Aquaculture industry resilience against climate change: Examining the effects of mild stress on heat tolerance in juvenile Chinook salmon (*Oncorhynchus tshawytscha*)

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Abstract

Climate change is already affecting aquaculture around the world and impacts are projected to worsen in many areas. Both Australia and New Zealand (NZ) have experienced multiple record-breaking heatwaves that have resulted in disease and mortality events in various aquaculture sectors. This thesis aimed to investigate cross-tolerance as a potential mechanism for increasing tolerance to heat stress in Chinook salmon (*Oncorhynchus tshawytscha*), a significant aquaculture species in NZ that may be impacted by climate change induced warming and weather events. Cross-tolerance describes a phenomenon where organism exposure to one stressor triggers a physiological stress response which confers increased resilience to a second subsequent stressor. Cross-tolerance has been observed in multiple teleosts, but in Chinook salmon has only been investigated in a single study on pre-swim life stages. In order to investigate the potential for cross-tolerance, two priming stressors, crowding (Chapter 2) and hypoxia (Chapter 3), were investigated in separate experiments on Chinook salmon smolts. Crowding (20-min at ~47.8 kg / m^{-3} density) had no effect on Chinook salmon heat tolerance, measured as critical thermal maximum (CT_{max}). The lack of effect was attributed to an insufficient crowding stressor severity. Moderate hypoxia stress priming (40% oxygen saturation for 2 h) resulted in cross-susceptibility to heat stress, which describes where stressor priming results in worsened tolerance to a second subsequent stressor. The physiological mechanisms by which cross-susceptibility occurs are largely unknown. Lastly, a survey investigating the climate change perceptions of Australian and NZ aquaculture industry members was completed to inform and support the cross-tolerance experiments and future research (Chapter 4). Industry members were asked which aspects of climate change were of most concern to their jobs and industry sectors, what they wanted from climate change research, and whether they would be willing to implement resilience tools, like cross-tolerance induction. Research investigating the perceptions of Australasian aquaculture industries on climate change threats has been lacking. Responses indicated that the Australian and NZ aquaculture industries are greatly concerned about the current and potential impacts of climate change. However, resilience tools with a growth trade-off had less likelihood of implementation. This thesis contributes novel insights into the potential for cross-tolerance solutions for increasing aquaculture species resilience to climate change threats.

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Chapter One - General Introduction

The problem – Climate change and thermal stress in ectotherms

Global climate change poses a ubiquitous threat to all ecosystems as it is driving increases in global temperature averages and extremes (Sage, 2020). Air and sea surface temperatures have risen globally resulting in the 5-year period between 2016-2020 being the warmest on record since 1851 (Arias et al., 2021). The observed warming has been consistent with historical projections (Arias et al., 2021) and is projected to continue (IPCC, 2019). Australasia alone has seen increased land surface temperatures, heat extremes, sea level rise, erosion, fire events, and marine heatwave frequency (IPCC, 2021). Australia is projected to experience increases in drought, dust storm frequency, marine heatwaves, and temperature extremes with all warming above 1.5°C, whilst New Zealand (NZ) will see increased rainfall, marine heatwaves, and glacial retreat (IPCC, 2021). In aquatic ecosystems, climate change is increasing the severity of numerous stressors, such as hypoxia, thermal stress, eutrophication, anthropogenic pollutants, species invasions, and acidification (Ficke et al., 2007; Hoegh-Guldberg et al., 2007; IPCC, 2019; Pörtner et al., 2017; Pörtner, 2002; Rahel & Olden, 2008; Sage, 2020). Temperature increases and related stressors are altering aquatic organisms and ecosystems by driving poleward biogeographical range shifts, altering food webs and lifecycles, increasing disease susceptibility, and altering metabolisms (Deutsch et al., 2015; Ficke et al., 2007; McBryan et al., 2013; Pörtner & Farrell, 2008; Yao & Somero, 2014). Climate projections indicate that aquatic ecosystems will continue to follow a warming trend and see heightened acidification due to carbon dioxide (CO_2) uptake. Additionally, oxygen (O_2) saturation in some surface waters is likely to continue decreasing (IPCC, 2019). Aquatic organisms are typically faced with multiple stressors simultaneously, potentially exacerbating their effects (Bopp et al., 2013; Crain et al., 2008; Ellis et al., 2015; Ficke et al., 2007; Fong et al., 2018; Petitjean et al., 2019; Przeslawski et al., 2015; Sage, 2020; Todgham & Stillman, 2013).

Ocean warming is of key concern as aquatic ectotherms are particularly susceptible to thermal stress (Atkinson, 1995; Pinsky et al., 2019). Ectotherms are unable to self-regulate their body temperature which fluctuates synchronously with environmental temperature (Zuo et al., 2012). The physiological performance of ectotherms typically relates to body temperature in the form of a curve, coined "thermal performance curves" (TPC) (Huey et al., 2012; Sinclair et al., 2016). The TPC describes the upper and lower thermal limits, and optimal body temperature (T_{opt}) as determined by physiological performance (fitness) (Huey et al., 2012; Sinclair et al., 2016). Fitness deteriorates rapidly as optimal body temperature, determined by environmental temperature, is exceeded. Small changes in environmental temperature can affect ectotherm metabolism, subsequently altering physiological functions (Paaijmans et al., 2013; Zuo et al., 2012). The inability of ectotherms to mitigate against environmental temperature changes makes them vulnerable to thermal stress from climate warming (Paaijmans et al., 2013). Protein denaturation, membrane instability, cell disruption, organ failure, altered metabolism, or altered reproductive capacities are all potential consequences of thermal stress, and can lead to changes in organism behaviour and fitness (Donaldson et al., 2008; Schulte, 2015; Somero, 2002; Verberk et al., 2016). Research has suggested numerous physiological mechanisms by which thermal tolerance could be limited in ectotherms such as failure of neural regulatory processes, cell membrane denaturation, and/or cardiovascular failure (Schulte, 2015). Tropical ectotherms or those already living near temperature extremes (e.g., rocky intertidal species, cold-adapted stenotherms) are considered to be at heightened risk as they already reside closer to their T_{opt}, whereas temperate ectotherms may initially experience positive effects as warming brings them closer to T_{opt} (Deutsch et al., 2008; Huey et al., 2012; Somero, 2010). As water temperatures rise, suitable habitats will compress, metabolic index distribution (O_2 supply : O_2 demand at rest) will shift, and poleward species displacement will occur on a global scale (Deutsch et al., 2015). The consequences of ocean warming for aquatic organisms and ecosystems will affect the availability of natural resources and the success of aquatic industries such as fishing and aquaculture (Gattuso et al., 2015; Sage, 2020).

Climate change effects on Aquaculture

The effect of ocean warming on the global aquaculture industry could be profound. In many farmed fish species, increased thermal stress can increase disease susceptibility, decrease growth, and increase mortality (Ahmed et al., 2019; Anyanwu et al., 2014; Prakoso et al., 2020; Reverter et al., 2020). Aquaculture farms will be exposed to numerous climate change driven stressors such as storms, drought, sea level rise, acidification, increased parasite loading, algal blooms, and species invasions (Anyanwu et al., 2014; Callaway et al., 2012; Mehvar et al., 2019; Prakoso et al., 2020). Unfortunately, many of these stressors are already impacting aquaculture worldwide (Froehlich et al., 2022; Lebel et al., 2016; Mehvar et al., 2019; Puspa et al., 2018). Disease and mortality events in teleost species in relation to temperature rise are already occurring in the wild and in aquaculture. For example, Sockeye salmon (*Oncorhynchus nerka*) in Canada are experiencing increased mortality during their river migration due to elevated river temperatures (Eliason et al., 2013). In Tasmania, Australia, a heat wave in the summer of 2015/2016 caused significant decline in growth and condition of farmed Atlantic salmon (*Salmo salar*) (Wade et al., 2019). Similarly, mass mortality events in the NZ salmon aquaculture industry have occurred due to heat waves (Salinger et al., 2019). Sea surface temperatures (SST) in Australasia are rising (Wijffels et al., 2018), and NZ has seen record breaking air temperatures over the last nine years (NIWA, 2022). The risk of future mortality and disease events due to increased water and air temperatures is high.

The deleterious effects of climate change on the health of farmed animals have ramifications for the productivity and economic success of aquaculture industries (Lebel et al., 2016; Tharanath et al., 2021; Yazdi & Fashandi, 2010). Fish is a primary protein source for billions of people worldwide (Farmery et al., 2017), and the economic effects of climate change are already being observed in aquaculture areas around the world (Lebel et al., 2016; Li et al., 2016; Tharanath et al., 2021). In 2018, global aquaculture production was 114.5 million tonnes and accounted for over half of all fish for human consumption (Bartley, 2022). Modelling suggests that not all aquaculture will be negatively affected, but that many species are vulnerable to climate change stress and alleviation strategies should be widely implemented (Cubillo et al., 2021; Li et al., 2016; Lorentzen, 2008; Oyinlola et al., 2020; Prakoso et al., 2020; Schrobback et al., 2018). Some research suggests that upscaling of aquaculture in small nations may alleviate some impacts of climate change on food availability by reducing reliance on wild fisheries (Dey et al., 2016; Merino et al., 2012; Rosegrant et al., 2016). However, increasing aquaculture production requires significant economic investment (Rosegrant et al., 2016) which many countries may be unable to afford. Additionally, the productivity of many aquaculture farms is also decreasing due to climate change pressures (Lebel et al., 2016; Li et al., 2016; Tharanath et al., 2021; Yazdi &

Fashandi, 2010). Furthermore, the suitability of sites for aquaculture development is shifting with climate change, altering the viability of both new ventures and existing farms (Mehvar et al., 2019; Oyinlola et al., 2020). Therefore, alleviation through aquaculture development will be dependent on availability of climate change resilient species, site suitability and availability, and sustainable aquaculture practices. Research predicts an increased reliance on imports of fish protein in some nations as local production continues to decrease (Dey et al., 2016). The socioeconomic impacts of decreased productivity could be extensive as the livelihoods and food security of thousands of people is at risk (Dey et al., 2016; Yazdi & Fashandi, 2010). There may be a disproportionate effect on poorer areas that rely heavily on the food and income derived from small-scale aquaculture, and are unable to implement expensive mitigation technology (Ahmed et al., 2019). The ubiquity of climate change impacts in aquaculture, and the potential socioeconomic consequences, necessitates the development of economically viable mitigation tools for implementation in standard aquaculture practice.

Environmental stressor interactions: cross-tolerance and cross-susceptibility

Whilst thermal stress alone can have significant physiological effects, it has become increasingly clear that climate change stressor research must recognise the interactions between simultaneously and sequentially occurring stressors, as it is unlikely that aquatic organisms will ever face stressors in isolation (Sage, 2020). Studying stressors in isolation can generate false estimations of the resilience of organisms. Synergistic or cumulative interactions between co-occurring stressors may cause decreased resilience when compared to single stressor resilience. Alternatively, stressors can exhibit antagonistic interactions, where the combined stressor effect is reduced when compared to the sum of the isolated effects of the individual stressors (Côté et al., 2016; Crain et al., 2008; Fong et al., 2018; Piggott et al., 2015). In the literature, the antagonism and synergism terminology has typically described the effects of concurrent stressors (Côté et al., 2016; Crain et al., 2008; Fong et al., 2018; Lin et al., 2018; Petitjean et al., 2019; Piggott et al., 2015; Rebl et al., 2020; Wen et al., 2018). However, not all stressor exposure will occur simultaneously, but may occur sequentially. Exposure to one stressor can sometimes confer “cross-tolerance” or “cross-susceptibility” effects on tolerance to a second stressor at a later time point (Rodgers & Gomez Isaza, 2021; Sinclair et al., 2013).

Cross-tolerance describes where exposure to an initial priming stressor will increase resilience to a different subsequent stressor, typically through the upregulation or activation of physiological protective mechanisms shared by both stressor responses (figure 1) (Rodgers & Gomez Isaza, 2021; Sinclair et al., 2013; Teets et al., 2020; Todgham et al., 2005). These protective mechanisms involve physiological changes that lead to increased stress resilience, such as an increase in heat shock protein (HSP) production (Borchel et al., 2018; Robinson et al., 2019; Sung et al., 2011; Todgham et al., 2005) or changes in cardiovascular capacity (Burleson & Silva, 2011; Opinion et al., 2021). Other terms used to describe cross-tolerance in the literature include hormesis, adaptive response, pre-conditioning, and pre-treatment (Berry & López-Martínez, 2020). Additionally, the term "cross-talk" is used to describe the same result achieved through shared signalling pathways leading to different protective mechanisms (Levesque et al., 2019; Rodgers & Gomez Isaza, 2021; Sinclair et al., 2013; Teets et al., 2020). Both of the terms "cross-talk" and "cross-tolerance" are encompassed within the umbrella term "cross-protection" (Rodgers & Gomez Isaza, 2021). In contrast, cross-susceptibility describes where exposure to a priming stressor decreases physiological resilience to a subsequently occurring stressor (Todgham & Stillman, 2013). The occurrence of cross-tolerance or cross-susceptibility varies with species and life stage, as well as stressor type, severity, and exposure duration (Rodgers & Gomez Isaza, 2021; Todgham et al., 2005).

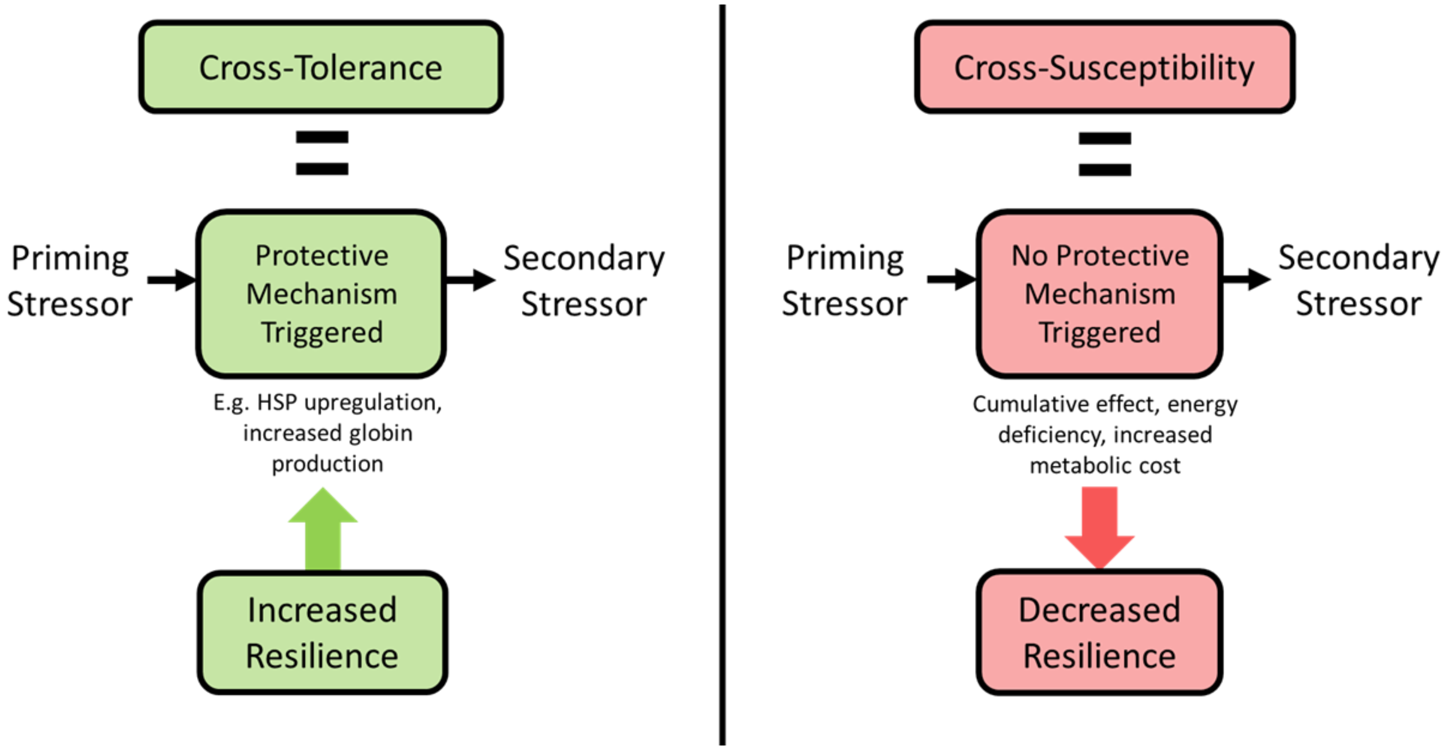


Figure 1. Defining cross-tolerance and cross-susceptibility. Exposure to a priming stressor can trigger physiological protective mechanisms, such as the production of heat shock proteins (HSP). Where this protective mechanism is shared by another stressor, resilience to that other stressor can be increased. Increased resilience through shared protective mechanisms is referred to as cross-tolerance. Alternatively, where a protective mechanism is not shared, stress to a priming stressor can result in energy deficits inhibiting the resilience to a secondary stressor. Where no protective mechanisms are shared, cross-susceptibility can occur.

There are numerous examples of cross-tolerance occurring in ectotherms, particularly in arthropods (Everatt et al., 2015; Sinclair et al., 2013; Sinclair et al., 2007; Teets et al., 2020). There is a commonly observed mechanistic interaction between desiccation stress and cold shock in numerous arthropod species (Everatt et al., 2015; Sinclair et al., 2013; Sinclair et al., 2007; Teets et al., 2020). Rapid cold hardening (RCH) refers to a plastic response to cold shock resulting in increased cold resilience. There are numerous associated physiological protective mechanisms including up- and down-regulation of HSP production, maintenance of cardiorespiratory function through regulation of apoptosis, oxidation and mitochondrial activity, and increased cryoprotectant production (e.g. glucose, glycerol, and sorbitol) (Teets et al., 2020). Cold shock and desiccation stress present similar physiological challenges and share protective mechanisms, enabling cross-tolerance. The cryoprotectants produced through RCH such as glycerol and sorbitol can confer increased desiccation tolerance as they can alter cellular water movement and retention (Everatt et al., 2015). The interactive relationship between desiccation and cold stress and the related physiological mechanisms has been thoroughly explored in the literature (Teets et al., 2020). Cross-tolerance between other stressors has also been observed in arthropods. For example, mild crowding increased thermal resilience in Drosophila flies (Henry et al., 2018).

Cross-tolerance stressor interactions in teleost fish is a relatively new area of research, but evidence has been found in a number of species. In tidepool sculpins (*Oligocottus maculosus*), exposure to sublethal heat shock has been shown to increase resilience to subsequent osmotic and thermal stress (Todgham et al., 2005). Similarly, Chinook salmon (*Oncorhynchus tshawytscha*) reared under hypoxic conditions showed improvements to heat tolerance later in life, although at the expense of growth and survival (Del Rio et al., 2019). Additionally, warming and nitrate exposure was found to improve tolerance to hypoxia in the common carp (*Cyprinus carpio*) (Opinion et al., 2021). Although there are numerous examples of cross-tolerance in teleosts, the studies have encompassed a broad range of species, life stage, stressors, stressor severities, and experimental designs (Burleson & Silva, 2011; Del Rio et al., 2019; Dolci et al., 2013; Dolci et al., 2017; Dolci et al., 2014; DuBeau et al., 1998; Gomez Isaza et al., 2021; Morgenroth et al., 2021; Ng et al., 2017; Opinion et al., 2021; Robinson et al., 2019; Rodgers & Gomez Isaza, 2021; Schreck, 2010; Todgham et al., 2005; Todgham & Stillman, 2013), resulting in a lack of cohesion and leaving significant knowledge gaps. For example, many studies refer to the role of HSP's in conferring resilience to various stressors, particularly thermal stress (DuBeau et al., 1998; Roberts et al., 2010; Todgham et al., 2005), however the magnitude and duration of effect, interacting stressors, study species, and methodology all vary. Consequently, there is no unanimous answer as to how to induce HSP driven resilience in teleosts, what effect it will have on resilience to other stressors, or how long the effect will persist. Some studies have attributed similar cross-tolerance effects to other protective mechanisms such as antioxidant production, mitochondrial refurbishment (Berry & López-Martínez, 2020), or cardiovascular performance enhancement (Burleson & Silva, 2011; Opinion et al., 2021). Whilst studies have observed cross-tolerance resulting in heightened heat tolerance in fish (Del Rio et al., 2019; Todgham et al., 2005), these studies are few and investigate only two priming stressors, heat shock and hypoxia. It is yet unknown which stressors could confer cross-tolerance to heat stress and investigation of various stressor interactions in numerous species is still required to provide a comprehensive understanding of cross-tolerance in teleosts.

The promise of cross-tolerance in aquaculture

The potential for increasing thermal resilience through the activation of protective physiological mechanisms is of particular interest in the face of global climate change. If a priming stressor could be identified that improves resilience to heat stress, it could be implemented as a resilience tool in the face of heat waves or other weather events, particularly in aquaculture settings where water parameters can be manipulated. The concept of utilising cross-tolerance as a tool for increasing stress resilience in aquaculture is not novel. Previous studies have discussed the potential of using stress to induce production of HSPs to increase resilience to disease and transport stress in aquaculture species (Roberts et al., 2010; Sung & MacRae, 2011; Sung et al., 2011). However, investigation into cross-tolerance for heat stress resilience as a mitigation tool in the face of climate change has not occurred. Heat stress is of significant concern as it is already impacting global aquaculture (Eliason et al., 2013; Salinger et al., 2019; Wade et al., 2019). Therefore, investigation into cross-tolerance as a protective tool against heat stress is of great value to industry.

Inducing cross-tolerance requires moderate, controlled exposure to a priming stressor (e.g., hypoxia) that shares a protective mechanism with the target stressor (e.g., heat stress) (Rodgers & Gomez Isaza, 2021; Sinclair et al., 2013; Todgham et al., 2005). Aquaculture standard practice already involves numerous stressors, such as crowding and hypoxia, as cultured species undergo netting, transportation, sampling, and harvesting (Brydges et al., 2009; Eissa & Wang, 2016; Wagner & Driscoll, 1994). Additionally, a significant portion of global aquaculture utilises closed systems where water parameters can be easily manipulated (Costa-Pierce et al., 2005; Otoshi et al., 2003; Terjesen et al., 2013). Inducing stressors already related to current aquaculture practice would require minimal monetary cost as no new infrastructure is required. Cross-tolerance could provide an economically viable and accessible tool for resilience to any unavoidable heat stress if a shared protective mechanism could be identified in existing production stress.

Measuring cross-tolerance to elevated temperatures

In cross-tolerance trials, exposure to a priming stressor occurs followed by a recovery period and subsequent exposure to a secondary stressor. For example, the effects of a priming stressor on the heat tolerance of an organism can be measured using critical thermal maximum (CT_{max}) as a measurement of upper thermal limits. CT_{max} describes the temperature that an animal loses locomotion or normal function (Burleson & Silva, 2011; Pörtner et al., 2017). In fish, CT_{max} is expressed as a loss of equilibrium (LOE) and serves as a proxy for 'ecological death' because it is indicative of the inability to move away from threats, or keep oneself alive in natural ecosystems (Becker & Genoway, 1979; Beitinger et al., 2000). Testing CT_{max} is typically achieved through organism exposure to incrementally increasing temperatures in a controlled environment. Once LOE is reached, the individual is immediately removed and returned to optimal conditions (Morgan et al., 2018). This process is typically non-lethal as organisms are removed from high temperature conditions immediately upon LOE and can fully recover from this state. Mortalities can occasionally arise due to confounding factors, such as additional treatments (Åsheim et al., 2020). A previously used marker of thermal tolerance in ectotherms was the temperature at which 50% mortality was observed (LT_{50}). Not only did this result in consistent mass mortality of study individuals, but was susceptible to influence from study design and exposure duration (Pörtner et al., 2017). Consequently, CT_{max} is commonly used to measure upper thermal limits.

The CT_{max} methodology can be performed before or after exposure to various conditions in order to identify how those conditions affect the thermal tolerance of the organism. An increase in CT_{max} is indicative of an increase in heat tolerance and is therefore a useful tool to assess if others stressors confer cross-tolerance to heat, especially in fishes. These tests assess whether an effect occurs, but do not provide insight as to the protective mechanisms (e.g., heat shock protein expression) being used (Becker & Genoway, 1979; Burleson & Silva, 2011; Pörtner et al., 2017).

The severity of the priming stressor is fundamental in determining whether cross-tolerance will result (Agathokleous, 2022; Rodgers & Gomez Isaza, 2021; Todgham et al., 2005). A study investigating cross-tolerance between heat and osmotic stress in tidepool sculpins (*Oligocottus maculosus*) found that heat shock of +12°C above ambient temperature induced cross-tolerance to subsequent osmotic shock (Todgham et al., 2005). However, variation of only ± 3°C in the heat shock treatment did not confer cross-tolerance. A lower 10°C shock had no effect on osmotic stress tolerance, whilst a 15°C shock conferred cross-susceptibility instead. Mild stress may be insufficient to trigger the necessary protective mechanisms for cross-tolerance to occur, whilst severe stress may incur additional metabolic costs overriding the potential physiological protection (Agathokleous, 2022; Schreck, 2010). Unlike cross-tolerance, the mechanisms by which cross-susceptibility occurs have not been explicitly explored in the literature.

Similarly, the recovery time between exposure to the priming and second stressors can also be critical in determining the magnitude or duration of cross-tolerance (Rodgers & Gomez Isaza, 2021). The same study by Todgham et al. (2005) found that the recovery period determined whether cross-tolerance would develop. Specifically, heat shock improved subsequent osmotic stress tolerance above controls, but only after a ≥ 6 h recovery period. Recovery periods less than 6 h resulted in reduced osmotic tolerance (i.e., cross-susceptibility). A recovery period of 24 h had the best effect, reaching 100% survival, whereas the control groups were approximately 50%. Sufficient recovery period may be necessary for the related protective mechanisms to be expressed.

The recovery period contributes to the practical applicability of cross-tolerance as a resilience tool. If a significant benefit can be derived from an extended recovery period, then a window of opportunity can be identified. Prior to a forecasted weather event, such as a heat wave, fish could be exposed to a priming stressor in preparation. For example, following exposure to a priming stressor, animals may require a 72-h recovery period to confer cross-tolerance benefits to an oncoming heatwave. Aquaculture farms could use this knowledge and prime fish with a sublethal dose of a stressor prior to a forecasted heatwave, knowing that within 72 h the fish will be better prepared for oncoming thermal stress. This could improve resilience to disease or mortality in the face of an incoming heatwave and provide security to sectors of the aquaculture industry. Heatwaves can be forecasted with increasing accuracy in NZ thanks to initiatives such as the Moana Project (https://www.moanaproject.org/) which began in

2018 (O'Callaghan et al., 2019). Accurate forecasting provides scope for tools such as cross-tolerance to be utilised effectively.

In addition to delayed cross-tolerance from recovery period, the duration of cross-tolerance may impact the applicability as a resilience tool. CT_{max} trials are repeatable on individual fish without negative consequences (Morgan et al., 2018), and research could investigate both the magnitude and duration of benefit for individual fish by completing multiple CT_{max} trials at various durations post exposure. If the maximum attainable duration of benefit is less than 24 hours the applicability in an aquaculture setting may be limited. Heat waves by definition span multiple days (Anderson & Bell, 2009; Oliver et al., 2018; Perkins & Alexander, 2013; Robinson, 2001), so cultured animals are likely to experience chronic exposure to heat stress during these events. However, if an optimum recovery period can be identified and the duration of effect is sufficient, there may be scope for cross-tolerance to be applied as a resilience tool.

Study species: Chinook salmon

Chinook salmon (*Oncorhynchus tshawytscha*) is a commercially valuable aquaculture species in NZ that has already been affected by global warming (Salinger et al., 2019). The NZ aquaculture industry employs over 3000 people, and salmon farming alone generates over 250 million dollars (NZD) in annual domestic and export revenue (Aquaculture New Zealand, 2020). The NZ government aims to make NZ aquaculture a three billion dollar industry by the year 2035 (New Zealand Government). Salmonids are stenothermic, meaning they have a narrow temperature range compared to other eurythermic fish species (Becker & Genoway, 1979). Salmonids are considered to be at high risk from climate change as they have low tolerance to thermal and oxidative stress coupled with a limited ability to acclimate (Anttila et al., 2013; Del Rio et al., 2019). Additionally, early life stages such as eggs and alevins are again more vulnerable to thermal stress. The effect of high temperature and hypoxia on the early-life stages of Chinook salmon is known to reduce the hatching success and growth of juveniles (Del Rio et al., 2019). Thermal limitation in salmon is thought to be linked to cardiorespiratory function and limitation (Eliason et al., 2013). Mass mortality events in NZ salmon farms have already occurred due to climate change. The summer of 2017/2018 saw a heatwave resulting in considerable salmon stock mortality in the Marlborough sounds (Salinger et al., 2019). Consequently, there is a vested interest in developing treatments and tools for improving heat tolerance in salmon. Research on cross-tolerance in Chinook salmon or its potential to increase thermal tolerance is limited to a single study on pre-swim life stages where cross-tolerance was achieved at the expense of growth and survivability (Del Rio et al., 2019). Chinook salmon were selected for investigation in this experiment due to their significance as an aquaculture species, their vulnerability to heat stress, and the existing knowledge gaps relating to cross-tolerance.

Thesis aims and structure

This thesis comprises of three experimental chapters (Chapters 2 - 4), which are written as independent manuscripts containing the following sections: introduction, materials and methods, results and discussion, followed by a General Discussion (Chapter 5). Chapter 3 is in preparation for submission to the *Journal of Fish Biology*. All experiments complied with The University of Canterbury's animal ethics requirements (Ref: 2020/07R). The aim of this thesis was to investigate cross-tolerance in juvenile Chinook salmon, with the primary goal of finding resilience tools against heat stress for the NZ aquaculture industry. It is unknown which priming stressors confer cross-tolerance to heat stress in Chinook salmon, so two priming stressors related to standard aquaculture practice were investigated in this thesis, crowding (Chapter 2) and hypoxia (Chapter 3). These stressors were selected for investigation owing to their shared physiological stress responses with heat stress (e.g., HSP production or cardiovascular improvements) (Berry & López-Martínez, 2020; Brijs et al., 2018; Burleson & Silva, 2011; Del Rio et al., 2019; Jensen & Benfey, 2022; Jung et al., 2020; Leeuwis et al., 2021; Morgenroth et al., 2021; Naderi et al., 2018; Todgham et al., 2005), and their achievability in standard aquaculture practices (see chapters 2 and 3).

The first experimental chapter (Chapter 2) investigated whether crowding stress confers cross-tolerance to heat stress. Crowding could easily be implemented as a resilience tool in aquaculture as standard farm practice already involves crowding of animals in fish transportation, health sampling, and harvest (Brydges et al., 2009; Eissa & Wang, 2016; Wagner & Driscoll, 1994; Warren-Myers et al., 2021). Implementation would require no additional infrastructure or alteration of standard practice, therefore being independent from monetary means and ubiquitously achievable throughout global aquaculture. Crowding stress is associated with stress responses related to improving cardiovascular function in fish under stress conditions, such as elevated blood glucose (Caipang et al., 2009; Chebaani et al., 2014; Hemre & Krogdahl, 1996; Ortuño et al., 2001). Similar stress responses can be expressed by fish under heat stress (Chebaani et al., 2014; Dengiz Balta et al., 2017; Dettleff et al., 2020; Zaragoza et al., 2008), suggesting that some protective mechanisms are shared between the two stressors. Therefore, it was predicted that crowding stress would increase heat tolerance in Chinook salmon. CT_{max} was used as a proxy for heat tolerance when investigating the effects of crowding stress.

Research questions:

(1) Does crowding stress confer cross-tolerance to heat stress in Chinook salmon?

(2) How does crowding stress severity impact resilience to heat stress in Chinook salmon?

(3) Does body mass affect cross-tolerance between crowding and heat stress in Chinook salmon?

The second experimental chapter (Chapter 3) examined whether hypoxia can be used as a priming stressor to improve heat tolerance in Chinook salmon. Cross-tolerance between hypoxia and temperature stress in fish is well documented (Berry & López-Martínez, 2020; Burleson & Silva, 2011; Del Rio et al., 2019; Jensen & Benfey, 2022; Todgham et al., 2005) and has been attributed to protective physiological mechanisms, such as increased HSP production (Berry & López-Martínez, 2020; Burleson & Silva, 2011; Todgham et al., 2005), cell wall alterations (Berry & López-Martínez, 2020), improved ventilatory capacity, and cardiovascular performance (Burleson & Silva, 2011; Del Rio et al., 2019; Jensen & Benfey, 2022; Morgenroth et al., 2021). Although there is a clear physiological link between hypoxia and heat stress, more information is required to fully understand how cross-tolerance can be achieved. Cross-tolerance research has shown that recovery period, stressor severity (magnitude and duration), species, and life stage are fundamental in determining whether cross-tolerance will be observed (Del Rio et al., 2019; Lu et al., 2019; McArley et al., 2020; Rodgers & Gomez Isaza, 2021; Todgham et al., 2005). However, studies rarely assess all of these variables, leaving significant knowledge gaps. Cross-tolerance research specific to Chinook salmon has only been completed on pre-swim life stages (Del Rio et al., 2019) leaving knowledge gaps about the effect in older life stages. For this reason, this study investigated the effect of hypoxia on heat stress in Chinook salmon smolts. Priming stressor severity, recovery period, and the duration of effect were also assessed as we do not yet know the ideal conditions for eliciting cross-tolerance. CT_{max} was the proxy for thermal tolerance following hypoxia stress exposure.

Another significant factor affecting the applicability of cross-tolerance as a resilience tool is the effect on fish growth. Whilst many studies have identified cross-tolerance in fish, very few have investigated the effect on fish growth following exposure to each stressor (Burleson & Silva, 2011; Jensen & Benfey, 2022; Opinion et al., 2021; Todgham et al., 2005). It is well documented that both hypoxia and heat stress can impact growth and survivability in fish, including salmonids (Aksakal & Ekinci, 2021; Chabot & Dutil, 1999; Dash et al., 2021; Del Rio et al., 2019; Handeland et al., 2008; Li et al., 2021; Remen et al., 2012; Vikeså et al., 2017; Wade et al., 2019; Yang et al., 2013; Zhu et al., 2019). Reduction in growth would impact productivity of aquaculture farms and would therefore limit the applicability of using cross-tolerance as a tool in production settings. Investigation into consequences for growth is fundamental to understanding cross-tolerance as a protective tool.

Research questions:

(1) Can hypoxia priming confer cross-tolerance to heat stress in Chinook salmon smolts?
(2) How does the severity of hypoxia priming affect resilience to heat stress?
(3) How does recovery period affect the development of cross-tolerance?
(4) How long do the effects of hypoxia priming last?
(5) Does hypoxia priming affect the growth of Chinook salmon?

The final experimental chapter (Chapter 4) surveyed individuals within the NZ and Australian aquaculture industries to gain an understanding of their perceptions of climate change, how their jobs might be affected by warming temperatures, and their main concerns regarding to global climate change. A significant research gap exists in understanding the opinions and perceptions of the Australian and NZ aquaculture industries in relating to the threats of climate change. Only two studies have assessed the perceptions of the Australian aquaculture industry in relation to climate change and both were limited to a small pool of expert industry representatives (Fleming et al., 2014; Lim-Camacho et al., 2015). No studies have assessed the perceptions of the NZ aquaculture industry on climate change threats and what they could mean for their industry sectors. This survey aimed to bring these research gaps by discovering what industry needs from climate change researchers to direct future research, what the primary areas of concern for industry are, and assess if a dissonance exists between industry and researchers and their understanding of climate change.

Research questions:

(1) Do individuals within the Australian and NZ aquaculture industries believe they will be impacted by climate change?
(2) Which aspect of climate change (e.g., extreme weather events, ocean warming, hypoxia, etc) is of most concern to the Australian and NZ aquaculture industries?
(3) Do perceptions and understanding of climate change threats vary between roles within the Australian and NZ aquaculture industries?
(4) Are the Australian and NZ aquaculture industries already seeing the effects of climate change?
(5) Do the Australian and NZ aquaculture industries want stress resilience tools and how likely are they to be implemented?

Chapter Two – The effect of crowding stress on heat tolerance in juvenile Chinook salmon

Introduction

Crowding of fish regularly occurs in standard aquaculture practice (Barcellos et al., 2004; Eissa & Wang, 2016; Hemre & Krogdahl, 1996; Hjelmstedt et al., 2021; Portz et al., 2006; Wagner & Driscoll, 1994). Research has primarily focused on assessing the effects of chronic (long-term) crowding stress associated with high stocking densities aimed at maximising farm outputs (Delfosse et al., 2021; Ellis et al., 2002; Liu et al., 2019; Liu et al., 2018; Montero et al., 1999; Ni et al., 2014; Onxayvieng et al., 2021; Oppedal et al., 2011; Rebl et al., 2020; Rowland et al., 2006; Sadhu et al., 2014; Treasurer et al., 2011). High stocking density can have adverse effects on animal feeding rates, growth, body condition, disease susceptibility, parasitic loading, intra-specific aggression, water quality, and oxygen (O_2) availability (Delfosse et al., 2021; Ellis et al., 2002; Liu et al., 2018; Montero et al., 1999; Portz et al., 2006; Rowland et al., 2006; Sadhu et al., 2014; Valenzuela et al., 2020). However, fish may also experience forms of temporary or acute (short duration) crowding during harvest, netting, transportation, or sampling (Barcellos et al., 2004; Eissa & Wang, 2016; Hjelmstedt et al., 2021; Skjervold et al., 2001; Warren-Myers et al., 2021). The adverse effects of acute crowding can be significant, particularly when the stressor is severe. For example, acute crowding stress involving netting and air exposure of multiple fish can cause increased parasitic loading (Delfosse et al., 2021).

Acute crowding stress can elicit immediate, sublethal, and long-lasting stress responses in fish for over 24 h, such as spikes in blood glucose (Caipang et al., 2009; Chebaani et al., 2014; Hemre & Krogdahl, 1996; Ortuño et al., 2001) and cortisol levels (Chebaani et al., 2014; Delfosse et al., 2021; Hemre & Krogdahl, 1996; Ortuño et al., 2001), reduced haematocrit, and elevated plasma lactate concentrations (Martins et al., 2018). There is a clear metabolic response to crowding stress similar to that of other stressors. Elevated blood glucose level is a response to increased metabolic activity indicative of physiological adaptive alterations (Hvas et al., 2018; Zaragoza et al., 2008), typically a shift from aerobic to anaerobic respiration (Naderi et al., 2018). Glucose is released from storage within the liver and tissues in order to meet the energy demands of heightened respiration under stress (Hvas et al., 2018; Zhang et al., 2017; Zhao et al., 2020). The increase in blood glucose from crowding indicates that there is an increased metabolic and respiratory demand whilst under crowding stress, as is the case with other stressors related to cardiorespiratory function. Spikes in blood glucose have been observed in fish under heat stress (Chebaani et al., 2014; Dengiz Balta et al., 2017; Dettleff et al., 2020; Zaragoza et al., 2008) and thermal tolerance is thought to be limited by cardiorespiratory capacity (Eliason et al., 2013). Other stressors such as exercise and hypoxia relate strongly to cardiorespiratory function and can also result in heightened blood plasma glucose level (Choi & Weber, 2015; Huang et al., 2015; Hvas et al., 2018; Sun et al., 2020). In addition to increased blood glucose, there are other physiological protective mechanisms induced by various stressors (e.g. hypoxia) related to increasing cardiovascular and cardiorespiratory capacity, including increased haemoglobin production (Del Rio et al., 2019; Giordano et al., 2021; Guan et al., 2011), cellular and morphological gill alteration (Fiorelini Pereira et al., 2017; Huang et al., 2015; Mohamad et al., 2021; Sinha et al., 2014), and altered blood pressure (Micheli-Campbell et al., 2009). Crowding may elicit similar cardiovascular and cardiorespiratory protective responses that could confer resilience to other related stressors (cross-tolerance). The shared protective mechanisms between multiple stressors, such as heat and hypoxia, indicate that cross-tolerance may be attainable through crowding stress. In rainbow trout (*Oncorhynchus mykiss*), crowding during standard aquaculture practice increased fish heart rate (Brijs et al., 2018), indicative of a metabolic increase. Additionally, rainbow trout exhibited changes in protein abundance (e.g. upregulated glycolysis-related and fatty acid binding proteins) under acute crowding stress indicative of a shift from aerobic to anaerobic metabolism (Naderi et al., 2018).

In practice, crowding has the potential to expose fish to multiple stressors simultaneously. Crowding events in aquaculture such as netting can involve additional stressors, such as hypoxia. Netting can involve the crowding of fish with nets within their normal pens or tanks. Fish being netted are exposed to hypoxia relative to the school density and netting duration (Handegard et al., 2017; Tenningen et al., 2012). Crowding and hypoxia share additional protective mechanisms in fish. For instance, in Amur sturgeon (*Acipenser schrenckii*), both crowding and hypoxia elicit upregulation of oxidation gene expression in the organs involved in immune response (e.g. kidney and liver) (Ni et al., 2014). Hypoxia shares numerous known protective mechanisms with heat stress relating to cardiovascular performance (see Chapter 3) and crowding may induce some of these responses through hypoxia. Therefore, hypoxia must be controlled for in experimental methodology to avoid confounding crowding stress effects. Exposure to multiple stressors can exacerbate alter stress responses and should be accounted for in climate change research (Todgham & Stillman, 2013). This is true for cross-tolerance experiments, as minor changes in the severity and magnitude of priming stressors can change whether or not cross-tolerance to other stress is developed (Rodgers & Gomez Isaza, 2021; Todgham et al., 2005). Cross-tolerance as a protective tool would require precise information on stressor severity and duration, so preliminary research for this purpose should aim to avoid conflating stressor interactions. If cross-tolerance is identified, further research should be undertaken into practical methodology and related stressors in practice.

There is the potential for crowding to confer cross-tolerance to heat stress if the correct stressor severity can be identified. Handling has been found to confer cross-tolerance to disease in gibel carp (*Carassius auratus gibelio*) (Yang et al., 2015). Specifically, repeat handling stress resulted in an increased antioxidant function and immune response, increasing the disease resilience of the fish under thermal stress compared to unhandled controls (Yang et al., 2015). Handling often occurs in relation to crowding, such as during netting or transportation (Barcellos et al., 2004). The handling stress in the study on gibel carp was relatively severe as it involved air exposure, which is known to be more stressful to fish than submerged crowding (Brydges et al., 2009). Although cross-tolerance has been observed in response to handling in carp, variation in stress response between species can be significant. For example, some species will experience increased heart rate in response to stress (tachycardia), whilst others lower their heart rate in response to similar stress (bradycardia) (Micheli-Campbell et al., 2009). At present, no research has investigated cross-tolerance between crowding stress and heat shock in teleosts, although there are numerous studies investigating the physiological consequences of crowding in aquaculture species, such as salmonids (Delfosse et al., 2021; Djordjevic et al., 2021; Ellis et al., 2002; Erikson et al., 2016; Naderi et al., 2018). Salmonids are stenothermic and are therefore vulnerable to heat stress (Anttila et al., 2013; Becker & Genoway, 1979; Del Rio et al., 2019). Salmonid stress responses to crowding have the potential to confer cross-tolerance to heat stress, potentially providing a resilience tool against heat waves for salmonid aquaculture.

As crowding is already a significant component of standard aquaculture practice, no new infrastructure would be required for its implementation as a protective tool should cross-tolerance be identified. Consequently, heightened heat tolerance conferred through crowding could be achieved ubiquitously throughout international industry, independent of monetary means. Crowding has the potential to provide an economically viable resilience tool for international fish aquaculture.

This study aims to investigate the sublethal effects of moderate, acute crowding stress, such that might occur throughout the rearing process during husbandry procedures such as netting (Barcellos et al., 2004; Brydges et al., 2009) and transportation (Barcellos et al., 2004; Wagner & Driscoll, 1994). This research aimed to investigate whether mild crowding stress could confer cross-tolerance to heat stress in Chinook salmon smolts as a resilience tool for aquaculture. Specifically, we aimed to address the following research questions:

(1) Does crowding stress confer cross-tolerance to heat stress in Chinook salmon?

(2) How does crowding stress severity impact resilience to heat stress in Chinook salmon?

It was hypothesised that (1), crowding stress would cause an increase in heat tolerance (CT_{max}) as it likely shares protective mechanisms relating to cardiovascular performance by which heat tolerance is limited, and (2), higher crowding stress severity would result in a stronger stress response, increasing the likelihood that cross-tolerance would be expressed.

Methods

Animal maintenance

All experimental methods complied with the New Zealand (NZ) Animal welfare act (1999) and were approved by the University of Canterbury's animal ethics committee (Ref: 2020/07R). Juvenile Chinook salmon (*Oncorhynchus tshawytscha*; single sex female smolts) were sourced from the New Zealand King Salmon Tentburn hatchery (Canterbury, NZ) and transported to the University of Canterbury (Christchurch, NZ). Fish were housed in three aerated ~60 litre holding tanks (maximum n = 10 / tank, ~9.1 kg / m^{-3}) in a ~2,200 litre closed artesian well water aquaria at 14 ± 0.5 °C with a 12 h light:12 h dark photoperiod. System filtration included a biofilter, sand filter, mechanical filtration via catchment nets, and water changes as required. Fish were housed for ~7 months prior to experimentation and were fed daily with commercial pellets (BioMar, 2 mm) at hatchery recommended rates (1% body mass / day).

Experimental design

There was a total of 3 treatment groups (control, mild crowding, and moderate crowding) of 6 – 9 individuals (Table 1). Each group underwent their respective crowding treatment, followed by a heat tolerance test 24 h later. Heat tolerance was measured using the critical thermal maximum (CT_{max}). All groups underwent the same CT_{max} trials and husbandry, but the priming stressor (crowding) differed in duration between treatments. All treatment groups individually underwent transfers to a crowding tank followed by a 30-minute adjustment period. Mild crowding consisted of crowding by lowering the water level for 10 minutes, and moderate crowding for 20 minutes, before being returned to their holding tanks. The control group received the same handling as the other groups and were moved to the crowding tank but were not crowded (water level not lowered). Experimental crowding has previously been achieved by lowering water level (Djordjevic et al., 2021; Hosseini & Hoseini, 2012; Naderi et al., 2018), using crowding structures in tanks (Anders et al., 2020), or transferring to smaller tanks (Silva-Brito et al., 2020). However, use of crowding structures and transfers typically involves additional netting or lowering of water level (Anders et al., 2020; Silva-Brito et al., 2020). In this experiment, lowered water level was used as a crowding treatment for ease of implementation and to limit multi-stressor exposure. All groups had a recovery period of 24 ± 2 h between their crowding treatment and their CT_{max} trial. Sample size was lower (moderate crowding, N = 6) in one treatment group as a result of oversized fish escaping during the heat tolerance trial.

Crowding treatment

Fish were transferred to a crowding tank where the water level could be rapidly lowered through the movement of a standpipe. Whilst full, the fish were in ~15 cm (~28 litres) of water (mean density: ~19.1 kg / m^{-3}). All treatment groups were maintained at this level for a 30-min adjustment period immediately following transfer and prior to crowding. When crowded, the water was lowered to ~6 cm (~11 litres), (mean density: ~47.8 kg / m^{-3}) a point at which dorsal fins frequently brushed the water's surface. Whilst crowded, fish had to actively avoid collisions with each other, but swimming remained free. The lowered water level was maintained for the treatment duration (10 or 20 min). Water was replaced at a rate of 3.6 L / min in order to maintain dissolved oxygen levels and avoid waste build up. A lid was placed on the crowding tank to avoid stress from observation for the duration of the crowding treatment. After the crowding treatment was complete, fish were immediately transferred back into their holding tanks to be fasted and begin their 24 h recovery period prior to heat tolerance being measured.

Heat tolerance - Critical thermal maximum trials

To determine if crowding (priming stressor) increases heat tolerance in juvenile chinook salmon, CT_{max} was measured in all treatment groups. At the conclusion of their recovery period (24 ± 2 h) following crowding, fasted (24 h fasted) fish were transferred into an aerated and custom-built waterbath (dimensions: 52 × 42 × 29 cm, A1 Figure 1) at the same temperature as their holding tanks (~13.5°C). Fish were divided evenly between two waterbaths in order to avoid additional crowding stress (mean density: ~8.6 kg / m^{-3}). After a 30-min adjustment period, temperature was increased by 0.3 ± 0.04 °C / min (recommended rate as per (Becker & Genoway, 1979; Morgan et al., 2018)) using combinations of submersible heaters (Grant T-series Heated Circulator, Shepreth, Cambridgeshire; Jebo 200w submersible heater, China). Temperature was constantly monitored using a digital thermometer (Fluke 51 II Handheld Digital Probe Thermometer, WA, USA, 0.05% + 0.3 °C). CT_{max} was identified as the temperature at which equilibrium was lost (inability to right themselves in the water column) as per recommendations by Becker and Genoway (1979). Once loss of equilibrium (LOE) was observed, each individual was immediately placed in an aerated recovery tub (~13.5°C, A1 Figure 2.), and the CT_{max} temperature was recorded. All fish remained in individual recovery tubs for ≥1 hour prior to being anaesthetised for body mass and length measurements. Post- CT_{max} survival was 100% across all treatment groups.

Statistical Analyses

Statistical analyses were carried out using RStudio (version 3.6.3; http://www.R-project.org/)) using the nlme (linear and non-linear mixed effects models; https://CRAN.R-project.org/package=nlme) package. A linear mixed effect models (LME) was used to determine the effect of crowding priming (three-level fixed factor) on CT_{max}. CT_{max} waterbath was included as a random effect, and fish body mass was included as a covariate. Statistical significance was accepted at $P < 0.05$.

Table 1. The crowding treatments, number of fish (replicates), and body mass (mean ± standard error, g) in each experimental group. TG1 received the same handling as the others, but were not crowded (water level remained high). TG2 and TG3 were crowded for 10 and 20 minutes respectively, with exposure time equating to treatment severity. Only the variables described differed, all other variables remained uniform across all groups. Low replicates in TG3 were a result of escapees during the experiment.

Treatment Groups	**Treatment Description**	**Number of fish**	**Body Mass (g)**
TG1	Control – handled, no crowding,	9	61.4 ± 9.6
TG2	Mild Crowding – 10 minutes	9	63.5 ± 9.1
TG3	Moderate Crowding – 20 minutes	6	51.7 ± 16.4
	Total number of fish	24	

Results

Effect of crowding priming stressor on CT_{max}

There was no effect of either mild (10 min) or medium (20 min) crowding exposure on CT_{max} in Chinook salmon smolts after a 24 h recovery period ($F_{2,19}$ = 0.10, *P* = 0.90, lme; figure 2). CT_{max} was independent of fish body mass ($F_{1,19}$ = 0.01, *P* = 0.92, lme).

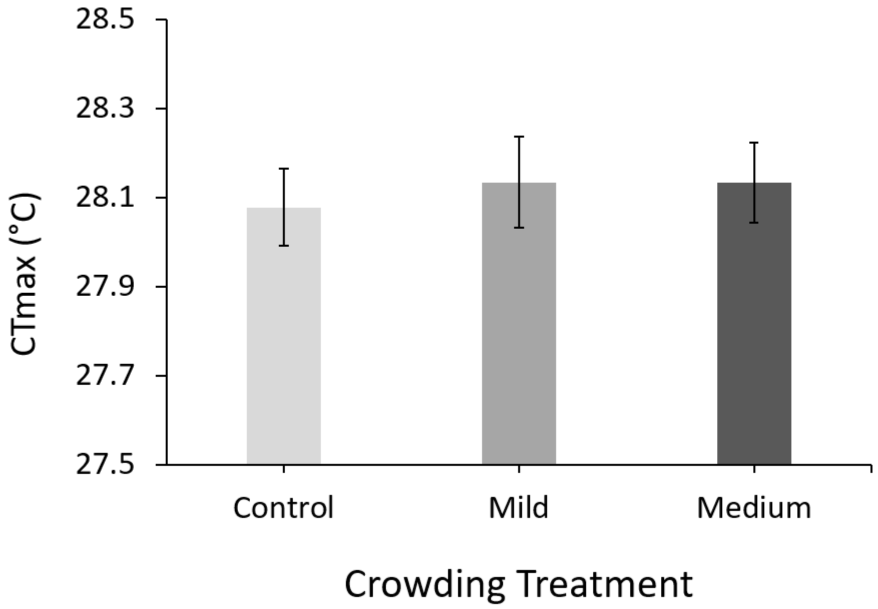


Figure 2. Effect of crowding as a priming stressor on heat tolerance of juvenile Chinook salmon (*Oncorhynchus tshawytscha*). No significant effect of mild (10 min) or medium (20 min) crowding was observed ($F_{2,19}$ = 0.10, *P* = 0.90, lme). Values are shown as mean ± s.e.m.

Discussion

The effect of crowding on heat tolerance

No effect of crowding on heat tolerance in Chinook salmon smolts was observed in this study. Neither the mild (10-min exposure to ~47.8 kg / m^{-3} density) or medium (20-min exposure to ~47.8 kg / m^{-3} density) treatments differed in CT_{max} from the controls (figure 2), rejecting the hypothesis that crowding would induce cross-tolerance to heat stress. It is possible that the lack of effect is due to the crowding densities being too mild to trigger the necessary stress response, as correct stressor severity is known to be fundamental to achieving cross-tolerance (Rodgers & Gomez Isaza, 2021). There is variation between studies about what is considered "low" and "high" severity density, usually in relation to crowding duration. In a study on largemouth bass (*Micropterus salmoides*), low density was set as 0.46 kg / m^{-3}, whilst high density was only 1.95 kg / m^{-3} (Jia et al., 2022). In gibel carp (*Carassius gibelio*), low and high density was set at 1.47 kg / m^{-3} and 10.85 kg / m^{-3} respectively (Onxayvieng et al., 2021), still substantially lower than the density used in this study (~47.8 kg / m^{-3}). However, both of these studies exposed fish to sustained crowding densities for up to 60 days. Alternatively, acute crowding stress studies used significantly higher densities. A study on acute crowding stress in rainbow trout (*Oncorhynchus mykiss*) used a density of 200 kg / m^{-3} for a 45 min duration (Naderi et al., 2018). Both the stressor severity and duration were more than double what was used as moderate crowding stress in this study. It is clear that the severity and duration of crowding stress used in this study were insufficient to trigger either positive or negative effects on heat tolerance. Additionally, CT_{max} was tested 24 h after crowding in this study. Some stress responses, such as the production of HSPs, are short-lived (DuBeau et al., 1998). It could be that a mild stress response did occur, but that the mild severity was insufficient to produce long-lasting physiological responses.

It should be noted that in this study fish were crowded to ~47.8 kg / m^{-3} without confounding stressors. Water quality and oxygenation was maintained throughout the crowding treatments so we could isolate the effect of crowding. Studies investigating stocking density in aquaculture often assess impacts in-situ, where fish are exposed to other stressors simultaneously (Oppedal et al., 2011). Confounding stressors may explain why deleterious effects can be seen at lower stocking densities in previous investigations (Oppedal et al., 2011; Treasurer et al., 2011). In practice, crowding is typically accompanied by handling, netting, or other treatments with their own associated stress responses (King, 2009; Rotllant & Tort, 1997). Crowding as an isolated stressor may require significantly higher severity to elicit a stress response, which may explain why no result was observed in this study. Additionally, the crowding in this experiment did not result in fish being exposed to air, struggling to swim upright, coming into regular physical contact with other fish, or rubbing against nets or walls. Air exposure in particular has been found to be significantly more stressful to some fish species than submerged crowding (Brydges et al., 2009).

Significance in aquaculture practice

Although no cross-tolerance was observed, neither was cross-susceptibility. Cross-susceptibility describes when exposure to a priming stress does not confer increased resilience to a secondary stressor, but instead decreases resilience (Todgham & Stillman, 2013). The lack of variation in CT_{max} indicated that the stocking densities and crowding duration used in this study have no lasting effects on the heat tolerance of Chinook salmon smolts. Optimal stocking density varies between species. A stocking density of 2 kg / m^{-3} was found to ameliorate aggressive behaviour between cod (*Gadus morhua*) conspecifics compared to hatchery stocking densities (up to 30–35 kg / m^{-3}) and low experiments densities (0.2 kg / m^{-3}) (Treasurer et al., 2011). In Atlantic salmon (*Salmo salar*) smolts, initial stocking densities of 5.6 ± 0.3 kg / m^{-3} were considered normal in sea pens, whilst 15.7 ± 0.5 kg / m^{-3} was considered high density (Oppedal et al., 2011). At stocking densities ≥ 20 kg / m^{-3} deleterious effects on welfare were observed. Of these examples, both species experienced negative consequences of stocking densities ≥ 20 kg / m^{-3}. The results of this study show that short-term exposure to densities as high as ~47.8 kg / m^{-3} has no effect on upper thermal tolerance in Chinook salmon. Further research is required to determine the duration this level of crowding could be sustained for without any effects, or what density is required for cross-tolerance or cross-susceptibility to be expressed. Further investigation could provide guidance on safe crowding densities for short-term production procedures.

Recommendations for future research

This study was a small pilot study designed to test methodology and complete a preliminary assessment of the potential for cross-tolerance to be conferred through crowding stress. This study determined that a crowding density of ~47.8 kg / m^{-3} for 20 min is insufficient to elicit a long-lasting physiological stress response in Chinook salmon smolts. Without a physiological stress response, neither cross-tolerance or cross-susceptibility can occur. However, the results of this study provide guidance for future research directions. Recommendations for future research are as follows:

(1) Investigation of numerous crowding densities (stressor severity). Identification of either positive or negative effects of a crowding stress response will provide further evidence of the possibility for cross-tolerance expression.

(2) Investigation of numerous crowding durations. Identifying thresholds for both acute and chronic crowding stress responses will provide guidance for both long-term and short-term crowding procedures in standard aquaculture practices.

(3) Comparison of crowding stress with and without additional stressor interactions (e.g., hypoxia and waste products). In aquaculture practice, crowding is likely to encompass numerous stressors and comparison with the effects of crowding in isolation will provide a more comprehensive understanding of thresholds for expression of protective mechanisms.

(4) Investigation of numerous crowding methods. Lowered water level was used to crowd in this experiment, but future studies could assess the differences in response to other crowding methods, such as netting.

(5) Investigation of how crowding stress affects fish growth. This study did not assess the effects of crowding stress on fish growth. The effect of stress on growth may determine whether industry would utilise a protective tool (see Chapter 4). Future studies should include growth measurements in order to accurately determine suitability as a potential resilience tool.

(6) Exploration of potential physiological mechanisms by which crowding stress may confer cross-tolerance in fish (e.g., HSP expression, cardiovascular remodelling, or improved energy storage).

Chapter Three – The effect of hypoxia stress on heat tolerance in juvenile Chinook salmon

Introduction

Global climate change is driving oceanic warming and hypoxic zone expansion and will expose aquatic ecosystems to increases in temperature extremes, subsequently affecting dissolved oxygen (O_2) availability (Bendtsen & Hansen, 2013; Breitburg et al., 2018; Keeling et al., 2009; Sage, 2020). As water temperatures increase, O_2 solubility and availability decreases. Consequently, the body temperature and concomitant metabolic demands of ectotherms increase in conjunction with increasing habitat temperatures and O_2 limitations (Farrell & Richards, 2009; Keeling et al., 2009; Meire et al., 2013; Pörtner Hans & Knust, 2007). Temperature and O_2 are considered two of the most important variables affecting the physiology and fitness of teleosts (Burleson & Silva, 2011). Temperature increases can be harmless to eurythermic species or life stages, or in areas where species reside distant from their optimal body temperature (T_{opt}). However, teleosts become vulnerable once specific environmental temperature thresholds beyond T_{opt} are exceeded (Pörtner & Peck, 2010). Increased body temperature is known to increase heart rate and metabolism (Burleson & Silva, 2011; Fry, 1971), which further increases O_2 demands (Nelson & Altieri, 2019). The combined effects of reduced O_2 solubility and increased O_2 demand exacerbates the effects of O_2 limitation in water. Increasing temperature drives an increase in hypoxia and therefore aquatic organisms are often exposed to both stressors. In aquaculture, the effects of climate change are already being observed. Sea surface temperatures (SST) are increasing in Australasia (Wijffels et al., 2018), and heat waves are already driving disease and mortality events in Australian and New Zealand (NZ) aquaculture species (Nguyen & Alfaro, 2020; Salinger et al., 2019; Wade et al., 2019).

Naturally occurring hypoxia events are widespread (Diaz & Rosenberg, 2008; Virtanen et al., 2019), and future expansion of hypoxic zones is likely to be gradual and broad in geographical scope. Oceanic hypoxic zone expansion typically results from ocean warming, increased thermal stratification, and the effects of anthropogenic nutrient pollutants from run-off (Breitburg et al., 2018; Gooday et al., 2009; Hou et al., 2020; Keeling et al., 2009; Mattiasen et al., 2020; Meire et al., 2013; Stramma et al., 2010).

Oceanic O_2 is predicted to decrease approximately 4% by 2090 (Bopp et al., 2013). This decrease will be gradual and generate chronic low intensity exposure to hypoxic conditions for many aquatic organisms. The sublethal effects of hypoxia on fish may be mitigated by species acclimation and adaptation, as seen in studies involving sustained stressor exposure (Burleson & Silva, 2011; Del Rio et al., 2019). Additionally, low level hypoxia exposure could increase resilience to temperature increases in some species through antagonistic or cross-tolerance responses, resulting in an overall increase in survival and resilience.

There is a physiological relationship between hypoxia and heat stress that has been explored in the literature (Berry & López-Martínez, 2020; Burleson & Silva, 2011; Del Rio et al., 2019; Jensen & Benfey, 2022; Jung et al., 2020; Leeuwis et al., 2021; Morgenroth et al., 2021; Todgham et al., 2005). Physiological resilience to both hypoxia and heat stress is linked to cardiovascular performance and aerobic capacity in fish (Jung et al., 2020; Leeuwis et al., 2021; Morgenroth et al., 2021), and many of the physiological mechanisms by which cross-tolerance could be achieved relate to increased cardiovascular capacity. Cross-tolerance refers to the increased stressor resilience conferred by exposure to a previous priming stressor (Sinclair et al., 2013; Todgham & Stillman, 2013). Both hypoxia and heat stress can induce increased expression of genes relating to production of globins (oxygen binding proteins), which correlate with thermal resilience (Giordano et al., 2021). Hypoxia is also known to cause an overall depression of energy metabolism (e.g., metabolic rate and supported functions such as heart rate), whilst temperature increases it (Burleson & Silva, 2011; Leeuwis et al., 2021), indicating that cardiovascular mechanisms may work in contradiction for varying resilience. In ectotherms, it has been hypothesised that thermal tolerance is limited by cardiorespiratory capacity (Eliason et al., 2013; Pörtner et al., 2017), further suggesting that there is potential that a protective mechanism may be shared between hypoxia and heat stress. A commonly observed stress response mechanism is the production of heat shock proteins (HSP). Multiple different stressors are associated with the upregulation of HSPs (e.g., hypoxia, heat, and osmotic stress), suggesting that they are associated with a generic stress response (Bowler, 2005; DuBeau et al., 1998; Malmendal et al., 2006; Roberts et al., 2010; Todgham et al., 2005). Resilience conferred by HSPs can be short-lived (DuBeau et al., 1998), therefore any cross-tolerance achieved through HSP mechanisms is likely to persist for only a short duration.

Recovery period is an important factor in determining whether cross-tolerance will occur (Rodgers & Gomez Isaza, 2021). Recovery period refers to the time between exposure to the priming stressor and exposure to the subsequent stressor. The delay in observable resilience can be referred to as delayed cross-protection (Rodgers & Gomez Isaza, 2021). Evidence of this delayed cross-protection has been seen in teleosts. For example, a study on tidepool sculpins (*Oligocottus maculosus*) found that heat stress increased resilience (i.e., higher survival rates) to subsequent osmotic shock, but only after a recovery period of ≥ 8 h (Todgham et al., 2005). Some protective mechanisms involve significant physiological refurbishment and are therefore not immediately evident. For example, cellular and morphological remodelling of the gills in fish can occur in response to many stressors, such as pollutant exposure, hypoxia, and temperature stress (Fiorelini Pereira et al., 2017; Huang et al., 2015; Mohamad et al., 2021; Sinha et al., 2014). Some of the slower remodelling can take days of exposure to occur (Sinha et al., 2014). Additionally, delayed cross-tolerance could be attributed to the time required for establishing associated cellular pathways for future stress responses (Todgham et al., 2005). It is appropriate that research investigating cross-tolerance also assesses the effect of recovery period, particularly when in the context of providing a protective tool for aquaculture. If insufficient time is allowed to observe the effect of stressor priming, inaccurate representation of resilience may occur. Additionally, if the observed cross-tolerance is short-lived, the benefits may be insufficient to increase resilience in an ecological context, where subsequent stressor exposure may be prolonged.

Evidence of cross-tolerance between hypoxia and heat stress has been observed in a number of teleosts (Berry & López-Martínez, 2020; Burleson & Silva, 2011; Del Rio et al., 2019; Jensen & Benfey, 2022; Todgham et al., 2005). Some of the observed cross-tolerance has been attributed to upregulation of HSPs (Todgham et al., 2005). However, cross-susceptibility between heat stress and hypoxia in teleosts has also been observed (McArley et al., 2020), leading to questions of inter-species variation in protective mechanisms. Cross-susceptibility describes where exposure to a priming stressor reduces resilience to a subsequent stressor (Todgham & Stillman, 2013). There is a significant knowledge gap around the potential for cross-tolerance between hypoxia and heat stress in Chinook salmon (*Oncorhynchus tshawytscha*). It remains unclear how hypoxia stress severity or subsequent recovery period may influence the expression of cross-tolerance to heat stress in fish. Additionally, in Chinook salmon the species-specific variations in physiological response to both hypoxia and heat stress are not well understood.

In Chinook salmon, only one cross-tolerance study has been completed and it was limited to pre-swim life stages (Del Rio et al., 2019). Stress treatments (e.g., hypoxia acclimation or cold shock) of embryonic or pre-swim life stages can have prolonged positive effects on stress tolerance in fish (Del Rio et al., 2019; Robinson et al., 2019). Prolonged improvement to heat stress resilience may require early life stage hypoxia treatment in order to trigger the necessary physiological changes. However, embryonic stress can result in decreased growth and survivability (Del Rio et al., 2019), a trade-off which industry has expressed concern over (see Chapter 4). Short duration resilience in the form of cross-tolerance in later life stages may be more readily implemented by industry where there is no impact on growth, as opposed to long-term increased resilience with a growth trade-off. Additionally, there is a knowledge gap around the potential for cross-tolerance in post-larval life stages in Chinook salmon. It is for this purpose that Chinook salmon smolts were selected to investigate the potential for cross-tolerance in this study, as opposed to earlier life stages.

Farmed fish are frequently exposed to hypoxia of varying degrees due to standard farming practices such as stocking, transportation, crowding, handling, and capture (Eissa & Wang, 2016). Oxygen availability in aquaculture farms can be reduced in comparison with naturally occurring O_2 levels due to high stocking densities, pen sizes, and water flow rates at farm sites (Oldham et al., 2019; Oldham et al., 2018). Oxygen saturation in sea pens has been found to reach as low as 30% Air saturation and display dramatic variation within a single pen (Solstorm et al., 2018). Fish in farms will attempt to avoid both hypoxia and elevated temperature conditions (Stehfest et al., 2017), but within the confines of a farm their escape behaviour is restricted, resulting in unavoidable stress exposure.

Chronic hypoxia exposure alters feeding behaviour in fish, resulting in stunted growth (Aksakal & Ekinci, 2021; Gamperl et al., 2020; Pichavant et al., 2000; Thorarensen et al., 2017; Vikeså et al., 2017).

Similar effects can be seen as a consequence of regular hypoxia shocks (20% Air saturation, 1 h / day, 5 d / week) (Person-Le Ruyet et al., 2003). Maintaining growth is of paramount importance to industry (see Chapter 4) and yet current farming practices, such as large sea cages and high stocking density, are resulting in frequent hypoxia exposure (Oldham et al., 2019; Oldham et al., 2018; Solstorm et al., 2018). The effect of acute hypoxia on fish growth rate is rarely investigated in the literature. Li et al. (2018) compared the effects of chronic and acute hypoxia on tilapia and assessed growth in relation to chronic exposure but not acute exposure. Thus, the impacts of acute hypoxia exposure on growth remain largely unknown.

The impact of both hypoxia and heat stress from climate change on farmed fish has the potential to become severe as conditions and weather extremes worsen. However, if cross-tolerance between hypoxia and temperature is occurring in farmed species, the outlook may be less dire than expected. Hypoxia was selected as a priming stressor to investigate the potential for cross-tolerance in Chinook salmon, as both hypoxia and heat stress are occurring in aquaculture and worsening due to climate change. Hypoxia is also an ideal candidate for potentially conferring cross-tolerance to heat stress due to their shared physiological pathways and responses (e.g., improved cardiovascular capacity (Jung et al., 2020; Leeuwis et al., 2021; Morgenroth et al., 2021)). This research aimed to assess whether mild hypoxia priming could induce cross-tolerance to heat stress in juvenile Chinook salmon.

Research questions and hypotheses were as follows:

(1) Can hypoxia priming confer cross-tolerance to heat stress in Chinook salmon smolts?

Hypothesis: Hypoxia priming will increase tolerance to subsequent heat stress.

(2) How does the severity of hypoxia priming affect resilience to heat stress?

Hypothesis: Priming hypoxia severity (Air saturation decrease) will correlate with fish performance under heat stress (CT_{max}), with higher severities resulting in greater improvements to heat tolerance.

(3) How does recovery period affect the development of cross-tolerance?

Hypothesis: A recovery period between hypoxia priming and heat stress (CT_{max}) will be required for cross-tolerance to be observed.

(4) How long do the effects of hypoxia priming last?

    Hypothesis: Observed cross-tolerance will be short-lived due to the ephemeral natures of common protective mechanisms, such as HSPs.

(5) Does hypoxia priming affect the growth of Chinook salmon?

    Hypothesis: Hypoxia priming will have a negative effect on fish growth.

Methods

Animal maintenance

All experimental methods complied with the New Zealand (NZ) Animal welfare act (1999) and were approved by the University of Canterbury's animal ethics committee (Ref: 2020/07R). Husbandry and maintenance methodology is similar to that of the crowding experiment (Chapter 2). Juvenile Chinook salmon (*Oncorhynchus tshawytscha*; single sex female smolts) were sourced from the NZ King Salmon Tentburn hatchery (Canterbury, NZ) and transported to the University of Canterbury (Christchurch, NZ). Fish were housed in 10 aerated, 60 litre holding tanks (n = 12 / tank) in a ~2,200 l closed artesian well water aquaria at 14 ± 0.5°C with a 12 h light:12 h dark photoperiod. System filtration included a biofilter, sand filter, mechanical filtration via catchment nets, and water changes as required. Fish were housed for ~4 months prior to experimentation and were fed daily with commercial pellets (BioMar, 2mm) at hatchery recommended rates (1% body mass / day).

Fish tagging

In order to track individuals, fish were tagged with visible implant elastomer (VIE, Northwest Marine Technology, Anacrotes, WA) tags. Fish were fasted 24 h prior to anaesthetisation to prevent regurgitation. Fasted fish were anaesthetised in buffered tricaine methanesulfonate (MS222; 50 mg / L, pH 7). Once anaesthetised, tags were inserted under the skin (as per manufacturer's instructions and Olsen and Vøllestad (2001)) with an insulin syringe mounted with a 29-gauge needle. Tags were made in one of six locations on the fish using one of two colours (red or orange). The tag locations were on either the left or right side of the fish in front of the dorsal fin, behind the dorsal fin, or on the side of the caudal peduncle (figure 3). This allowed for the identification of up to 12 individuals per tank/treatment group. Tag retention was 100% throughout the experiment.

Figure 3. Visible implant elastomer (VIE) tag locations used for the identification of individual fish. Orange bars represent approximate tag location and size. Individual fish only received a single tag. Red- or orange-coloured tags were placed on either the left or right side of the fish, enabling a total of 12 tag variations per treatment group.

The tags were externally visible through the translucent skin tissue when exposed to ultraviolet light. Tagging of individual fish was completed within 1 min after removal from anaesthetic. Afterwards, fish stayed in a recovery tank until they regained normal movements. They were then randomly allocated to treatment groups and sorted into the appropriate holding tanks. There was a 2-month recovery period after tagging and sorting prior to the experiment.

Hypoxia priming and recovery periods

Fish were exposed to one of three levels of hypoxia (40%, 60% or 100% [control, normoxia] Air saturation) for 2 h as a priming stressor and, after a set recovery period, heat tolerance was measured in the form of a critical thermal maximum (CT_{max}) trial (figure 4). Hypoxia exposure was limited to 2 h in order to test for cross-tolerance at durations that are not likely to impair growth rates. Nitrogen (N_2) gas was bubbled into a tank (dimensions: 35 x 35 x 50 cm; volume: ~60 l; water temperature: 13.5 ± 1 °C) to reduce the dissolved oxygen (DO) concentrations to target levels (± 5%). An oxygen probe (Pyroscience, FireSting, Ohio, USA) was used to monitor DO concentrations throughout the 2 h exposure period and the DO profiles were recorded using FireSting software.

Following hypoxia exposure, fish were returned to holding tanks for a recovery period. For each hypoxia treatment group there were two different recovery periods initially investigated (24 h or 72 h, table 2). CT_{max} was measured after the recovery period had concluded, and then again one week later to represent a second recovery period per treatment group (figure 4). CT_{max} can be repeated on the same individuals after one week without any effect on growth or survival (Morgan et al., 2018; O'Donnell et al., 2020), allowing for repeat CT_{max} measurements to be used to assess multiple recovery periods. Repeat measurements allowed for the assessment of four recovery periods in total (1, 3, 8, and 10 days), enabling examination of the longevity of any observed effects.

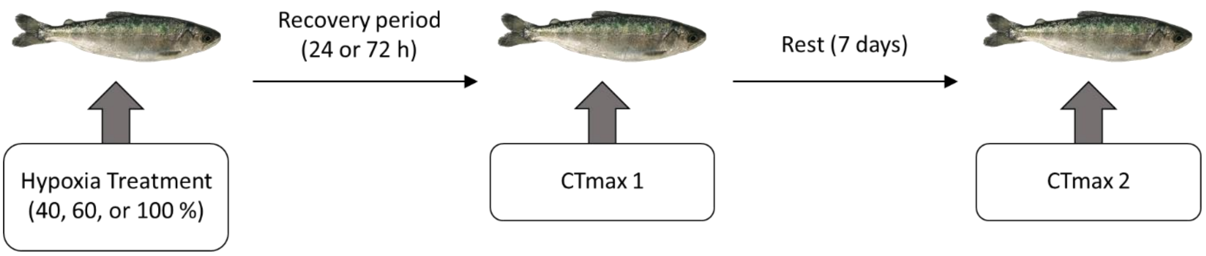


Figure 4. Experimental procedure for each fish from initial priming stressor exposure to final CT_{max} trial. Fish underwent repeat CT_{max} trials to expand the number of recovery periods. CT_{max} 1 was completed 24 or 72 h after hypoxia priming, representing the 1- and 3-day recovery periods. CT_{max} 2 was completed 1 week after CT_{max} 1, adding 7 days to recovery time since hypoxia priming, therefore representing the 8- and 10-day recovery periods.

Table 2. Overview of treatment groups and the number of fish (replicates, N) in each group for the experiment (40 ± 5% Air saturation represents medium hypoxia, 60 ± 5% Air saturation represents mild hypoxia, Normoxia = 100 ± 5% Air saturation). Recovery period describes time period between hypoxia exposure (hypoxia) and CT_{max} trials.

Treatment Groups	Treatment Description	Number of Fish	Mean ± SD Body Mass (g)
TG1	Handling Control – No handling, No hypoxia (Normoxia)	11	34.6 ± 13.1
TG2	Hypoxia Control – Normoxia (100 ± 5% saturation), 24 h recovery period	12	28.5 ± 11.7
TG3	Hypoxia Control – Normoxia (100 ± 5% saturation), 72 h recovery period	11	29.2 ± 7.5
TG4	Mild Hypoxia (60 ± 5% saturation), 24 h recovery period	12	29.2 ± 13.2
TG5	Mild Hypoxia (60 ± 5% saturation), 72 h recovery period	11	28.3 ± 9.6
TG6	Medium Hypoxia (40 ± 5% saturation), 24 h recovery period	12	33.8 ± 14.3
TG7	Medium Hypoxia (40 ± 5% saturation), 72 h recovery period	11	28.4 ± 9.2
	Total number of fish	84	

A handling control treatment was also included, where fish did not undergo any handling or hypoxia priming prior to their CT_{max} trial. Inclusion of a handling control group meant the effect of handling stress on CT_{max} could be determined. In total, there were seven treatment groups (N = 9 – 12 fish / treatment, Table 2). All hypoxia treatments resulted in 100% fish survival.

Heat tolerance - CT_{max} trials

To determine if hypoxia exposure (priming stressor) increases heat tolerance in juvenile Chinook salmon, CT_{max} was measured in all treatment groups. CT_{max} methodology was identical to that of the crowding experiment (Chapter 2, page 20).

At the conclusion of their recovery period (i.e., 1, 3, 8 or 10 days) following hypoxia exposure, fasted (24 h fasted) fish were transferred into an aerated and custom-built waterbath (dimensions: 52 × 42 × 29 cm, A1 Figure 1) at the same temperature as their holding tanks (~13.5 °C). Fish were divided evenly between two waterbaths in order to avoid additional crowding stress (density: ~0.24 kg / m^{-3}). After a 30-min adjustment period, temperature was increased by 0.3 ± 0.04 °C / min (recommended rate (Becker & Genoway, 1979; Morgan et al., 2018)) using combinations of submersible heaters (Grant T-series Heated Circulator, Shepreth, Cambridgeshire; Jebo 200 w submersible heater, China). Temperature was constantly monitored using a digital thermometer (Fluke 51 II Handheld Digital Probe Thermometer, WA, USA, 0.05 % + 0.3 °C). CT_{max} was identified as loss of equilibrium (LOE, inability to right themselves in the water column) as per recommendations by Becker and Genoway (1979). Once LOE was observed, each individual was immediately placed in an aerated recovery tub (~13.5 °C, A1 Figure 2) and the CT_{max} temperature recorded. All fish remained in individual recovery tubs for ≥1 h prior to being anaesthetised for body mass and length measurements, by which time normal movement and behaviour had resumed. Post-CT_{max} survival was 100% across all treatment groups. Data were omitted for any individual fish that jumped out of the CT_{max} tubs.

Growth rates and body condition

To assess the potential effects of hypoxia priming on growth rates, body mass and total length was measured at two time points for each treatment group. Measurements were taken 1 h after their first CT_{max} trial when equilibrium was re-established. The final measurements were recorded 3 weeks after their final CT_{max} trial, resulting in a 4-week total growth period. Prior to measuring growth, fish were fasted for 24 h to reduce the risk of regurgitation under anaesthetic. Fish were then anaesthetised using buffered tricaine methanesulfonate (MS222; 50 mg/L, pH 7) and placed on a damp sponge on a scale (Sartorius TE153S model, Göttingen, Germany) to be weighed. Total length was calculated from photographs taken of each individual against a 1 x 1 cm grid. Once measurements were complete, fish were placed in a recovery tank until anaesthetic had worn off and then returned to their holding tanks.

Growth was calculated as specific growth rate (SGR_{BM}) using the following formula:

$$SGR_{BM} = 100\ (\ln(BM_f) - \ln(BM_i) / t)$$

Where BM_f represents final mass (g) per individual fish, BM_i is initial mass, and t is the growth period (days).

Body condition was also calculated at trial conclusion using Fulton's condition factor:

$$K = 100 \times (BM / L^3)$$

where K represents body condition, BM is body mass (g) at final weigh, and L is standard length (cm) at final weigh.

Statistical analyses

Statistical analyses were carried out using RStudio (version 3.6.3; http://www.R-project.org/) using the nlme (linear and non-linear mixed effects models; https://CRAN.R-project.org/package=nlme) and lsmeans (Least-Squares Means, version 2.30-0; https://cran.r-project.org/web/packages/lsmeans/lsmeans.pdf) packages. A linear mixed effect model (LME) was used to determine the effect of hypoxia priming (three-level fixed factor) and recovery period (four-level fixed factor) on CT_{max}. Fish ID was included as a random effect and fish body mass was included as a covariate. Another LME was used to determine if heat hardening occurred between repeated CT_{max} tests. Separate Analysis of variance (ANOVA) models were used to discern the effect of hypoxia priming on SGR_{BM}, K, and the effect of handling on CT_{max}. A Tukey HSD post-hoc analysis was used to highlight the variation between hypoxia treatment groups. Statistical significance was accepted at $P < 0.05$. Fish that showed developmental deformities were omitted from analyses (n = 5).

Results

Effect of handling and repeated CT_{max} trials on heat tolerance

There was no difference in CT_{max} between fish in the normoxia (100% Air saturation) control (CT_{max} = 28.6 ± 0.1 °C, mean ± s.e.m.) and the handling control (CT_{max} = 28.5 ± 0.1 °C, mean ± s.e.m.) treatments (figure 5), indicating that handling had no effect on heat tolerance ($F_{1,17}$ = 0.76, *P* = 0.40, aov). This pattern was observed at all recovery timepoints (A2 Figure 1).

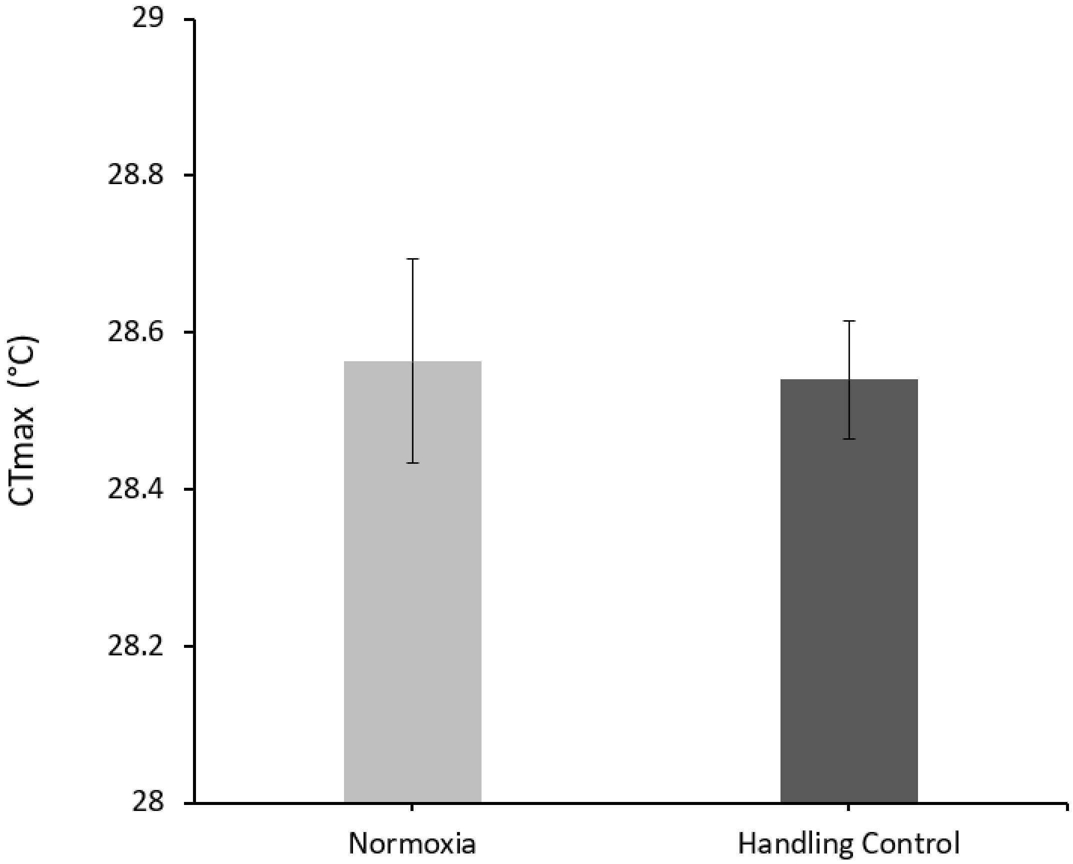


Figure 5. The effect of handling on critical thermal maximum (CT_{max}; ºC) in juvenile Chinook salmon (*Oncorhynchus tshawytscha*). There was no difference between the normoxia control (100% Air saturation, n = 23) and the handling control groups ($F_{1,17}$ = 0.76, *P* = 0.40, aov, *n* = 11). Values are shown as mean ± s.e.m.

Fish underwent repeat CT_{max} trials one week apart and no increase in heat tolerance (CT_{max}) was observed at the second time point (figure 6), indicating that heat hardening did not occur ($F_{1,3}$ = 1.63, *P* = 0.29, lme). Heat hardening models were run only on groups that did not undergo any hypoxia priming (controls, n = 23).

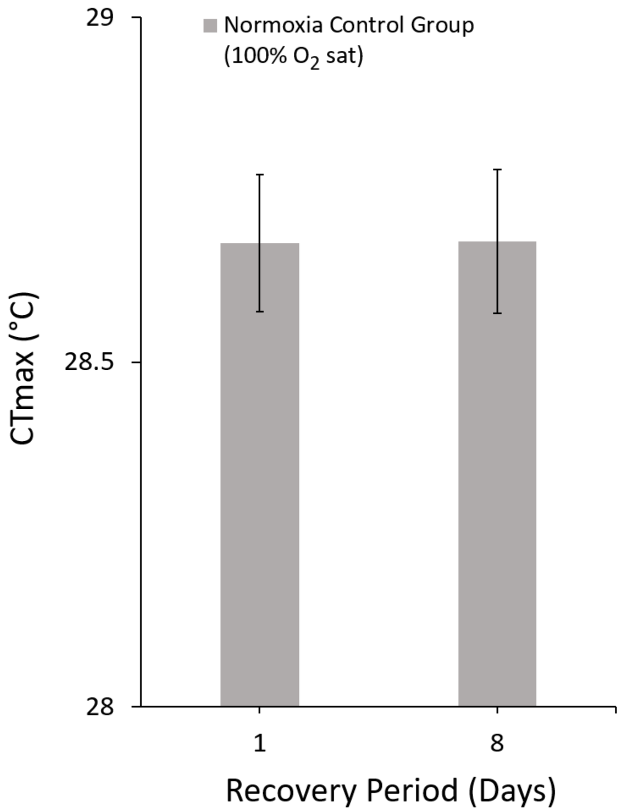


Figure 6. Critical thermal maximum (CT_{max}; ºC) of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) measured twice, one week apart. Figure depicts a control group which did not undergo any hypoxia treatment. No evidence of heat hardening was observed between the two CT_{max} trials ($F_{1,3} = 1.63$, $P = 0.29$, n = 23, lme). Values are shown as mean ± s.e.m.

Effect of hypoxia priming and recovery period on CT_{max}

Hypoxia exposure resulted in a significant decrease in CT_{max} ($F_{2,98} = 6.1$, $P < 0.01$, lme), indicating cross-susceptibility. No evidence of cross-tolerance between hypoxia and heat occurred under any treatment. A Tukey Post-hoc analysis revealed that the deleterious effects were consistently observed in fish exposed to the highest severity of hypoxia (40% Air saturation), and no significant effects resulted from the mild hypoxia treatment (60 % Air saturation).

At 1-day post-hypoxia exposure, a stepwise pattern of decline in CT_{max} was observed between treatments in order of severity (Figure 7A) with a 0.23°C reduction in CT_{max} in the 40% hypoxia treatment group compared to the control. The 40% Air saturation treatment at the 8-day recovery period showed the largest reduction in CT_{max} compared to the control (0.31°C, Figure 7C). After 10 days of recovery from hypoxia exposure, the negative deviation from the control reduced to only 0.07°C (figure

7D), a 0.24°C improvement from the lowest CT_{max} at day 8. CT_{max} was independent of body mass ($F_{1,24}$ = 0.36, P = 0.56, lme).

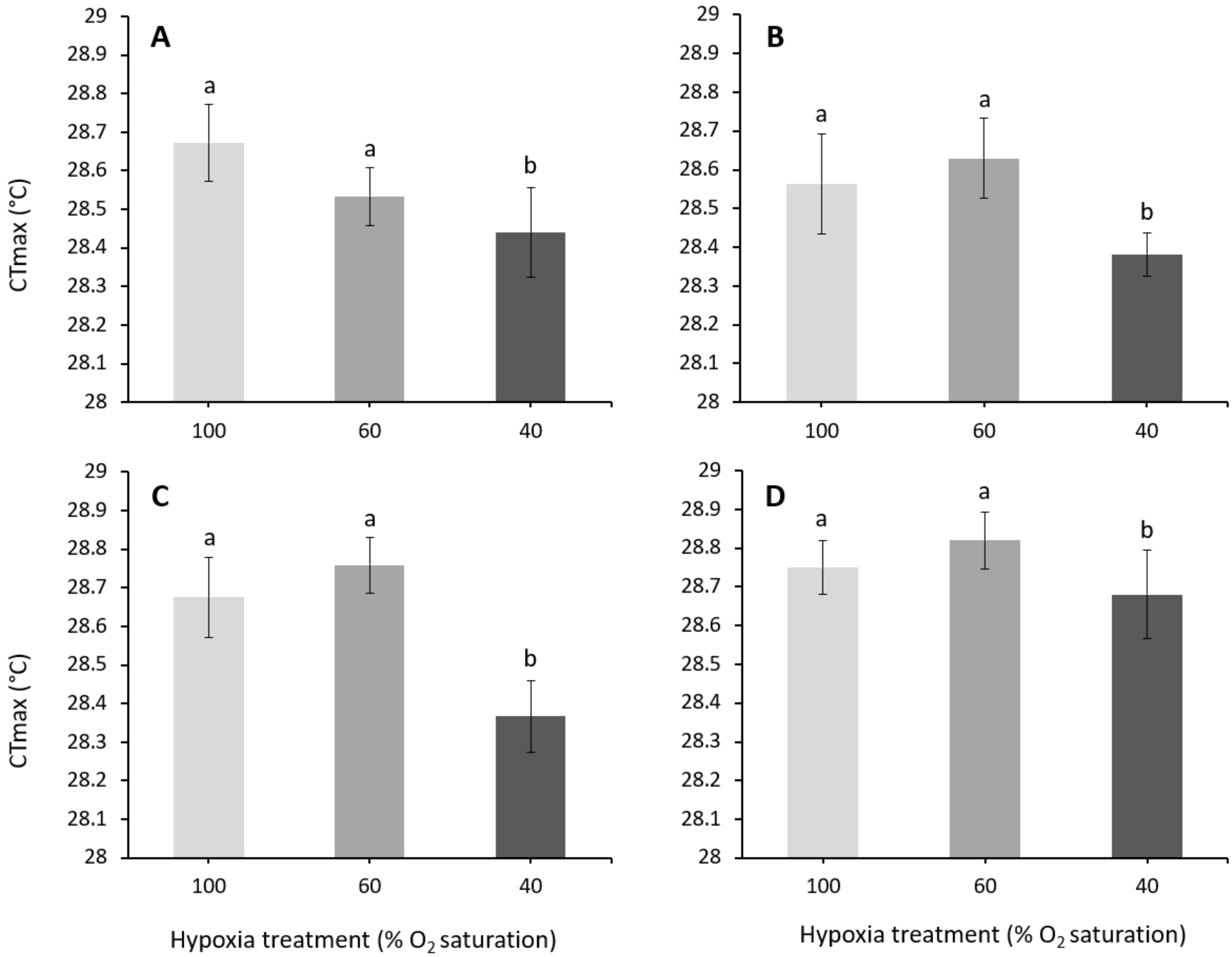


Figure 7. Effect of hypoxia exposure (priming stressor) and recovery period (i.e., days post hypoxia exposure) on critical thermal maximum (CT_{max}) in juvenile Chinook salmon (*Oncorhynchus tshawytscha*). Each graph represents a different recovery period between hypoxia and CT_{max} (A = 1 day, B = 3 days, C = 8 days, D = 10 days). Fish were exposed to normoxia (>85% Air saturation, n = 23), mild hypoxia (60% Air saturation, n = 23) or moderate hypoxia (40% Air saturation, n = 23) for 2 h, and subsequently left to recover for 1, 3, 8 or 10 days before CT_{max} was measured. Values are shown as mean ± s.e.m. and different lowercase letters indicate statistical differences among treatment groups (P < 0.05).

Effect of hypoxia priming on fish growth and condition

There was no significant difference in fish body mass between treatment groups at the start of the experiment ($F_{6,72}$ = 0.49, P = 0.81, ANOVA). ANOVA models showed that hypoxia priming had no effect on mean body mass ($F_{2,65} = 0.31$, $P = 0.74$), mean body length ($F_{2,65} = 0.12$, $P = 0.89$), specific growth rate ($F_{2,65} = 0.27$, $P = 0.77$, figure 8), or condition factor ($F_{2,65} = 1.14$, $P = 0.33$, figure 9) over the 4-week experimental period.

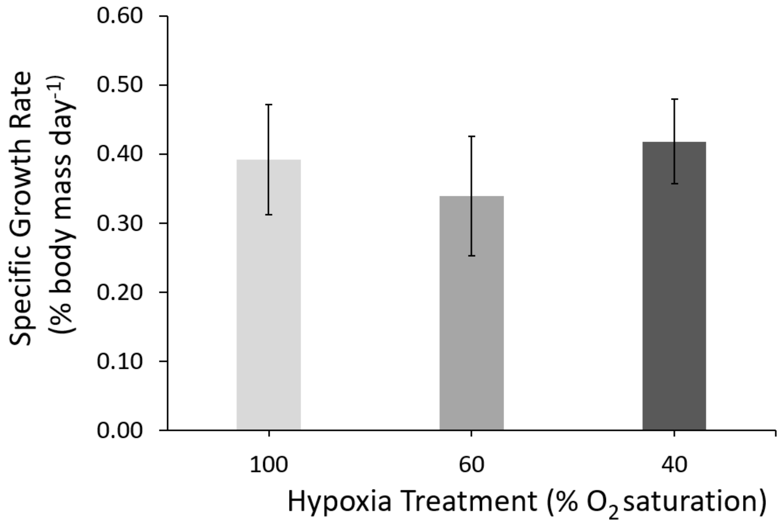


Figure 8. Effect of hypoxia priming on specific growth rate in juvenile Chinook salmon (*Oncorhynchus tshawytscha*). Growth was calculated for the 4-week experimental period. There was no significant difference found in specific growth rate (% body mass day^{-1}) between hypoxia priming treatments ($F_{2,65} = 0.27$, $P = 0.77$, n = 23 / treatment). Values are shown as mean ± s.e.m.

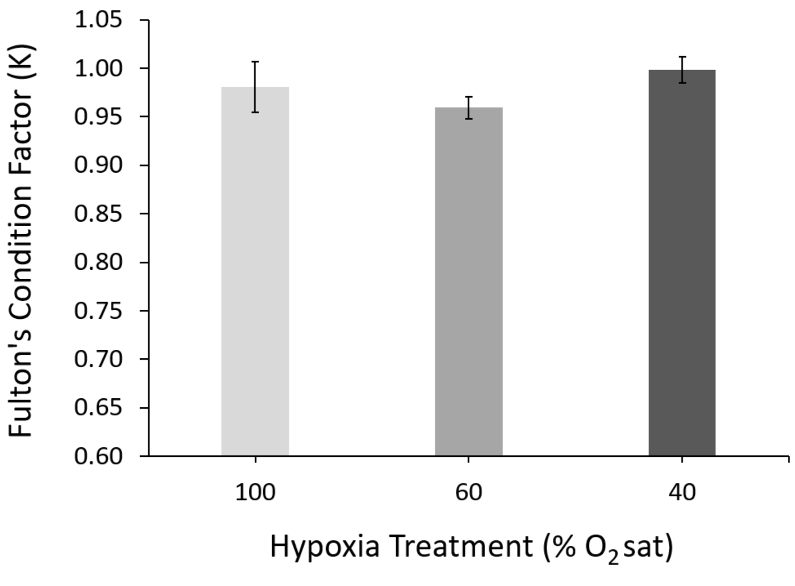


Figure 9. Effect of hypoxia priming on body condition of juvenile Chinook salmon (*Oncorhynchus tshawytscha*). Body condition was calculated as Fulton's condition factor (K). No significant difference was found in K between hypoxia priming treatments ($F_{2,65} = 1.14$, $P = 0.33$, n = 23 / treatment). Values are shown as mean ± s.e.m.

Discussion

Effect of hypoxia on heat tolerance

A significant reduction in CT_{max} was observed in fish exposed to moderate hypoxia treatment (2 h at 40% Air saturation), whereas the CT_{max} of fish exposed to mild hypoxia (2 h at 60% Air saturation) was unaffected. Where synergism typically describes the increased negative impact of two simultaneously occurring stressors (Côté et al., 2016; Crain et al., 2008; Piggott et al., 2015), cross-susceptibility specifically describes how an initial priming stressor increases susceptibility to a secondary stressor occurring at a later time point (Todgham & Stillman, 2013). The deleterious effects of hypoxia observed in this study are indicative of cross-susceptibility, as opposed to the hypothesised cross-tolerance.

There have been examples where hypoxia was found to both temporarily and permanently improve heat tolerance in teleosts (Burleson & Silva, 2011; Del Rio et al., 2019). However, hypoxia can inhibit physiological responses to heat stress, such as preventing increases in heart rate to meet cardiovascular demands under rising temperature (Leeuwis et al., 2021). Additionally, severe hypoxia (water oxygen tensions (P_wO_2) ≤ 35 mmHg) reduced the CT_{max} of multiple stenothermic reef fish species (Ern et al., 2017). The physiological effects of chronic hypoxia exposure in fish differ to those induced by acute exposure (Li et al., 2018). It has been suggested acute exposure may be insufficient to achieve cross-tolerance via physiological remodelling (Rodgers & Gomez Isaza, 2021). Chronic hypoxia exposure may be necessary to stimulate the physiological changes required for improved thermal tolerance, for example, priming cells with HSP production related mRNA and proteins (Todgham et al., 2005).

Research pertaining to cross-tolerance and cross-susceptibility in ectotherms is extensive, particularly in relation to terrestrial arthropods. In ectotherms, there are numerous examples of shared physiological protective mechanisms that may require sustained or chronic exposure to a priming stressor to manifest. Studies that have found evidence of cross-tolerance in teleost species have involved chronic or sustained exposure to a priming stressor. A study on channel catfish (*Ictalurus punctatus*), for example, found that moderate hypoxia (50% Air saturation, 75 Torr P O_2) exposure for a total of seven days improved cardiovascular performance under subsequent heat stress compared to control fish held under normoxic conditions (Burleson & Silva, 2011). It was suggested that hypoxia priming induced changes to cellular processes related to O_2 uptake, which directly affects thermal stress tolerance (Burleson & Silva, 2011). Additionally, cross-tolerance to heat was observed in Chinook salmon (*Oncorhynchus tshawytscha*) that were reared from fertilisation to fry stage under heat and hypoxia stress conditions (Del Rio et al., 2019). It was again suggested that the changes could be attributed to an increase in respiratory capacity. The mechanisms potentially involved included altered gill morphology for increased O_2 uptake, increased availability of haemoglobin, and altered cellular metabolism (Del Rio et al., 2019). These studies involved priming stressor exposure spanning many days, and attributed increases in resilience to physiological alterations that would take time to achieve. In contrast, a cross-tolerance study on triplefin fish (*Bellapiscis medius*) found a negative correlation between acute heat shock priming stressor exposure and subsequent hypoxia resilience (McArley et al., 2020). The study used a priming stressor exposure duration of only five hours that resulted in deleterious effects on hypoxia tolerance indicative of cross-susceptibility. It was proposed that the adverse effects could be attributed to insufficient tissue fuel stores (e.g., glycogen) for meeting increased cardiorespiratory demands under hypoxia (McArley et al., 2020). Elevated blood glucose is a known response to heightened metabolic activity (Hvas et al., 2018; Naderi et al., 2018; Zaragoza et al., 2008) such as would occur under hypoxia. Increased blood glucose levels indicate that glucose has been released from energy stores in the liver, gills, and tissues (stored as glycogen) in response to increased energy demands (Hvas et al., 2018; Sinha et al., 2014; Zhang et al., 2017; Zhao et al., 2020) and a shift from anaerobic to aerobic metabolism (Naderi et al., 2018). Blood glucose spikes have been seen in response to heat stress in fish (Chebaani et al., 2014; Dengiz Balta et al., 2017; Dettleff et al., 2020; Zaragoza et al., 2008). Additionally, glycogen may be a key source of energy at the gills under stress (Huang et al., 2015), therefore limiting respiratory capacity. McArley et al. (2020) suggested that the observed cross-susceptibility could have been due to the experimental recovery period not reflecting the natural exposure patterns occurring in natural habitats to which the fish were adapted. Cross-susceptibility may occur where fish have limited energy stores in tissues, have insufficient time (recovery period) to make morphological changes to enhance metabolic efficiency, or where recovery period does not reflect species-specific natural adaptations.

Alternatively, the observed decrease in heat tolerance in this study may be attributed to a stress-induced energy deficiency, as described by the energy-limited tolerance concept, first proposed by Sokolova et al. (2012). The energy-limited tolerance concept suggests that stress can alter the storage and supply of Adenosine triphosphate (ATP), sometimes resulting in an energy deficit, reducing aerobic scope. Consequently, physiological processes such as reproduction, growth, locomotion, and tolerance to subsequent stress are reduced (Sokolova, 2013). An ATP deficit or restricted aerobic scope could explain the reduced heat tolerance seen here in hypoxia primed juvenile salmon. A chronic mild hypoxia exposure may be necessary to develop the physiological protective mechanisms required to overcome or address the stress induced reduction in aerobic scope.

The hypoxia exposure used in this experiment was of short duration (2 h) and low severity. Acute lethal hypoxia levels for freshwater teleosts can range anywhere between 0.25 – 2 mg l^{-1} (~3 - 24.2 % Air saturation) depending on species specific natural tolerance and duration of exposure (Hrycik et al., 2017; Small et al., 2014). Hypoxia levels as mild as 4 mg O_2 l^{-1} (~48.4 % Air saturation) can also be lethal after prolonged exposure (weeks to months) in some species (Gilmore et al., 2018). Studies have typically defined acute hypoxia exposure duration as < 6 h and chronic exposure as ≥ 24 h (Li et al., 2018; Ma et al., 2020). The most severe hypoxia level used in this experiment was only 40% Air saturation at ~13.5 °C (~4.17 mg l^{-1}) for 2 h, which is very mild when compared to lethal or chronic hypoxia levels. Even mild exposure still resulted in significant reductions in CT_{max}. It has been suggested that sublethal effects of hypoxia can be observed at or below 4.5 mg l^{-1} (Hrycik et al., 2017), which is supported by the observed reduction in heat tolerance in this study.

Heat hardening

Cross-tolerance describes where one stressor shares a protective mechanism with a different independent stressor, therefore enabling the increase of resilience through mild exposure to the first stressor (Todgham & Stillman, 2013). In contrast, heat hardening describes the way in which exposure to thermal shock can temporarily increase resilience to subsequent thermal stress, usually through upregulation of protective genes, particularly those involved in producing heat shock proteins (HSPs) (Bowler, 2005; Malmendal et al., 2006). There are numerous studies observing the effects of heat hardening in ectotherms, particularly in arthropods (Alemu et al., 2017; Borchel et al., 2018; Hoffmann et al., 2013; Malmendal et al., 2006) and some teleosts (Bard & Kieffer, 2019; Bilyk et al., 2012). In this experiment, fish underwent two sequential CT_{max} trials, exposing them to upper thermal limits, one week apart. It was expected that heat hardening would be observed, however, there was no significant increase in heat tolerance between repeat CT_{max} trials (figure 6). As a result, observed negative changes in heat tolerance could be attributed solely to cross-susceptibility.

Heat hardening in fish is typically observed where thermal shocks are delivered in relatively quick succession. In a study on Antarctic notothenioid fishes, heat hardening was observed 24 h after the initial thermal shock (Bilyk et al., 2012). Heat hardening has also been observed in shortnose sturgeon (*Acipenser brevirostrum*) which underwent repeat CT_{max} trials only one hour apart (Bard & Kieffer, 2019). Additionally, a study investigating cross-tolerance between heat and osmotic shock in Atlantic salmon fry (*Salmo salar*; Penobscot strain) found that HSP70 was only produced for 3 h post heat shock treatment, and after 6 h was no longer present (DuBeau et al., 1998). It is likely in this study that heat hardening did occur in juvenile salmon, but that HSP gene expression had reverted to normal levels after 1 week when the repeat CT_{max} trial occurred.

The short duration increase to HSP gene expression from heat hardening suggests that its applicability as a practical tool for increasing heat tolerance in an aquaculture setting is limited. Cross-tolerance has potential for long duration effects and provides more scope for practical use. However, it should be acknowledged that cross-tolerance induced HSP expression will also have limited applicability. Longer lasting protective mechanisms would likely be required for successful implementation as a resilience tool for heat waves.

Effect of hypoxia on growth and body condition

Growth is of significant concern to the aquaculture industry and any protective treatments that negatively impact growth are unlikely to be implemented in practice (see Chapter 4, page 57). Studies have found that whilst sustained or chronic high temperatures will affect growth rates in fish (Dash et al., 2021; Handeland et al., 2008; Zhu et al., 2019), heat shock in the form of CT_{max} has no effect on fish growth (Desforges et al., 2021) and can be repeated at one week intervals without effect (Morgan et al., 2018). CT_{max} can therefore be an effective method of testing upper thermal limits without confounding growth data. For this reason, repeat CT_{max} measurements were spaced one week apart in this study and changes in growth could be attributed to hypoxia exposure.

Oxidative stress tolerance and the consequential effects on growth rate varies dramatically between species. Numerous studies have found that both intermittent and sustained hypoxia exposure in fish results in a marked decrease in feeding rate, growth, and body condition (Aksakal & Ekinci, 2021; Chabot & Dutil, 1999; Li et al., 2018; Pichavant et al., 2000; Remen et al., 2012; Vikeså et al., 2017; Yang et al., 2013). However, in some species, hypoxia has no effect on growth except at hypoxia extremes (Lifavi et al., 2017; McNatt & Rice, 2004; Stierhoff et al., 2009) and in others, acclimation to hypoxia can increase growth rates (Dan et al., 2014). In salmonid species growth rate is typically reduced under hypoxic conditions (Aksakal & Ekinci, 2021; Gamperl et al., 2020; Hou et al., 2020; Remen et al., 2012; Vikeså et al., 2017), suggesting that salmonids have a relatively low tolerance to oxidative stress. A short duration exposure (2 h) was used in this study to avoid specific growth rate (SGR) reduction in relation to hypoxia exposure. Analyses showed that acute hypoxia had no effect on growth or body condition at four weeks post-exposure.

Studies finding negative impacts of hypoxia on growth typically involve chronic hypoxia exposure spanning multiple days or weeks (Aksakal & Ekinci, 2021; Gamperl et al., 2020; Pichavant et al., 2000; Vikeså et al., 2017; Yang et al., 2013), whereas in this study, fish were exposed to hypoxia for only two hours. Few studies have found that acute hypoxia can affect growth and related physiological parameters (Hou et al., 2020). However, decreased feed rates and subsequent reduction in growth and body condition are typically attributed to hypoxia driven reductions in metabolic scope (Claireaux et al., 2000; Remen et al., 2012; Wang et al., 2009), and fish limiting their metabolic costs by reducing feed rates under hypoxic conditions (Chabot & Dutil, 1999; Vikeså et al., 2017). Fish feed rates have been found to increase once dissolved oxygen levels stabilise after hypoxia (Remen et al., 2012), therefore feeding is only severely reduced during hypoxic conditions. Consequently, acute hypoxia is unlikely to have a significant effect on growth as feeding is only interrupted for a short period. In this study, hypoxia treatments did not coincide with feeding times, and fish were able to feed normally before and after hypoxia exposure. The low severity and duration of hypoxia exposure is unlikely to have driven noticeable changes in metabolic scope, and feeding was unaffected. Therefore, the primary causes of growth retardation driven by hypoxia were reduced, potentially explaining the lack of effect observed.

Implications for natural ecosystems

Coastal benthic, estuarine, and fresh water ecosystems could be facing severe hypoxia events, particularly due to the effects of eutrophication driven by anthropogenic pollutants (Caballero-Alfonso et al., 2015; Gooday et al., 2009; Justić et al., 2003; Levin et al., 2009; Meire et al., 2013; Rabalais et al., 2010). Additionally, whilst many natural ecosystems may not be dealing with acute hypoxia, they will be exposed to numerous other climate change driven stressors simultaneously, such as algal blooms, acidification, exotic species invasion, food web shifts, over predation, heavy metal contamination, and food scarcity (Rabalais et al., 2010; Sage, 2020). The results of this research show that short duration, moderate severity hypoxia stress exposure negatively impacted the heat tolerance of Chinook salmon. The effects observed were isolated from other naturally occurring stressors such as predation, food scarcity, or pollutants. Additional stressor interactions would potentially worsen the impacts observed in this study. However, global climate change driven marine hypoxia expansion is likely to be gradual and chronic (Bopp et al., 2013). Where acute stressor exposure may be insufficient to elicit cross-tolerance, chronic exposure may successfully improve resilience (Li et al., 2018). Alternatively, the effects on shallow water ecosystems such as estuaries or rockpools may be significant, as the inhabiting species are already living with regular extreme stress exposure which is predicted to worsen (McArley et al., 2018). Further research would be necessary to determine whether cross-tolerance could be expressed under naturally occurring hypoxia levels and with additional confounding stressors.

Implications for the aquaculture industry

The significance that these findings may have for the aquaculture industry should not be understated. Standard industry practices involve crowding of fish, harvesting, grading, stocking, catching, and transportation. The likelihood of mild hypoxia occurring during these processes is increased as O_2 is often further restricted by the procedures (e.g. reduced water availability) and fish O_2 uptake increases due to stress (Eissa & Wang, 2016). Additionally, O_2 levels in sea pens are reduced in relation to high stocking density and water flow at farm sites (Oldham et al., 2018). Our results show that even a very mild and short hypoxia exposure can negatively impact the ability of fish to tolerate heat stress. Aquaculture activities involving hypoxic conditions, like crowding, should therefore be carefully considered in summer months or in relation to heat wave warnings where heat stress is more likely. Additionally, farm stocking densities may require re-assessment or reduction in summer months.

The duration of negative effects may also be significant to the aquaculture industry. Results showed that the detrimental effect on heat tolerance did not exhibit any improvement until recovery period day 10 (figure 7D), which showed a 0.24°C improvement compared to day 1. These findings suggest that it could take over a week for fish to physiologically recover from a mild and short duration hypoxia event. This is an exceptionally long time in relation to aquaculture practices in summer months, where heat waves could be prolonged in duration. In order for industry to maximise fish survival and welfare, exposing fish to even mild hypoxia may need to be avoided for extended periods during hot weather. Additionally, more severe reductions in CT_{max} could be expected from prolonged hypoxia events, as the extended recovery duration observed was triggered by very mild and short duration hypoxia.

Already we have seen significant effects of climate warming on the NZ aquaculture industry. A 2018 heatwave resulted in a 1.5°C increase from average historical sea surface temperature (SST) and mass mortalities of Chinook salmon were recorded (Salinger et al., 2019). Additionally, NZ has just recorded its warmest year on record and seven of the past nine years have been the warmest since recording started in 1909 (NIWA, 2022). The increase of warm weather events and extremes is of great concern to industry and may require cautionary practices during high-risk summer months, particularly in relation to hypoxia events. The results of this study contribute to the overall understanding of climate change related multi-stressor interactions, provide baseline knowledge for further investigation of cross-tolerance as a protective tool, and contributes to the understanding of how the aquaculture industry may be impacted by climate change.

Chapter Four – Survey: The perceptions of the aquaculture industry on the impacts of climate change in Australia and Aotearoa New Zealand

Introduction

Global aquaculture has steadily expanded since the 1980's, reaching over 90 million tonnes in global production by 2013 (Nadarajah & Flaaten, 2017). In Australia and New Zealand (NZ), the aquaculture industries are economically significant and provide a huge source of marine dietary protein. The Australian aquaculture industry employs 7000 people nationwide and produces over 100,000 tonnes of aquaculture product annually (Steven et al., 2021). Salmonids, oysters, prawns, and tunas are among the species with the highest production quantity and value, with salmonids being the largest at over 65,000 tonnes produced each year (Steven et al., 2021). By comparison, the NZ aquaculture industry employs over 3000 people nationwide, and produces over 100,000 tonnes worth over $650 million per annum (Aquaculture New Zealand, 2020). Greenshell™ mussels (*Perna canaliculus*) is the largest production sector in NZ aquaculture (> 100,000 tonne / year, $3700 / tonne), whilst Chinook salmon (*Oncorhynchus tshawytscha*) is the most valuable by weight (> 15,000 tonne / year, $16,000 / tonne) (Aquaculture New Zealand, 2020).

The aquaculture industries of both developed and developing nations around the world are at risk due to the effects of climate change (Handisyde et al., 2017). The potential effects of climate change on Australian aquaculture have been broadly investigated in the literature (Doubleday et al., 2013; Fleming et al., 2014; Hobday et al., 2018; Hobday et al., 2016; Lim-Camacho et al., 2015; Ling & Hobday, 2019; Morash & Alter, 2016; Richards et al., 2015; Robbins et al., 2017; Schrobback et al., 2018; Spillman et al., 2015; van Putten et al., 2014), although many impacts on farmed species are already being observed. In 2015/2016 there was a record breaking heatwave in Australasian waters which caused mortality in cultured Tasmanian oysters and abalone and reduced the production of Atlantic Salmon (*Salmo salar*) (Oliver et al., 2017). Research suggests that the south-east Australian oyster industry is at heightened risk of mortality events compared to other local industry species due to the oyster's susceptibility to heat waves (Doubleday et al., 2013). Elevated temperature and carbon dioxide (CO_2) have been found to alter bacterial composition within the haemolymph microbiome of the Sydney rock oyster (*Saccostrea glomerata*), potentially resulting in heightened disease susceptibility (Scanes et al., 2021). In 2013, a marine heatwave in South Australia caused algal blooms and mortality events in numerous fish and abalone species (Roberts et al., 2019). Temperature, hypoxia, and osmotic stress all contribute to immunocompromisation in abalone and are expected to cause increased disease events in Australian abalone aquaculture (Morash & Alter, 2016). Additionally, both wild and farmed scallops and prawns are expected to be somewhat vulnerable to ocean acidification due to climate change (Richards et al., 2015). Australian aquaculture businesses are expecting climate change to negatively affect their product quality, business reputation, and profitability (van Putten et al., 2014).

New Zealand is seeing ongoing evidence of increased temperature averages and sea level rise (Hopkins et al., 2015). Sea Surface Temperature (SST) has risen in the Pelorus Sound in Marlborough, NZ, since 2002 and is projected to continue to slowly rise (Broekhuizen et al., 2021). Pelorus Sound and surrounding waters in the Marlborough region house the majority of NZ's farmed Chinook salmon (56%) and Greenshell™ mussel farms (65%), as well as a small number of Pacific oyster farms (1%) (Aquaculture New Zealand, 2020). Disease and heat related mortality events have already occurred in shellfish species throughout the world, including in key NZ aquaculture species such as the Greenshell™ mussel and Pacific oyster (Nguyen & Alfaro, 2020). Additionally, mortality events have occurred in NZ finfish aquaculture. A heatwave in the summer of 2017/2018 caused extensive loss of Chinook salmon farm stock which resulted in salmon being imported to meet local demand (Salinger et al., 2019). Climate change can exacerbate the severity and impact of parasitism and is a huge problem facing global finfish aquaculture (Costello, 2006, 2009; Delfosse et al., 2021; Torrissen et al., 2013). In 2009, an estimated global cost of parasitism in salmon aquaculture alone was up to US $480 million (Costello, 2009). Climate change will potentially drive increased susceptibility to disease and parasites in the NZ aquaculture industry, although the scale of this threat is not yet fully understood (Lane et al., 2022). Additionally, marine based aquaculture may be faced with species invasions, as harmful species, like jellyfish, are proliferating under climate change conditions (Purcell, 2011). Increasing offshore aquaculture has been suggested as a possible resilience strategy for industry in the face of climate change in NZ (Heasman et al., 2020), as has diversification of culture species and methodology (Metian et al., 2020). However, diversification or relocation may not be possible for many established farmers so in-situ resilience tools may be required.

There are a number of research institutes in Australia and NZ dedicated to the improvement of the aquaculture industry. In Australia, state governments typically work with state specific aquaculture research groups, for example, the South Australian Research and Development Institute (SARDI, https://pir.sa.gov.au/research). Australia also has research institutes such as the Fisheries research and Development corporation (FRDC, https://www.frdc.com.au/), and the Commonwealth Scientific and Industrial Research Organisation Aquaculture division (CSIRO Aquaculture, https://research.csiro.au/aquaculture/). Additionally, many Australian universities have dedicated aquaculture research groups (e.g., James Cook University Marine and Aquaculture Research Facility, JCU MARF, https://www.jcu.edu.au/marf). In NZ, aquaculture research is typically completed by universities or organisations such as Plant and Food (https://www.plantandfood.com/en-nz/), the Cawthron Institute (https://www.cawthron.org.nz/), or the National Institute of Water and Atmospheric Research (NIWA, https://niwa.co.nz/aquaculture). Collaboration between researchers and industry is necessary for the generation of impactful science, but there is currently a recognised disconnect between parties (Engle, 2017).

The local perceptions on climate change and how it will impact aquaculture have been investigated in published literature in a variety of nations, such as Canada (Steeves & Filgueira, 2019), Ghana (Asiedu et al., 2017), Denmark (Ahsan & Brandt, 2015), India (Udmale et al., 2014), Bangladesh (Ahsan & Brandt, 2015), Turkey (Rad et al., 2018), and Australia (Fleming et al., 2014; Lim-Camacho et al., 2015). Australian industry perceptions research has focussed on the effect of climate change on the resource supply chain and how impacts on production may affect other industry areas (Fleming et al., 2014; Lim-Camacho et al., 2015). During phone interviews, the most commonly discussed climate change impact by Australian industry experts was the rising cost of fuel and energy. The most common topics relating to climate change adaptation were industry restructure and improved marketing. Only key representatives of industry sectors with extensive expertise were selected for participation (Fleming et al., 2014).

The public perceptions about the risks of climate change in NZ have been widely addressed in the literature and considered in relation to management planning and socio-economic impacts (Archie et al., 2018; Gartin et al., 2020; Hopkins et al., 2016; Hopkins et al., 2015; Linde, 2020; Manning et al., 2015; Talwar, 2021). Research has typically found that a majority of the public in NZ believe that climate change is real and needs to be addressed (Hopkins et al., 2015). Additionally, public perceptions of the aquaculture industry itself have been investigated (Robertson & Comfort, 2014). However, the perceptions of individuals in the NZ aquaculture industry about industry specific impacts of climate change are lacking in the literature. A 2019 study surveyed the NZ marine science community about their research priories and highlighted their suggestions as to how future climate change research should be focussed (Jarvis & Young, 2019). None of the research directions suggested by the NZ marine science community encompassed both climate change and aquaculture. Climate change directions primarily focussed on ecological effects, whilst directions relating to aquaculture were few, even when prompted. The responses indicate that there may be a disconnect between marine scientists and the needs and concerns of the aquaculture industry in NZ.

Australia has had few studies related to industry perceptions of climate change but they were restricted to surveys of the perceptions of a few key industry representatives (Fleming et al., 2014; Lim-Camacho et al., 2015). In NZ, research has been completed on public perceptions of climate change impacts and significance (Archie et al., 2018; Hopkins et al., 2015; Talwar, 2021), but not the perceptions of industry members. The lack of research in this area has resulted in knowledge gaps around whether industry has a comprehensive understanding of the nature of climate change threats, such as multi-stressor effects. Additionally, it is unknown whether industry wants or needs resilience tools for combating the effects of climate change, such as might be achieved through cross-tolerance. Cross-tolerance describes how resilience to one stressor (e.g., heat stress) can be improved through previous exposure to another stressor (e.g., hypoxia or crowding) (Rodgers & Gomez Isaza, 2021; Sinclair et al., 2013; Todgham & Stillman, 2013). The aim of this study was to address the research gaps around industry perceptions and understanding of climate change threats in Australia and NZ and how their areas of work may be affected. Additionally, it aimed to establish whether there is a need for the development of resilience tools, and how likely industry is to implement them.

Using a survey tool, this research aimed to address the following research questions:

(1) Do individuals within the Australian and NZ aquaculture industries believe they will be impacted by climate change?
(2) Which aspect of climate change (e.g., extreme weather events, ocean warming, hypoxia, etc) is of most concern to the Australian and NZ aquaculture industries?
(3) Do perceptions and understanding of climate change threats vary between roles within the Australian and NZ aquaculture industries?
(4) Are the Australian and NZ aquaculture industries already seeing the effects of climate change?
(5) Do the Australian and NZ aquaculture industries want stress resilience tools and how likely are they to be implemented?

The results of this survey will contribute to understanding the concerns of the Australasian aquaculture industries and provide guidance for future industry related research.

Methods

Survey design and distribution

All methodology for this survey was approved by the University of Canterbury's Human ethics committee (Ref: HEC 2021/52/LR-PS) and the survey was run through the programme "Qualtrics". This study was based on data from participants contacted through public channels such as contact emails and social media (LinkedIn and Twitter). The survey was open to anyone employed in any position within the aquaculture industries of Australia and NZ. Participation was open to all research and production roles at any level, including, but not limited to, farmhands, managers, aquaculture scientists, and owners. The survey was anonymous and allowed participants to abort at any time. Participants were given the option to provide contact information separately from their responses if they desired a summary of the results. A total of 100 participants or organisations were invited to complete this survey in total. Of those contacted, 31 were industry individuals with publicly available contact information or who had contacted us in response to public survey invitations. The other 69 were organisations contacted through websites or emails.

Participants were questioned on their broad roles within the industry in order to assess the demographics of respondents. Incomplete responses and respondents not employed in the aquaculture industry were omitted from results. The survey was comprised of 23 questions including multi-choice, Likert scale, and open answer question formats. Survey questions assessed participant's understanding of climate change threats, opinions on how climate change may affect industry, and how future climate change research should be directed (see A3 Table 1., pages 71-74, for survey questions). Many questions allowed open answers in addition to multi-choice (e.g., "Other, please specify"). Written responses were allocated to an appropriate multi-choice category wherever possible.

Statistical analyses

Survey data was extracted from Qualtrics into Microsoft Excel where it was compiled and assessed. Descriptive and inferential analyses were used where appropriate to assess survey results. Participants were invited to privately request a results summary if they wished it at the end of the survey (see A3 Table 1., Q24).

Results

Participant demographics

Of the complete survey responses, 68% were from NZ and 32% from Australia (Total n = 22). The highest proportion of participants was comprised of aquaculture scientists and science technicians (n = 9, 41%, table 3). The roles with least representation were company owners (n = 1), aquaculture educators (n = 1), and aquaculture farm workers (n = 1). Within the "other" category were the roles "hobby farmer" and "director of aquaculture".

Table 3. The proportion of survey participants working in various industry roles.

Role Description	Number of Participants
Researcher / research technician	9
Aquaculture educator	1
Farm worker / technician	1
Farm manager	5
Company owner	1
Industry representative / public relations professional	3
Other	2

The majority of participants worked in finfish aquaculture (59%) followed by shellfish (36%), crustaceans (14%), and algae (9%) (figure 10A). Almost 90% of participants who worked with shellfish, and 100% who worked with algae, were from NZ. The largest proportion of participants (~70%) said they worked with land-based aquaculture systems, which included raceways, ponds, and canals (figure 10B). Ocean based aquaculture included sea pens and lines and was selected by 45% of participants. Alternatively, 32% of participants said that they worked with closed recirculating aquaculture systems (RAS). Just over half of the participants said that they had previously been involved in research relating to the effects of climate change (n = 12, 55%).

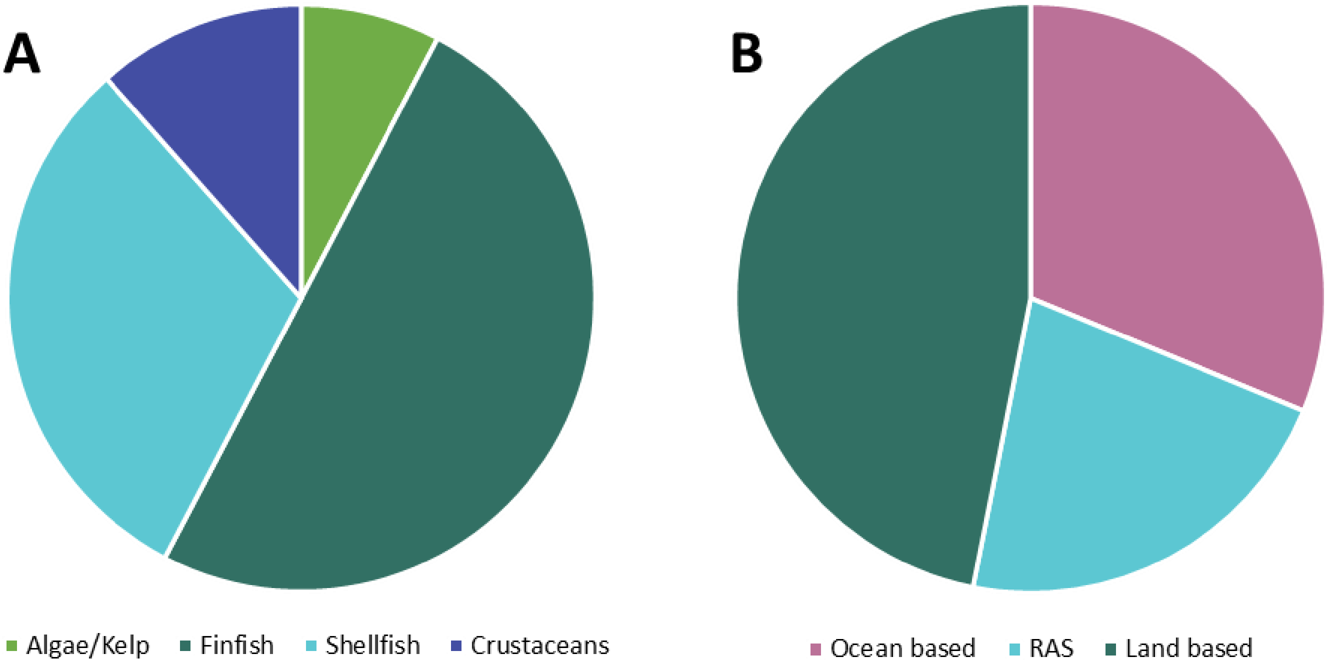


Figure 10. Visualisation of the proportion of survey participants who work with selected species groups (**A**) and aquaculture systems (**B**). Some participants worked with multiple species and systems and were included in multiple tallies. RAS represents Recirculating Aquaculture Systems.

Climate change perceptions

Five categories were used for assessing understanding of climate change impacts: Sea level rise, Ocean warming, Hypoxia, Increasing temperature extremes, and Extreme weather events. The majority of participants believed that they understand ocean warming sufficiently to describe it to other people (~91%, n = 20), whilst hypoxia was the least understood of the five categories (~41%, n = 9). Only 18% (n = 4) of participants said that they had never professionally discussed the potential impacts of climate change on their area of industry, and only 5% (n = 1) said it had never come up in casual conversation. The three most discussed categories were ocean warming (73%, n = 16), increasing temperature extremes (68%, n = 15), and extreme weather events (68%, n = 15). When asked whether discussions had addressed the effects of multi-stressor exposure, 50% assented and 50% were either unsure, or had not discussed multi-stressor effects. The majority of participants indicated that the largest perceived threat to their industries was ocean warming (32%, n = 7), and increased temperature extremes (32%, n = 7). Hypoxia and sea level rise were not considered to be the largest threat by any participants (n = 0).

Participants were asked to indicate their levels of concern that climate change will negatively affect their jobs (table 4). The majority of participants (91% , n = 20) said that their industry areas would likely be affected by climate change to some degree. Only 23% of participants said they were certain to be affected (n = 5), 9% were unsure (n = 2), and only 5% said they were unconcerned and unlikely to be affected (n = 1). Responses indicating the perceived threat to aquaculture animals were mixed, but the average response showed that a moderate negative impact is expected (table 4).

Table 4. Average responses out of 10 to Likert scale survey questions. 1 and 10 scale values are defined for each topic. Average responses displayed as mean ± SD (n = 22).

Question Topic	**Average Response**
Concern that climate change will negatively impact their job (*1 = no concern/not affected, 10 = great concern / definitely affected*)	7.8 ± 1.9
Impact of climate change on animals in their care (*1 = no impact, 10 = severe negative impact*)	6.4 ± 2.5
Likelihood to implement resilience tools with no impact on growth (*1 = very unlikely, 10 = certain*)	8.7 ± 1.9
Likelihood to implement resilience tools with small negative impact on growth (*1 = very unlikely, 10 = certain*)	6.8 ± 2.5
Availability/abundance of climate change research related to their area / species (*1 = none, 10 = lots*)	5.7 ± 2.8

The perceived likelihood of implementing a protective tool (see A3 Table 1, Q17 & Q18) against climate change threats (e.g., cross-tolerance) varied with impact on growth, as the average response decreased by -1.9 (Likert scale) once a small negative impact on growth was included. Only 23% (n = 5) of participants said that they were very likely or certain to implement tools with an impact on growth. Of these responses, the majority were by Australian participants (n = 4). Of the other participants, 27% indicated that they were unlikely to implement or unsure, and 5% indicated that they were very unlikely to implement (n = 1). Only one participant had a response lower than seven (out of 10) to the likelihood of implementation with no impact on growth, but the same participant had also indicated that they expected climate change to have little to no impact on the animals in their care.

There were large variations in perceptions of how much research was available in relation to the effects of climate change on their specific industry sectors. The mean score out of 10 (1 = no available research, 10 = abundant available research) was 5.7 and responses showed the largest variation of all scored questions (table 4). The highest proportion of participants scored 7 or higher (n = 8, 36%) indicating that they believed there was lots of available research. Alternatively, 27% indicated that they thought there was little to no research available (n = 6), whilst another 27% were either unsure or believed that some research was available (n = 6).

Participants were asked which area of research is of greatest importance in relation to climate change. Areas included disease management, medicine production, hypoxia tolerance, animal growth, heat tolerance, and animal welfare (figure 11). Heat tolerance was the most common response (45%, n = 10), whilst medicine production was the least (14%, n = 3).

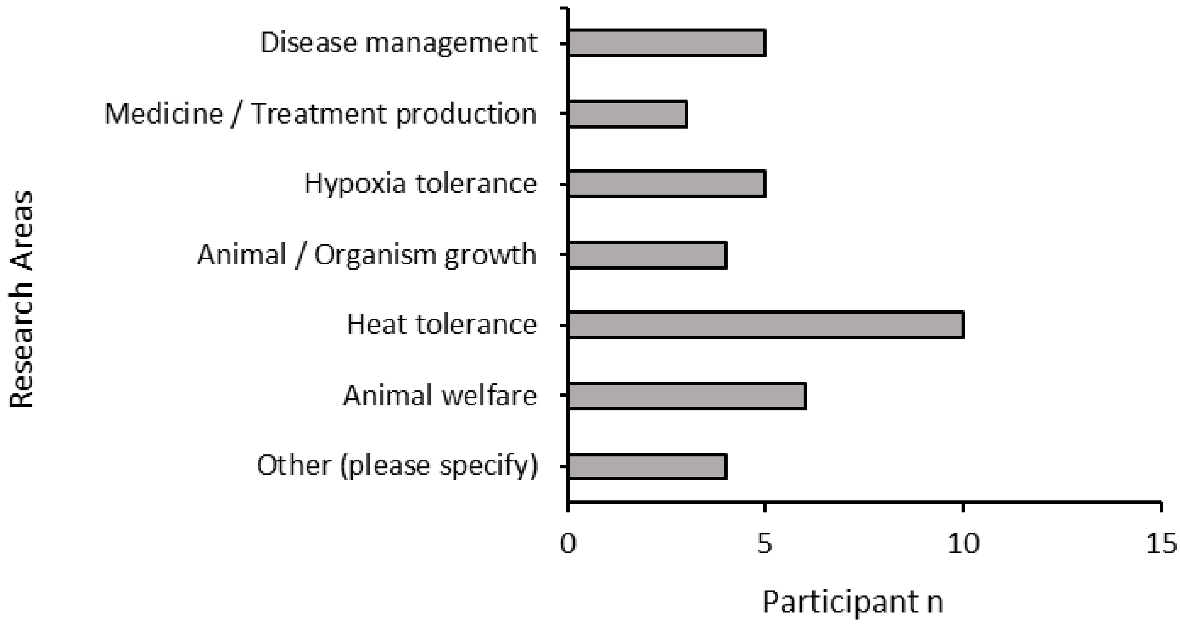


Figure 11. Number of participants who selected each research area in response to the question: "*What do you think the key focus of climate change/aquaculture research should be*?" (Participant n = 22).

The majority of participants believed that their work areas are certain to be affected by increased disease susceptibility and/or mass mortality of stock if climate change patterns do not change (64%, n = 14, figure 12). Additionally, 55% believed that farm site suitability is certain to decrease under current climate change progression (n = 12). Only one participant believed that they will be unaffected by climate change.

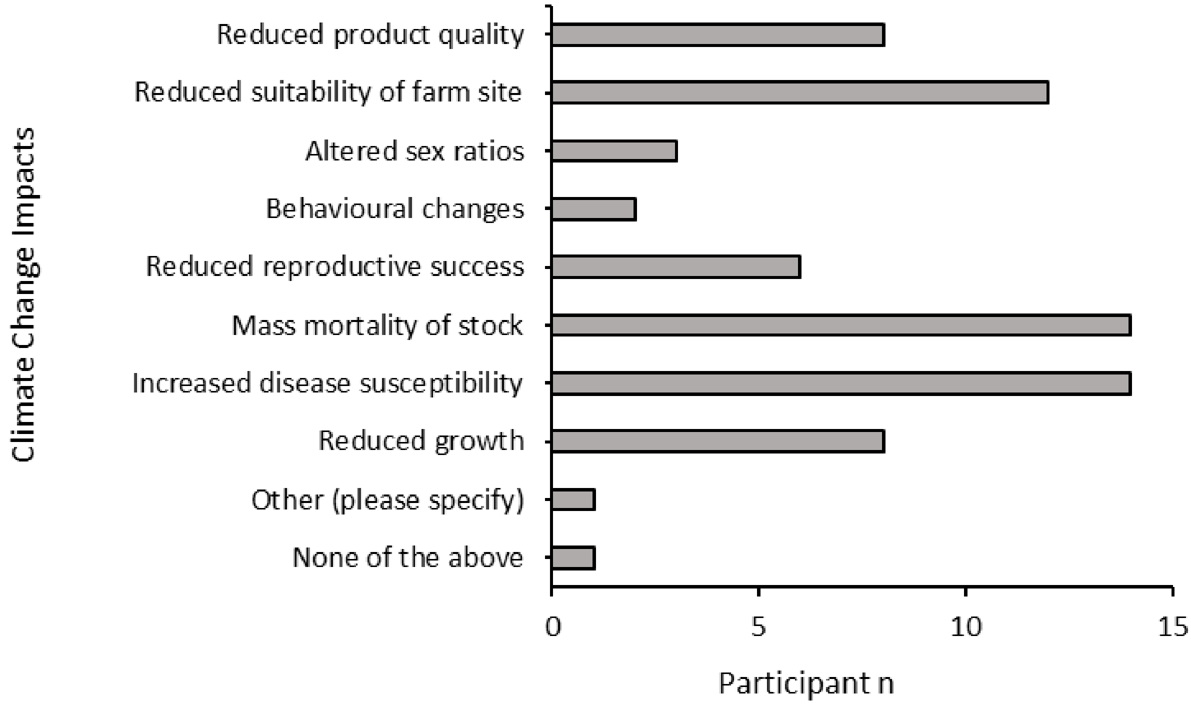


Figure 12. Number of participants who selected each climate change impact in response to the question: "*Which impacts of climate change do you think WILL impact your work/area of industry if nothing changes*?" (Participant n = 22).

Current impacts of climate change and future research

The majority of participants said that they were already being impacted by climate change in their industry areas (68%, n = 15), and that they had already implemented changes in response to climate change effects (64%, n = 14). The examples of changes being implemented provided by participants included selective breeding for resilience, reassessment and relocation of farm sites, carbon reduction programmes, investigating tools for stressor mitigation, and altering farm set up and species. Recommendations for future research focusses provided by survey participants were varied, but encompassed shared themes. Notable research directions are listed below:

(1) Selective breeding for heat stress resilience, hypoxia resilience, and increased genetic plasticity (adaptability).

(2) Impacts of heat and hypoxia stress on animal growth, maturation, and welfare.

(3) Multi-stressor research and mitigation of additional external influences such as land run-off.

(4) Collaboration with industry for in-situ research relevant to standard practice.

Discussion

The results of this survey indicate that members of the Australian and NZ aquaculture industries have immediate and serious concerns about the impact climate change will have on their work. The majority of survey participants believed climate change would negatively affect their industry areas to some degree. Additionally, the majority indicated that they were already seeing impacts of climate change in their areas and implementing changes in response. Ocean warming and increased temperature extremes were perceived as the largest threats to industry and most respondents believed that they are certain to witness increased disease and mortality events if current climate change projections and trends continue. Most participants had specific requests and recommendations for future research relating to their industry areas, including the recommendation for collaborative research between scientists and industry stakeholders. However, when asked how much research was available in relation to their areas, participant responses were mixed, with some believing there to be an abundance, whilst others believed there was almost none. Most participants indicated that they were already implementing changes in response to climate change threats, including investigating possible tools for increasing resilience and assessing tools for mitigating against climate change stressors. There is an evident desire for science-backed solutions to climate change threats such as heat stress, and the tools investigated in this thesis could provide some of these solutions.

Climate change research on Chinook salmon has primarily focussed on the impacts on wild stocks and in-situ mitigation strategies (Crozier et al., 2021; Crozier & Zabel, 2006; Crozier et al., 2008; Honea et al., 2016; Irvine & Fukuwaka, 2011; Justice et al., 2017; Lawrence et al., 2014; Thompson Lisa et al., 2012; Yates et al., 2008). Climate change research relating to impacts specific to Chinook salmon aquaculture is more limited and rarely addresses protection or resilience tools or solutions (Broekhuizen et al., 2021; Hanson & Peterson, 2014; Salinger et al., 2019). Research pertaining to the effects of climate change on Greenshell™ mussels investigates avenues potentially related to industry adaptation, such as assessment of thermal resilience mechanisms (e.g., HSP production) (Dunphy et al., 2013), metabolic mechanism alterations related to a heat induced mortality event (Nguyen & Alfaro, 2020), and resilience to ecologically relevant acidification levels (Ericson & Ragg, 2022). Research directions requested by participants of this survey involved multi-stressor research, in-situ farm research, and selective breeding, all of which are under-represented in published literature relating to NZ aquaculture species.

Heat tolerance was identified as being the area of most concern to industry members. In addition to the cross-tolerance experiments completed for this thesis (chapters 2 and 3), there are numerous studies investigating heat tolerance in aquaculture species (Froehlich et al., 2016), such as salmonids (Anttila et al., 2013; Bartlett et al., 2022; Calado et al., 2021; Gamperl et al., 2020; Hines et al., 2019; Lulijwa et al., 2021; Nuez-Ortín et al., 2018; Quinn et al., 2011; Wade et al., 2019), mussels (Cheng et al., 2018; Steeves et al., 2018), abalone (Chen et al., 2019; Chen et al., 2016; Liang et al., 2014; Wang et al., 2022), prawns (Aishi et al., 2019; Dong et al., 2020; Meng et al., 2019; Shi et al., 2020; Song et al., 2020), and other finfish (He et al., 2014; Larios-Soriano et al., 2021; Newton et al., 2010; Velasco-Blanco et al., 2019). This survey indicates that the results from the cross-tolerance research completed in this thesis (chapters 2 and 3) may be of great interest to the aquaculture industry. Survey results suggest there is an evident desire from industry for solutions and resilience tools in response to climate change driven heat stress. Consequently, the likelihood of industry implementing science-backed resilience tools is high.

The growth trade-off

One finding of this survey with particular significance to climate change resilience research is the reluctance of industry to implement tools that impact growth. Participants believed that it was considerably less likely that resilience tools would be implemented in standard aquaculture practice if even a small growth trade-off was identified. Only 23% of participants thought that resilience tools would be implemented in spite of a growth trade-off, and most of those participants were from Australia. There have been more disease and mortality events in Australia (Doubleday et al., 2013; Oliver et al., 2017; Roberts et al., 2019) than there have been in NZ (Salinger et al., 2019). Additionally, projections suggest that Australia is going to experience more severe effects of climate change than will occur in NZ (IPCC, 2021). The severity of current and predicted impacts of climate change in Australia may contribute to an increased willingness of Australian industry members to consider greater trade-offs.

The overall unwillingness of industry to implement resilience tools with growth trade-offs indicates that it is essential for evaluations of cross-tolerance tools to include assessment of growth effects, such as was completed in the second cross-tolerance experiment of this thesis (see Chapter 3, page 41).

Survey participation

Participation in this survey was relatively low compared to the number of people invited to participate. A total of 100 individuals and organisations from both Australia and NZ were contacted directly and invited to participate, in addition to publicly announced invitations on social media. A total of 22 complete survey responses were recorded, 68% of which were from NZ. Whilst the survey was open to all industry roles, the majority of participants were either researchers or farm managers, with only 1 farm worker. This survey relied on public communication channels and word of mouth for distribution which may be responsible for the lack of engagement from a broader range of positions. Farm workers would need to be informed of the survey by managers and employees and encouraged to complete it. Larger buy in from managers and industry representatives is likely required for increased responses. The representation bias towards people in research related roles may be indicative of low research engagement from industry employees.

Industry engagement

Survey responses showed some clear trends in perspectives from industry members. Ocean warming and temperature extremes were of most concern, and most participants believed that climate change would result in decreased stock health and survival. Additionally, almost all participants had requests for future research and expressed a desire for scientific solutions. These responses suggest that there is a clear desire for scientific research in relation to climate change impacts on aquaculture from the industry itself. There may also be an economic interest as participants believed they were already seeing, or likely to see, the negative effects of climate change on their businesses. However, the mixed perceptions on the amounts of available research may be indicative of a disconnect between researchers and industry members. Direct collaboration may be necessary to bridge the resulting communication gap. Unfortunately, engagement with this survey was low despite businesses and industry members being contacted directly. Whilst the responses indicated that there is a definite desire from industry for more research and increased collaboration, when given the opportunity to contribute to research, industry members did not participate. Additionally, it is clear that whilst managers and researchers were aware of the survey and willing to contribute, they likely did not distribute it to other employees and peers or encourage their participation, effectively eliminating the perspectives of the larger workforce within the industry. Alternatively, industry members in other positions may not have been willing or able to participate in the survey, or may not have understood their eligibility to contribute.

Conclusions

The results of this survey indicate that resilience tools against climate change stressors, such as cross-tolerance, are both wanted and needed by the aquaculture industries of Australia and NZ. The timeliness and relevance of the research completed for this thesis should not be understated. Industry desires collaboration with scientists for development of stressor mitigation and resilience tools relevant to industry practice. Additionally, there is no consensus among industry members as to how much climate change research is available relevant to their respective industry areas. A large portion of participants believe that there is a significant lack of relevant literature available to them. The results of this thesis will be made available to industry members in order to contribute to increasing collaboration and awareness of research findings in industry.

Chapter Five - General Discussion

In the aquaculture industry there is a pressing need for resilience tools against the impacts of climate change. Industry is already witnessing numerous mortality and disease events due to increasing temperature extremes and weather events (Eliason et al., 2013; Froehlich et al., 2022; Lebel et al., 2016; Mehvar et al., 2019; Puspa et al., 2018; Salinger et al., 2019; Wade et al., 2019), including NZ's Chinook salmon industry (Salinger et al., 2019). This thesis aimed to investigate potential avenues for inducing cross-tolerance in Chinook salmon as a resilience tool against heat stress as might occur during heatwave events. Chapter two investigated crowding as a potential priming stressor for cross-tolerance to heat stress. Results indicated that the crowding stress severity used was insufficient for cross-tolerance to be expressed. Chapter three involved a larger study investigating hypoxia as a priming stressor for cross-tolerance to heat stress. However, instead of conferring cross-tolerance, evidence of cross-susceptibility was observed. The observed cross-susceptibility had significant implications for industry practice as it resulted from relatively mild hypoxia stress. Finally, Chapter four involved a survey of members of the aquaculture industries of NZ and Australia for the purpose of gaining insight into their concerns, priorities, and needs in relation to climate change. Survey results showed that the NZ and Australian aquaculture industries have immediate concerns about climate change threats and that they desire science-backed solutions.

The effects of stressor severity and recovery period

The results of the two cross-tolerance experiments completed for this thesis highlighted the variable impact that recovery period and stressor severity have on stress response and resilience in fish. The stressor severity in the crowding experiment was insufficient to elicit any significant changes in heat tolerance in Chinook salmon. Other cross-tolerance studies have identified priming stressor thresholds or combinations necessary to confer increased resilience to subsequent stress (Del Rio et al., 2019; DuBeau et al., 1998; Opinion et al., 2021; Todgham et al., 2005). Todgham et al. (2005) found that a variation of just 2°C in priming heat shock temperature could determine whether HSP production would increase and confer cross-tolerance to hypoxia stress in tidepool sculpins (*Oligocottus maculosus*). Whilst the treatment in the crowding experiment was insufficient to elicit a response, a threshold was identified in the hypoxia experiment. The mild hypoxia treatment (60% Air saturation for 2 h) had no effect on subsequent heat stress tolerance, however the moderate hypoxia treatment (40% Air saturation for 2 h) resulted in cross-susceptibility to heat stress being observed. This identified a lower threshold of stressor severity before negative impacts of stress were observed. The identified threshold may supply industry with valuable insight as to the duration and severity of hypoxia farmed fish can be exposed to without an effect on heat tolerance. Unfortunately, the threshold at which negative effects on heat tolerance were observed was low in severity and short in duration relative to the thresholds for sublethal effects identified in the literature (Gilmore et al., 2018; Hrycik et al., 2017; Li et al., 2018; Ma et al., 2020; Small et al., 2014), indicating that Chinook salmon may be vulnerable to hypoxia stress than previously thought.

Cross-susceptibility mechanisms

Moderate hypoxia priming in Chinook salmon smolts resulted in the expression of cross-susceptibility to heat tolerance, observed as a reduction in CT_{max}. Cross-tolerance mechanisms (e.g., HSP upregulation, antioxidant production, mitochondrial refurbishment, and cardiovascular enhancement) have been explored and discussed in the literature (Berry & López-Martínez, 2020; Burleson & Silva, 2011; DuBeau et al., 1998; Opinion et al., 2021; Roberts et al., 2010; Todgham et al., 2005; Todgham & Stillman, 2013), but the physiological mechanisms by which cross-susceptibility occurs are largely unexplored. Cross-susceptibility may related to stress driven energy deficiencies counteracting any conferred resilience as described by the energy-limited tolerance concept (Sokolova, 2013), however it has yet to be experimentally investigated. Additionally, the cross-susceptibility observed in this study may have related to hypoxia stress conditions not reflecting the physiological nature of the study species determined by their natural environment, as was suggested by McArley et al. (2020) who found cross-susceptibility between heat shock and hypoxia in triplefins (*Bellapiscis medius*). Further research would be required to assess the mechanisms by which moderate hypoxia has conferred cross-susceptibility to heat stress in Chinook salmon.

The growth trade-off

The survey of aquaculture industry members highlighted a number of areas of particular interest to industry, including improving heat tolerance of aquaculture species, engaging in collaborative research, and the development of selective breeding. There were high levels of concern regarding the current and predicted impacts of climate change on the Australian and NZ aquaculture industries. However, results indicated that industry were far less likely to implement protective mechanisms and tools if there was even a mild trade-off with growth rates of stock. Whilst the hypoxia experiment assessed the impacts of stress on fish growth, the crowding experiment did not. Protective tools developed with no understanding of growth impacts are less likely to be of interest to the industry for which they are developed. However, the majority of the participants who said likelihood of implementation remained high were from Australia, indicating a potential variation in attitude or desperation for protective tools between the two countries. Australia is predicted to experience severe drought and temperature extremes under current climate change projections, whereas NZ will primarily be affected by increased precipitation (IPCC, 2021). The severity of potential impacts from climate change may influence industry willingness to accept larger trade-offs for increased protection.

Study limitations and recommendations for future research

Cross-tolerance was not observed in either of the stressor studies completed for this thesis. Stressor type, stressor severity, recovery period, fish life stage, and experimental methodology may all have contributed to determining whether cross-tolerance would or would not be expressed. The crowding and hypoxia experiments were designed to require as few individual fish as possible without compromising statistical tests of cross-tolerances. However, the scope for expanding this research is significant and would require increased sample sizes and stressor variations. Increasing the range of recovery periods investigated would inform increasingly accurate observations of stressor thresholds for cross-tolerance and the protective mechanisms involved. Additional investigation of recovery periods would aid in assessing the applicability as a protective tool in standard aquaculture practice in relation to resilience duration and implementation methodology. Moreover, increasing the range of stressor severities would increase the accuracy of any identified resilience or cross-tolerance thresholds.

Crowding and hypoxia were selected for assessment as priming stressors for heat stress due to their shared physiological responses with heat stress (e.g., HSP production or cardiovascular improvements) (Berry & López-Martínez, 2020; Brijs et al., 2018; Burleson & Silva, 2011; Del Rio et al., 2019; Jensen & Benfey, 2022; Jung et al., 2020; Leeuwis et al., 2021; Morgenroth et al., 2021; Naderi et al., 2018; Todgham et al., 2005), and the ease of implementation in standard aquaculture practice. However, there are other stressors that could confer cross-tolerance to heat stress in aquaculture. Research has shown that air exposure results in a similar but heightened stress response when compared to submerged crowding in fish (Brydges et al., 2009). Air exposure can also be achieved easily through netting of fish (Barcellos et al., 2004; Brydges et al., 2009). As a result, air exposure could provide another avenue for investigation in the search for a primer for cross-tolerance to heat stress in fish. Cross-tolerance between heat stress and osmotic shock has already been observed in teleost species (Todgham et al., 2005), including salmonids (DuBeau et al., 1998). As there is an established physiological connection between osmotic and heat stress responses in fish, it is likely that osmotic stress priming could confer cross-tolerance to heat stress in Chinook salmon.

The cross-tolerance experiments completed for this thesis were completed exclusively using Chinook salmon smolts. Cross-tolerance has previously been observed in Chinook salmon primed during the pre-swim life stages, although at the expense of growth and survivability (Del Rio et al., 2019). The observed cross-tolerance in pre-swim life stages could provide an avenue for selective breeding for heat resilience in salmon, an area which industry members expressed an interest in during the climate change perceptions survey. Additional research into the effects of stress at different life stages of Chinook salmon would present a more complete understanding of their capacity for increasing stress resilience, and the consequences of stress throughout development.

Post-mortem physiology assessments could be applied to investigate the mechanisms driving cross-tolerance and cross-susceptibility. Studies that have investigated physiological protective mechanisms have posthumously assessed the impacts of stress on blood parameters, tissue functionality and structure, body morphology, and genetics (Dolci et al., 2014; DuBeau et al., 1998; Morgenroth et al., 2021; Opinion et al., 2021; Robinson et al., 2019; Todgham et al., 2005). Pre- and post-mortem assessment of physiological parameters would be required in order to investigate the protective mechanisms underlying the development of cross-tolerance or cross-susceptibility in Chinook salmon.

Below is a summary of future research recommendations based on the results of this thesis:

(1) Investigation into the effects of additional hypoxia and crowding stress severities and recovery periods on the development of cross-susceptibility or cross-tolerance to heat in Chinook salmon smolts. This would provide improved understanding of stress response thresholds.

(2) Investigation into the underlying mechanisms of cross-tolerance or cross-susceptibility from hypoxia priming in Chinook salmon through assessment of physiological parameters such as blood chemistry, heat shock proteins, tissue functions, morphological alterations, cardiovascular responses, and genetics.

(3) Investigation of additional priming stressors with the potential to confer cross-tolerance to heat stress in Chinook salmon, such as air exposure and osmotic shock.

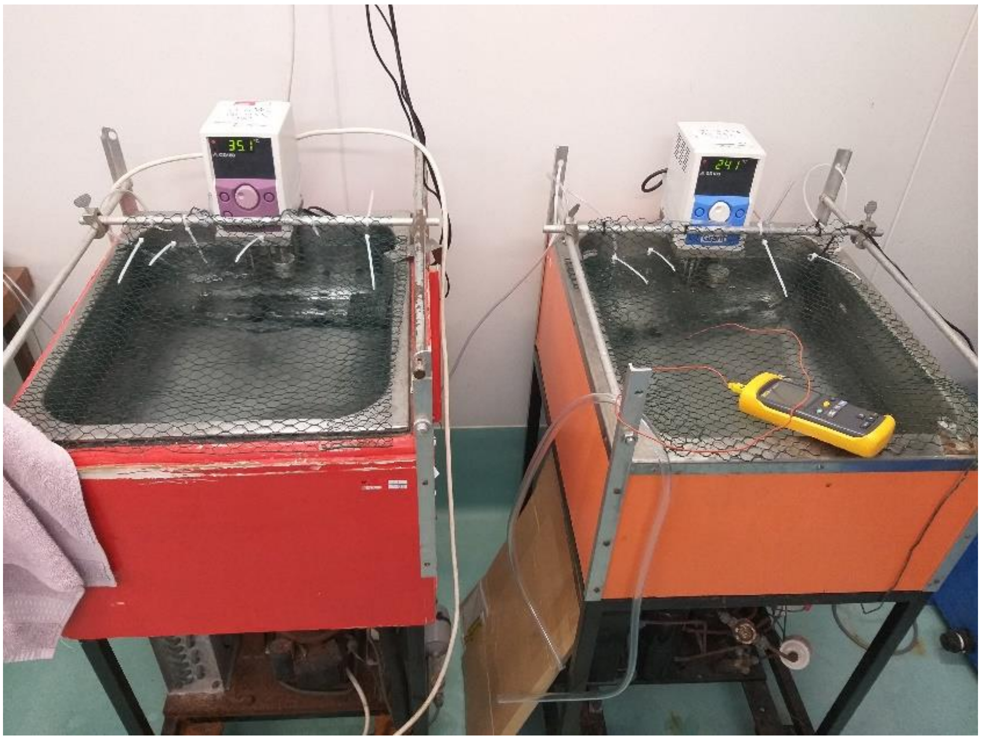
(4) Investigation of cross-tolerance across a variety of life stages for comparison of effect and assessment of long-term consequences.

Summary and conclusions

Results from the industry perceptions survey indicate that the Australian and NZ aquaculture industries are deeply concerned about the current and projected impacts of climate change. Industry members desire more research on how to mitigate the effects of climate change in their work areas. Resilience tools are likely to be implemented, but implementation likelihood decreases when a growth trade-off is required. Cross-tolerance has the potential to provide a resilience tool in the face of climate change. Mild crowding (20-min at ~47.8 kg / m^{-3} density) was found to be insufficient to confer cross-tolerance to heat stress. Alternatively, moderate hypoxia exposure (40% Air saturation for 2 h) resulted in cross-susceptibility to subsequent heat stress. The cross-susceptibility between hypoxia and heat stress could have significant implications for aquaculture standard practice. Although the stressors investigated here were not found to induce cross-tolerance to heat, we provide valuable baseline data on the effects of mild stressors, common to aquaculture practices, on heat tolerance.

Appendices

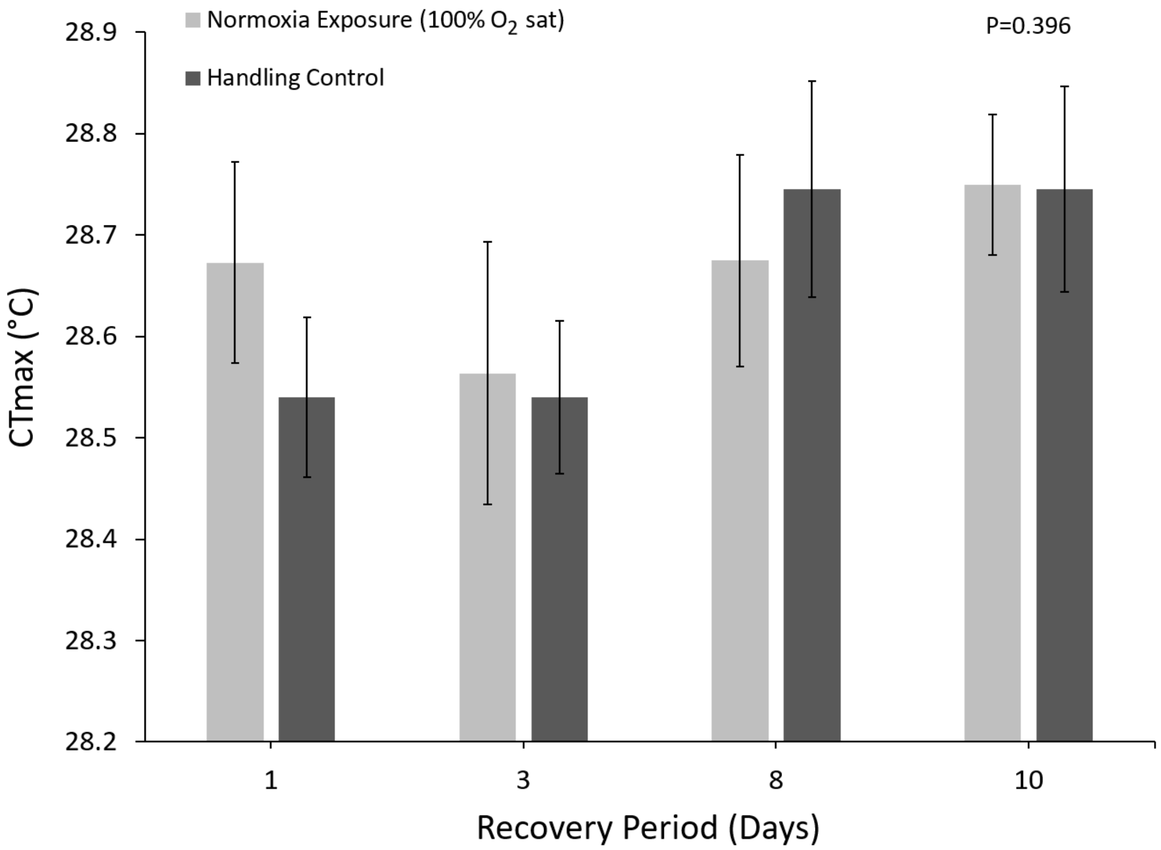
Appendix I



A1 Figure 1. Custom water baths used for controlled temperature ramping during CT_{max} trials.

A1 Figure 2. Aerated and temperature-controlled recovery tubs in which fish regained equilibrium post-CT_{max}

Appendix II



A2 Figure 1. The effect of handling on critical thermal maximum (CT_{max}; ºC) in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) after a recovery period of 1, 3, 8, or 10 days. There was no difference between the normoxia control (100% Air saturation, n = 23) and the handling control groups ($F_{1,17}$ = 0.76, P = 0.40, n = 11) at any recovery period. Values are shown as mean ± s.e.m.

Appendix III

A3 Table 1. Survey questions and response options given to participants during the Chapter 4 study: “The perceptions of the aquaculture industry on the impacts of climate change in Australia and Aotearoa, New Zealand”

Q1. What is your role within the aquaculture industry? (Select best fit):
- Farm manager - Farm worker/technician - Researcher/research technician - Animal health/welfare professional - Biosecurity professional - Company owner - Industry representative/ public relations professional - Aquaculture educator - I have no role in the aquaculture industry - Prefer not to say - Other (please specify)
Q2. Are you based in Aotearoa, New Zealand or Australia?
- Aotearoa, New Zealand - Australia - Neither
Q3. What species/area do you work in? (Select best fit):
- Shellfish - Finfish - Crustaceans - Algae/kelp - Prefer not to say - Other (please specify)
Q4. What kinds of aquaculture systems do you work with ?
- Ocean based (nets, pens, lines, etc) - Land based (ponds, raceways, etc) - Land based Recirculating Aquaculture systems (RAS) - Prefer not to say - Other (please specify)
Q5. Which of these facets of climate change do you understand sufficiently that you could you describe it simply to another person? Select all that apply:
- Sea level rise - Ocean warming - Hypoxia (i.e., low oxygen, ‘dead zone’) expansion - Increasing temperature extremes - Increasing extreme weather events - None of the above
Q6. Within your professional role, have you ever discussed the potential impacts of climate change on your industry or work in professional conversation? (e.g., meetings, conferences, etc) If so, what areas have you discussed? Select all that apply:

- No, it has not come up in my profession
- The potential impact of sea level rise on your industry/job
- The potential impact of ocean warming on your industry/job
- The potential impact of increased temperature extremes on your industry/job
- The potential impact of hypoxia on your industry/job
- The potential impact of weather extremes on your industry/job
- Other (please specify)

Q7. Within your professional role, have you ever discussed the potential impacts of climate change on your industry or work in casual conversation? (e.g., jokes between workers, break room discussions, etc.) If so, what areas have you discussed? Select all that apply:

- No, it has not come up in my profession
- The potential impact of sea level rise on your industry/job
- The potential impact of ocean warming on your industry/job
- The potential impact of increased temperature extremes on your industry/job
- The potential impact of hypoxia on your industry/job
- The potential impact of weather extremes on your industry/job
- Other (please specify)

Q8. Have your discussions on climate change threats ever addressed the combined effects of multiple stressors? For example, have you discussed how two threats at once (e.g., temperature extremes and hypoxia) both acting simultaneously could affect your work/the animals in your care?

- No, we have never discussed the combined impacts of multiple stressors
- Unsure
- Prefer not to say
- Yes we have discussed combined stressor effects (provide details if possible)

Q9. On a scale from 1 to 10, indicate how concerned you are that climate change will negatively affect your job/industry area.

- 1 = no concern / will not be affected
- 5 = unsure
- 10 = extreme concern / will definitely be affected

Q10. On a scale from 1 to 10, indicate what impact you believe climate change will have on the animals/organisms in your care/area of industry. Select no impact if not applicable.

- 1 = No impact (no change in production)
- 5 = Significant impact (reduced survivability, health, welfare, and/or success of organisms)
- 10 = Severe negative impact (complete collapse of stock)

Q11. Which ONE area of climate change do you think poses the LARGEST direct risk to your industry/job?

- Sea level rise
- Ocean warming
- Temperature extremes
- Hypoxia
- Extreme weather events
- None of the above
- Other (please specify)

Q12. Have you previously been involved in any industry related research about the effects of climate change?

- Yes
- No
- Prefer not to say

Q13. Do you believe that you have already witnessed the potential effects of climate change within your work area/job?

- Yes (provide details – optional)
- No
- Prefer not to say

Q14. Are you already implementing changes in your area of work/industry in response to climate change threats and/or research? If so, what kinds of changes you are making?

- No, we are not yet making any climate change related changes.
- I don't know.
- Prefer not to say
- Yes (please specify the types of changes you are making)

Q15. What do you think the key focus of climate change/aquaculture research should be?

- Animal welfare
- Heat tolerance
- Animal/Organism growth
- Hypoxia (low oxygen) tolerance
- Medicine/treatment production
- Disease management
- Other (please specify)
- Prefer not to say

Q16. Below are various areas of research that relate to understanding the biology and culture of species. Select which ones you believe are important for understanding how your specific farmed species/work will be affected by climate change. (select as many as you like).

- Genetics
- Animal physiology
- Animal behaviour
- Immunology
- Ecology
- None of the above
- All of the above
- Other (please specify)

Q17. If scientists were to discover a way to increase heat tolerance or resilience to temperature extremes that had no impact on growth or production yields, how likely do you think your industry is to implement this research?

- 1 = Very unlikely to implement
- 5 = Unsure
- 10 = Certain to implement

Q18. If scientists were to discover a way to increase heat tolerance or resilience to temperature extremes that had a small negative impact on growth, how likely do you think your industry is to implement this research?

- 1 = Very unlikely to implement
- 5 = Unsure

- 10 = Certain to implement
Q19. On a scale of 1 to 10, how does the stress levels of animals/organisms affect their ability to cope with climate change threats?
- 1 = has no effect on ability to cope with threats - 5 = unsure - 10 = has large effect on ability to cope with threats
Q20. Which impacts of climate change do you think WILL impact your work/area of industry if nothing changes?
- Reduced growth - Increased disease susceptibility - Mass mortality of stock - Reduced reproductive success - Behavioural changes (e.g., heightened aggression, etc) - Altered sex ratios - Reduced suitability of farm site - Reduced product quality - None of the above - Prefer not to say - Other (please specify)
Q21. Which impacts of climate change do you think are of most immediate concern in your work/area of industry?
- Reduced growth - Increased disease susceptibility - Mass mortality of stock - Reduced reproductive success - Behavioural changes (e.g., heightened aggression, etc) - Altered sex ratios - Reduced suitability of farm site - Reduced product quality - None of the above - Prefer not to say - Other (please specify)
Q22. On a scale from 1 to 10, indicate how much research do you believe is available on the effects of climate change on your work species/industry area.
- 1 = No research exists for this species/industry area - 5 = Some research / Unsure - 10 = Lots, this is a heavily researched species/industry area
Q23. Is there one research project on your industry area/farmed species in relation to climate change that you would request if you could? If so, what would this be?
- Prefer not to say - None – there is no research I would want. - Yes (please describe your research request)
Q24. Please let us know if you have any other comments relating to the possible impacts of climate change on the aquaculture industry in NZ and Australia, and research on this topic (optional).

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