies have investigated the OCLTT hypothesis at low temperatures, providing little support for the universal role of O_2 in setting the lower thermal limits of aquatic invertebrates (Verberck *et al.,* 2016). This knowledge gap is even more pronounced for tropical marine and freshwater species of ornamental shrimp. To date, no studies have examined the physiological responses of these species to decreasing temperatures. Consequently, our understanding of their lower thermal tolerance, critical limits, and complete thermal tolerance ranges is severely limited. To conclude, it remains uncertain whether the OCLTT hypothesis is a unifying model for species of tropical marine and freshwater shrimp.

Investigations into the physiological responses of tropical shrimp to rising temperatures has only just begun, with a considerable gap in our understanding of what shapes tolerances and sensitivity to both increasing and declining temperatures. Increasing our understanding of how species of tropical shrimp respond to changes in temperature is not only essential for scientific inquiry but also holds practical relevance to live trade industries including the ornamental trade. As such, ecophysiological research addressing responses to temperature should incorporate ‘real world’ scenarios to ensure data is relevant and applicable to industry. For example, great benefit could be brought about by identifying species variable ‘safety margin’ between ambient temperature extremes and lethal temperatures, to inform the control of conditions during transportation, and depict whether declines in temperature during international transport by air freight contribute to reported cases of poor health and mortality of traded shrimp. Ultimately, the growth of ecophysiological knowledge holds the potential to underpin evidence-based decision-making by industry stakeholders, supporting efforts to further enhance husbandry practice, and the overall sustainability of the trade.

Overall, a change in temperature can drive a shift in physiological rate(s) as the organism attempts to compensates for changes in its thermal environment. How physiology responds to the change is driven by a complex amalgamation of physical properties of the change e.g. period of exposure, and intensity of temperature change, as well as the thermal history of the organism, all of which contribute to the thermal tolerance of an organism. However, the precise underlying mechanisms governing thermal tolerance is debated, and while the OCLTT hypothesis provides a conceptual framework for comprehending thermal limits, it remains unclear whether the thermal tolerance of tropical marine and freshwater species of shrimp is driven by insufficient O_2 supply for basal maintenance or the alternative, where tissue O_2 supply does not drive the collapse of vital functions. As research continues to progress, it is crucial for researchers to strive for practical applicability within the ornamental industry. By pinpointing the tolerances and sensitivities of various species of tropical marine and freshwater shrimp to temperature change, we can begin to optimise conditions during the trade and transportation of livestock. Overall, supporting the enhancement of health, and reducing the risk of mortality.

1.5.2 Dissolved O_2

Put simply, hypoxia is a shortage of O_2, anoxia is the complete absence of O_2, and hyperoxia is when O_2 is more abundant than naturally found in the atmosphere. When air or O_2 is introduced into transportation bags (Lim *et al.,* 2003; Correia and Rodrigues, 2017), individuals experience acute heightened levels of O_2. During transportation, O_2 levels within the sealed bag(s) declines, and in severe cases, organisms are exposed to hypoxic conditions. Exposure to declining levels of oxygen, particularly hypoxia, is considered a significant threat to survival (Lim *et al*., 2003; Mandal *et al.,* 2010; Sampaio *et al.,* 2019; Vanderzwalmen *et al.,* 2021). To assess the validity of this assumption, we will focus our review on the current literature on the physiological impacts of hypoxia on shrimp.

While hypoxic events naturally occur in a range of aquatic systems, anthropogenic disturbance, climate change and eutrophication have led to an increase in the severity and frequency of hypoxic and anoxic events, posing considerable ecological threat (Vaquer-Sunyer and Duarte, 2008; Diaz and Breitburg, 2009; Spicer, 2016). The adaptive adjustment of physiology in response to hypoxia determines tolerance and survival in aquatic organisms (Frederich and Portner, 2000; Portner, 2001; Verbeck *et al.,* 2016). Many species of shrimp, have demonstrated the ability to regulate their oxygen consumption, modulating their rates of O_2 uptake to sustain aerobic metabolism in low O_2 environments at least during acute exposure. However, an absence of knowledge concerning the regulatory capacity and tolerance of tropical marine and freshwater ornamental shrimp to hypoxia prevails.

Aquatic hypoxia is typically defined as dissolved O_2 concentrations below 2-3 mg O_2 L^{-1} in marine and estuarine environments and below 5-6 mg $O_2.L^{-1}$ in freshwater environments (Diaz and Rosenberg, 1995; Vaquer-Sunyer and Duarte, 2008). Albeit such singular classifications, while useful for the ecological monitoring of environments, omit marked differences in hypoxia tolerance between species (Seibel, 2011; Spicer, 2016), as many marine and freshwater organisms possess empirical sublethal and lethal O_2 thresholds above the described values of environmental hypoxia in their respective environments (Vaquer-Sunyer and Duarte, 2008).

Adaptations of organisms to hypoxia and low dissolved O_2 are largely driven by strong selective pressures to maintain aerobic metabolism (Seibel, 2011). The regulation of O_2 consumption (MO_2) can be dependant or independent of the environmental partial pressure of O_2 ($P_{I}O_2$), and this dependency varies across species (McMahon and Wilkens, 1983; Taylor and Spicer, 2009; Spicer, 2016). Most organisms facing low or decreasing O_2 concentration respond by either decreasing their rates of O_2 consumption (oxyconformity) or maintain a constant O_2 uptake, irrespective of ambient oxygen levels (oxyregulation) (Tremblay *et al.,* 2020). Perfect examples of oxyconformity and oxyregulation can seldom be found (Wood, 2018), as species' metabolic responses to declining O_2 levels often lay somewhere between the two (Mueller and Symour, 2011). Many species of shrimp have demonstrated at least some ability to oxyregulate, and can maintain aerobic metabolism in hypoxic conditions, to a point. When individuals are unable to maintain their normoxic metabolic rate, some resort to the utilisation of anaerobic metabolism, the point at which anaerobic respiration is recruited assumed to coincide with the point at which metabolic energy requires supersede aerobic uptake capacity (Tremblay *et al.,* 2020). When dissolved O_2 reaches the point in which oxyregulation can no longer be maintained, termed the critical O_2 tension (or P_c), the rate of O_2 uptake declines, conforming with decreasing environmental P_IO_2. For instance, the common UK shrimp, *Palemon serratus* and *Palemon elegans*, can oxyregulate until the P_c is reached (Taylor and Spicer, 1987; 1989). Hypoxia tolerance is phylogenetically rooted within an enhanced O_2 uptake capacity (Mandic *et al.,* 2008). Essentially, a species tolerance to acute and chronic hypoxia is contingent upon its capacity to obtain adequate O_2 to fulfil metabolic demands (Frederich and Portner, 2000; Portner, 2001; Verbeck *et al.,* 2016). Organisms that possess a greater capacity for O_2 extraction can maintain lower basal metabolic rates, thus, thus, are more capable of sustaining themselves in low O_2 environments (Mandic *et al.,* 2009).

As far as we are aware no investigations into the responses of ornamental shrimp to hypoxia have been completed, however a substantial body of research across various species of shrimp reveals similarities in underlying morphological features such as the presence of scaphognathites and pleopods, structures both used for ventilation of the gill chamber (McMahon and Wilkens, 1983). This shared morphological foundation suggests common physiological function and can be used to inform predictions of the responses of marine and freshwater ornamental shrimp to low O_2 environments, whilst avoiding overreaching generalisations. When confronted with hypoxic conditions, shrimp employ a variety of physiological mechanisms to cope with the reduction in O_2 availability. This can include increasing the frequency of movements of ventilation appendages (scaphognathites) to increase the rate of O_2 uptake (Abercrombie, 1941; Mika, 2014), modulation of the circulatory system, changes to the O_2 carrying capacity of respiratory pigments, reducing metabolic O_2 requirements through decreasing energy expenditure below the standard metabolic rate, and the utilisation of anaerobic metabolism (Gannon, 1999; Spicer *et al.,* 2014; Spicer, 2016; Cobbs and Alexander, 2018). Typically, evaluations of hypoxia differentiate between acute (short-term) or chronic (long-term) exposures, where differences in physiological responses can be observed depending on the period and intensity of exposure (Burnett and Stickle, 2001). In a broad context, immediate responses to hypoxia frequently entail heightened ventilation and heart rate (Imray *et al.,* 2011), with an overall change in locomotion (Domenici *et al.,* 2007), growth (Wei *et al.,* 2008; Han *et al.,* 2017), feeding (Peruzza *et al.,* 2018) and reproductive rates (Peterson *et al.,* 2008) observed with continuous exposure. For example, a study by Re and Diaz (2011) revealed that maintaining the tropical shrimp *Litopenaeus stylirostris* at hypoxic levels (2 mg L-1) increased the rate of O_2 consumption, faecal production, and declined growth rate and scope for growth, although the species was able to survive such hypoxic levels it suggest prolonged exposure should be avoided for optimal growth in aquaculture.

More recently documented, are the molecular mechanisms underlying responses to hypoxia in particular the hypoxia-inducible factor 1 (HIF-1) signalling pathway, the adenosine 5′-monophosphate-activated protein kinase (AMPK) signalling pathway, and apoptosis. Recent studies have revealed that HIF-1, the main transcription factor that regulates gene responses to hypoxia in vertebrates (Semenza, 2012), also regulates the synthesis of several genes controlling glycolysis, gluconeogenesis, antioxidant proteins including metallothionein (MT) and apoptosis in two species of shrimp, the white legged shrimp (*Litopenaeus vannamei*) (Cota-Ruiz *et al.*, 2016;

Camacho-Jimenez *et al.,* 2019; Duarte-Gutierrez *et al.,* 2021) and a mantis shrimp (*Oratosquilla oratoria*) (Kodama *et al.,* 2012). Such studies have confirmed a difference in HIF-1a transcript levels post hypoxic exposure, with the relative expression varying across different tissues. Kodama *et al.,* (2012) revealed a marked upregulation of HIF-1a in the cardiovascular system of *O. oratoria*, reflecting a HIF-induced enhancement of cardiovascular system function to achieve efficient O_2 transport under chronic hypoxia. A recent study by Huang *et al.,* (2023) also demonstrated the pivotal role HIF-1a plays in safeguarding haemocytes from cellular death (apoptosis), through the upregulation of heat shock protein HSP70, contributing to *L. vannamei* enduring hypoxia (air exposure) during transport.

Dissolved O_2 stands as a primary limiting factor in aquatic environments (Mandal *et al.,* 2010). Ensuring sufficient O_2 supply during transportation is essential for the success of shipments and the survival of organisms (Sampaio and Freire, 2016). While well-maintained aquariums seldom face hypoxic challenges, O_2 decline remains a significant risk during the transportation of livestock (Lim *et al*., 2003; Mandal *et al.,* 2010; Sampaio *et al.,* 2019; Vanderzwalmen *et al.,* 2021). In ornamental fish, several factors influence respiration rates during transport, including environmental stressors such as temperature, salinity, ammonia, and pH, alongside mechanical stress, body weight and feeding level (Mandal *et al.,* 2010; Zarantoniello *et al.,* 2021; Fang *et al.,* 2023). Consequently, O_2 consumption rates during transport are typically elevated (Mandal *et al.,* 2010). To compensate for declines in dissolved O_2 during international transport, pure O_2 is often introduced into transport bags (Lim *et al.,* 2003; Correia and Rodrigues, 2017), and organism may be starved for 12-24 hours to reduce metabolic processes prior to shipment (Mandal *et al.,* 2010). However, the efficacy of these methods in preserving the health and survival of shrimp during transport remains uncertain. These challenges are exacerbated by the lack of species-specific ecophysiological knowledge concerning ornamental shrimp, including O_2 consumption rates and tolerances to low or declining oxygen levels. Acquiring such knowledge could inform and optimise industry practices, thereby addressing transportation challenges more effectively. For instance, by identifying rates of MO_2 and hypoxia tolerance through indices such as P_c, we can better avoid suboptimal conditions and tailor O_2 levels more precisely in transport bags before shipment. This approach minimises the excessive use of O_2, enhancing both the cost-efficiency of businesses as well as the health of traded organisms. As the frequency and severity of hypoxic events in natural ecosystems increases, it also becomes imperative to understand the potential risk posed to wild livestock. In the absence of critical physiological information on the effects of hypoxia on ornamental shrimp species, we remain unable to fully grasp the risks they face. Therefore, advancing out understanding of ornamental shrimp ecophysiology, in the context of hypoxia, is essential to effectively manage and conserve traded populations.

In summary, hypoxia poses a growing threat to aquatic ecosystems. To comprehensively assess its impact on wild aquatic populations, scientific research must address species-specific responses and tolerances to fluctuations in dissolved O_2 levels. The regulation of O_2 consumption varies across species. Many species of shrimp a demonstrate the ability to regulate O_2 consumption, maintaining aerobic metabolism in hypoxic conditions up to critical limits. Hypoxia tolerance hinges on an enhanced O_2 uptake capacity, allowing certain species to survive in O_2-depleted environments. When faced with low O_2 levels, shrimp deploy various adaptive strategies to ensure O_2 uptake meets metabolic demands. These strategies include increasing ventilation rates, adjusting circulatory system processes, and resorting to anaerobic metabolism for survival.

As our understanding of the physiological responses and underlying mechanisms governing hypoxia tolerance in shrimp continues to advance, the necessity for dedicated research targeting species of ornamental shrimp becomes increasingly imperative. While hypoxia is rarely a problem in well maintained aquariums, declines in O_2 continue to be seen as a risk to livestock during transportation. However, the role of declining O_2 and hypoxia in driving mortality of ornamental shrimp remains ambigious, as to date, scientific knowledge concerning the responses of ornamental shrimp to hypoxia, species-specific tolerances, rates of O_2 consumption and underlying mechanisms driving tolerance is limited. Addressing this knowledge gap is crucial to support the enhancement of industry practices related to the transportation of ornamental shrimp, and, thereby, the health and survival of organisms during transit.

1.5.3 Carbonate Chemistry (Carbon Dioxide (CO_2) and pH)

Carbon dioxide gas (CO_2) does not only dissolve in water but chemically reacts with elements already present, and so increased CO_2 plays a significant role in shaping carbonate water chemistry. Since industrialisation, increasing levels of atmospheric pCO_2, have caused a gradual lowering of pH in aquatic water bodies (Caldeira and Wickett, 2003; Orr *et al.,* 2005). This phenomenon, termed acidification, poses significant challenges to marine and freshwater ecosystems alike (Orr *et al.,* 2005; Whiteley, 2011). Changes in pCO_2, and the subsequent declines in pH are known to affect the physiology, health and survival of many marine (Melzner *et al.,* 2009) and freshwater organisms (Thomas *et al.,* 2022). Among this, the susceptibility of shrimp remains unclear.

Alterations in physiology act as vital indicators to determine the tolerance and sensitivity of organisms to acidification (Prakash *et al.,* 2022). For example, metabolic rate may increase (Wang *et al.,* 2018) or decrease (Rosa and Seibel, 2008) from elevations of CO_2 and/or subsequent, declines in pH in aquatic environments. Ecophysiological approaches have tended to study the effects of acidification on physiology through measuring changes in growth, survival and reproduction (Rosa and Seibel, 2008; Rosa *et al*., 2014; Khan *et al.,* 2021; Prakash *et al.,* 2022), with experiments split across the measurement of responses to acute and chronic periods of exposure. Among this, acidification has shown to affect the regulation of acid-base balance, metabolism, calcification of exoskeleton, growth, immunity and survival of a variety of aquatic organisms (Hofmann *et al.,* 2010; Kroeker *et al.,* 2010; Whiteley, 2011; Mollica *et al.,* 2018; Melzner *et al.,* 2020) such as copepods (Weydmann *et al.,* 2012), lobsters (Keppel *et al.,* 2012), crabs (Quijada-Rodriguez *et al.,* 2021) and shrimp (deVries *et al.,* 2016). A growing body of literature addressing the effects of acidification on crustacean physiology has demonstrated overwhelming evidence that many marine and freshwater organisms are negatively affected by acidification (Kroeker *et al.,* 2013). Although, our understanding is, comparatively, less comprehensive for freshwater organisms.

In marine environments, the average pH of surface ocean water has decreased by 0.1 pH units (8.2 to 8.1) (Caldeira and Wickett, 2003; Orr *et al.,* 2005), and continuing with current emission rates, further declines of 0.3-0.5 pH units are expected by 2100 (Beaufort, *et al.,* 2011; Turley and Gattuso, 2012). As a consequence of declining pH, the relative abundance of dissolved inorganic carbon

(DIC) shifts, causing an increase in aqueous CO_2 and carbonic acid (H_2CO_3) concentrations. Carbonic acid rapidly dissociates into bicarbonate ions (HCO_3^-) and protons (H+), leading to a decrease in pH, and a concurrent decrease in carbonate ions (CO_3^{2}). Overall, this reduces the calcium carbonate ($CaCO_3$) saturation state and carbonate saturation depth ($\Omega < 1$) (Orr *et al.,* 2005). Substantive evidence has shown that with increasing anthropogenic CO_2, the rate of dissolution of marine carbonates can increase, as the seawater attempts to buffer changes to pH, leading to neutralisation of CO_2 and a slight increase in total alkalinity (Feely *et al.,* 2004). This can increase the dissolution of the calcified exoskeleton of marine species, and reduce calcification rate (Haese *et al.,* 2014), altering the critical structuring, growth and survival of marine calcifying organisms (Feely et al., 2004; Andersson *et al.,* 2008; Kroeker *et al.,* 2010; Roleda *et al.,* 2012). However, the dissolution of calcified exoskeletons and a reduction in calcification rate has not been, conclusively, demonstrated, for many groups of crustacea, including shrimp (Taylor *et al.,* 2015). As where the calcification process of an organism is not dependent on environmental CO_3 saturation, but on bicarbonate ions (HCO_3^-) and metabolic CO_2 or where calcite, a less soluble form of $CaCO_3$, or chitin is used for exoskeleton formation, the exoskeleton should, theoretically, provide more resilience to decreasing levels of carbonate saturation, and thus, tolerate a larger variation of carbonate chemistry (Boßelmann *et al*., 2007; Roleda *et al*., 2012). Acid-base regulation is also thought to support the buffering of disruptions to internal pH, thereby reducing the susceptibility of the calcification process in crustacea, to changes in water chemistry in relation to pCO_2 (Wheatly and Henry, 1992).

At present, our understanding of the physiological responses to declines in pH in shrimp is limited to a handful of species, and the mechanisms driving such tolerance are a subject of debate. Several studies have emerged both in support and against the role of acidification driving adverse physiological, morphological and behavioural shifts in various species of shrimp (Bhadury, 2015). Where the evidence supports the notion, consistent patterns in responses have been observed across an array of species of shrimp. For example, a study conducted by Taylor *et al.,* (2015) on the effects of $p\text{CO}_2$-induced pH reduction on the exoskeleton structure and biophotonic properties of the red rock shrimp (*Lysmata californica*) [3]found that a reduction in pH had no effect on exoskeletal growth (percentage increase in carapace length) or mean cuticle thickness, but an increase in the calcium weight percent of the cuticle which resulted in a greater Ca:Mg ratio, and a five-fold decrease in animal transparency. Despite no effect on exoskeleton growth, the results show increased calcification during acute exposure to a reduction pH, evidencing that a reduction in pH can affect exoskeleteon mineralisation and biophotonics of a temperate-tropical shrimp.

Interestingly, similar dynamics have been found amongst the substantive proportion of research conducted on white legged shrimp (*Litopenaeus vannamei*). Several studies have demonstrated the occurrence of hyper-calcification, and elevated particulate inorganic carbon (PIC): particulate organic carbon (POC) ratios in exoskeletons exposed to low pH (Weerathunga *et al.,* 2023). When exposure to acidification (7.6 to 7.0 pH units) was chronic, broader adverse implications on feeding, moulting, growth, and overall survival were found (Muralisankar *et al.,* 2021). The work of Muralisankar *et al.,* (2021) revealed that *L. vannamei* experienced a decrease in survival, growth rate, and protein efficiency, alongside

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[3] *Lysmata californica* although is rarely available to purchase under its own species name, it is one of the many species commonly traded under the common name peppermint shrimp. It is often mistaken for the true peppermint shrimp, *Lysmata wurdemmani*.

increased muscle antioxidants, lipid peroxidation and metabolic enzymes in the muscles. These findings support the suggestion that seawater acidification in disrupting the physiological regulation of shrimp. Elevated antioxidants, lipid peroxidations, and metabolic enzymes suggest exposure of shrimp to oxidative, free radical, and metabolic stress, henceforth, conclude that seawater acidification can produce harmful effect on *L.vannamei*. Notably, the significant decrease in chitin content raises concerns about the effects of seawater acidification on chitin synthesis, a crucial process for exoskeleton formation, and structural integrity in shrimp (Gao *et al.,* 2015). These finding are consistent with the previous work of Mustafa *et al.* (2015), who also reported a reduced shell chitin content in *L. vannamei* exposed to acidified seawater. The occurrence of hyper-calcification in shrimp is believed to be arise from physiological changes that occur during exposure to low pH including improved acid-base regulation, production and maintenance of high extracellular CO_2 level, and the recycling of bicarbonate and calcium ions from previously shed exoskeletons (Weerathunga *et al.,* 2023). This is supported by the findings of several studies including Liu *et al.,* (2020) which have demonstrated the ability of shrimp to maintain high extracellular pH (>8.2) at sites of calcification, under conditions of low seawater pH (7.85 to 7.31), as well as for a variety of other marine crustaceans (Spicer *et al.,* 2007; Small *et al.,* 2010). Interestingly, consistent physiological changes to decreasing pH are not observed across all species of shrimp. In fact, in species such as the intertidal mantis shrimp (*Neogonodactylus bredini*), no changes in growth, molting, exoskeleteon morphology, calcium content, mechanical properties of shell, or enzymatic and protein indicators of oxidative stress are observed in response to a chronic seawater acidification and thermal stress (deVries *et al.,* 2016). It is believed that variations in the responses of different species of shrimp, to declines in pH, could be attributed to their natural environment. For example, organisms inhabiting environments with broader pH ranges, as found in intertidal zones, may exhibit lower susceptibility to atmospheric $p\text{CO}_2$ induced acidification, due to physiological adaptation to a wider scale of pH (Sunday *et al.,* 2011; Engstrom-Ost *et al.,* 2014).

Surprisingly, given the commercial value of tropical marine ornamental shrimp, and the susceptibility of their natural habitat to rising acidification (Mollica *et al.,* 2018), little is known about species-specific responses and tolerances of this group to ocean acidification. Within the highly limited pool of literature, the physiological effects of ocean acidification have been addressed within glass shrimp (*Ancylocaris brevicarpalis*). Prakash *et al.,* (2022) reported significant increases in concentrations of lactate, succinate ($p<0.05$), ASP and APX antioxidant metabolite activity, and in glutathione S-transferase (GSH) enzymes ($p<0.05$), suggesting oxidative stress, as redox alteration of antioxidant metabolites and enzymes occurred during exposure to reduced pH (ambient: 8.1 ± 0.05; experimental 7.7 ± 0.02). The results demonstrated that the physiology of glass shrimp (*A. brevicarpalis*) will be adversely affected by predicted declines in pH, posing threats to their long-term survival across tropical reef environments. While this study represents just a singular piece of evidence, these findings emphasise the need to address our current lack of understanding regarding the potential risks posed by ocean acidification to commercially valuable species. Understanding the physiological impacts of acidification on marine ornamental shrimp is essential to ensuring the long-term sustainability of the marine ornamental trade.

It is generally acknowledged that freshwater systems also experience acidification, however, less is known about the impact of freshwater acidification on aquatic organisms (Thomas *et al.,* 2022) due to the highly variable biogeochemistry between freshwater systems that has created challenges in modelling future freshwater scenarios (Hasler *et al.,* 2016; Weiss *et al.,* 2018). Sources of $p\mathrm{CO_2}$ in freshwater systems range from decomposing organic and inorganic matter, producing a net excess of carbon dioxide (Phillips *et al.,* 2015). Natural sources of elevated $p\mathrm{CO_2}$ in combination with increasing anthropogenic $p\mathrm{CO_2}$ contributions are predicted to create substantial shifts in the pH of large freshwater bodies (0.3-0.5 pH units), at rates equal to or greater than ocean acidification (Weiss *et al.,* 2018) emphasising the need to understand the biological implications for freshwater species. To date, very few studies have investigated the various physiological effects of acidification in freshwater species of shrimp. Among those that have, broadly, similar adverse physiological effects to those found in marine shrimp are demonstrated. For example, a study by Kawamura *et al.,* (2015) found that low pH (ambient: 7.5; experimental: 4, 5 and 6 pH) negatively affected survival, growth, size distribution and carapace quality of the giant freshwater prawns (*Macrobrachium rosenbergii*), which is synonymous with the findings of the various marine studies discussed earlier. Regrettably, no literature addressing the effects of freshwater acidification on species of tropical freshwater ornamental shrimp species could be found. Given that freshwater environments are anticipated to undergo significant acidification, at least equivalent to that of marine environments, it is clear research needs to address whether species of tropical freshwater shrimp are at risk.

Developing a comprehensive understanding of the physiological impacts, tolerances, and sensitivities of shrimp species holds key importance for both commercial interests and broader conservation efforts. In absence of such understanding, the potential ramifications of climate changed-induced pH shifts on the health and survival of these species remains unclear. Moreover, within live trade industries, such as the marine ornamental trade, information concerning the sensitivity of ornamental shrimp to declines in pH is invaluable to support commercial operations and transportation logistics. For example, during international transportation ornamental shrimp are placed into bags, respire and $p\text{CO}_2$ in the water increases (Manan *et al.,* 2019), resulting in a potential pH shift. However, we hold little knowledge on respiration rates and ventilation capabilities of many species of ornamental shrimp, thus, it remains unclear whether changes in $p\text{CO}_2$, due to respiration, have a substantive or negligible effect on pH in water in bagged conditions. Therefore, the effects of such changes on the health and survival of shrimp remain ambiguous.

Overall, the pervasive influence of $p\text{CO}_2$ on aquatic ecosystems poses profound challenges to marine and freshwater aquatic ecosystems. While calcifying organisms are, generally, considered highly vulnerable, mounting evidence suggests acidification also adversely affects numerous species of crustacean. Studies investigating the physiological effects of acidification on shrimp have revealed significant changes in exoskeletal calcification and mineralisation, metabolic processes, growth, and survival, among other factors. Nevertheless, no consensus on the susceptibility of different crustaceans yet exist, and notable gaps in our understanding exist regarding the impacts of both saltwater and freshwater acidification on tropical shrimp species, highlighting the need for expanded research efforts.

1.5.4 Salinity

Salinity is one of the most fundamental abiotic factors affecting growth, metabolism, immunity and survival of aquatic species (Rahi *et al.,* 2021). A variety of units are used to measure salinity (S) including psu (one practical salinity unit), ppt (parts per thousand), ppm (parts per million) across industry and science (Hutter *et al.,* 2014). Within this chapter we have adopted the approach of referring to the relevant unit reported within the literature. Fluctuations in salinity of aquatic bodies arise due to variations in the input of freshwater, from sources such as precipitation, river run off, and ice melting (Koblinsky *et al.,* 2003), as well as the loss of water through evaporation from rising temperatures (Seggel *et al.,* 2016). In freshwater environments, many natural ecosystems are facing increased salinisation through anthropogenic salt inputs (Valesco *et al*., 2018; Cañedo-Argüelles *et al.,* 2019). To address salinity fluctuations in water, organisms must adapt their physiological mechanisms accordingly (Moshtaghi *et al.,* 2016). For many species of crustacean, maintaining ionic balance (osmoregulation) between the hemolymph and the surrounding medium is the primary challenge for survival (Mantel and Farmer, 1983; Freire *et al.,* 2008; Rahi *et al.,* 2020). When salinity deviates beyond the physiological tolerance of an organism, it can result in mortality (Jahan *et al.,* 2019). To date, research conducted on different species of shrimp has shown that many are osmoregulators (Charmantier, 1998; Walker *et al.,* 2009). However, our understanding is subjectively biased towards food-based aquacultured species, and little is known on the species-specific osmoregulatory ability and tolerance of tropical marine and freshwater ornamental shrimp to salinity.

Salinity fluctuations, can trigger an organism’s adaptive response, affecting different physiological functions and rates of performance, and ultimately, growth and survival (Young *et al.,* 1989). Omsoregulation is a key physiological function, in which aquatic organisms adopt different osmoregulatory adaptive strategies to maintain the osmolarity of their extracellular fluids according to their surroundings (Pequeux *et al.,* 2006). Osmoregulatory patterns range from osmoconformation to hyper-hypo osmoregulation (Charmantier *et al.,* 2009, Péqueux *et al.,* 2006). Typically, during post-embryonic development crustacean exhibit three ontogenic osmoregulatory patters including osmoregulation that varies little with developmental stage, or examples where adults are osmoconformers, the adult model of osmoregulation being established in the first post-embryonic stage, and metamorphosis marking the appearance of the adult type of osmoregulation strategy (Charmantier, 1998). By the point of recruitment, individuals usually have developed their physiological capabilities to overcome the fluctuating nature of their environment (McKenney and Celestial, 1995). The quantification of haemolymph osmotic pressure (hOP) is used to understand osmoregulation patterns and capacities of crustaceans acclimated to different salinities. Measuring the difference between haemolymph and external medium osmolality (OC) it allows researchers to determine the effect of salinity on the physiology of an individual and compare osmoregulatory abilities across developmental stages (Lignot *et al.,* 2000). Osmoregulation is energetically expensive, but necessary in many species of shrimp (Chong-Robles *et al*., 2014). Osmoregulatory patterns, the OC, and eurohalinity strategies has only been studied in a handful of shrimp, but where this has been undertaken much attention has been paid to commercially important aquacultured shrimp, including the Panaeids, such as the giant tiger prawn (*P. monodon*) (Ferraris *et al.,* 1986) and Litopenaeids such as white legged shrimp (*L. vannamei*) (Chong-Robles *et al*., 2014). Panaeids and Litopenaeids genera are, generally, considered efficient osmoregulators (Charmantier, 1998; Walker *et al.,* 2009), with numerous studies have shown the capacity of many species to tolerate wide changes to environmental salinity (Kutty *et al.,* 1971; Stern *et al.,* 1990; Rosas *et al.,* 1997). For example, *L. vannamei* can hyper-hypo osmoregulate (Chong-Robles *et al*., 2014), tolerating a wide range of salinities, from slightly brackish (S=1-2ppt) up to hypersaline (S=40ppt) (Stern *et al.,* 1990). Salinity tolerance is thought to increase with development in *L. vannamei*, through the gradual acquisition of specialised epithelium and organs involved in osmoregulation (Charmantier *et al.,* 1988; Chong-Robles *et al*., 2014). Many species of freshwater shrimp are also efficient osmoregulators, such as the bristled river shrimp (*Macrobrachium olfersii*) which is a hyperosmotic osmoregulator (Santos and McNamara, 1996), and Amazon river shrimp (*M. amazonicum*) (Boudour-Boucheker *et al.,* 2013).

Salt movement in an organism occurs across permeable surfaces such as excretory organs and gills, which are considered the primary site for ionic and osmotic regulation in crustacean (Charmantier, 1998). Osmoregulators typically have considerable differences in ionic and osmolality concentrations of their hemolympth compared to surrounding medium. This state of equilibrium is maintained by buffering mechanisms of ion uptake, reabsorption, and excretion of urine which can be adjusted to maintain equilibrium when saline conditions in the surrounding water changes (Santos and McNamara, 1996). However, the specific nature of the physiological processes driving tolerance remains poorly defined for many species. In particular, very little is known about osmoregulation capabilities, and tolerances in many commercially important species of tropical marine and freshwater ornamental shrimp. Questions also arise around the duration over which physiological adjustments can be maintained, particularly when chronic shifts in environmental salinity occurs, and whether sustaining these adjustments becomes detrimental to long-term performance.

Any change in environmental salinity imposes osmotic stress, in which the physiological response to the stress depends on the intensity of salinity change and period of exposure (Freire *et al*., 2018; Rahi *et al.,* 2021). Fundamental changes in osmoregulatory function can occur, in shrimp, as a result of both an increase or decrease in salinity, these include changes in the ionic content (uptake or release of ions) of body fluid (change in hemolympth osmolality), mRNA production (Rahi *et al.,* 2017; 2018; Moshtaghi *et al.,* 2018), gill structure (Fregoso-López *et al.,* 2016), respiration, contraction or extension of gill lamellae, and an increase in regulatory cell volume (RVI) or decrease (RVD) (Rahi *et al.,* 2017; 2018; 2021; Moshtaghi *et al.,* 2016). In high salinity (hypotonic) environments organism face the loss of water through osmosis, so to maintain osmotic pressure an increase in haemolympth osmolality occurs, while the activity and mRNA expression of Na+/K+-ATPase in the gills decreases (Wang *et al.,* 2022). Osmoregulatory ability in shrimp has been shown to greatly decline in increasing salinity, as individuals' approach osmoconformation (Huong *et al.,* 2010; Wang *et al.,* 2023). For instance, studies in *M. rosenbergii* revealed osmolality can be maintained around 420 mOsmolkg-1 at S < 18‰ by gradually increasing the salinities, but that the osmotic capacity becomes severely challenged as salinity increases (Huong *et al.,* 2010). In low salinity (hypoosmotic) environments, shrimp face the opposite challenge. To regulate water influx, physiology often responds by decreasing osmolality and upregulating metabolic ions (Gao *et al.,* 2016; Cuenca *et al.,* 2021). A current bias exists towards reporting the effects of high salinity, due to its climate-related relevance (Maulu *et al.,* 2021). However, research addressing the effects of low salinity on shrimp physiology has been undertaken by Gao *et al.,* (2016) who revealed that in *L. vannamei*, mRNA levels of Na^+-K^+-ATPase α-subunit and carbonic anhydrase (CA), within the gills, increased significantly ($p<0.05$) in shrimp reared in low salinity environments (experimental: S= 2psu and 10psu), compared to the experimental groups with relatively higher salinities (experimental: S= 20 and 30 psu). Conversely, compared to animals maintained at a S= 30, chymotrypsin mRNA expression was decreased by 15% and 36%, in conspecifics maintained at S= 10 and 2 respectively, whilst trypsin mRNA expression decreased significantly by 20%, 72% and 45%, at S= 2, 10 and 20 respectively. Moreover, higher ($p<0.05$) growth performance (final weight, weight gain and specific growth ratio) and survival rates were found at S= 20 and 30, compared to shrimp cultivated at 10 and 2. This indicates that exposure to low salinity can induce an osmotic stress response in *L. vannamei*, as reflected by the up-regulation of Na^+-K^+-ATPase α-subunit, CA, trypsin and chymotrypsin mRNA. The up regulation of metabolic ions and mRNA indicates an adaptive response to this increased demand for ion transport and osmotic adjustment in response to decreased salinity. The study also evidenced that chronic exposure to low salinity can affect both growth performance and survival, which is suggested to be related to immunodepression through changes in digestive enzyme activity. In freshwater species, McNamara *et al.,* (1986) demonstrated that acute exposure to different salinities (0, 7, 14, 21, 28 and 35%) can affect the respiratory metabolism of bristled river shrimp (*M. olfersii*) across different developmental stages (zoeae, postlarvae and adults) finding that in adults rates of oxygen consumption decrease with salinity increase. Metabolic rates were also found to vary across developmental stage, being salinity independent from 14-28% in zoeas 1-4 but increasing with rising salinity in zoeae 5 and 8, and with postlarvae showing peak metabolic rates in moderate salinities. The differential responses observed across different stages of *M. olfersii* supports the notion that the sensitivity to salinity can change throughout a shrimp's life cycle, underscoring the importance of considering ontogenetic differences when assessing the physiological responses of shrimp to environmental salinity stress.

Biochemical alterations observed in shrimp exposed to a change in salinity have also included shifts in the concentrations of free fatty acids (FFA) and free amino acids (FAA) in the haemolympth and gill (Huong *et al.,* 2001), the number of haemocytes within the haemolympth (Tantulo and Fotedar, 2017), and increased glucose and serotonin levels in the haemolympth (Rahi *et al.,* 2021). Further indicating metabolic adjustment occurs in response to changes in salinity. For instance, Rahi *et al.,* (2021) demonstrated that varied salinity treatments (experimental: S= 0, 2.5, 5, 10, 20 and 30‰) significantly ($p<0.05$) affected a broad suite of physiological parameters in giant tiger prawn (*P. monodon*) including growth, survival performance, O_2 consumption and haemolympth osmolality, biochemical markers such as FFA and FAA levels, glucose, serotonin and haemocystes, and gene expression patterns. Specifically, reductions in salinity led to elevated FFA levels but reduced FAA levels, while lower salinity treatments (S= 0-10‰) decreased haemolympth osmolality, which is in contrast to an initial increase observed in osmolality, glucose, and serotonin levels, as well as O_2 consumption at S= 30‰ followed by stabilisation. This data aligns with previous studies that have indicated shrimp regulate ion balance through gill mechanisms, where increased FFA levels in the gills minimise ion loss by forming an impermeable membrane (Huong *et al.,* 2001; McNamara *et al.,* 2015). Overall, supporting the notion that alterations in salinity can induce an osmotic compensatory physiological stress response in shrimp that aids their ability to withstand environmental change. However, if environmental stress exceeds an organism’s tolerance, free radical production and removal within the body becomes imbalanced, triggering tissue cell apoptosis (Wang *et al.,* 2023). This can occur during exposure to both low and high salinity, and can lead to organismal death (Li *et al.,* 2008).

Only a highly limited number of studies have investigated the osmoregulatory capacity and salinity tolerance of tropical marine and freshwater ornamental shrimp. Among these, Sandifer (1983) is one of the few instances where such investigations have been conducted. Specifically, exploring the effects of salinity (experimental: S= 5, 10, 15, 20, 25, and 30%.) and temperature on larval development of grass shrimp (*Palaemonetes vulgaris*), a popular species within the tropical freshwater trade. The findings showed a significant effect ($p<0.05$) of salinity on larval survival and development, across all temperature conditions. The lowest survival rates were observed at 5% salinity, survival rates exceeded 60% at higher salinity levels in 20°C and 25°C temperature groups. Additionally, the study revealed a surpressing effect on development at low salinity (S= 5 and 10%). Indeed, the findings support the notion that salinity can play a crucial role in shaping the survival and development of shrimp. The effects observed highlight the need to develop our understanding of how these species respond to varying salinity levels.

When managed correctly, salinity should not be an issue in well maintained aquariums (Hunziker, 2005; Kioke, 2013). However, in saltwater aquariums, the reality of maintaining a constant salinity can be a challenge, due to the constant evaporation of water, necessitating freshwater top-ups (Spotte, 1993; Skomal, 2006; Carter, 2023) which can result in minor fluctuations in salinity within aquariums. In many cases, efforts are made to ensure the salinity of the transportation medium is kept identical to the medium in which they are maintained in (Mandal *et al.,* 2010).

Whether this state is maintained during the period of transport is unknown, as there is little quantitative documentation of how salinity changes over periods during exportations and importation transportation. Salinity also remains an unquantified and undetermined threat for wild ornamental shrimp livestock, which is particularly concerning given the increasing occurrence of environmental salinity fluctuations in marine and freshwater habitats (Koblinsky *et al.,* 2003; Seggel *et al.,* 2016; Valesco *et al*., 2018; Cañedo-Argüelles *et al.,* 2019). Until a comprehensive understanding on the effects of salinity on shrimp physiology, including the underlying mechanisms that drive tolerance, and define species-specific tolerances is achieved, we cannot determine how salinity may affect the health and survival of traded shrimp and wild livestock; something we presently, know very little about.

In conclusion, salinity stands as a crucial abiotic factor affecting various aspects of shrimp physiology including growth, metabolism, immunity, and survival. Many species of shrimp have demonstrated osmoregulatory abilities, in which evidence has demonstrated their adaptive responses to diverse salinity conditions. Studies have revealed that osmoregulatory capabilities vary across developmental stages, emphasising the importance of considering ontogenetic differences in responses to salinity stress. To date, the majority of our understanding of the effects of salinity on shrimp physiology primarily revolves around food-based aquacultured species. Little information is held on species-specific osmoregulatory abilities and tolerance in tropical marine and freshwater ornamental shrimp. Consequently, changes in environmental salinity poses and unquantified and undetermined threat to shrimp populations. In the context of the ornamental trade, maintaining optimal salinity is believed to be crucial for the health and survival of shrimp. Henceforth, comprehensive research is required to determine the effects of salinity on shrimp physiology, the underlying mechanisms that drive tolerance, and species-specific tolerances. For instance, identifying the tolerance levels of specific ornamental shrimp could help to inform suitable and avoidable conditions for transportation. Additionally, such research could shed light on whether wild shrimp populations are vulnerable to the increasing occurrence of salinity changes in natural habitats. Ultimately, only with a deeper understanding of these factors can effective management and conservation strategies be developed to support the health of ornamental shrimp species.

1.5.5 Ammonia & Nitrogenous Waste

Ammonia and nitrogenous waste are naturally found in aquatic ecosystems, existing primarily in the form of dissolved inorganic nitrogen ions ammonium (NH_4^+), nitrate (NO_2^-) and nitrate (NO_3^-) (Romano and Zeng, 2013). When ionised, they constitute the most common forms of dissolved inorganic nitrogen available to aquatic ecosystems, however, in excess these ions can affect physiological responses, abundance, and distribution of many aquatic species (Zhao *et al.,* 2020). This is a looming issue when considering the increase in global anthropogenic activities augmenting nitrogen availability (Romano and Zeng, 2013).

Ammonia is found in aquatic environments primarily generated through protein catabolism, and the decomposition of unconsumed feed and faecal waste excreted by aquatic animals (Randall and Tsui, 2002; Chang *et al.,* 2015). Urea, being one source, is a minor N-excretion product which accounts for around ≈20% of total N-excretion (Weihrauch *et al.,* 1999). Crustaceans, including shrimp, predominately excrete ammonia through specialised organs involved in osmoregulatory ion transport such as the gills and pleopods (Romano and Zeng, 2013). The process of ammonia excretion in crustacean is believed to be an active process, due to their ability to excrete ammonia against an inwardly directed gradient (Weihrauch *et al.,* 2004b). A natural pH-dependant equilibrium exists between NH_4^+: NH_3 ratio in water. At a pH of <8.75, ammonia predominately exists in the form of ammonium (NH_4^+), when the pH is <9.75 ammonia exists as ammonia (NH_3) (Molins-Legua *et al.,* 2006). Biological oxidation breaks ammonia and ammonium into the nitrogenous forms of nitrate (NO_2^-) and nitrate (NO_3^-) through the process known as nitrification, facilitated by ammonia-oxidizing and nitrite-oxidizing microorganisms (Stein and Klotz, 2016; Stein, 2019). Though all nitrogenous ions, in certain concentrations, can be toxic (Hadfield, 2021), toxicity decreases along the process of oxidation. Ammonia is considered more toxic as it easily diffuses across plasma membranes and into the blood or hemolymph (Spotte, 1993; Weihrauch *et al.,* 2004b). As pH is increases, ammonia toxicity increases (Armstrong *et al.,* 1978).

Studies investigating the effects of ammonia, nitrite and nitrate toxicity on shrimp have tended to adopt a multifaceted approach, encompassing the use of various physiological mechanisms, each offering distinct insight into the organism's physiological responses to nitrogenous ions. Gene expression and enzyme activity analysis have been used to assess changes in key molecular biomarkers associated with stress, detoxification and immune responses following exposure to nitrogenous ions (Sun *et al.,* 2018), respirometry to study changes in metabolic activity and energy expenditure (Chen and Nan, 1993), evaluations of ionoregulatory mechanisms such as the regulation of sodium and potassium to identify osmoregulatory disturbances, and histological examinations to detect cellular damage indicative of respiratory and osmoregulatory dysfunction (Zhao *et al.,* 2020).

By employing such techniques, researchers can grow the understanding of species-specific responses to toxicity at a molecular, cellular and organismal level, and the factors underlying their tolerance to change. However, comprehensive reviews of the effects of ammonia and other nitrogenous ions on physiology and adaptive mechanisms are few are far between (Romano and Zeng, 2013).

Exposure to toxic concentrations of nitrogenous ions in the water has been shown to induce direct and indirect physiological changes in shrimp, such as changes to metabolic processes (Zhang *et al.,* 2023). To date, the lethal effects of ammonia have been studied in only a handful of shrimp species, predominately in marine species, including white legged shrimp (*L. vannamei*) (Lin and Chen, 2003), pink shrimp (*Farfantepenaeus paulensis)* (Miranda-Filho *et al.,* 2009), giant tiger prawn (*Penaeus monodon*) (Chen *et al.,* 1990), green tiger shrimp (*Penaeus semisulcatus*) (Wajsbrot *et al.,* 1990), white shrimp (*Penaeus setiferus*) (Alcaraz, 1999), São Paulo shrimp, (*Penaeus paulensis*) (Ostrensky and Wasielesky, 1995), chinese white shrimp (*Penaeus chinensis*) (Chen and Nan, 1993), greasy back shrimp (*Metapenaeus ensis*) (Li *et al.,* 2023), and the blue shrimp (*Litopenaeus stylirostris*) (Mugnier *et al.,* 2008). Of the few freshwater species studied, research has included the giant freshwater prawn (*Macrobrachium rosenbergii)*, and the Amazon river shrimp (*Macrobrachium amazonicum)*. In many of these species, ammonia has been reported to adversely affect physiological and pathophysiological processes including osmotic regulation, respiration, metabolism, growth, metamorphosis, immunity, reproduction, and survival. (Hong *et al.,* 2007; Chang *et al.*, 2015; Lu *et al.,* 2016; Cui *et al.,* 2017). Conversely to the above species, there is a significant dearth of literature on the effects of ammonia and other nitrogenous ions on the physiology of tropical marine and freshwater ornamental shrimp species.

When levels of nitrogenous wastes in the water exceed the tolerance limit of a species, it can cause direct damage to tissue structures, notably affecting vital organs including gills and hepatopancreas, resulting in anoxia (Zhao *et al.,* 2020; Zhang *et al.,* 2023). Results have been most heavily documented within *L.vannamei*. For example, the work of Zhang *et al.,* (2023) reported that chronic exposure to ammonia reduced growth, caused histological structural damage and vacuolation in the hepatopancrease and gill tissues, and changed enzymatic activity (Superoxide dismutase (SOD) and Na^+/K^+-ATPase) associated with oxidative stress. Oxidative damage and apoptosis of organs, induced by increased levels of free reactive O_2 species in response to elevated ammonia, has been frequently documented in many species of shrimp (Liang *et al.,* 2016; Qui *et al.,* 2018). Similar findings in support of the adverse effects of ammonia toxicity in *L. vannamei* have been documented by several authors, Lu *et al.,* (2016), Duan *et al.,* (2018), and Fregoso-López *et al.,* (2016) among others. Whilst a number of studies have also shown that exposure to high ammonia induces oxidative stress in the endoplasmic reticulum (ED stress) (Liang *et al.,* 2016), supporting the evidence of a the role of ammonia toxicity in causing cellular dysfunction, and likely triggering cascading implications on broader physiological functioning. For example, shifts in metabolic processes, and the structural integrity of morphological features caused by exposure to high ammonia have been shown to reduce the intake of food, disrupting energy metabolism by lowering body lipids, and increasing carbohydrate content, eventually leading to slower rates of growth in pink shrimp (*Farfantepenaeus paulensis*) (Miranda-Filho *et al.,* 2009). The multiple physiological changes induced by ammonia also poses a threat to the full functioning of the immune system, increasing the susceptibility of shrimp to pathogens (Qui *et al.,* 2018). Liu and Chen (2004) showed that mortality rates of *Vibrio alginolyticus*-injected *L. vannamei* exposed to ammonia (1.10, 5.24, 11.10 and 21.60mgL^{-1}) were significant higher ($p<0.05$) than control groups (0.01mgL^{-1}), evidencing that exposure to elevated ammonia in water can cause a depression in the immune response, resulting in increased mortality in shrimp. Of the limited literature conducted on the physiological responses of freshwater shrimp to ammonia, the likes of *M. rosenbergii* and *M. amazonicum* have been studied, with adverse impacts of increased ammonia concentrations on physiological properties being demonstrated in both species. Pinto *et al.,* (2016) showed that increasing total ammonia-N concentrations resulted in changes in oxidative stress enzyme activity, immune system effects and alterations in gene expression in *M. amazonicum*, with a 2-fold increase (V_M = 316.9 ± 15.8 nmol P_i min^{-1} mg^{-1}) in gill (Na^+, K^+)-ATPase activity, and a decrease in haemolymph haemocyte count of ≈30% after 72 hours ammonia exposure to 2.0 mmol L^{-1} total ammonia nitrogen (TAN). These findings are similar to those reported after exposure to high ammonia concentrations for many other species of shrimp (Jiang *et al.,* 2004; Rodríguez-Ramos *et al.,* 2008), but contrast with the findings of unchanged haemocytes numbers or functionality in *L. vannamei* (Liu and Chen, 2004).

Ammonia stress tolerance of shrimp can vary significantly between breeding families (Lu *et al.,* 2017; Wang *et al.,* 2017). Several mechanisms exist, by which aquatic organism detoxify exogenous ammonia (Randall and Tsui, 2002) and reduce body ammonia loads (Chen and Cheng, 1993; Durand et al., 1999). These include the excretion of haemolymph ammonia-N via regular osmoregulatory function (Kormanik and Cameron, 2004), and the detoxification of exogenous ammonia via transformation into urea and free amino acids, mediated by enzymes such as glutamate dehydrogenase (GDHase). As both ammonia conversion and ion transport are high energy consuming processes (Leone *et al.,* 2017), individual energy reserves are also crucial for supporting ammonia excretion function (Tong *et al.,* 2023). Despite this knowledge of specific mechanisms, the actual mechanisms driving ammonia tolerance and adaptability among different species and families of shrimp are still poorly understood. In *L. vannamei*, degree of ammonia tolerance has shown to be heritable, suggesting the potential for genetic improvement of ammonia tolerance (Lu *et al.,* 2017; Xiao *et al.,* 2019). Therefore, gaining greater understanding of the ammonia tolerance of different species and families is crucial to ensure the optimal selection of shrimp in food-based aquaculture (Tong *et al.,* 2023). Similarly, this holds exciting applications to other live-based industries such as the ornamental trade, in which distinguishing differences in tolerances across groups of shrimps may help streamline the selection of more optimal shrimp for collection and breeding, and support the enhancement of adaptive strategies to safeguard the health of those identified more susceptible to ammonia-induced stress.

As traded ornamentals are packed for shipping in small volumes of water, sometimes at high loading density, metabolic waste can accumulate rapidly in transport water (Lim *et al.,* 2003). When unionised ammonia surpasses tolerable levels, mortality can occur (Lin *et al.,* 2003; Cobo *et al.*, 2014; Sun *et al.,* 2018). Elevated ammonia during transportation is cited as the key driver of fish mortality during long journeys (>8 hours) (Sampaio and Freire, 2016), but the relationship between ammonia and mortality remains unclear for ornamental shrimp. While anecdotal evidence, within the ornamental industry, suggest that many shrimp are sensitive to ammonia, fundamental empirical evidence supporting this claim of shrimp is weak. To mitigate the deterioration of water quality, resulting from the accumulation of metabolic waste, various techniques are employed including the starvation of organisms prior to packaging (Phillips and Brockway, 1954; Nemato, 1957), lowering temperatures of transport water (Phillips and Brockway, 1954; Nemato, 1957; Teo and Chen, 1993), buffers (McFarland and Norris 1958), anaesthetics (Teo *et al.,* 1989), and for freshwater organisms, the addition of drugs in the transport water (Ling *et al.,* 2000). However, the utilisation of such techniques may impose additional strain on the organisms. Until we gain a comprehensive understanding on the effect of ammonia on shrimp physiology, the underlying mechanisms that drive tolerance, and define species-specific tolerances, then we cannot truly understand whether ammonia significantly influences the health and survival of shrimp during trade and transportation, and whether such mitigation techniques are required, effective and cost-worthy efforts to make.

Overall, the presence of ammonia and nitrogenous waste in aquatic ecosystems can pose significant challenges to the survival of shrimp populations, particularly in the context of live trade industries. Understanding physiological responses to high levels of nitrogenous ions that interrupts homeostatic performance can provide insight into the tolerance of a species. While research is beginning to elucidate the impact of ammonia, nitrite and nitrate on shrimp physiology, there remains a notable gap in the literature regarding tropical marine and freshwater ornamental shrimp. Despite anecdotal knowledge within the ornamental industry suggest sensitivity of shrimp to ammonia, and, thus, recommending its avoidance, empirical evidence supporting this claim is lacking. Currently, science understands very little about the species-specific tolerances and sensitivities of ornamental shrimp to nitrogenous ions. Therefore, there is an urgent need to systematically investigate the physiological effects of ammonia, nitrite and nitrate on such species, as well as the underlying mechanisms driving tolerance, and adaptability across different families and species of shrimp.

1.6 Conclusion and Aims & Objectives of this Thesis

Ecophysiology emerges from this review as a dynamic and interdisciplinary field that offers important insight into the intricate relationship between organisms and the environment. Defined as the study of species' physiological responses to abiotic stimuli, ecophysiological serves as a bridge between diverse and developing disciplines, incorporating comparative animal physiology, evolutionary physiology, and molecular techniques. Such multi-disciplinary approaches are crucial to unravelling the complexities of how animals' function and adapt to environmental changes. The interplay between physiology and health raises fundamental questions around the definition of health and its current utilisation in industry welfare assessments. Overall, the effects of environmental stimuli on physiological stress responses can provide insight into organismal health, henceforth, making it an appropriate advancement from current disease-centric approaches.

We have used Hatch's (1962) conceptual framework was used as a foundation to delineate and construct an operation definition of health. This emphasises an organisms continued ability to adaptively respond to stress, in absence of chronic physiological damage. We explored the applications of ecophysiological approaches to improving the management of organismal health within live trade industries, and conducted a review of the existing knowledge, or lack thereof, on the physiological effects of key physico-chemical parameters of ornamental shrimp. Overall, we lack the sufficient knowledge and understanding required to predict how changes in physio-chemical parameters can affect the physiology of ornamental shrimp, and, therefore, how these changes may be associated with poor health and mortality within the trade. What is true, however, is that temperature emerges as an interesting and fundamental parameter to explore. Such investigations into the physiological responses by tropical shrimp temperatures have only just begun. To date, no studies have examined the physiological responses of tropical shrimp to decreasing temperatures. Consequently, our understanding of their lower thermal tolerance, critical limits, and complete thermal tolerance ranges is also severely limited. As such, it also remains uncertain whether the OCLTT hypothesis is a unifying model in setting both upper and lower thermal tolerances and critical limits of ornamental shrimp, and indeed tropical species generally.

Therefore, the aim of the following (experimental) chapter is to investigate how the respiratory physiology of tropical shrimp react to continuous warming and cooling of their immediate environment. The study commences with an investigation of the thermal changes that take place in the waters of in which shrimp are transported long distances by air. This is done by using sensor technology to record temperature from within live material transport containers shipped by air over a 38-hour period. This information is then used to inform experiments which investigate the effects of thermal change (warming and cooling) on scaphognathite ventilation, and, using various physiological indices, in order to establish critical upper and lower thermal limits of a popular tropical ornamental shrimp; cleaner shrimp (*Lysmata amboinensis)*. By establishing a baseline thermal performance curve and examining thermal tolerance, through parameters such as CT_{max}, CT_{min}, and T_{opt} the study provides fundamental insight into understanding how *L. amboinensis* tolerates thermal change, and the underlying metabolic processes that shapes their response. Furthermore, the study lays the groundwork for integrating rigorous scientific study with ornamental industry-driven agendas, to not only enhance our understanding of their biology, but provide data that contributes to the development of evidence-based strategies to optimise the health of shrimp during their trade.

Chapter Two:

The Thermal Performance and Tolerance of Tropical Cleaner Shrimp (*Lysmata amboinensis*)

Abstract

Understanding thermal performance and tolerance is essential for predicting how species respond to temperature change. While the responses of temperate species to thermal change is relatively well understood, tropical species remain understudied. This knowledge gap hinders our ability to accurately predict ecological responses, and effectively manage tropical species in aquaculture. Here we investigated the physiological performance and tolerance of a tropical marine shrimp, *Lysmata amboinensis* (cleaner shrimp), under thermal warming and cooling scenarios. This was done by quantifying scaphognathite ventilation rate (beat.min^{-1}) along a thermal gradient, and determining critical thermal limits (CT_{max}, CT_{min}, and T_{opt} values) using ventilation, together with other set points, namely the cessation of sensory antennules, and the release of pleopods. The onset of anaerobic metabolism towards thermal extremes was determined by measuring changes in lactate, glucose and ATP concentrations. A significant effect of temperature on mean ventilation rate in both warming and cooling treatments was detected. Peak performance (P_{max}) in ventilation reached 381.5 beats.min^{-1} at T_{opt} of 30.25°C. CT_{max} (35.25°C) and CT_{min} (7.5°C) values indicated a broad thermal tolerance consistent with findings on other tropical shrimp. Cardiorespiratory system failure coincided with the upper thermal limit, but it failed to comprehensively explain the complete collapse of ventilation at critically low limits. Temperature had a significant effect on mean L-lactate, glucose, and ATP concentrations. The utilisation of anaerobic metabolism

was not exclusive to temperatures approaching CT_{max} or CT_{min}. This study is the first to investigate the upper and lower thermal performance and tolerance of any tropical marine ornamental shrimp.

2.1 Introduction

In ectotherms, ambient temperature (T_A) is thought to shape physiological, biochemical and behavioural processes (Nguyen *et al.,* 2011), influencing the distribution and survival of many ectothermic species (Deutsch *et al.,* 2008; Chen *et al.,* 2011). In ectotherms, physiological performance traits vary with T_A (Angilletta, 2009; Huey and Berrigan, 2001), as T_A plays a significant role in determining body temperature (T_B) (Hockachka and Somero, 2002). The effects of temperature on organismal physiology, at any level of organisation, has been described in numerous ways, but perhaps most commonly using the concept of thermal performance and tolerance (Fry, 1971; Beitinger and Lutterschmidt, 2011; Ern *et al.,* 2023). Thermal performance describes the effects of temperature on the capacity of physiological systems, while thermal tolerance defines the range of water temperatures over which physiological systems can be maintained at a predefined capacity (Ern *et al.,* 2023). Thermal performance is commonly assessed using performance traits, such as scaphognathite ventilation, in which changes in physiological rate(s) can be graphed against a thermodynamic temperature gradient, producing a physiological thermal performance curve (TPC) (Figure 2.1) (Angilletta, 2009; Schulte *et al.,* 2011; Molnar *et al.,* 2017; Kellermann *et al.,* 2019; Little and Seebacher, 2021). Through examining shifts in physiological rate(s), these curves offer valuable insight into how an organism may adjust their underling physiology to cope in a changing thermal environment (Little and Seebacher, 2021). Given that ventilation of respiratory surfaces is a good measure or proxy of overall respiratory performance, it is reasonable to hypothesise that temperature will affect scaphognathite ventilation, producing a TPC similar to Figure 2.1.

The thermal tolerance limits of the time-limited zones can be defined through the application of models including the Arrhenius break point and critical thermal maximum, minimum and optima (CT_{max}, CT_{min}, and T_{opt}) (Killen *et al.,* 2021), which shed light on the capacity of the organism to cope with extreme environmental conditions. Specifically, CT_{max} and CT_{min} represent the T_A above and below which performance is at a minimum, and T_{opt} represents the T_A at which performance is maximum (Kingsolver and Umbanhowar, 2018; Bacigalupe *et al.,* 2018). Pre-defined sublethal endpoints, rather than death, are used to ascertain critical temperature thresholds (Lutterschmidt and Hutchison, 1997). Physiological endpoints from heat failure at CT_{max} can be deduced from the complete cessation of the physiological performance trait measured, or for fish, the loss of equilibrium (Lutterschmidt and Hutchison*,* 1997; Jutfelt *et al.,* 2019; Ern *et al.,* 2023). For ectotherms, where loss of equilibrium is less obvious, the cessation of physiological performance traits or sensory stimulation including heart rate (Chung *et al.,* 2012), scaphognathite beating (ventilation) (Nakanishi and Nishiyama, 1932; Mika, 2013), pleopod movement (Korhonen and Lagerspetz, 1996; Verberk *et al.,* 2018), and sensory antennule movement (Rosa *et al.,* 2014) are commonly used to derive CT_{max} values. These end points represent a state where the organism loses its ability to escape from conditions, ultimately leading to mortality (Cowles and Bogert 1944; Beitinger *et al.,* 2000). While the suitability of these different traits, as a reliable endpoint for assessing CT_{max} in ectotherms, is debated, suitable traits for establishing endpoint at CT_{min} remain less well defined, mainly due to the lack of understanding on the physiological processes that occurs as ectothermic organisms approach their lower thermal limits.

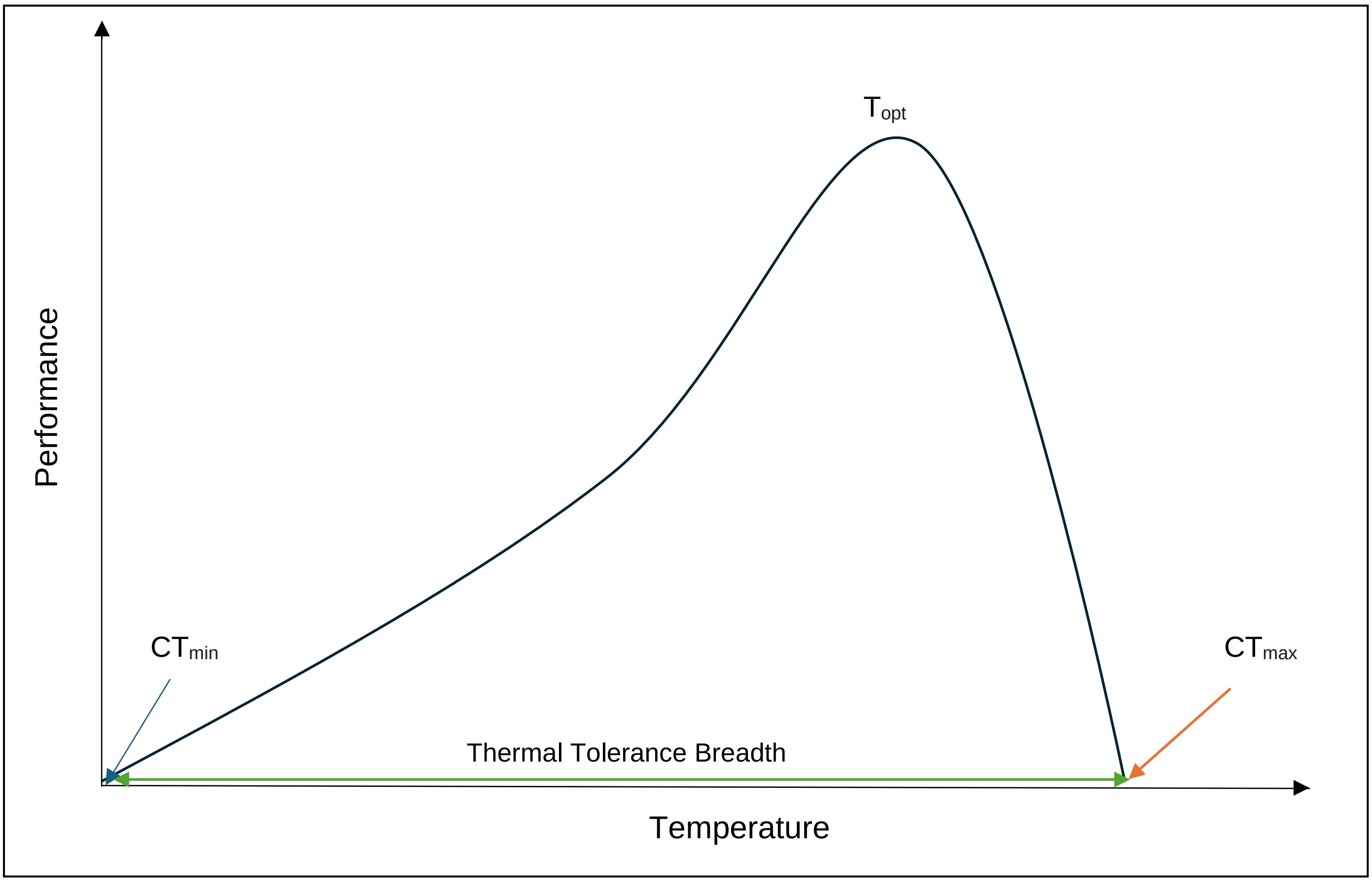

Figure 2.1: A generalised standard thermal performance curve (TCP) modelling the relationship between environmental temperature and a physiological rate of an ectotherm. TCP’s are typically characterised by an initial slow increase in performance with increasing temperatures, reaching maximum rate at the optimal temperature (T_{opt}), followed by a rapid decrease until the point of complete cessation (Huey and Stevenson, 1979; Huey and Kingsolver, 1989; Angilletta, 2009; Ern *et al.,* 2023). Fry (1971) divided the tolerance range of an organism into the zone of tolerance, the range of water temperature which functional capacity is maintained, and zones of resistance (upper and lower incipient lethal thermal limits). The upper and lower incipient lethal temperatures are analogous to upper and lower critical temperatures, used to broadly define the temperatures beyond which physiological systems can maintain capacity in a time-limited manner (Richard *et al.,* 2012; Ern *et al.,* 2023).

In aquatic environments, rising water temperatures reduce O_2 availability while, simultaneously, increasing standard metabolic O_2 demand of ectotherms (Ern *et al.,* 2014). This has led several authors to propose that cardiorespiratory performance drives thermal tolerance, where insufficient O_2 supply for basal maintenance drives CT_{max} and $CT_{min,}$ as explained by the Oxygen and Capacity Limited Thermal Tolerance (OCLTT) hypothesis (Frederich and Pörtner, 2000; Pörtner, 2002; 2010). An organism's capacity to maintain aerobic scope and supply O_2 to tissues, under the OCLTT hypothesis, is limited by the functional capacity of the ventilatory and circulatory systems (Pörtner, 2002; Hochachka and Somero, 2002; Pörtner, 2010). In the absence of a sufficient supply of O_2, organisms may recruit alternative means of acutely supplying energy to tissues through anaerobic respiration (Pörtner, 2010; Sokolova *et al.,* 2012), resulting in an increase in metabolites such as glucose and lactate, and a decrease in ATP (Müller, *et al.,* 2012). When this can no longer be maintained and exposure continues, mortality occurs (Pörtner, 2001; 2002; 2010). Alternative theories suggest insufficient O_2 delivery in relation to metabolic demand may not be the key driver in jeopardising performance at thermal limits, but one of several contributing factors. For example, exposure to thermal extremes may cause the loss of structural integrity of proteins, leading to their denaturation and loss of functionality, prior to the onset of tissue hypoxia (Pörtner, 2010; Clark *et al.,* 2013; Ern *et al.,* 2015; Schulte, 2015). However, the efficacy of the OCLTT hypothesis in predicting cardiorespiratory performance in organisms is heavily debated, particularly, as little evidence has been found for the universal role of O_2 in setting the lower thermal limits of many aquatic invertebrates (Verbeck *et al.,* 2016). Given this, we hypothesise that cardiorespiratory performance will solely drive the upper thermal tolerance of *L. amboinensis*, reflected in the recruitment of anaerobic metabolism at high temperatures. We predict concentrations of lactate and glucose concentrations will increase, and ATP will decrease in thermal warming scenarios.

Currently, research in this field has focussed on temperate ectotherms, with tropical ectothermic thermal physiology understudied by comparison (Seebacher *et al.,* 2015). Where tropical examples can be found, studies have typically focused on upper thermal performance and tolerance, in an effort to predict the effects of global warming on physiology and survival (Somero, 2010). In the context of climate change, the impacts of increasingly frequent warming events and higher thermal extremes are expected to be particularly severe for many ectotherms, including species of shrimp (Kingsolever *et al.,* 2013; Seebacher *et al.,* 2015; Burraco *et al.,* 2020). Similar to other ectotherms, many shrimp have a constrained capacity to regulate their body temperature (Kir *et al.,* 2023), relying instead on ambient conditions for thermal regulation (Angilletta, 2009). This dependency means they can be vulnerable to changes in temperature (Stillman, 2003; Deutsch *et al.,* 2008; Vinagre *et al.,* 2016). Tropical shrimp are thought to live closer to their upper thermal limits than their temperate conterparts, making them particularly vulnerable to small increases in temperature (Ern *et al.,* 2014). However, for the handful of tropical species of shrimp studied, data is equivocal. For example, *M. rosenbergii*, can tolerate a wide range of temperatures, adapting to diverse thermal environments (Ern *et al.,* 2014; Chaurasia *et al.,* 2016; Tay *et al.,* 2022), providing evidence that eurythermal tropical shrimp may have evolved a more thermoresistant cardiorespiratory system than originally thought. This has sparked debates on the vulnerability of tropical shrimp to thermal change (Tewksbury *et al.,* 2008). Currently, our understanding of the physiological responses and tolerances of tropical shrimp to high temperatures is limited, with even less known about their responses and tolerances to low and declining temperatures. Questions also arise around the role of cardiorespiratory performance in determining thermal tolerance of eurythermal tropical shrimp. There is insufficient studies to either support or falsify the predictions made by the OCLTT hypothesis in setting both upper and lower thermal tolerances and critical thermal limits of tropical shrimp. As far as we are aware, no studies have examined the physiological responses of a tropical marine shrimp to both increasing and decreasing temperatures, along a thermal gradient. Consequently, our understanding of their complete thermal tolerance range, critical thermal limits and the underlying processes driving their response is incomplete. Therefore, the aim of this study is to investigate the performance and tolerance of a tropical shrimp across a wide temperature range, using a thermal ramping rate that is representative of conditions experienced by aquatic ornamentals during transportation. Our objectives were to:

a) Measure the temperature regime experienced by tropical ornamentals during a long-haul flight to produce a realistic thermal ramping rate when constructing thermal performance curves.
b) Quantify scaphognathite ventilation (BPM) along the thermal gradient (cooling and warming) informed by point a.
c) To determine critical thermal limits (CT_{max}, CT_{min}, and T_{opt} values) using ventilation, together with other set points, namely the cessation of sensory antennule movement, and the release of pleopods.
d) To investigate the onset of anaerobic metabolism towards thermal extremes by measuring changes in lactate, glucose and ATP concentrations at those extremes.

Cleaner shrimp *(L. amboinensis)* were chosen as a model species. Despite its significant commercial value and popularity within the marine ornamental trade (Calado, 2017), there is little known about their physiology. In particular, their thermal performance and tolerance to changes in temperature is poorly understood. This impedes on our understanding of their susceptibility to temperature change, such as accompanying climate change, and hinders efforts to optimise their management and transport for optimal health during their trade. Few studies have investigated the upper critical limits of *L. amboinensis*, and to our knowledge there are no published studies investigating lower thermal performance and tolerance. Therefore, the current study is the first to investigate the upper and lower thermal performance and tolerance for any tropical marine ornamental shrimp.

2.2 Methods

2.2.1 Transport of Shrimp and Temperature Conditions During Transit

Cleaner shrimp, *Lysmata amboinensis*, (n=120, mean length: 3.60±1.9cm) were air-freighted from Sri Lanka on the 19th of February 2023 to Tropical Marine Centre (TMC) Chorleywood, and transported by van approximately 48 hours later, arriving at The University of Plymouth on the 22nd of February 2023. Shrimp were individually shipped in double-sealed plastic bags containing seawater (salinity= 35ppt).

To experimentally replicate a ramping rate that reflects the type of thermal variation marine ornamentals may be exposed to, across a popular marine ornamental trading route, temperature loggers (ElectricBlue, model: T2.4 27mm Envologger, origin: Portugal) were placed in four shipment boxes containing *L. amboinensis* being shipped for our experiment. Loggers were placed both within sealed bags containing equal amounts of seawater (n= 8) and within boxes exposed to air (n=7), but bagged separately from *L. amboinensis*, at 5:55am +15 minutes (local Sri Lankan time) on 19th Feb 2023. This was done to account for potential thermal variation between the water and air inside each box resulting from the heat packs. The loggers were preprogrammed to record temperature every 5 min (sample resolution: 0.1℃), capturing the thermal change that occurred from the onset of organisms being bagged in Sri Lanka to the point of unboxing at TMC, Chorleywood, approx. 38 hours later. From the data collected and presented in Sect 3.** (Figure 2.3) below we used a ramping rate of 0.3℃.h^{-1} in the experiments described in Sect. 2.**.

2.2.2 Animal Husbandry

Upon arrival shrimp were drip acclimatised, haphazardly split across four separate recirculating aquaculture systems (RAS) and placed individually into isolation containers within a 12-L tank (length: 21cm, width: 37cm, diameter: 15cm). Separation avoided aggression and additional stress. Each RAS system comprised of x10 15-L plastic tanks and a 50L sump filled with natural seawater (S= 35±1, T= 25℃ ± 0.5℃, pH= 8.2± 0.2, DO= 92%± 5%), equipped with biological, mechanical and chemical (activated carbon) filtration that included an external filter (make: EHEIM, model: Ecco Pro 200, origin: Germany), protein skimmer (make: EHEIM, model: Skim 350, origin: Germany), UV steriliser (make: TMC, model: Vecton 400, origin: United Kingdom) and a heater (make: EHEIM, model: Thermocontrol+e 200w, origin: Germany) (Figure 2). Each tank was also supplied with 15 small pieces of live rock to create a more natural environment.

Shrimp were maintained in a temperature-controlled environment (T=25℃) on 12h light:12h dark rotation. Testing kits (JBL Pro Aquatest Marin Complete Testing Kits) were used to measure water quality biweekly and water changes were conducted as required to maintain healthy conditions for the shrimp (ammonia: 0, nitrites: 0, nitrates: 0-5, calcium: 420, phosphates:0.05 all data expresses as mg.L $^{-1}$). Temperature and dissolved O_2 were monitored daily using a thermocouple (Omega, model: HH806, New Jersey) and O_2 probe (YSI, model:Pro2030 DO Probe, Ohio) respectively. Shrimp were acclimated to these laboratory conditions for two months prior to experimental use. Shrimp were individually fed on a rotated diet of small Vitalis Marine Pellets (n=3), frozen Gamma mysis and brine shrimp. Feeding began the day after arrival and occurred thereafter every few days, ensuring a 24-hour starvation period prior to any experimental use.

Figure 2.2: Image of two of the four RAS systems that were used maintain *L. amboinensis* (n=120). Each RAS system comprised of x10 15-litre plastic tanks and a 50L sump filled natural seawater. Each RAS system maintained 30 individual cleaner shrimp.

2.2.3 Thermal Performance and Tolerance

2.2.3.1. Construction of thermal performance curves

Thermal performance curves were constructed as follows. Shrimp (n=24) were haphazardly selected and divided between two experimental groups (n=8/group) (group 1: warming, group 2: cooling) and two control groups (n=4/group). Individuals were placed inside glass vials vol= 35ml), inserted with a mesh strip to minimise movement (spacing: 0.1cm, length: 6cm, width:1.5cm). Mesh was also secured on the openings of the chambers, to allow O_2 exchange. Vials were placed in individual beakers (vol=1000ml) filled with 900ml of autoclaved natural seawater (see Section 2.2.2) and single air stones placed in each beaker maintained dissolved oxygen concentrations (DO) at 95% air saturation. Experimental groups were then placed into thermostatted water baths (TXF200-R52, Grant Instruments, UK), pre-programmed to maintain the water at 25℃ for one hour, after which temperature was changed at a ramping rate of 0.3℃. h^1 either warming (group 1) or cooling (group 2) the water in the respective treatment groups. Control groups were placed into 20L plastic tubs maintained at a constant temperature of 25℃ using a heater (75watt, EHEIM, Germany). Thermocouples (Omega HH806) were fitted across beakers to monitor the internal temperature of the beaker water. To maintain consistent cooling for experimental group 2 a 1:1 glycol: water mixture was used in tandem with a cooling coil. Each thermal ramp was run separate to one another, over the course of two weeks, but simultaneous to one control group.

2.2.3.2 Measurement of rate of ventilation and behavioural end points

Scaphognathite beating (ventilation) was quantified as a function of increasing or decreasing temperature in order to construct a TPC for each shrimp. Both full and partial beating of the scaphognathite were recorded as a ‘beat’. Scaphognathite measurements were repeated measurements on the same individual. A macro camera (make: Samsung, magnification: x3) was used to record scaphognathite beating. Videos were obtained every 0.5℃ for 30sec for each individual (control and experimental), and then every 0.25℃ interval once 30℃ was reached. Towards upper thermal limits rapid cessation of physiological performance was observed during preliminary experiments. Video playbacks were watched at half speed. both intra- and inter-observer reliability was considered when counting scaphognathite beats. Videos were taken until individuals were deceased, which was defined as when physiological shutdown had occurred, determined by the cessation of scaphognathite beating, internal bailers, heart and sensory antennules, as well as the release of pleopods when appropriate). Once mortality had occurred (~7°C in cooling treatment, ~37°C in warming treatment), individuals were gently blotted dry with a tissue and dry mass (g) was measured using a mass balance (PPS413, Fisher Scientific, USA). Control individuals (n=8) were recorded up until the final video of the experimental group was complete. Each video was independently reviewed three times and an average taken for each shrimp at each temperature interval, to minimise human error and count bias. This average was then doubled to provide scaphognathite beats per minute (beats.min ^{1}) for each recording.

Thermal tolerance was determined using the dynamic method described in Mora and Ospina (2001). The parameters measured included CT$_{max}$, CT$_{min}$, and T$_{opt}$ values (given in ℃). Three physiological traits were used to determine critical thermal limits (CT$_{max}$ and CT$_{min}$) including cessation of scaphognathite activity, movement of sensory antennules, and the release of pleopods.

2.2.3.3 Collection and Preparation of Tissue Extracts for Metabolite Analysis

Following a review of the mean TPC (Appendix 1), four temperatures (T= 10, 29, 31 and 34℃) and a control (T=25) were chosen as points to sample metabolites (glucose, L-lactate and ATP). Samples were taken at two temperatures on either side of the thermal optimum (30.25℃), excluding the control group (25℃). These temperatures were chosen to cover critical points along the curve, accounting for patterns observed nearing CT_{max}, CT_{min}, and T_{opt}. The experimental protocol described above was repeated. When treatment groups reached their designated temperature, individuals were sampled, their abdomen was rapidly dissected and immediately frozen in liquid N_2 (mean sampling time= 18.9 sec. Samples were labelled and stored in aluminium foil (T= -80℃) until required. All subsequent tissue preparation was carried out in a controlled temperature environment (4℃). Frozen abdomen tissue (mass= 100-150 mg.individual^{-1}) was separated from the carapace by grinding in a pre-chilled (-70℃) ceramic mortar filled with liquid N_2. Ground tissue was transferred to prechilled (T=-70℃) microcentrifuge tubes (vol=1.5mL Eppendorf™) and homogenised with four parts ice cold $HClO_4$ (0.9 mol.L^{-1}) using a mixer mill (Mixer Mill Retsch MM400 with one 5mm stainless steel and one UFO 3.5mm bead at 5℃) for 4min. Homogenate was centrifuged (Eppendorf™, model: 5425R) at maximum RPm (4>12000) for 20min at T= 4℃. The resultant supernatant was then transferred to chilled microcentrifuge tubes (1.5 mL Eppendorf™). A known amount (ul) of supernatant was then transferred to an empty microcentrifuge tube (1.5 mL Eppendorf™) and four parts ice-cold K_2CO_3 (3.75 mol.L^{-1}) was added to one $HClO_4$ to neutralise the acid. Gases were allowed to escape, and the samples were centrifuged using the same settings as given above. The resulting supernatant was then stored at T= -80℃ until they were, samples were thawed out on ice immediately before use in the assay described below.

2.2.4.2 Metabolite Assay

Concentrations of three key metabolites, D-glucose, L-lactate and ATP were determined in tissue homogenates using spectrophotometric assays. All assays followed enzymatic analysis as described in Bergmeyer (1965). Glucose concentrations were measured using the hexokinase method, ATP concentrations were determined using NADH linked assays8 and L-lactate determined as described in Bergmeyer and Bernt (1974). Samples were measured in duplicates. All measurements were made using a microplate reader (Greiner 96 well micro titer plate reader) at λ= 340 nm.

2.2.5. Statistical Analysis

Untreated data was handled in Microsoft Excel v2307 and exported to RStudio v2023.12.0+369 (R Core Development, 2023) for analysis and presentation. All statistical analysis was performed using a significance level of 0.05, or 0.0001 where values were highly significant. Packages used included “ggplot2” (Wickham *et al.,* 2016), “rTPC” (Padfield, 2023), “tidyverse” (Wickham *et al.,* 2019), and “dunn.test” (Dinno, 2024).

2.2.5.1 Thermal Performance and Tolerance

Ventilation data for both experimental groups (warming and cooling) were treated separately. The effect of time, batch and mass effects on scaphognathite beats were tested and showed no significant effect of any of these factors. Attempts were made to fit various rTCP models to empirical data with none proving a suitable fit. As residuals of transformed linear model data did not confirm to assumptions of normality (Shapiro-Wilks) and variance of heterogeneity (Levene's test) ($p<0.05$) a non-parametric alternative was adopted. The Kruskal-Wallis χ^2 -squared test was used to a test for an effect of temperature on ventilation rate, followed by unadjusted Dunn posthoc test to identify differences between temperatures.

As no rTCP models fitted the recorded pattern, CT_{max} and CT_{min} values were taken as the temperature at which physiological performance was lost (cessation of scaphognathite beating). T_{opt} was taken as the temperature at which peak performance (P_{max}) of scaphognathite beating was reached.

2.2.5.2 Metabolites

A one-way ANOVA was performed to determine the effect of temperature on L-lactate and ATP (after square root transformation), and glucose (after $\log_{10}$ transformation) concentrations. Tukey's HSD posthoc test was used to indicate the significance between mean values with temperature.

2.3 Results

2.3.1 Thermal Performance and Tolerance

On average, shrimp experienced a temperature decline of 5.9℃ during the 38-hour shipment period. Mean rate of decline was $0.3℃.h^{-1}$ (Figure 2.3).

Mean CT_{max} and CT_{min} for ventilation rate, were 35.25°C and 7.5°C respectively. A peak performance (P_{max}) of 381.5 $beats.min^{-1}$ was observed at the thermal optimum (T_{opt}= 30.25°C). For the cessation of sensory antennule movement, mean CT_{max} and CT_{min} values were 34.25°C and 11.44°C. The mean CT_{max} value based on the temperature pleopods were released was 34.9°C, but no CT_{min} value could be determined as pleopods were not released at low temperatures.

Temperature significantly affected mean ventilation rate in both warming (H(31)=84.103 , p=<0.0001) (Figure 2.4) and cooling treatment groups (H(35)=139.81, p=<0.0001) (Figure 2.5). As the temperature increased, so did ventilation until mean peak performance (P_{max}) was reached (381.5 beats.min^{-1}) at T_{opt}= 30.25°C. Beyond T_{opt}, ventilation rate declined, with a small peak in rate observed between 32.00-33.75°C. Physiological performance was lost at 7.5°C (CT_{min}) and 35.25°C (CT_{max}) respectively.

Results of the posthoc test (Dunn) revealed significant differences across both the warming and cooling treatments. In the warming treatment (Figure 2.4) 114 comparisons were statistically significant. For example, ventilation rate at 35.25°C differed significantly to all other temperatures, with differences in ventilation rate were also observed between the thermal optimum (30.25°C) and temperatures ranging between 25.0-28.5°C and 30.75-35.25°C, and the control (25°C) to 25.5°C and 26.5-30.75°C. In the cooling treatment (Figure 2.5), 124 comparisons were significantly different. For example, ventilation rate at 7.5°C significantly differed from that between 10.5-25°C. Ventilation rate at temperatures around CT_{min} (8-9.5°C) were significantly different to temperatures 17°C+. Significant differences were also detected between ventilation at the control (25°C) to 7.5-10°C and 12.5-16.5°C respectively.

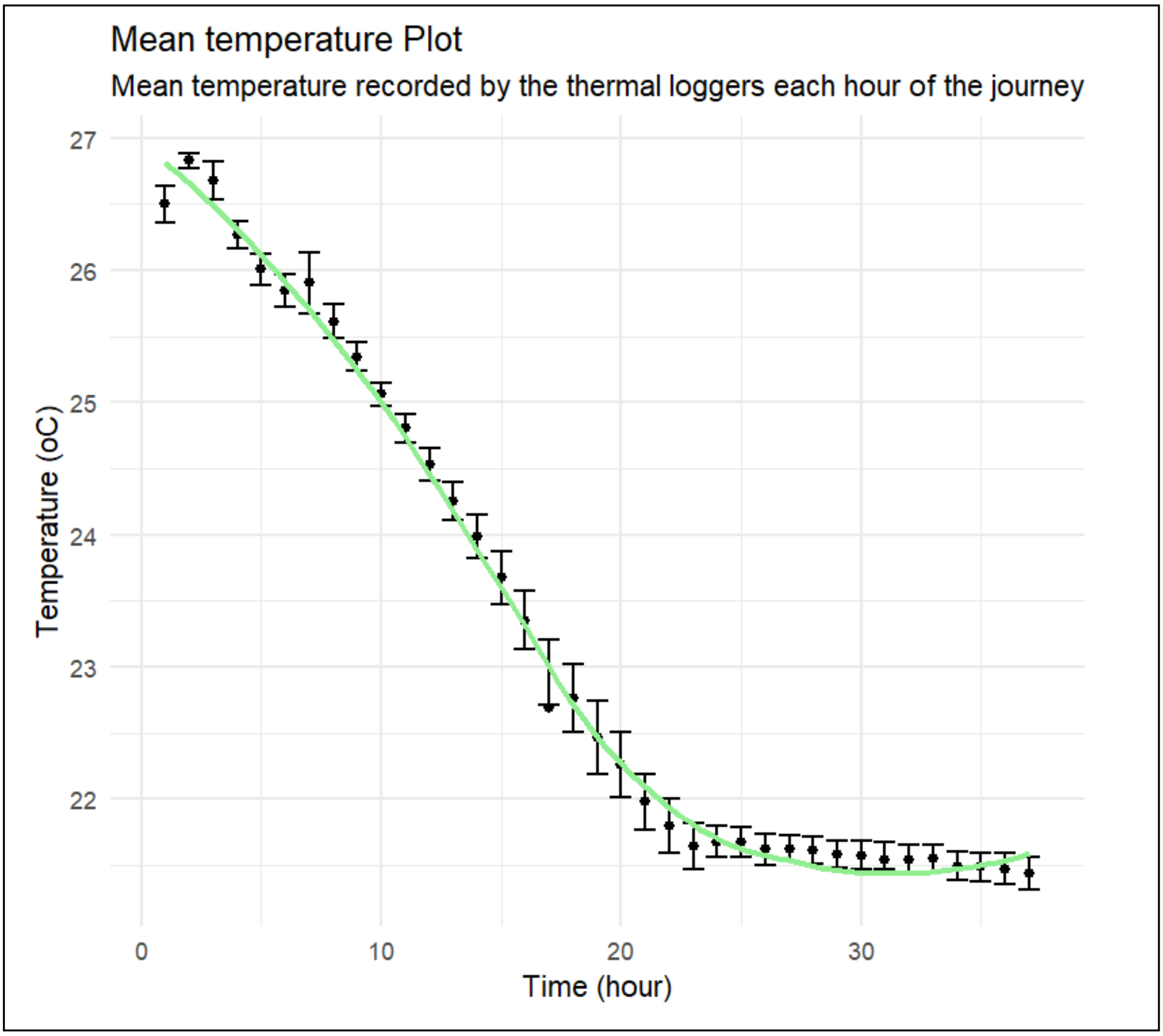

Figure 2.3: Rate of temperature decline *L. amboinensis* experienced per hour during an approx. 38-hour shipment journey, from the point of bagging in Sri Lanka, to their arrival and unbagging at Tropical Marine Centre, London. To track the thermal change the marine ornamentals experienced along this trade route thermal loggers (n=15) were divided amongst four shipment boxes (box 19, 20, 21 and 22), placed in and out of water, containing *L. amboinensis*. The mean rate of thermal decline over this period was calculated to be 0.3℃.h^{-1}. The rate of decline for each logger was calculated by taking the total degree decline÷ total time taken to reach the lowest temperature) e.g. Logger 9, Box 19: (maximum temperature 27.5℃, minimum temperature 21.7℃) 5.8 ÷ 22h 30 mins = **0.26℃.h^{-1}**

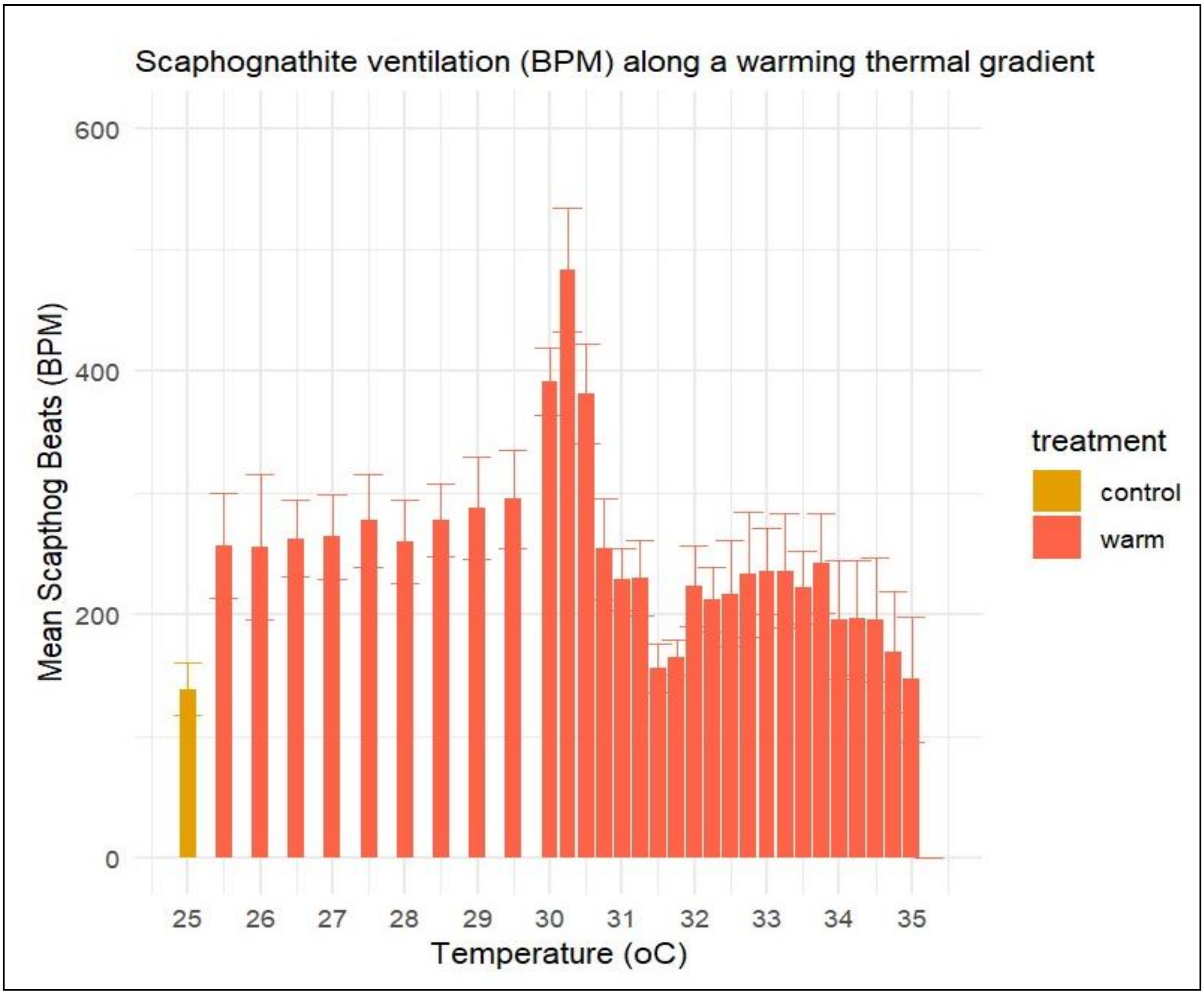

Figure 2.4: The effect of temperature (explored as a gradual thermal warming, ramping at a rate of 0.3°C.h^{-1}) on mean ventilation rate in *L. amboinensis*, illustrated as a 0.5°C and 0.25 thermal gradient increase (x-axis). Starting temperature was 25°C. Sampling increased from every 0.5°C rise to 0.25°C once temperatures reached 30°C. Mean beating of the control group (25°C) was 138.75 beats.min^{-1}. Peak performance (P_{max}) (381.5 beats.min^{-1}) was reached at the thermal optimum (T_{opt}) of 30.25°C. Physiological performance (ventilation) was lost once shrimp reached 35.25°C. Error bars represent ±1 standard error.

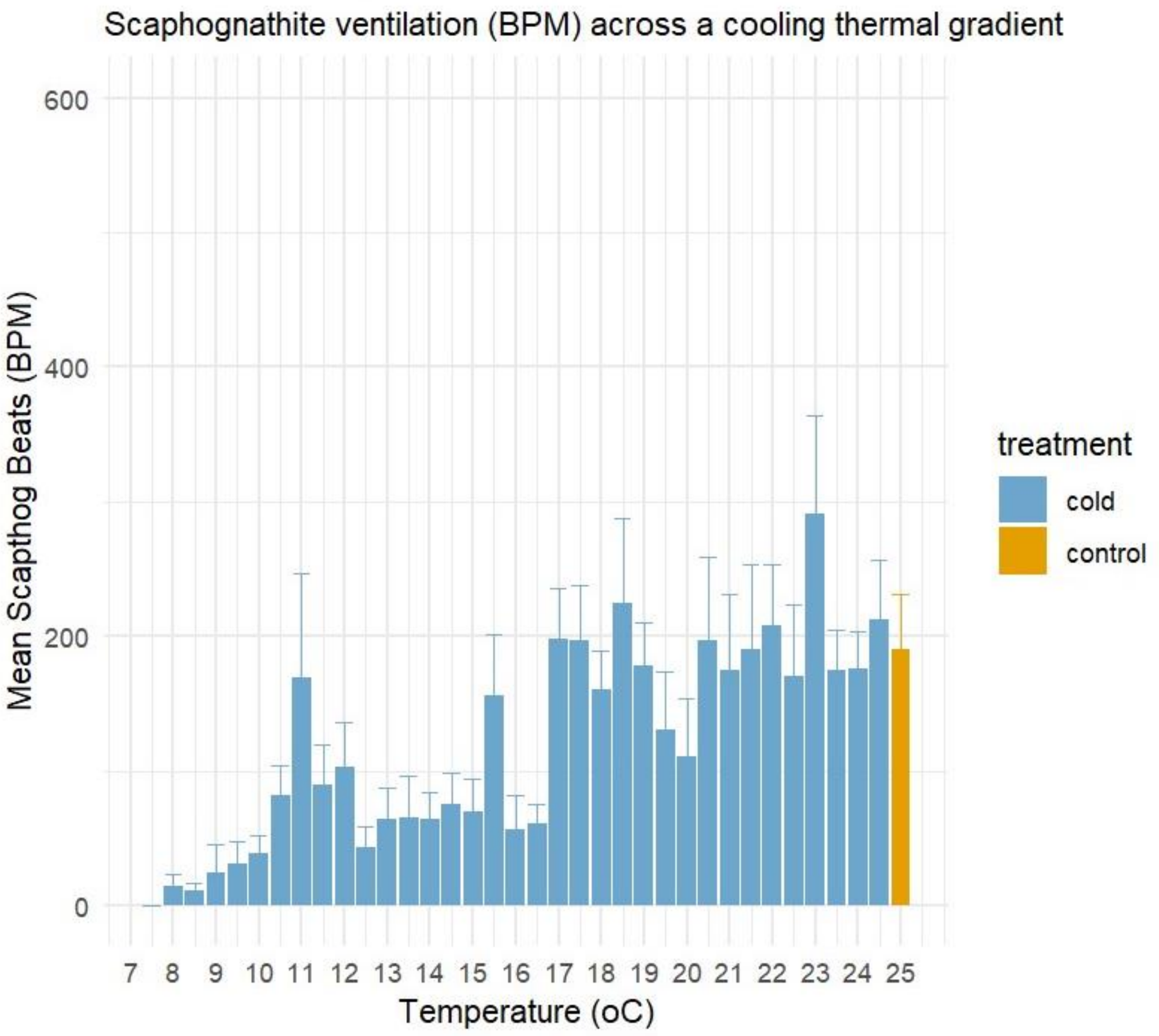

Figure 2.5: The effect of temperature (explored as a gradual thermal cooling, ramping at a rate of 0.3°C. hour^{-1}) on mean ventilation rate (beats.min^{-1}) in *L. amboinensis*, illustrated as a 0.5°C thermal gradient increase (x-axis). Starting temperature was 25°C. Mean beating of the control group (25°C) was 190 beats.min^{-1}. Physiological performance (ventilation) was lost once shrimp reached 7.5°C. Error bars represent ±1 standard error.

2.3.2 Metabolites

Results of the one-way ANOVA revealed a significant effect of temperature on mean L-lactate (F(4,35)= 8.7719, p<0.0001), glucose (F(4,33)= 3.7268, p<0.05), and ATP (F(4,31)= 5.9002, p<0.005). Tukey's HSD Test for multiple comparisons found that mean values of L-lactate concentrations (mmol.L^{-1}) were significantly different between 10°C and all other treatments (25, 29, 31 and 34°C) ($p<0.05$, 95% C.I.). As demonstrated in Figure 2.6, lactate concentrations rose significantly from 10°C with increasing temperatures. Similarly, glucose concentrations (mmol/kg) increased with temperature, with a significant difference found between 25°C and 34°C ($p<0.05$, 95% C.I. = [0.678, 0.039]) (Figure 2.7). ATP concentrations showed a decline with rising temperatures, with significant differences found between the cooling and warming treatments including 10°C and 25°C ($p<0.05$, 95% C.I. =[-0.801, -1.584]), 10°C and 31°C ($p<0.05$, 95% C.I. =[-1.181, -1.992]), and 10°C and 34°C ($p<0.05$, 95% CI.I.= [-1.179, -2.072]) (Figure 2.8).

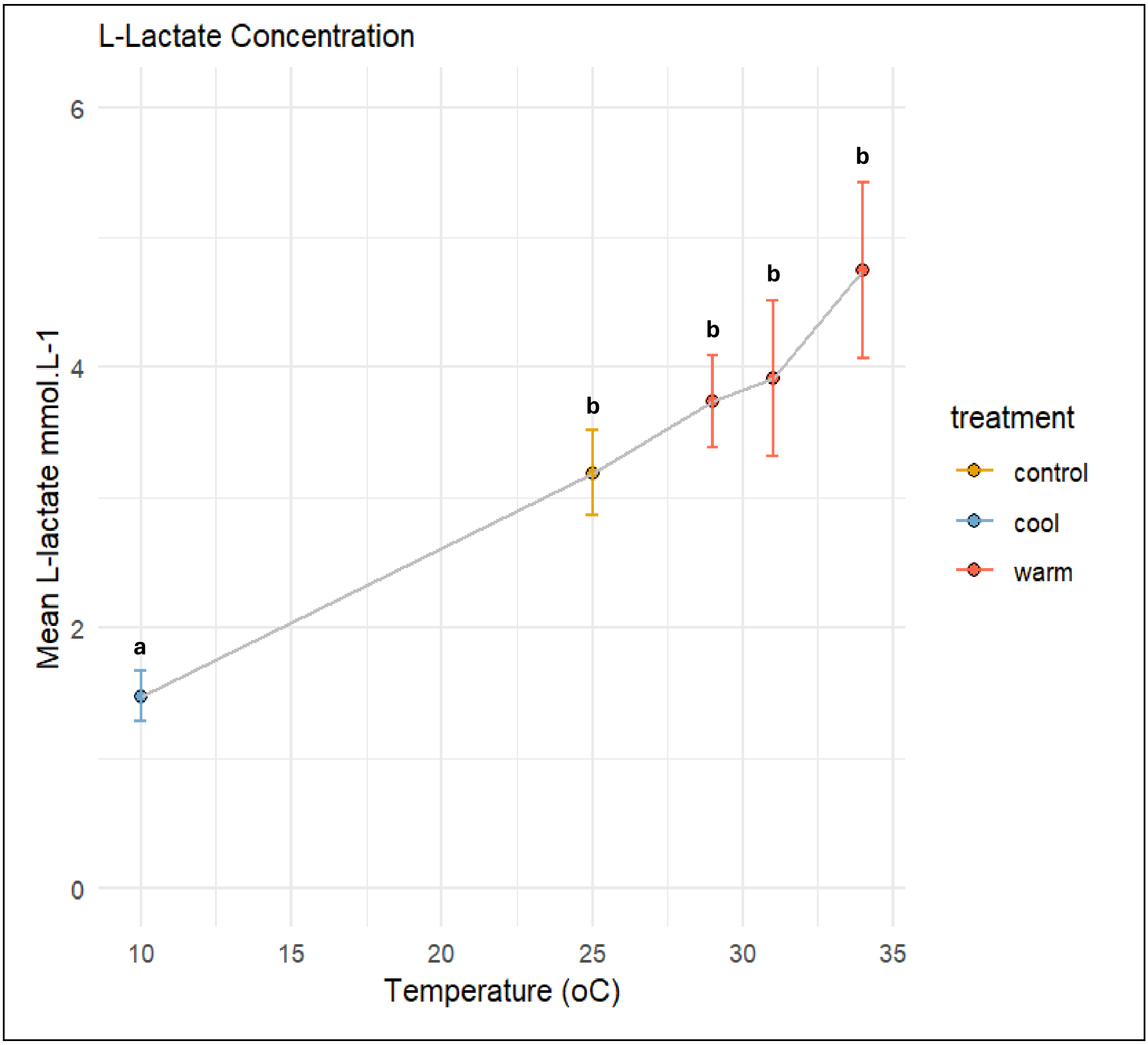

Figure 2.6: The effect of temperature on mean L-lactate concentrations (mmol.L^{-1}) in *L. amboinensis* muscle tissue across five temperature treatments (T=10, 25, 29, 31 and 34°C). All temperature ramping (0.3°C. h^{-1}) commenced at T= 25°C and the temperature increased or decreased until the desired temperature was achieved, then muscle samples were taken. Mean values were calculated as 1.48 (10°C), 3.19 (25°C), 3.74 (29°C), 3.92 (31°C), and 4.75 mmol.L^{-1} (34°C). Significant differences are indicated by the lower-case letters on the graph.

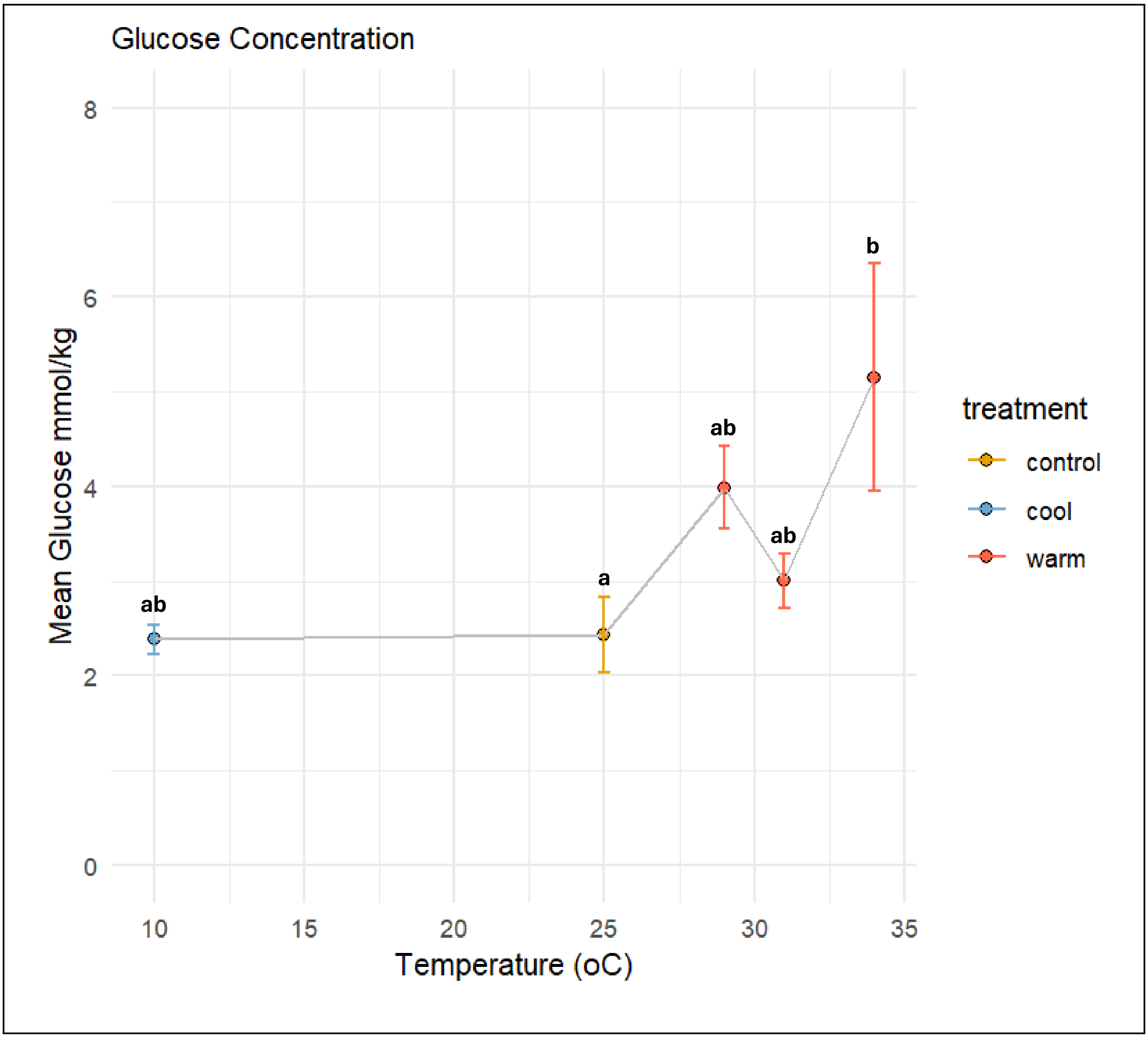

Figure 2.7: The effect of temperature on mean glucose concentrations (mmol.kg^{-1}) in *L. amboinensis* muscle tissue across five temperature treatments (T=10, 25, 29, 31 and 34°C). All temperature ramping (0.3°C. h^{-1}) commenced at T= 25°C and the temperature increased or decreased until the desired temperature was achieved, then muscle samples were taken. Mean values (2dp) were calculated as 2.34 (10°C), 2.44 (25°C), 3.99 (29°C), 3.00 (31°C), and 5.15 mmol.kg^{-1} (34°C). Significant differences are indicated by the lower-case letters on the graph.

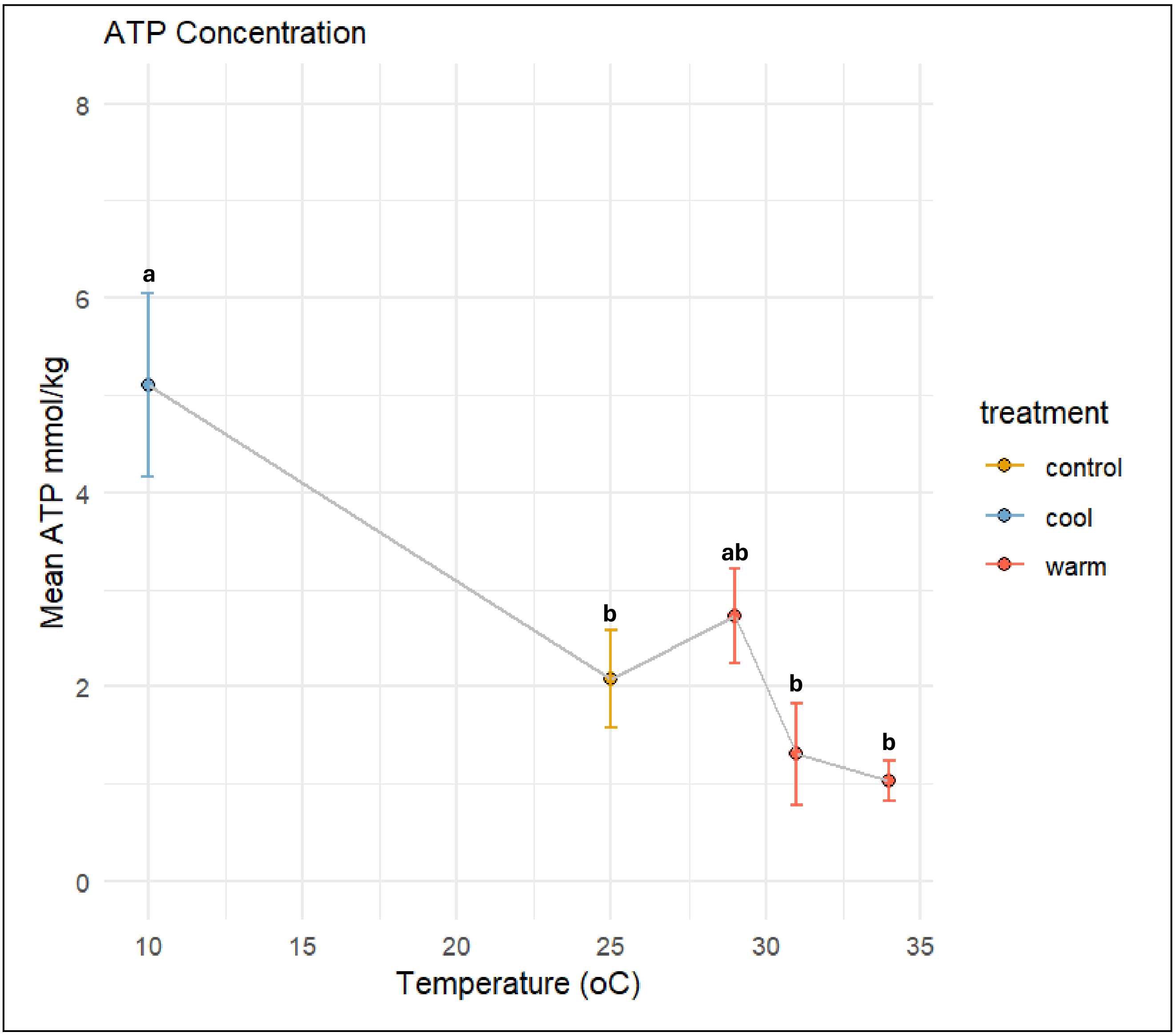

Figure 2.8: The effect of temperature on mean ATP concentrations (mmol.kg^{-1}) across five treatments (10, 25, 29, 31 and 34°C) in *L. amboinensis* muscle tissue across five temperature treatments (T=10, 25, 29, 31 and 34°C). All temperature ramping (0.3°C. h^{-1}) commenced at T= 25°C and the temperature increased or decreased until the desired temperature was achieved, then muscle samples were taken. Mean values (2dp) were calculated as 5.11 (10°C), 2.08 (25°C), 2.74 (29°C), 1.31 (31°C), and 1.04 mmol.kg^{-1} (34°C). Significant differences are indicated by the lower-case letters on the graph.

2.4 Discussion

This is the first study to quantify the thermal performance and tolerance in a marine ornamental invertebrate, in this case using the cleaner shrimp (*L. amboinensis*). The thermal challenges of low and high temperatures were applied at an industry relevant rate of change.

As predicted, temperature significantly affected scaphognathite beating. The TPC of *L. amboinensis* (Appendix 1) largely followed a 'typical' TPC shape, showing a gradual increase in performance with increasing temperatures, reaching maximum rate at the optimal temperature (T_{opt}), and then declining. However, unlike 'typical' TPC's, performance did not consistently decline after T_{opt}. Instead, performance increased between 32-33.75°C prior to the onset of decline, reaching complete cessation at 35.25°C. Interestingly, a peak in performance was also observed in cold temperatures towards CT_{min} between 10-11°C. Although these peaks in performance nearing CT_{max} and CT_{min} were not predicted, the results of the study are not dissimilar to the recorded patterns of ventilation of tropical blue crabs (*Callinectes sapidus*) (Garcia-Rueda *et al.,* 2020), and cardiac performance in spider crabs (*Maja squinado*) (Frederich and Pörtner, 2000) under resting conditions, where spikes in performance rate can be observed in both species nearing critical thermal limits.

Scaphognathite beating was used to determine mean critical thermal limits and thermal optima. Derived CT_{max} (35.25°C), CT_{min} (7.5°C), and T_{opt} (30.25°C) values reveal a broad thermal tolerance range consistent with findings on other tropical shrimp species, including *M. rosenbergii* (Ern *et al.,* 2014; Chaurasia *et al.,* 2016; Tay *et al.,* 2022). Our data supports the idea that eurythermal tropical shrimp may have evolved a more thermoresistant cardiorespiratory system than originally thought. It shows that *L. amboinensis* are currently maintained in temperatures (24-25°C) below their optimum for peak ventilation (30.25°C). Other traits were also measured to determine critical thermal limits, including the cessation of sensory antennules and the release of pleopods. The equivalent mean values were calculated as 34.25°C (CT_{max}) and 11.44°C (CT_{min}) for the cessation of sensory antennules, and 34.9°C (CT_{max}) for the release of pleopods. Pleopods were not released upon reaching their lower critical thermal limit. Instead, as temperature declined, the shrimp appeared to enter a state of torpor, noted by a gradual reduction in physiological movement.

One other study has determined the upper critical limits of *L. amboinensis*. The findings by Rosa *et al.,* (2014) on *L. amboinensis*, and *L. seticaudata* (a temperate-tropical species) presented CT_{max} values not dissimilar to those presented here. Reported CT_{max} values were 35.4 °C (*L. amboinensis*) and 35.0 °C (*L. seticaudata*) (acclimation T=24 °C, ramping rate: 2°C. hour $^{-1}$) and the cessation of sensory antennules to determine endpoint. Experimental groups acclimated to different temperatures (*L. seticaudata*: T= 18, 21, 24°C and *L. amboinensis*: T= 24, 27, 30°C) showed an increase in HSP70/HSC70 levels in muscle tissue with warming in both species (*L. seticaudata*: 266–294 ng mg−1, *L. amboinensis*: 134–161 ng mg−1 total protein; $p > 0.05$) where upregulation of chaperone proteins occurred to compensate the changes in temperature. L-Lactate concentrations significant increased within the muscle tissue under higher temperatures (*L. seticaudata:* 6.2–7.23 μmol g−1 dw, *L. amboinensis*: 3.4–4.4 μmol g−1 dw). MDA (malondialdehyde) levels in *L. amboinensis* also increased dramatically, indicating extreme cellular lipid peroxidation occurred. Evidence across both studies indicates that although *L.*

amboinensis can withstand warming scenarios, rising temperatures induces a stress response that can significantly affect physiological and metabolic functioning. Our data indicates *L. amboinensis* can endure a temperature rise of +5.25°C to reach the optimum for scaphognathite ventilation, and +9.25-10.25°C prior to complete loss of physiological function (all traits accounted for).

Results of the thermal cooling experiment revealed CT_{min} values (ventilation= 7.5°C, sensory antennules= 11.44°C) to be similar to thresholds seen in other tropical shrimp. For example, CT_{min} values for *L. vannamei* shrimp, calculated as the point in which locomotory movement became disorganised, range between 7.82-10.96°C for post-larvae, and 7.5°C-10.8°C for juveniles (acclimation T= 15°C, 20°C, 25°C, and 30°C, ramping rate: 1°C. hour $^{-1}$) (Kumlu *et al.,* 2010). CT_{min} values of riverine *M. rosenbergii* lie between 9.3°C to 13°C (Herrera *et al.,* 1998). *L. amboinensis* has shown to have a high tolerance to low temperatures, arguably somewhat more than what we predict for a tropical species. The results indicate *L. amboinensis* sit closer towards their upper thermal limit than lower, and although they have a degree of adaptive plasticity to both warming and cooling, they have a greater ability to withstand more significant declines in temperatures than increases. The ability of *L. amboinensis* to withstand low temperatures, could be an evolutionary adaptation attributed to conditions experienced in their natural habitat. Individuals have been found and collected at depths varying between 4-40m (Chen *et al.,* 2012) across tropical reef environments in the Indo-Pacific region (Wabnitz *et al.,* 2003), likely exposing individuals to temperature variation. More broadly, it has been proposed that certain mechanisms may exist that enable the species to withstand future environmental change (Chevin *et al.,* 2010). These include the adjustment of cellular and membrane properties, such as the composition and density of mitochondria, and the types of enzyme present (Rosa *et al.,* 2014), which may eventually enhance the efficiency of mitochondrial respiration, thereby extending the species' thermal tolerance (Pörtner, 2002). However, this hypothesis has yet to be tested.

The determined critical thermal limit of *L. amboinensis*, along with the observed differences in their ability to endure thermal change, prompts further investigation into the mechanisms underlying these responses. In particular, questions arise around the role of cardiorespiratory performance driving thermal tolerance of eurythermal tropical shrimp. The spikes in performance observed *in L. amboinensis* nearing its critical thermal limits suggest that individuals either a) receive insufficient O_2 supply to meet metabolic demands (Frederich and Pörtner, 2000; Pörtner, 2002; 2010), or b) experienced impaired metabolic function and/or efficiency such as through the loss of structural integrity nearing critical thermal limits (Pörtner, 2010; Clark *et al.,* 2013; Ern *et al.,* 2015; Schulte, 2015); resulting in increases in ventilation rate. Evidence supporting the role of cardiorespiratory performance driving thermal tolerance- where insufficient O_2 supply for basal maintenance drives critical thermal limits (CT_{max} and $CT_{min,}$)- is typically observed through a decline in cardiorespiratory performance, and the subsequent recruitment of anaerobic metabolism approaching these limits (Frederich and Pörtner, 2000; Pörtner, 2002; 2010). The utilisation of anaerobic respiration is indicated by increased concentrations of metabolites such as lactate and glucose, and decreased ATP (Müller, *et al.,* 2012). In a contradiction to the predictions made by the OCLTT hypothesis, this study indicates that 1) cardiorespiratory system failure, driven by lack of sufficient O_2, was only a principal determinant of the upper thermal limit, as we hypothesised. 2) Anaerobic metabolism was not exclusive to temperatures towards CT_{max} or CT_{min}. Although we rightly hypothesised that evidence of anaerobic metabolism would be present in thermal warming scenarios, with the recruitment of anaerobic metabolism at high temperatures, we did not predict metabolic changes associated with anaerobic metabolism would be found in cooling scenarios between 10 and 25°C.

Mean L-lactate concentration significantly increased from 1.48 (10°C) to 4.75 mmol.L^{-1} (34°C). The increase in L-lactate concentrations from 10°C suggests the efficiency of aerobic pathways declined with increasing temperatures, resulting in a growing reliance on anaerobic metabolism. ATP concentrations were significantly higher at 10°C than 25°C ($p<0.05$, 95%), 31°C ($p<0.05$, 95%), and 34°C ($p<0.05$, 95%), further supporting the idea that aerobic scope declined as temperature rises. No significant differences in glucose concentrations were detected between 10 and 25°C (mean values: 2.34 and 2.44 mmol.kg^{-1} respectively), but a significant increase was found at 34°C (concentration= 5.15 mmol.kg^{-1}). The increased glucose concentration observed in warming scenarios (29°C+) indicates a threshold where metabolic demand may surpass the availability of energy from immediate sources. This may prompt increased glucose mobilisation, or further, stimulate the release of glucose to meet heightened metabolic demands. This suggests that while aerobic scope declines with increasing temperatures, leading to a growing reliance on anaerobic metabolism from 10°C+, anaerobic metabolism becomes the primary means to obtain O_2 in temperatures 29°C+. Adequate O_2 delivery was found in low temperatures to T_{opt}. Cardiorespiratory performance did decline towards thermal extremes, but as no spikes in metabolites e.g. L-lactate and glucose, were detected at greater concentrations at 10°C compared to 25°C, suggesting that aerobic metabolism was predominant in low temperatures. Overall, our study suggests that anaerobiosis is associated with thermal warming and the upper thermal limits of

L.amboinensis, but that cardiorespiratory performance does not limit lower thermal tolerance.

Usually, high temperature causes the recruitment of anaerobic metabolism and eventual collapse of the cardiorespiratory systems in temperate or Arctic species (Pörtner, 2010; Rosa *et al.,* 2014), whereas it is not considered a limiting factor to upper thermal tolerance in some tropical species including *M. rosenbergii* (Ern *et al.,* 2014) and *Penaeus monodon* (Ern *et al.,* 2015). Our findings for *L. amboinensis*, akin to Rosa *et al.,* (2014), contradict this pattern by showing that tropical *L.amboinensis* are more capable of withstanding greater reductions in temperature more than increases, and that cardiorespiratory system failure, associated with insufficient oxygen supply, appears to drive their upper thermal tolerance limit. Rosa *et al.,* (2014) proposed that the relationship between O_2 supply capacity, animal fitness and thermal tolerance in tropical species is influenced by the heat-induced loss of protein structure or membrane integrity, leading to a sharp decline in metabolic and physiological functions at high temperatures (40°C). However, given our data shows the CT_{max} of *L. amboinensis* is significantly below this threshold (40°C), and the evidence of anaerobic metabolic recruitment in warming scenarios, we hypothesise that metabolic and physiological shutdown occurs due to decreased efficiency in obtaining the necessary O_2 through anaerobic means as metabolic demands rise.

The few studies that have investigated the OCLTT hypothesis at low temperatures, mainly lend little support the universal role of O_2 setting the lower limits of many crustacean (Verbeck *et al.,* 2016). Whether oxygen delivery is sufficient depends on the capacity of O_2 to supply oxygen in relation to O_2 demand (energy expenditure) (Frederich and Pörtner, 2000; Pörtner, 2002; 2010; Pörtner *et al.,* 2017). Ectotherms may increase O_2 delivery through increasing heart and ventilation rates or decrease O_2 demands by altering the use of energy, and entering a hypometabolic state (Verbeck *et al.,* 2016). As previously stated, *L. amboinensis* appeared to enter a state of torpor with declining temperatures, which likely would have reduced their metabolic requirements. Evidence of this has also been observed in the spider crab (*Maja squinado*). *M. squinado* enter a hypometabolic state when exposed to thermal cooling, which reduces their metabolic demands and delivery processes e.g. ventilation rate and haemolymph circulation decreases (Frederich and Pörtner, 2000). Ultimately, anaerobiosis and tissue hypoxia from insufficient performance at low temperatures lead to mortality in *M. squinado*. Our results indicate that although cardiorespiratory performance declines in decreasing temperatures, anaerobic metabolism is not recruited at critical lower thermal limits. Alternatively, we hypothesise that the mortality of *L. amboinensis* at low temperatures is likely due to a combination of impaired and disrupted cellular activity from a hypometabolic state induced by cold-water shock. At low temperatures, enzymatic reactions and cellular activity slows down, leading to metabolic imbalances and energy deficiencies. For example, cold temperatures can decrease membrane fluidity and impair ion channel function that disrupts cellular homeostasis (Amanto and Christner, 2008). The peak in scaphognathite activity observed prior to complete cessation in critically low temperatures may represent a 'last-ditch' attempt to maintain critical physiological function under extreme stress and/or inadequate cellular respiration efficiency. Although we recognise that insufficient O_2 delivery is likely a contributing factor in diving physiological collapse at low temperatures, other drivers such as the loss of functionality in proteins, and enzyme, together with metabolic imbalances likely jeopardises performance (Pörtner, 2010; Clark *et al.,* 2013; Ern *et al.,* 2015; Schulte, 2015), ultimately, resulting in mortality of *L. amboinensis*.

2.5 Conclusion and Implications for the Industry

This paper presents the first TPC produced for a tropical crustacean, the shrimp *L. amboinensis*, in response to both thermal warming and cooling, Ventilation in *L. amboinensis* was significantly affected by temperature, with the TCP generally following the predicted pattern. However, there were unpredicted peaks in scaphognathite beating near critical thermal limits resembling similar patterns observed in the blue crab (*Callinectes sapidus*) (Garcia-Rueda *et al.,* 2020), and the spider crab (*Maja squinado*) (Frederich and Pörtner, 2000). Peak performance (P_{max}) reached 381.5 beats.min^{-1} at T_{opt}= 30.25°C. *L. amboinensis* are currently maintained in temperatures 5.25°C below their optimum for peak scaphognathite ventilation. Derived CT_{max} (35.25°C) and CT_{min} (7.5°C) values showed a broad thermal tolerance range consistent with findings on other tropical shrimp. *L. amboinensis* sit closer towards their upper thermal limit than lower, showing a greater ability to withstand a more significant decline in temperatures than rise. Further investigation into the role of cardiorespiratory performance driving thermal tolerance revealed that while cardiorespiratory system failure drove upper thermal limits, it failed to explain the complete collapse of physiology at critical low temperatures. Our results indicated that although cardiorespiratory performance declined in decreasing temperatures, anaerobic metabolism was not recruited at critically low thermal limits.

Overall, our study contributes to a relatively understudied area of thermal biology; tropical species. By showcasing the unique thermal characteristics of *L. amboinensis* we highlight the potential risk of the species to future thermal warming challenges. While this study has provided a foundation for understanding the thermal performance and tolerance of *L. amboinensis*, further investigation into the molecular and cellular pathways involved in determining both the upper and lower thermal limits of the species is required. For example, exploring the pathways involved in thermal acclimation and adaption would depict the specific changes that enables this species to withstand such extreme temperatures.

The insight gained from determining the lower thermal tolerance of *L. amboinensis* holds significant relevance to industries that trade this species i.e. the marine ornamental trade. By subjecting the shrimp to industry-relevant rates of thermal change, we aimed to re-create the thermal conditions encountered during international transportation from a representative trading route from Sri Lanka to the United Kingdom. From this study we determined a) the mean rate of decline experienced during a key trading route was 0.3°C.h^{-1}, b) the significant affect this rate of thermal change has on scaphognathite ventilation, c) that the critical thermal limits of *L. amboinensis* supersede the likely thermal change experienced during international transport- and therefore, in isolation, temperature decline is unlikely to be a driver of mortality during their trade, and finally d) that the optimum temperature to maximise scaphognathite ventilation is 30.25°C. Therefore, existing methods for regulating temperatures during international transportation, such as heat packs, can be considered effective in maintaining suitable thermal conditions for the species. At present, it seems that little to no additional intervention is necessary beyond these measures. The findings of this study warrant further investigation into what environmental parameters, or combinations of, such as hypoxia, salinity, pH, temperature, and ammonia drive cases of associated poor health and mortality in the trade of *L. amboinensis*.

Chapter Three: Summative Conclusion and Recommendations

3.1 Recommendations

To support ongoing development of the ornamental trade, it is essential to integrate techniques that broaden our approaches and understanding in order to optimise the health and welfare of traded animals. Chapter one reveals that the integration of ecophysiology has the potential to support a more systematic, evidence-based approach to welfare, offering tailored strategies for optimising health, reducing stress, and enhancing the overall welfare and sustainability of the ornamental trade. Chapter two acts as a fundamental example for integrating rigorous scientific study with ornamental industry-driven agendas. As we navigate the challenges posed by environmental change and global trade, ecophysiological approaches become imperative for informed decision-making, conservation efforts and to ensure the health and wellbeing of species in our care. Long overdue is the call for a shift from disease-centric methodologies to more comprehensive ecophysiological approaches, providing foundations for purposeful, effective and positive collaborative industry-science efforts.

The literature review in Chapter One revealed a significant gap in industry-informed ecophysiological research. We currently lack sufficient knowledge of how changes in physio-chemical parameters can affect the physiology of ornamental shrimp, and how these changes may be associated with poor health and mortality within the trade. Therefore, we recommend

1) An increased focus on **industry-informed ecophysiology research**. This entails conducting studies on ornamental species that integrates industry

agendas into research aims and/or outputs, to deliver pragmatic advice to industry. For example, research could address species-specific responses and tolerances to industry-relevant physio-chemical parameters and multi-stressors. Chapter One highlights a particular need for understanding how temperature, dissolved oxygen (specifically hypoxia), carbonate chemistry, salinity, ammonia and other parameters affect the physiological health of ornamental shrimp.

2) **Research and Development (R&D) initiatives** should be introduced to support this work and encourage collaboration between researchers, industry stakeholders, and regulatory bodies to develop practical guidelines and policies based on ecophysiological research findings. Work undertaken should produce bi-directional benefits for both research and industry, and incorporate practical, anecdotal knowledge held by industry. Individuals working within the industries should be valued for the expertise they bring. Chapter Two demonstrated the potential for such collaborations to produce data that is directly beneficial to industry practices, such as determining optimal thermal conditions for species like *Lysmata amboinensis*.

Chapter one emphasised the central role physiological homeostasis plays in maintaining organismal health. The chapter advocated for a transition away from disease-centric approaches towards a broader perspective that includes ecophysiology, thereby fostering a more comprehensive understanding of organismal health. Currently, there is no established framework guiding the wider adoption of physiological approaches to health assessments, particularly for the assessment of aquatic livestock. This highlights the need for a move towards standardised protocols and best practices that incorporates physiological perspectives to form comprehensive health assessments. Therefore, we recommend:

3) The development and standardisation of ecophysiologically informed **health indicators** for key ornamental species. For example, the use of biomarkers to produce reference ranges and thresholds that indicate optimal health versus a stress response. In Chapter Two, measurements of ventilation rate were used to determine the thermal optimum and critical limits of *L. amboinensis*, to infer whether the isolated effects of thermal change during air freight poses a risk to survival, and, thus, require remediation actions.

4) The development of **species-specific guidelines** and standardised protocols detailing optimum, safe or unsafe physio-chemical conditions, identifiers of poor health, and what to do when conditions or health deviate away from optimum for key species. Subsequent, species-specific guidelines, indicators and/or recommendations must be tailored to ensure that they are appropriate and pragmatic for the sector. Considerations must be given to the practical application and accessibility of these recommendations to fishermen, exporters, importers, and regulators etc., that should naturally arise through true holistic and cooperative workings with industry stakeholders.

5) Adopt more **holistic approaches to health** by integrating ecophysiological knowledge into existing health protocols, such as those set by regulatory bodies for imported livestock. This ensures more comprehensive and informed assessments of both physiological and pathological aspects of

health in ornamentals. Practical applications could include developing dynamic checklists or health scoring systems that incorporate physical, behaviour, physiological and environmental factors specific to key species, and/or maintaining logs of environmental conditions and correlating these with health outcomes and allocating resources to monitor and maintain the most critical parameters for key species during transportation.

6) Finally, we recommend establishing **long-term research monitoring programs** to track physiological parameters in ornamental species over extended periods of time. This will help to develop our understanding of how organisms respond to chronic exposure to key physio-chemical parameters.

Overall, this thesis is a call for collaborative research initiatives that align scientific inquiry with industry goals to produce pragmatic outcomes. It recommends the development of standardised health indicators, and species-specific guidelines to bolster the management of traded species. While this thesis contributes to a relatively understudied area of the thermal biology of tropical species it also serves as a test-case for integrating scientific study with ornamental industry-driven agendas and. the development of evidence-based strategies to optimise the health of traded shrimp.

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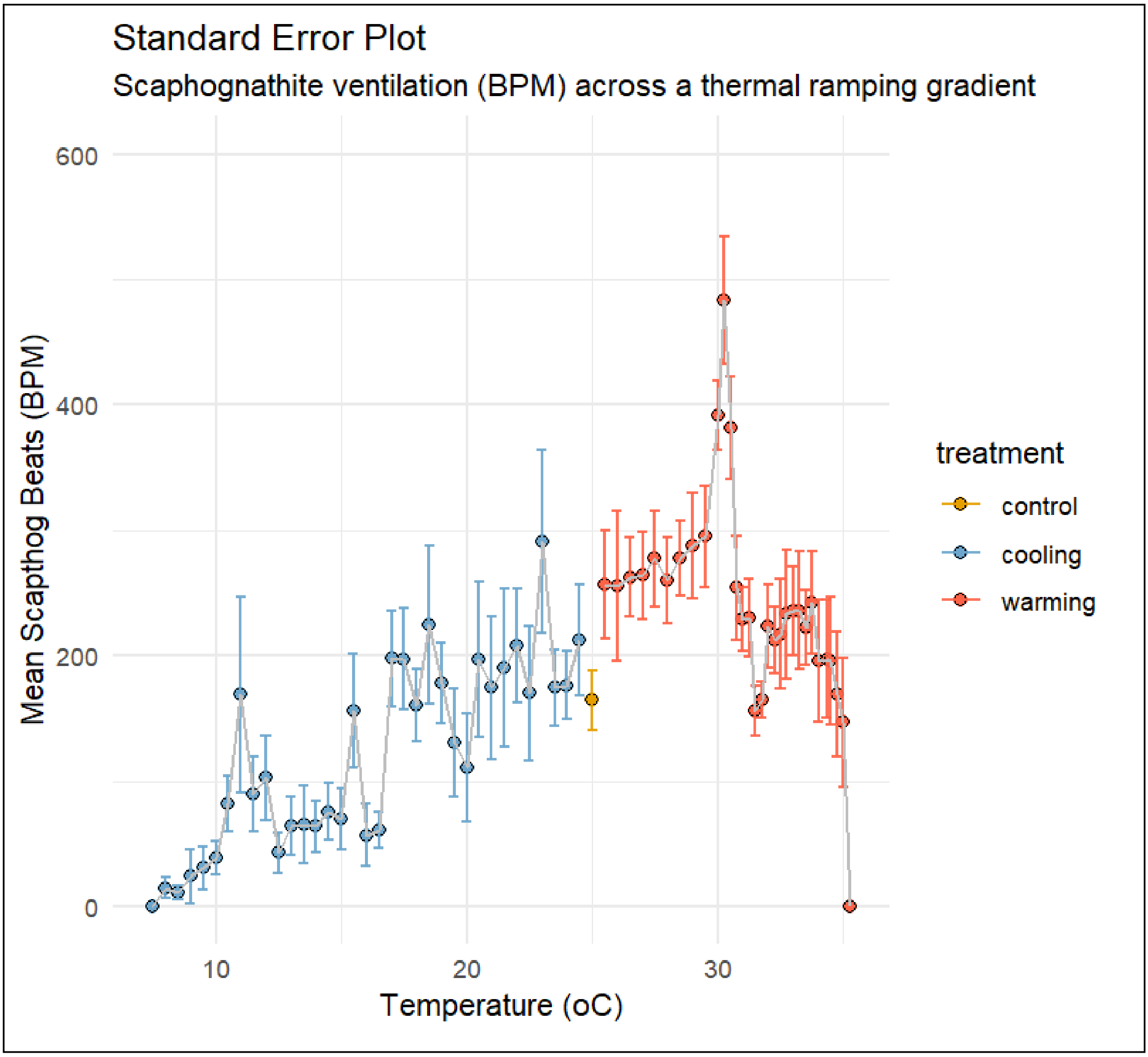
Appendices



Appendix 1: The effect of temperature (°C) (explored as two separate warming and cooling thermal ramps) on mean scaphognathite beats (beats.min^{-1}). Error bars represent ±1 standard error. Peak performance (P_{max}) was 381.5 BPM at the thermal optimum (T_{opt}) of 30.25°C. The upper and lower thermal limits (CT_{max} and CT_{min}), as the point at which performance is lost (cessation of scaphognathite beating), were calculated as 35.25°C and 7.5°C respectively. Here, the mean value for the control group (25°C) were calculated accounting for values from both the warming and cooling ramp.