



Effects of climate change on reproduction,  
larval development, and adult health of coral  
trout (*Plectropomus* spp.)

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## 1.0 NON TECHNICAL SUMMARY

**2010/554** Effects of climate change on reproduction, larval development, and population growth of coral trout (*Plectropomus* spp.)

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### OBJECTIVES:

The overarching objective of this study was to test the sensitivity of coral trout (mainly, *Plectropomus leopardus*) to changes in habitat and environmental conditions (specifically, increasing temperature and ocean acidification) linked to ongoing climate change. Comprehensive understanding of all direct and indirect effects of climate change on coral trout, and across all different life stages, will require extensive ongoing research. This study comprised four independent projects intended as the first step towards understanding whether large, commercially important fisheries species will be more or less vulnerable compared to small site-attached reef fishes on which prior research has been conducted. These four projects explored:

1. Effects of increasing temperature and ocean acidification on fertilisation and egg development using gametes obtained *P. leopardus* that were spawned in captivity
2. Effects of ocean acidification (declining pH) on the sensory discrimination and behaviour of early post-settlement *P. leopardus*
3. Variation in sensitivities and responses of adult *P. leopardus*, directly comparing between fishes from northern and southern sections of the Great Barrier Reef
4. Reliance on live corals for settlement and post-settlement survivorship, to test whether coral trout will be adversely affected by climate-induced bleaching and coral loss

### OUTCOMES ACHIEVED TO DATE:

This project has unequivocally shown that coral trout and particularly, *Plectropomus leopardus*, are sensitive to changes in habitat and environmental conditions expected to occur as a consequence of sustained and ongoing climate change. More specifically, coral trout will negatively affected by degradation of coral reef habitats (specifically, declines in abundance



of *Acropora* colonies), increasing temperature, and ocean acidification. It is unclear if, when, or how these changes will impact the size, abundance and catchability of coral trout, which are key to understanding effects on the productivity and profitability of wild fisheries and particularly, commercial fisheries. However, this project shows that coral trout are vulnerable to climate change and so, more research is urgently required to understand the specific effects on wild stocks and associated fisheries, as well as exploring appropriate adaptation options within each of the distinct fisheries sectors; commercial, charter, recreational and indigenous fisheries

### KEY BIOLOGICAL FINDINGS:

1. Development, growth and survival of larval coral trout (*Plectropomus leopardus*) were severely impacted at 2-3°C above current ambient temperatures, at 30-32°C. Unless *P. leopardus* is able to adapt to increasing temperatures (e.g., by spawning earlier in the year) fertilisation success and larval development and survival are likely to be much lower with ongoing temperature increases, especially in the northern GBR.
2. Although ocean acidification did not seem to have an effect on larval growth and survival, it greatly altered the behaviour of common coral trout larvae. In more acidic water expected by the end of the century, *P. leopardus* larvae spent a lot less time in the safety of shelter and were attracted to predator odour instead of avoiding predators in current conditions. This is expected to lead to much lower levels of survivorship in newly settled larvae and juveniles.
3. Juvenile coral trout (specifically, *P. maculatus*) had a strong reliance on live corals at settlement. Importantly, >70% of trout that were <15cm TL were found living in close association with live colonies of *Acropora* corals in the Keppel Islands. This strong affinity with one specific habitat, suggests that live coral must offer a significant fitness advantage in terms of growth and survivorship. Therefore, ongoing declines in the abundance of *Acropora* would be expected to reduce successful recruitment. Tests of the generality of these findings for other coral trout (e.g., *P. leopardus*) are ongoing.
4. Temperature had a strong effect on the metabolism (aerobic scope) and survival of adult *P. leopardus*. The cost of staying alive increases with temperature, but energy available for activities such as foraging were greatly reduced at 33°C.



Furthermore, coral trout were unable to survive exhausting physical activities at 30°C and 33°C, but were not affected at 24°C and 27°C.

5. Populations of *P. leopardus* from the northern and southern GBR seemed to be equally affected by absolute (not relative) temperature increases. If coral trout are adapted to local temperature regimes the northern population would be expected to be able to tolerate higher temperatures than southern populations. Our findings suggest that the common coral trout has a very limited capacity to adapt to increasing temperatures.
6. Although higher temperatures require fish to spend more energy to meet metabolic demands, individual fishes did not compensate by increasing food intake. Unless fish are able to behaviourally adapt to increasing temperatures (e.g., by moving to deeper cooler waters), they are likely to lose condition during hot summer months, which would lead to reduced growth and reproductive output.

#### **IMPLICATIONS FOR FISHERIES MANAGEMENT:**

There are several different ways in which climate change will affect coral trout, but the most immediate and direct impacts relate to effects of increasing temperature on larval and juvenile life stages. Our research suggests that wild populations of coral trout on Australia's Great Barrier Reef will be severely compromised due to limited survivorship of larval trout whenever or wherever ocean temperatures exceed 30°C. Tank-based studies of aerobic scope, suggest that these effects will be further compounded by declines in the size, growth and reproductive capacity of adult fishes regularly exposed to these temperatures. Average reef-wide sea surface temperatures (SST) on the Great Barrier Reef are expected to exceed 30°C between 2065-2080, though maximum summer-time temperatures already approach (if not exceed) this threshold in the northern GBR. If coral trout are unable to adapt or acclimate to increasing temperatures (behaviourally or physiologically) then it is very likely that the sustainability of fisheries for coral trout on the GBR will be undermined by sustained and ongoing increases in ocean temperatures, and these effects will be felt first and worst in the northern GBR, where temperatures are already much closer to the 30°C threshold. The key however, is to understand how individual fish and populations as a whole respond to fluctuations in ocean temperatures, both on very short (days to weeks) and longer time-frames (generations). It may be that tank-based studies conducted here, overestimate the vulnerability of coral trout to increasing temperatures and other climate-induced changes in environmental and habitat-conditions by failing to account for behavioural responses that can occur in the



wild. For example, large mobile fishes, such as coral trout, would be expected to have increased capacity (compared to small site-attached reef fishes) to escape areas of warm water by moving offshore and to greater depths.

#### **FURTHER RESEARCH & DEVELOPMENT:**

This project represents the very first step in assessing the vulnerability of coral trout to climate change, using manipulative experiments to assess the sensitivity of fishes to projected changes in environmental and habitat conditions. Given the limited time and budget, research was focussed on several processes and life-stages (e.g., survivorship of zygotes immediately after fertilization) that were expected to be extremely vulnerable. Even so, the sensitivity of coral trout to increasing temperature, ocean acidification and climate-induced habitat degradation was far greater than was expected. The imperative now is to understand how these effects will manifest in terms of the productivity and sustainability of fisheries for wild stocks on the GBR. Essential future research falls in to three key areas:

1. Explore the comprehensive range of effects that climate change will have on the individual condition and demography and population dynamics of coral trout, considering not only *P. leopardus*, but also *P. maculatus*.
2. Explore behavioural and physiological responses of individuals and populations to environmental extremes, in order to better understand the adaptive capacity of coral trout. This is key to understanding the consequences of climate change on wild stocks and associated fisheries.
3. Explore adaptation options for fishers and fisheries that rely heavily on coral trout. While it is not yet clear if or when coral trout populations will be compromised, there are significant advantages (in maximising the range of adaptation options available) to initiating research in to the adaptive capacity of the fishery as soon as possible.

#### **KEYWORDS:**

Serranidae, *Plectropomus*, climate change, Coral Reef Fin Fish Fishery, larval development, aerobic scope, gene expression, vulnerability, sensitivity, exposure, adaptive capacity, temperature, habitat degradation, ocean acidification



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### 3.0 BACKGROUND

Climate change is emerging as the single greatest threat to coral-reef ecosystems and reef-associated fishes (Munday et al. 2008, Pratchett et al. 2008, Bell et al. 2013). The most immediate impacts will be a loss of diversity and changes to fish community composition as a result of coral bleaching and climate-induced habitat degradation (Pratchett et al. 2008). Such effects are likely to become increasingly important in the future, and may lead to eventual declines in abundance and productivity of key fisheries species (e.g., Pratchett et al. 2011c, Bell et al. 2013). However, increasing experimental studies are also showing that fishes will be directly affected by increasing temperature (e.g., Pörtner 2001, Perry et al. 2005) and ocean acidification (e.g., Pörtner and Farrell 2008, Munday et al. 2009a,b), linked to global climate change. Additional environmental changes and severe climatic events, such as increased severity of tropical cyclones, more extreme rainfall events and sea level rise, could have further important effects for some taxa or life-stages (Munday et al. 2008, 2009a), but these effects will be mostly due to changes in habitat structure. Tropical cyclones, for example, are expected to become more intense in a warmer world (Webster et al. 2005) contributing to increased incidence and severity of disturbances within coastal environments. Tropical cyclones often cause a temporary decline in the abundance of some fishes (Halford et al. 2004), but this is mostly due to the loss of critical habitat or food (Wilson et al. 2006). Higher intensity cyclones will cause greater damage to key habitat-forming species, such as corals and sea grasses (Madin and Connolly 2006), compounding upon other climatic disturbances, such as coral bleaching.

Effects of climate change on marine organisms will vary depending on their specific biology and ecology (Munday et al. 2008). For example, recent effects of climate-induced coral loss on reef fishes vary depending upon their reliance on live corals for food, shelter and/ or recruitment (Pratchett et al. 2008). Other fishes may also be susceptible to climate change owing to their specific sensitivities to changes in temperature or ocean chemistry. However, research on ecological effects of climate change on coral reef fishes is mostly limited to small, strongly habitat-associated species, such as anemone fishes (e.g., Munday et al. 2009a), which have little relevance to food fishes and fisheries. In order to establish the economic consequences of climate change based on potential effects on important fishery species, there is definite need to test effects of increasing temperature and ocean acidification on larger, piscivorous species.



### 3.1 Family Serranidae

The family Serranidae is one of the largest and most diverse families of perciform fishes, comprising nearly 500 species. The family includes very large carnivorous species (e.g., *Epinephelus lanceolatus*) as well as several smaller planktivorous species (e.g., *Pseudanthias* spp.). However, the best-known species, and most conspicuous components of reef fish assemblages, are the larger *Epinephelus* and *Plectropomus* species (subfamily Epinephelinae) that are among the largest coral reef fishes. As dominant predators, the epinepheline serranids (groupers) also play a major ecological role in structuring reef fish assemblages (e.g., Almany 2003).

Epinepheline serranids are among the most important and valuable tropical fisheries species, and have been heavily exploited throughout the world (e.g., Morris et al. 2000). In particular, groupers are the most intensively exploited group for the live fish trade (Morris et al. 2000), and the high prices paid for individual fishes (up to US\$10,256) places considerable pressure on target species (Sadovy and Vincent 2002). Since the late 1960's the demand for groupers (and other high-value species, such as *Cheilinus undulatus*) has resulted in sequential over-exploitation of reefs and locations at increasing distances from the central market (in Hong Kong), including the Philippines, Indonesia, Solomon Islands, and Fiji. Many epinepheline serranids also have biological characteristics that make them particularly vulnerable to fishing. In particular, many of the most heavily exploited species, tend to form very large spawning aggregations that can be readily targeted by local fishers (Morris et al. 2002). The Nassau grouper (*Epinephelus striatus*), for example, may form aggregations of tens of thousands of individuals, and individuals migrate up to 240 km to form aggregations. Heavy fishing of spawning aggregations is the major reason for the catastrophic decline in populations of *E. striatus*, which is now regarded as Endangered throughout the West Indies (Beets and Hixon 1994).

Reproductive biology of fishes in the family Serranidae is diverse and complex. Most serranids are protogynous hermaphrodites, changing sex from female to male, as has been shown for two species of coral trout *Plectropomus laevis* and *P. leopardus* (Adams 2003). There are however, a number of exceptions and variations to the generalised reproductive pattern. For example, Sadovy and Colin (1995) deduced that the *E. striatus* is gonochoristic, whereby juvenile fishes become males or females upon maturity, and there is no evidence of



sex change in the field. As a consequence, it is difficult to generalise about the reproductive biology for the large number of species (75%) for which nothing is currently known about reproductive biology. Also, most of the biological research on serranids has been conducted in the Caribbean and for species that do not occur in Australia (e.g., Sadovy and Colin 1995), even though the highest diversity of serranids is found within the Indo-Pacific.

### 3.2 *Plectropomus leopardus*



Figure 3.2.1. The common coral trout or leopard grouper, *Plectropomus leopardus*.

The single most important fishery species on Australia's Great Barrier Reef is the common coral trout, *Plectropomus leopardus* Lacepède 1802 (Figure 3.2.1). This species is found mainly in the western Pacific from southern Japan and Australia, eastward to Fiji. *Plectropomus leopardus* is ecologically and economically important, representing major target species of commercial reef-based fin-fish fisheries in Australia and accounts for > 50% of the total commercial catch (Mapstone et al. 1996). Historically (prior to 1998), most *P. leopardus* were caught and filleted for the Australian domestic market, but >90% of the catch is now exported live to the lucrative Southeast Asian markets. Exports of coral trout are worth as much as \$67.3 million per annum, and coral trout are the economic mainstay reef-based fisheries in the western Pacific (Welch et al. 2008). While the biomass and abundance of coral trout are certainly higher on reefs closed to fishing, compared to fished reefs (e.g., Williamson et al. 2004), commercial fisheries for *P. leopardus* on the Great Barrier Reef are generally



considered to be sustainable (Mapstone et al. 1996). If however, coral trout are sensitive to increases in temperature and/or ocean acidification, ongoing climate change could greatly undermine the sustainability of these, and many other reef-based fisheries in the Pacific (Pratchett et al. 2011).

### ***3.3 Effects of temperature on fish***

Every organism thrives within a limited range of environmental conditions, for which it is optimally adapted. Optimal conditions and the range of conditions an organism can tolerate vary greatly between species and species groups. For ectotherms (e.g., fishes) temperature plays a vital role, as their internal temperature directly reflects what they experience in their environment. Temperature determines the distribution of a species and influences all levels of biological organisation, including nearly all biochemical, physiological and life history activities (e.g., Feldhaus et al. 2010, Beitinger et al. 2000). Virtually all cellular processes, including protein stability and enzymatic activity are influenced by temperature (Hochachka and Somero 2002), but the thermal environment also affects habitat choice and behaviour (Gamperl et al. 2002). Fish exposed to thermal conditions outside their optimal range are able to allocate less energy to development, growth, reproduction, foraging and overall fitness is reduced. Once a certain threshold is exceeded, passive survival is only possible for a limited time period (Pörtner and Farrell 2008). Although some studies have shown that different populations of the same species can adapt to their local thermal environment (Edmunds et al. 2010, Gardiner et al. 2010, Eliason et al. 2011), whether or not this is true for other species and whether these populations will be able to adapt/acclimate to changing environmental conditions is less well known.

Fishes are poikilotherms and rely on the thermal performance of vital physiological processes to survive (Pörtner and Farrell 2008, Pörtner et al. 2010). When occupying habitats with optimum temperatures fish may benefit from increased metabolic scope (i.e. the difference between resting and maximum oxygen uptake, equivalent to the energy available for activities), increased oxygen delivery to tissues, stronger and faster muscle contraction, and increased swimming performance (Luiker and Stevens 1994, Pörtner 2009, Pörtner et al. 2010, Johansen and Jones 2011). In turn, this can improve foraging, predator evasion and reproduction (Wood and McDonald 1997, Pörtner et al. 2010). However, at temperatures above optimum, tissues and muscles require more oxygen to function while metabolic scope may be severely reduced and activities must then be prioritized within the remaining available



energy (Pörtner et al. 2010). Under these conditions, individuals may be forced to compromise between food-intake and energetically demanding activities such as swimming. As temperatures increase due to global warming it is predicted that populations of thermally sensitive marine organisms will shift to higher latitudes, while populations that endure warmer waters may exhibit changes in their life history traits, such as growth rates and average longevity (Munday et al. 2008). Global warming is likely, therefore, to have significant consequences for the distribution and abundance of key fisheries species, as well as productivity and composition of coastal fisheries.

Fishes from tropical waters, such as coral reefs, have evolved in a comparatively stable environment and may therefore only be tolerant to a narrow range of temperatures. As a result, coral reef fishes are often expected to be particularly sensitive to global warming (e.g., Deutsch et al. 2008, Munday et al. 2008). The response of coral reef fish species to climate change will depend on how close a species already is to their thermal limit in their natural environment and how the species will respond to increasing temperatures (Stillman 2003). To what degree a species will be able to adapt or acclimatise its thermal tolerance will determine the persistence of populations in regions where the temperature will exceed their current thermal limits.

### ***3.4 Effects of ocean acidification on fish***

Ocean acidification, caused by the uptake of additional carbon dioxide (CO<sub>2</sub>) at the ocean surface, is a serious threat to marine ecosystems (Hoegh-Guldberg et al. 2007, Fabry et al. 2008) and associated fisheries (e.g., Cooley and Doney 2009). Additional CO<sub>2</sub> dissolved in the ocean reacts with seawater to form weak carbonic acid, causing pH to decline and reducing the availability of dissolved carbonate ions that are required by many marine calcifying organisms (particularly corals and other invertebrates) to build their shells or skeletons (Orr et al. 2005). Ongoing ocean acidification will reduce growth and survivorship of many calcifying organisms and thereby impact on food webs that depend on them (Fabry et al. 2008, Doney et al 2009). The reduction in ocean pH could also have a range of direct effects on marine organisms, from increased metabolic demands to developmental problems (Pörtner et al. 2004).



Increased CO<sub>2</sub> not only acidifies the ocean, it also acts to decrease the pH of animal tissue (Ishimatsu et al. 2005, 2008, Pörtner et al. 2004). Fishes compensate for this acidification with bicarbonate accumulation and counter-exchange of ion across the gills (Heisler 1989, Claiborne et al. 2002). Consequently, most fishes appear to be tolerant to a wide range of dissolved CO<sub>2</sub> levels and water pH (Ishimatsu et al. 2005, Pörtner et al. 2004, 2005). Compensation of acidosis is not detrimental in the short-term, but may impose long-term physiological costs, especially for species or life stages with high metabolic demands (Pörtner et al. 2004) or when compounded by higher metabolic demands at elevated temperatures (Pörtner and Farrell 2008). Direct effects of elevated CO<sub>2</sub> (hypercapnia) are likely to be greatest during the early life history phases of marine animals (Ishimatsu et al. 2004, Pörtner et al. 2004). The only study conducted to date on reef fishes using CO<sub>2</sub> levels relevant to climate change prediction (up to 1000ppm) did not detect any negative effects on the growth or development of clownfish larvae (Munday et al. 2009a). However, more studies are urgently needed to test the effect of elevated CO<sub>2</sub> across a broader range of species, and to test for possible synergistic effects of elevated temperature and CO<sub>2</sub>.

A greater concern for marine fishes is the effect that elevated CO<sub>2</sub> levels could have on the sensory ability of larvae (e.g., Munday et al. 2009b). Recent research has shown that clownfish larvae exposed to CO<sub>2</sub>-acidified water lost their ability to distinguish olfactory cues of preferred settlement habitat (Munday et al. 2009b), or to detect and avoid the smell of predators (Dixson et al. 2010), at the end of their larval phase. When reared in water treated with 1000ppm CO<sub>2</sub> the larvae became strongly attracted to chemical cues that they avoided in control water. The larvae of many marine fishes use chemical cues for a wide range of important behaviour decisions, including navigation to reefs and selection of settlement sites (Atema et al. 2002, Gerlach et al. 2007). Impairment of this process by ocean acidification could have serious implications for the replenishment of adult populations and patterns of population connectivity in coral reef ecosystems.



#### 4.0 NEED

This research is critical to the National Climate Change Adaptation Research Plan, and addresses several of the information needs and research gaps identified under sectoral sub-themes of i) Aquaculture, ii) Commercial and recreational fishing, iii) Conservation management, and iv) Tourism and recreational uses.

Specifically this study:

- i) Addresses the severity of likely impacts of climate change on coral trout, which are the No. 1 commercial and recreational fin-fish fisheries species caught within coral reef waters, and account for 41% of wild-caught fish in Queensland waters;
- ii) Will predict spatial and temporal changes in the fisheries production of coral trout populations across the entire area of the Great Barrier Reef, which is critical for spatial zoning of fisheries closures and assessing the immediacy of required intervention;
- iii) Identifies environmental tolerances of coral trout, especially during highly sensitive larval stages, which impacts natural recruitment of wild populations, and grow-out of juveniles in open aquaculture systems, which is necessary for further development of coral trout production;
- iv) Explores ontogenetic changes in the habitat requirements of newly-settled, juvenile and sub-adult coral trout, thought to rely on habitat structure provided by good coral growth. If so, this will help to establish coral reef habitats of high conservation priority, and
- v) Significantly advances understanding of climate impacts on coral reef fishes, which are critical for both fisheries and tourism industries on the Great Barrier Reef, currently worth in excess of \$6 billion to the Australian economy.

The research is critically important for commercial and recreational fisheries, and has strong endorsement by the Great Barrier Reef Marine Park Authority (GBRMPA), which is responsible for conserving biodiversity of the Great Barrier Reef and ensuring ecologically sustainable use of its resources. The proposed research is of considerable benefit to GBRMPA in that it addresses several of the action strategies identified in the GBRMPA Climate Change Action Plan, including i) to explore specific sensitivities of ecologically and economically



important species (e.g., top predators and key fisheries species) to climate change, ii) identifying areas of low and high resilience to climate change to prioritize investment of management effort, and iii) identify thresholds beyond which climate change causes irreversible damage to vulnerable species. Ultimately, this research will help to fill key knowledge gaps about climate change impacts on larger fishes, which are fundamental to optimizing resilience-based management, and in turn improve the adaptive capacity of industries and communities along the Great Barrier Reef.





## 5.0 OBJECTIVES

This project was intended to provide a holistic overview of the vulnerability of coral trout (mainly, *Plectropomus leopardus*) to sustained and ongoing climate change on Australia's Great Barrier Reef. This involved studies to test both direct (e.g., effects of increasing temperature and ocean acidification) and indirect (climate-induced habitat degradation) effects of climate change at all stages in the life cycle of these fishes. This study used a comprehensive, multi-disciplinary approach to assess the effects of climate change on *Plectropomus* spp. Methods used encompassed physiology (metabolic rate, blood parameters, physical activity, critical thermal maximum), genetics (gene expression), biology (fertilisation, development) and ecology (habitat use).

Preliminary objectives of this study were fourfold.

5. To assess sensitivities of coral trout to climate-related changes in temperature and seawater chemistry, during fertilisation and early larval development
6. To test the effects of ocean acidification on behaviour of early post-settlement coral trout
7. To test for spatial variation in sensitivities to increasing temperatures among adult populations of coral trout along the Great Barrier Reef
8. To measure coral-dependence at different ontogenetic stages, to test whether coral trout will be adversely affected by climate-induced bleaching and coral loss



## 6.0 METHODS

### *6.1 Effects of temperature and ocean acidification on larval survival*

Effects of climate change on large, piscivorous fishes (e.g., *Plectropomus leopardus*) are expected to be most pronounced during early life-history stages, from fertilisation through to first feeding. This is because of the small size of individuals (Ospina and Mora 2004), but also the critical effects of temperature and other environmental conditions on developmental rates and the incidence of abnormalities. Tests of the effects of increased temperatures and pH were conducted using eggs and larvae obtained from captive broodstock of common coral trout (*Plectropomus leopardus*) at the Northern Fisheries Centre (NFC), Queensland Department of Agriculture, Fisheries and Forestry.

Captive broodstock (1 male : 4 females sex ratio) were maintained in 30,000L tanks and reproductive condition controlled by manipulating the photo-thermal regime ( $27.5 \pm 0.3^{\circ}\text{C}$ ; 13:11 hours ratio of day and night; 28-day lunar cycle). All broodstock are conditioned on a diet of pilchard, squid and vitamin/mineral supplement. Spawning occurred monthly over a 7-12 day period prior to the new moon. The fertilised eggs are collected in external nets due to partial flow-through of the recirculation system then harvested either immediately at 2-cell stage or 9-10 hours later at early neurula stage.

#### *6.1.1 Larval growth and survival during the endogenous nutrition phase*

Trials for the effect of increased temperature on larvae during the endogenous nutrition phase required spawned eggs to be collected at early neurula stage (4-6 myomeres) to prevent mortalities associated with handling eggs at gastrula and late neurula stages. All fertilised eggs were ozone washed (disinfection) prior to transfer to a replicate 300L experimental system. Three temperature treatments were selected at  $28^{\circ}\text{C}$ ,  $30^{\circ}\text{C}$  and  $32^{\circ}\text{C}$  in a triplicate randomised design. Fertilised eggs were stocked at 30 per litre in ambient hatchery conditions ( $27.5^{\circ}\text{C}$ ) before tank temperatures were increased approximately by  $0.5^{\circ}\text{C}$  every 30 minutes to desired levels and maintained for the duration of temperature trials. All replicates were sampled at initial hatch then every 300 degree-hours until eye pigmentation was present in all larvae. The presence of eye-pigmentation is a developmental stage prior to the mouth opening and the requirement for exogenous feeding. Treatment groups were terminated based on developmental stage at sampling intervals to prevent mortalities associated with starvation, as



developmental rates increase with temperature. All larvae were collected at completion of each trial for morphological measurements (total length and yolk sac volume) and percentage survival data. In addition, the effect of increased temperature on hatch rate was assessed after 600 degree-hour (<24hour), after which all larvae were also collected for morphological measurements (total length and yolk sac volume) and percentage survival data. Treatment effect on survival and morphology were tested for significance by analysis of variance.

### ***6.1.2 Sperm activation, fertilisation and egg development***

Coral trout males (n=7) were sedated with benzocaine and milt was collected by catheter to assess the effects of increased temperature on sperm activity. A droplet of milt was added to 5ml vials immersed in a temperature bath and activated by addition of seawater (pH 8.1) at each temperature treatment (28°C, 30°C and 32°C). A sample of activated milt was placed on a cavity slide with a coverslip and observed under 400 x magnifications to record activation levels. Observations were recorded immediately after activation (T=0) and again at time intervals until the cessation of >99% of movement. Motility was defined as any visible movement, not necessarily resulting in progressive movement. The intensity was assessed on a relative scale of 0 to 5 (Hogan and Nicholson 1987) as follows:

- 5** Most active sample observed; sperm creating currents
- 4** Very active sample; all sperm progressing rapidly
- 3** Less energetic head and tail movement; most in forward motion
- 2** Slow head and tail movement; some sperm progressing slowly
- 1** Head vibration only
- 0** No activity

All milt samples were activated at each temperature and a replicate sample collected a month later from each male.

The effects of increased temperatures on egg development required harvesting of naturally spawned eggs from external collection nets after spawning and assessing fertilisation rate and developmental stage. Fertilised eggs at the 2-cell stage (30-45 minutes after fertilisation) were selected by pipette using a stereomicroscope and transferred to 70ml vials (50 per replicate) and placed in a temperature block to acclimate to treatment temperature over a 15-25 minute period. Developmental rate and mortality were recorded every 3 hours until late stage embryo



development. A total of 4 temperature treatments (28°C, 30°C, 32°C and 34°C) were selected to establish maximum thermal limit for egg development.

Trials were also conducted on the synergistic effects of increased temperature and ocean acidification on fertilisation and egg development required to strip spawn fish and assess fertilisation rates under controlled environmental conditions. Female fish were selected based on the presence of tertiary (mature) eggs in a catheter sample and given a hormone injection (GnRH) 36 hours prior to strip spawning to induce final maturation of eggs. Viable milt was collected by catheter from running males (<8 hrs prior trial) and stored. 10µl of an equal mixture of milt from 2-3 males was used to fertilise 0.5ml of eggs. Both milt and eggs were incubated at treatment temperature (3 minutes) during dry mixing, after which sperm was activated with seawater of the desired temperature/pH regime. A further incubation of 5 minutes allowed fertilisation to occur before rinsing into larger 70ml vials and transfer to treatment vessels in the heat block. All three temperature and pH regimes (28 °C at pH 8.1; 30 °C at pH 7.8 and 32 °C at pH 7.6) were tested in triplicates. Initially fertilisation rate was counted after 1 hour to differentiate fertilised eggs (multiple cell division). Unfertilised eggs were then removed before development and mortality rates were recorded as per earlier development trial.

## ***6.2 Effects of ocean acidification on larval development and olfactory discrimination***

Larval coral trout (*Plectropomus leopardus*) were reared from naturally spawned eggs from captive broodstock at the Northern Fisheries Centre in Cairns, Australia (described in section 6.1). Buoyant fertilized eggs were harvested from incubators, and ozone disinfected prior to stocking at 30 eggs L<sup>-1</sup> in larval rearing tanks. Larval rearing included a sequential diet of copepods, rotifers, and *Artemia*. Metamorphosis commenced from 35 days posthatch and coincided with weaning onto a commercial pellet diet.

At approximately 45 days posthatching, juvenile coral trout were transported to James Cook University's experimental aquarium facility in Townsville (50 min by air). One hundred and sixty-six juveniles were randomly assigned to one of 16 replicate 60 L rearing tanks in groups of 10–12 fish per tank. Four tanks were assigned to each of the four CO<sub>2</sub> treatments and continuously diffused with air or CO<sub>2</sub>- enriched air as described below. Aerated rearing tanks had no water flow during the day, but were flushed for 40 min each night with clean, filtered, seawater that had been aerated all day with the same concentration of CO<sub>2</sub> as the rearing tanks



(see Munday et al. 2009b for details). Juveniles were fed twice daily with NRD diet (INVE Aquaculture). Tanks were checked daily for any fish mortality. At day 28, after completing behavioural trials, all remaining fish were euthanized, weighed ( $\pm 1$  mg), and standard length (SL = length from tip of nose to base of tail) measured with a micrometer ( $\pm 0.1$  mm).

Rearing tanks were continuously diffused with one of four concentration of CO<sub>2</sub> in air: average 397 (unmanipulated air), 550, 750 or 1050 ppm CO<sub>2</sub>. Control or CO<sub>2</sub>-enriched air was bubbled into each tank at 1.5 l m<sup>-1</sup> through a fine-pore airstone. The concentration of CO<sub>2</sub>-enriched air for each treatment was controlled by a scientific-grade pressure regulator and precision needle valve and measured continuously with an in-line infrared CO<sub>2</sub> probe (Vaisalla GMT222). Rearing tanks were sealed on top with a clear acrylic lid to limit CO<sub>2</sub> exchange with the atmosphere. The pHNBS in each rearing tank was measured daily with a portable meter (Hach HQ40D) calibrated with fresh buffers (Merck). pH was measured between 7-8am, approximately 12 hours after tanks had been flushed with clean treatment seawater the previous evening. Water temperature was maintained at 28.5°C with electronic heaters. Salinity was measured weekly and total alkalinity was determined weekly by titration. Seawater pCO<sub>2</sub> was estimated in the program CO<sub>2</sub>SYS using the constants of Mehrbach et al. (1973) refit by Dickson & Millero (1987). Average pCO<sub>2</sub> was estimated to be 490, 570, 700, and 960  $\mu$ atm (Table 1), therefore we report values of approximately 450, 550, 750, 1000  $\mu$ atm. Oxygen levels were checked regularly with an oxygen probe (CellOx 325 WTW, Germany) and were always above 90% saturation. A summer light cycle of 13 h light/ 11 h dark was simulated with fluorescent lights.

Previous experiments have found that the response of juvenile fish to predator odour is dramatically altered after 2-4 days exposure to elevated CO<sub>2</sub> (Dixson et al. 2010, Munday et al. 2010). The response of juvenile coral trout to the odour of a common predator, the rockcod *Cephalopholis cyanostigma*, was tested after 4 days in treatment and again after 28 days in treatment. This comparison allowed us to test if olfactory responses were affected by elevated CO<sub>2</sub> in a similar way to other species, and if they became acclimated following longer-term CO<sub>2</sub> exposure. A total of 20 fish from each CO<sub>2</sub> treatment (5 from each tank) were tested at each time period. Fish tested at day 4 were returned to their rearing tanks after the flume trials.



The response of juveniles to predator odour was tested in an Atema Flume, a two channel choice flume (13cm x 4cm) described in Gerlach et al. (2007). This apparatus was designed to conduct pair-wise choice experiments, with fish able to freely choose between water flowing from two different sources. Water from the two different sources was gravity fed into the choice flume, which was partitioned along half of its length. A constant flow of 100 ml min<sup>-1</sup> per channel was maintained using flow meters (Gerlach et al. 2007). One water source contained seawater with predator odour, the other contained untreated seawater. Water containing the chemical cue of a predator was created by soaking a single *C. cyanostigma* in 60 l seawater for two hours prior to testing. Both water sources were at the same CO<sub>2</sub> level as the rearing treatment of the fish. At the start of each trial a single fish was released at the downstream end of the flume where it was free to move to either side or swim toward the preferred water source. Each trial consisted of a two-minute acclimation period, followed by a two-minute testing period where the position of the fish, on either the right or left side of the chamber was recoded at five-second intervals. Then, there was a one-minute rest period and the water sources were switched from one side to the other, to ensure a side preference was not being displayed. The two-minute acclimation period and two-minute testing period were then repeated. Dye tests were conducted at each water change to ensure that the two flow channels exhibited parallel water flow with no areas of turbulence or eddies.

### ***6.3 Effects of body size on aerobic scope***

The performance of an animal is supported by aerobic scope, which is defined as the difference between oxygen consumption at rest and oxygen consumption at maximal activity. Resting metabolic rate indicates the amount of energy necessary to maintain basic body functions in a non-feeding, non-reproducing and non-motile state and is directly affected by body temperature and typically doubles or triples with a 10°C acute increase in temperature (Farrell 2009). As temperature increases, oxygen consumption in a resting fish therefore increases to meet the demands of higher metabolic rates. However, the capacity to increase oxygen consumption and maximal activity is limited by the oxygen transport limitations of the circulatory system (Clark et al. 2008). As a result, aerobic scope decreases when temperatures exceed optimal conditions (e.g., Munday et al. 2009a).

A total of 32 adult and 16 juvenile *P. leopardus* were collected during April 2012 on reefs within 15km of the Lizard Island Research Station (LIRS) on the Great Barrier Reef, Queensland, Australia (14°41'S, 145°27'E). Adult fish were caught using hand lines with a



single hook and pilchards (*Sardinops neopilchardus*) as bait and their size ranged from 360 to 570mm (total length) or 0.5 to 2.4kg. Juvenile fish were 130 to 210mm long (0.025 to 0.100kg) and were collected on SCUBA using barrier nets and an anaesthetic (clove oil). Upon arrival at the research station, fish were treated in a freshwater bath for two minutes to reduce the risks of skin infection and disease after catching and handling the fish. Each adult received a T-bar anchor tag (Hallprint, Hindmarsh Valley, SA, Australia) for individual identification. As these tags were too large for juvenile trout, elastomer tags injected under the skin were used instead. Adult fish were placed into 2000L plastic tanks, whereas juveniles were kept in 60L plastic tubs. All holding tanks were aerated and connected to the station's flow-through system. The ambient seawater temperature, with which the tanks were filled during the experimental period, was approximately 28.5°C ( $\pm 1^\circ\text{C}$ ). After an acclimation period to captivity for 2 weeks, the temperature in the 'hot' treatment tanks was increased at a rate of 0.5°C per day until 33°C was reached. Fish were fed pilchards every second day to satiation, but starved for a minimum of 48 hours before respirometry trials.

Minimum and maximum metabolic rate were determined using static chambers (respirometers). Metabolic rates were measured at ambient temperature within 3 to 5 days after capture at ambient temperature (28.5°C), as well as after 4 weeks to determine whether captivity was having an effect on metabolic rate. Trials at 33°C were run within 2 to 5 days after the temperature in the holding tanks reached 33°C, which coincided with the 4-week post capture trials at ambient temperature. To measure maximum metabolic rate, each fish was chased for 3 minutes, followed by 60 seconds air exposure and immediately placed into the chambers. Measurements were taken over a 20 to 24 hour period to record metabolic recovery and achieve resting metabolic rates. Background respiration was routinely checked, but undetectable at all times.

Respirometers were custom built of cylindrical PVC pipes to suit the size of the fish (Fig 6.3.1). Large respirometers were 25cm in diameter and approximately 70cm long with a total volume of 29.5 to 31L. Small respirometers for juvenile fish had a diameter of 11cm, were 25cm long and a volume of 2.5L. All respirometers were equipped with a closed circuit recirculation pump ensuring adequate water mixing inside the chamber and an automated flush pump. The flush pump replaced the respirometer water with naturally oxygenated seawater for 3 to 7 minutes, every 10 to 15 minutes. Oxygen consumption was measured between the flush cycles for 5 to 8 minutes. The length of the closed versus flush cycles was adjusted depending on the size of the fish inside the chambers to ensure the dissolved oxygen



inside the chambers never fell below 80% saturation. Dissolved oxygen inside the respirometer was measured continuously using fibre-optic sensors (PyroScience, Aachen, Germany), which were incorporated into the closed loop of the recirculation pump (Fig 6.3.2).

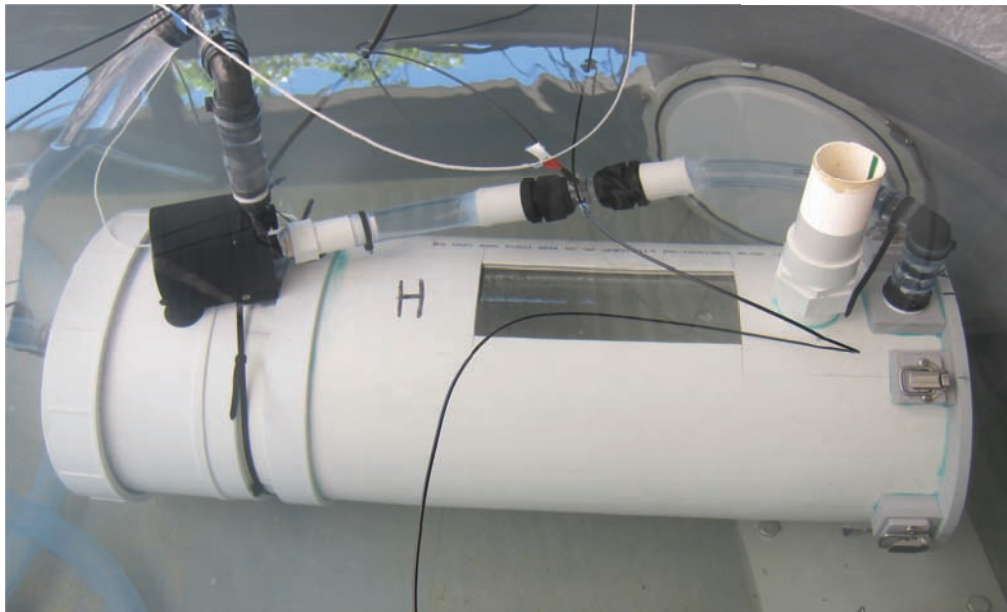


Fig 6.3.1: Custom built large respirometer used for adult fish. The recirculation pump can be seen attached to the respirometer, the flush pump is out of view.

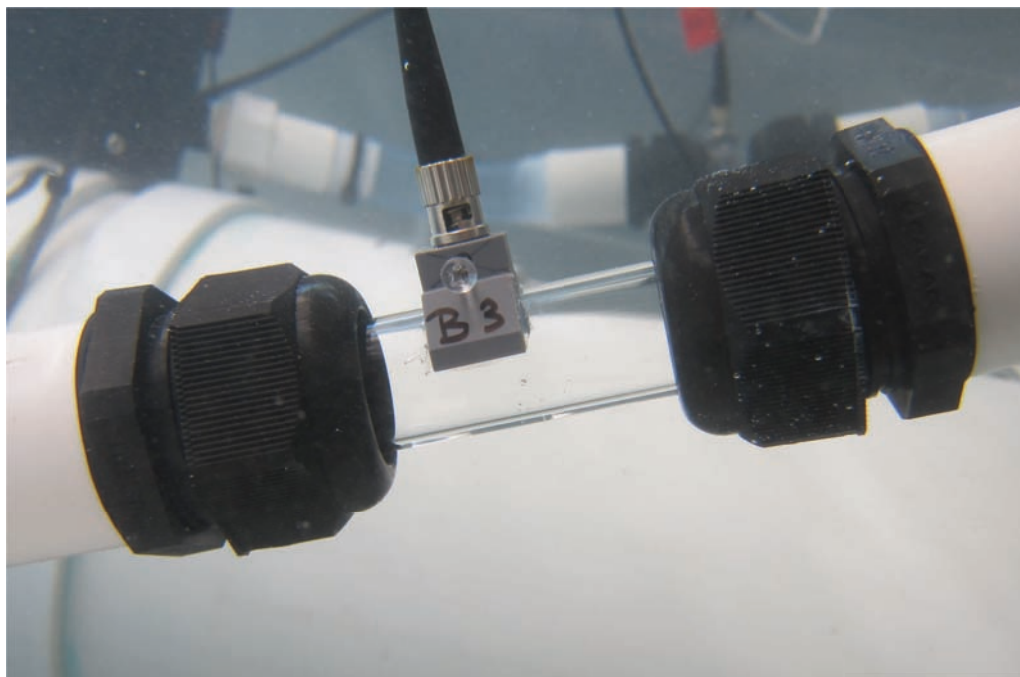


Fig 6.3.2: Fibre-optic sensor attached to glass tube in the recirculation loop measuring dissolved oxygen at a rate of once per second.





#### **6.4 Effects of temperature on aerobic scope**

To test the thermal tolerances of *P. leopardus* from the northern and southern GBR, 85 fish were sourced from commercial fishermen at each location in late June / early July 2012 and kept in eight 2000L tanks at 25°C to 26°C at the Marine and Aquaculture Research Facilities Unit (MARFU) at James Cook University. All fish were given a 2-min freshwater bath upon arrival, and two more within the first week to remove any external parasites, combat any signs of fin rot and other transport-related skin irritations and encourage production of the protective mucous layer to reduce risk of further infections and disease. Every individual was measured and weighed and received two unique T-bar anchor tags (Hallprint, Hindmarsh Valley, SA, Australia) for individual identification.

The fish were allowed to settle into the tank environment for 6 to 8 weeks to ensure the majority were weaned onto frozen fish and any compromised fish were eliminated. Fish were fed to satiation 5 to 6 times per week and the amount of food eaten by each fish was recorded. After the acclimation period, the remaining 151 fish were allocated to four temperature treatments with two replicate tanks per temperature: 24°C, 27°C, 30°C and 33°C. Ten fish from each location were placed in each tank, except for the two tanks at 27°C where only 7 or 8 fish per population were left (see experimental design in Fig 6.4.1). The temperature in each tank was slowly increased or decreased by 0.5°C per day until the experimental temperature was reached.

To test the acclimation capacity of the northern and southern populations to the different temperature treatments, minimum and maximum metabolic rates were measured after one week acclimation to 24°C and 30°C, as well as after six weeks to all experimental temperatures. Maximum and minimum metabolic rates were determined using the same static chambers as described in section 6.3. Eight large respirometers were set up in two 1000L oval tanks (similar to set up in Fig 6.4.2) and fitted with fibre-optic sensors (PyroScience, Aachen, Germany) to measure dissolved oxygen inside the chambers. The temperature in the respirometers was controlled to match the experimental temperatures in the holding tanks.

To measure maximum metabolic rate, each fish was chased in a 500L round tub for 3 minutes, followed by a 60 seconds air exposure and immediately placed into the chambers. Measurements were taken over a 20 to 24 hour period to record metabolic recovery and achieve resting metabolic rates. Background respiration was routinely checked, but was found



to be negligible at all times. Fish were given a two-minute freshwater bath after the respirometry trials before being returned into their holding tanks to encourage production of the protective mucous layer after handling. The text files from each fibre-optic probe were imported into LabChart software (ADInstruments, Sydney, Australia).

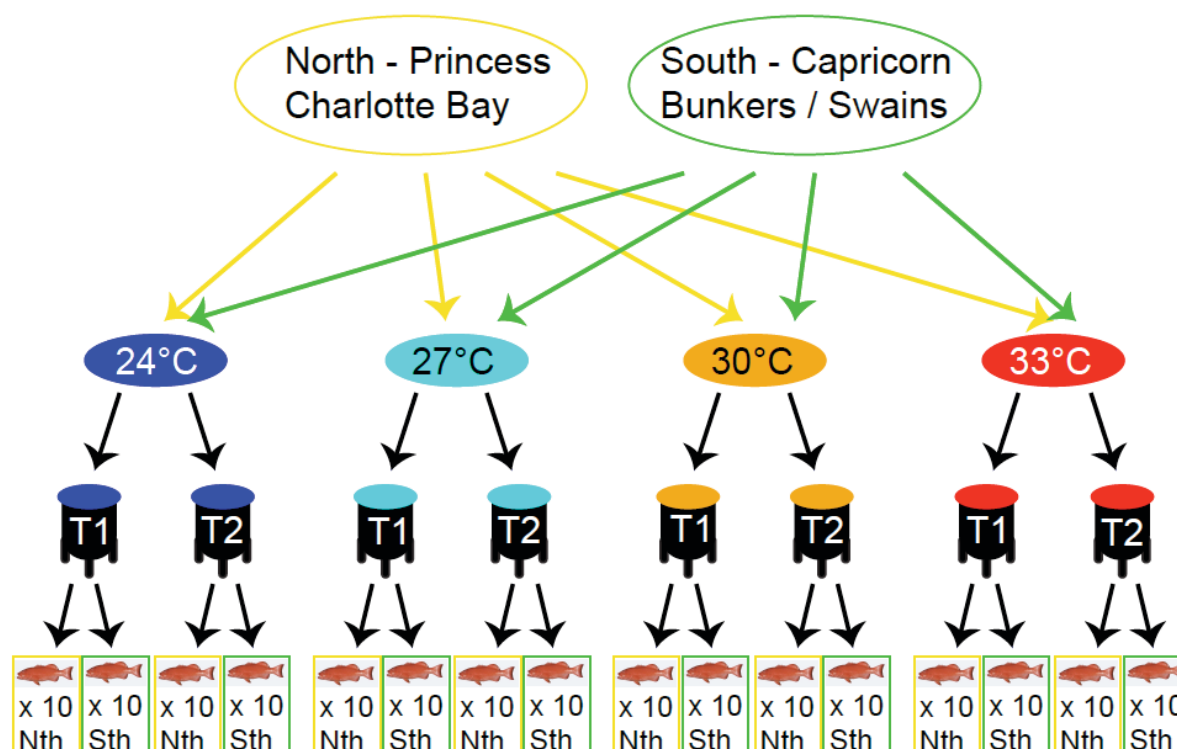


Fig 6.4.1: Experimental design testing the thermal tolerance of *P. leopardus* from the northern (Princess Charlotte Bay) and southern (Capricorn Bunkers / Swains) GBR. 10 fish from each population were placed into each of two replicate tanks for each temperature.

### ***Tissue sampling***

After an acclimation period of eight weeks, the following tissues from the remaining 103 fish were sampled and snap frozen in liquid nitrogen:

- *liver* for gene expression (*LdhB* and *Hsp70*) and liver fat content
- *heart* for ventricular mass
- *spleen* for Erythropoietin
- *muscle* for cholinesterase activity, potentially gene expression (*Hsp70*)

Blood samples were analysed for haemoglobin concentrations, haematocrit, lactate and glucose.



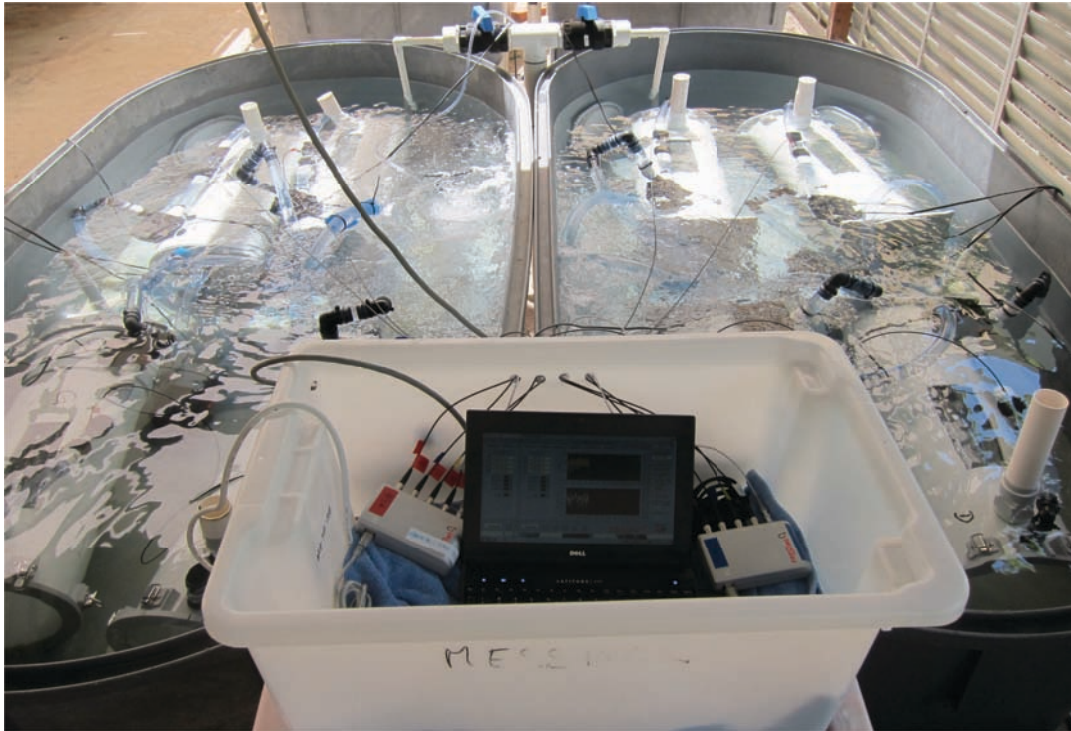


Fig 6.4.2: Example set up of four large respirometers (approx. 30L) in each of two 1000L oval tanks. Two firesting units with four fibre-optic sensors (PyroScience, Aachen, Germany) were used to measure dissolved oxygen in the respirometers.

### ***6.5 Comparisons of thermal sensitivity among widely separated populations***

Prior research on coral reef fishes (albeit relatively small and non-commercial species) has often shown that there are marked differences in thermal sensitivities of geographically separated fish populations (e.g., Gardiner et al. 2010). Populations that are naturally exposed to higher maximum temperatures (e.g., tropical versus temperate species) are likely to have a higher optimal temperature, whereby physiological performance (growth, reproduction, aerobic scope etc.) is greatest at a higher temperature compared to fishes that normally experience lower maximum temperatures. Pörtner and Farrell (2008) argued that thermal windows of fishes are evolved to be as narrow as possible, and should therefore vary spatially with differences in natural thermal regimes. Therefore, we compared coral trout (*Plectropomus leopardus*) collected from reefs off Princess Charlotte Bay (14°S) versus the Capricorn Bunkers / Swains Reefs (23°S). These two locations are separated by 1,200km, and there is a 2.2°C difference in average maximum sea surface temperatures. It was expected therefore, that populations of coral trout from these two regions would exhibit marked differences in their thermal sensitivity (Figure 6.5.1).



Although genetic analyses based on mitochondrial DNA of *P. leopardus* did not show any structure along the GBR (van Herwerden et al. 2009), high levels of local larval retention (Harrison et al. 2012) seem to indicate that gene flow between the northern and southern GBR is probably not very high. We would therefore expect *P. leopardus* of our two locations to at least acclimate, if not adapt, to their local conditions over generations and therefore exhibit marked differences in their thermal sensitivity.

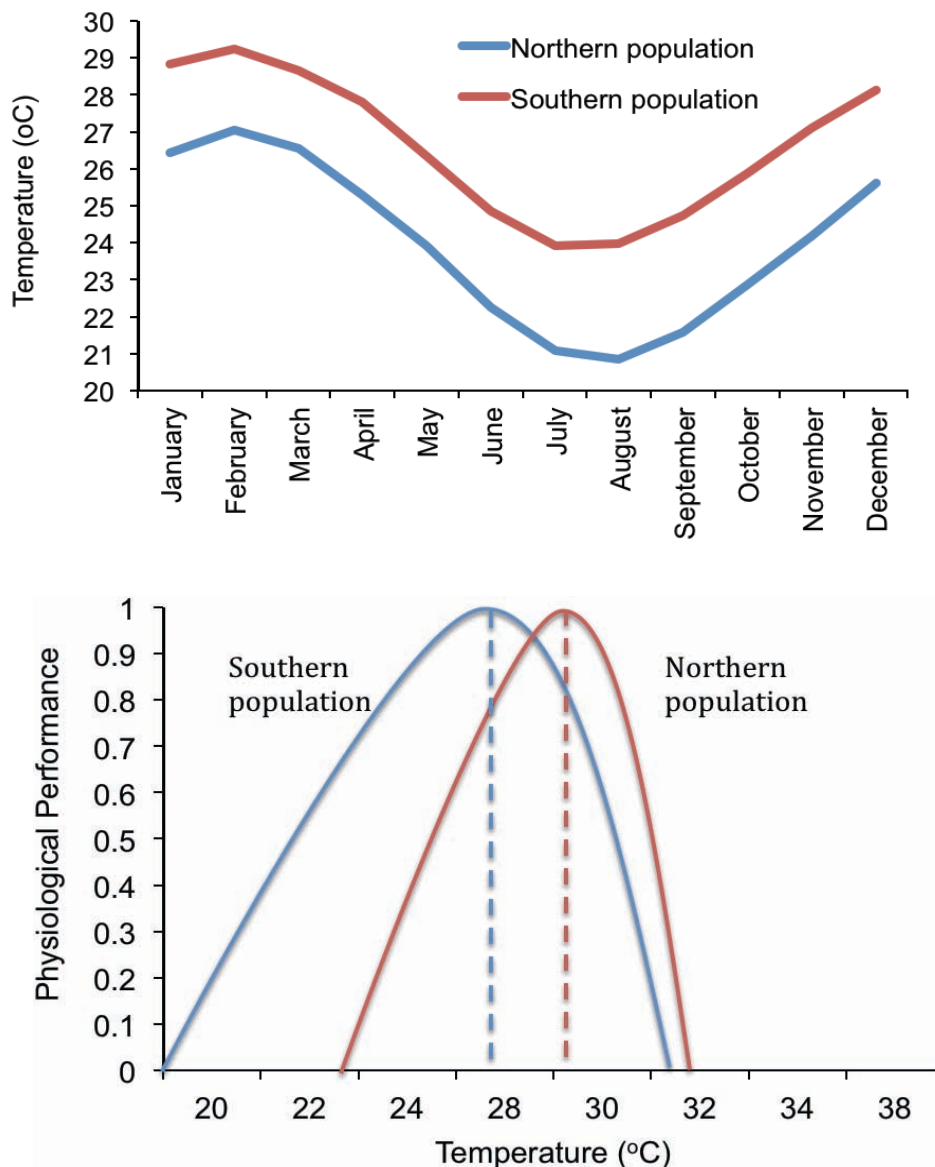


Figure 6.5.1 a) Mean monthly temperatures at 14°S versus 23°S on the Great Barrier Reef, from AIMS temperature loggers. B) Theoretical temperature performance curves for two distinct populations of tropical reef fishes on the Australia's Great Barrier Reef (GBR). Populations from the northern GBR (14°S) are naturally exposed to summer time maxima 2°C higher compared to conspecifics living the southern extent of the GBR (23°S), which is expected to result in higher thermal optima (indicated by the vertical dashed line), if not higher critical maxima (the upper temperatures at which basic metabolic functions can be maintained).



Identifying a single gene to use as a marker for thermal acclimation would be useful for reef managers as a fast and effective way to determine possible perseverance of a species in the face of climate change. Several genes have been evaluated in fishes as a putative means for assessing thermal acclimation, including lactate dehydrogenase-B (*Ldh-b*) and heat shock protein 70 (*Hsp70*).

*Ldh-b* encodes a tetrameric enzyme (LDH-B) involved in maintaining aerobic metabolism. During normal metabolism, pyruvate is converted to lactate in skeletal muscle by LDH-A (an isoenzyme of LDH-B) and then, in the liver and other tissues, lactate is converted back to pyruvate and glucose by LDH-B, continuing the cycle (Markert et al. 1975). In conditions of high anaerobic activity, poor swimming performance by rainbow trout (*Oncorhynchus mykiss*) is correlated with elevations in plasma lactate (Jain and Farrell 2003), illustrating the function of LDH-B and the requirement of adequate expression of the protein. LDH-B, therefore, functions to sustain aerobic activity for extended periods of time in fishes (Powers et al. 1991; Powers and Schulte 1998).

In the tropical fish barramundi (*Lates calcarifer*), southern (cold-adapted) and northern (warm-adapted) populations exposed to normal temperatures or elevated temperatures (+10°C, +5°C respectively), showed a significant increase in *Ldh-b* expression in all cases (Edmunds et al. 2012). In another fish species, *Fundulus heteroclitus* found along the East Coast of North America, there is an adaptive difference in *Ldh-b* expression between northern (cold-adapted) and southern (warm-adapted) populations: the northern fish express 2-fold more LDH-B in liver even after both populations are acclimated long-term to a common temperature (Crawford and Powers 1989). Schulte et al. (2000) discovered a repressor element upstream of the transcription start site that is present in the southern alleles, but one base pair different in the northern alleles. This repressor allows for variable transcription of *Ldh-b* in the presence /absence of stress conditions in southern fish. In northern fish, however, the modification of just one base pair eliminates repression of the gene, allowing perpetual expression of *Ldh-b*, regardless of stress conditions.

Unfolding of proteins can occur in situations of thermal, physiological or chemical stresses. In response, molecular chaperones called heat shock proteins (*HSPs*) are expressed to protect proteins and help refold them or, when the protein is too damaged, remove it through



proteolysis (Parsell and Lindquist 1993, Hartl 1996, Bukau and Horwich 1998, Morimoto 1998, Frydman 2001). For example, laboratory reared annual killifish, *Austrofundulus limnaeus*, that were maintained at constant temperature were exposed to either natural daily fluctuations in temperature of the wild population or chronically elevated or lowered temperature. For the temperature cycling fish, there was an initial increase in a number of molecular chaperones expressed but their levels returned to baseline after two weeks of cycling. Small HSPs (Hsc70, Hsp22) were strongly induced while the larger HSPs (Hsp70, Hsp90) were only mildly induced. In contrast, the chronically elevated temperature caused a strong induction of Hsp70 and Hsp90 (Podrabsky and Somero 2004), suggesting that these HSPs are required during times of chronic, increased stress to the organism.

In this study, total RNA was extracted from approximately 25ng of liver from 21 individuals using the PerfectPure<sup>TM</sup> RNA Tissue Kit (5 PRIME) as per manufacturer instructions: six from the northern and five from the southern population exposed to 24°C for two months and four from the northern and six from the southern population exposed to 33°C for two months. Total RNA quality was determined by absorbance readings with a NanoDrop Spectrophotometer (Invitrogen Australia Pty) and an RNase-free 1% agarose gel.

After normalising the quantity of RNA based on the NanoDrop readings, 1µg of each RNA sample was used to synthesise cDNA with iScript Reverse Transcription Supermix (Bio-Rad Laboratories) containing a blend of oligo(dT) and random primers. qRT-PCR was performed in triplicate 12µL reactions using 1x SsoFast EvaGreen Supermix (Bio-Rad Laboratories), 0.3mM forward and reverse primers (Table 1) and 2.5ng cDNA. Primers for *Hsp70* and two housekeeping genes, *Efla* and *Rpl7*, were designed using 3Prime (Leis et al., 2011) based on conserved regions of relevant fish genes obtained from GenBank. *LdhB* primers were obtained from Newton *et al.* (2012). Using the Chromo4 detector (Bio-Rad Laboratories) on a 96-well Thermal Alpha Block (MJ Research), qRT-PCR was performed with the following program: 95°C for 30s, 40 cycles of 95°C for 5s and 56.5°C for 15s. A melting curve was then performed to test for reaction specificity and determine the presence of primer-dimer. A standard curve was generated from five 1:5 serial dilutions of an equal mixture of all cDNA samples and, from this curve, qPCR efficiency was assessed using CFX Manager software (Bio-Rad Laboratories). The qbasePLUS<sup>TM</sup> software (Biogazelle) was used to validate the housekeeping genes (*Rpl7* and *Efla*) and normalise target gene (*LdhB* and *Hsp70*) expression. Fisher's LSD *post hoc* tests were completed where necessary.



## ***6.6 Temperature effects on daily food intake***

Throughout the 58 day experimental period, individuals were fed ad-libitum five to six times per week, with ~24g pieces of *Pagrus auratus*, and live *Acanthachromis polyacanthus* (~10g per individual). During every feeding session, the amount and type of food eaten by each individual was recorded.

To standardise for size-differences between individuals, the amount of food eaten by each individual was calculated as a proportion of body-weight (i.e. total grams of food eaten / body-weight in grams \* 100). Data on daily food intake was  $\log(x+1)$  transformed to comply with assumptions of normality and homogeneity of variance for ANOVA (Kolmogorov–Smirnov test on residuals and Levene’s test for homogeneity of variance; Meal size: K–Sd = 0.099,  $P < 0.2$ , Levene’s  $F_{1,117} = 1.216$ ,  $P = 0.299$ ; Feeding frequency: K–Sd = 0.117,  $P < 0.1$ , Levene’s  $F_{1,117} = 0.963$ ,  $P = 0.461$ ; Daily intake: K–Sd = 0.096,  $P < 0.2$ , Levene’s  $F_{1,123} = 1.478$ ,  $P = 0.181$ ). Individual differences in meal size and daily intake were then compared between temperatures using a set of 2-way ANOVAs, with temperature and population as the fixed factors. This was followed by post-hoc Tukey for specific differences within and among temperatures and populations. Analyses were restricted to food records obtained after holding tanks had stabilized on the treatment temperature. All data were analysed using SigmaPlot v12 and Statistica v10.0.

## ***6.7 Effects of temperature on spontaneous swimming speeds and activity patterns***

A single digital Go-Pro Hero 2 camera was fixed below the water line inside each holding tank, at a height of 110cm above the centre of the bottom. This allowed all individuals swimming near the bottom to be recorded by the camera. To standardise the distance between the camera lens and the test subjects, a central stand pipe was placed in each tank with four vertical arms at 25 cm above the bottom. Only those individuals swimming between these vertical arms and the bottom were included in the analysis.

The activity patterns of test subjects within each holding tank were videotaped for 2-4 hrs per day over a period of 12 days. All video recordings were conducted between 9am and 3pm, thereby focussing strictly on the daily activity patterns of this species. Videos were saved in 720p quality (1280 x 960), MPEG4 H.264 format at 29.971 frames per second.



The spontaneous swimming speeds of randomly chosen test subjects were analysed using Logger Pro v.3.8.6 (Vernier software) and Microsoft Excel. In short, based on the dorsal view of the fish, the x and y coordinates of the geometric centre of one of the eyes of each test subject was manually marked on every 10<sup>th</sup> frame over no less than 150 frames and exported into Excel for further analysis. This provided a record of the change in the x and y coordinates over a period of no less than 5 seconds. The average rate of spontaneous swimming speed (i.e. the cumulative distance swum in cm per second) was then calculated from the positional (x and y) data relative to a 10cm X 10cm grid drawn across the bottom of treatment tanks.

Resting activity of fishes within each temperature treatment was analysed by counting the number of individuals resting motionless on the bottom at random intervals of each video recording. An average of 336 individual observations (range: 319-376; total: 1345) was conducted within each temperature treatment. Resting patterns were then calculated as the average number of individuals resting on the bottom relative to the total number of fish in each treatment tank.

We used linear mixed effects models fit by restricted maximum likelihood (LME) to compare the spontaneous swimming speeds and activity patterns of individuals within each of the four temperatures (24, 27, 30 and 33°C). Linear mixed models are highly robust to non-independence of data points obtained on the same individuals and can produce unbiased estimates of variance and covariance (Bolker et al. 2009). Temperature and population (southern or northern) were treated as fixed effects, while holding tank nested within temperature, and observation nested within date, were treated as random effects. To assess the validity of these mixed effects analyses, we performed likelihood ratio tests by comparing the models with fixed effects to the null models with only the random effects. We rejected results in which the model including fixed effects did not differ significantly from the null model. Following square-root transformations on the swimming data and arcsine-sqrt transformation of the resting data, we checked for normality and homogeneity by visual inspections of plots of residuals against fitted values. Significance of main effects were estimated using Markov Chain Monte Carlo (MCMC) p-values at  $\alpha = 0.05$  (Bates and Maechler 2009). MCMC is robust to the fact that the exact degrees of freedom cannot be calculated in linear mixed model designs (Bates and Maechler 2009). Linear Mixed Model Tukey's were used to examine specific differences within and among temperatures, populations and holding tanks. False





detection rate (FDR) was used to correct for Type I errors following Benjamini and Hochberg (1995).

All data were analysed using SigmaPlot v12 and the R packages *lme4* (Bates and Maechler, 2009), *languageR* (Baayen et al. 2009), *LMERConvenienceFunctions* (Tremblay 2011) and *Multcomp* (Hothorn et al. 2008) (R Development Core Team, v2.15.2, 2012).

### ***6.8 Microhabitat preferences and reliance on corals***

A large proportion of coral reef fishes have very specific microhabitat requirements. Jones et al. (2004) suggested that up to 75 % of reef fish species rely on individual coral heads for food, shelter or recruitment substrata. Moreover, both abundance (e.g., Carpenter 1981) and diversity (e.g., Bell and Galzin 1984, Sano et al. 1984, Bouchon-Navaro and Bouchon 1989, Chabanet et al. 1997, Munday 2004) of coral reef fishes is often positively correlated with live coral cover. Declines in live coral cover, reported for many coral reef ecosystems around the world (Gardner et al. 2003, Bellwood et al. 2004, Bruno and Selig 2007,) will therefore affect many species of coral reef fishes (Sano et al. 1987, Jones et al. 2004, Pratchett et al. 2008). Effects of coral loss on fishes are most apparent among highly specialised coral-dependent species, including butterflyfishes, damselfishes and gobies (Pratchett et al. 2008). However, some larger, carnivorous species such as coral trout (*Plectropomus* spp.) also decline in abundance following extensive coral loss (Graham et al. 2007; Russ et al. 2008). On the Great Barrier Reef (GBR), for example, densities of coral trout (*Plectropomus* spp.) declined >20 % following severe coral loss at the Keppel Islands in 2006 (Russ et al. 2008). Mechanisms underlying these effects are unclear because there is little known about specific habitat requirements of such fishes.

In order to test for coral reliance among coral trout, field sampling was conducted at the Keppel Islands (23°10'S, 151°00'E), in the southern section of the Great Barrier Reef (GBR). Patterns of microhabitat use by juvenile (young of the year) coral trout (*Plectropomus maculatus*) were recorded at six different locations; North Keppel, Clam Bay, Halfway Island, Middle Island, Mial Island and Humpy Island (Wen et al. 2013). Four replicate 30-min swims were conducted at each location. For each fish, we recorded the specific microhabitat in which it was initially sighted, considering both the structural microhabitat and the underlying substrate (Wen et al. 2013). Availability of different microhabitat types was quantified using



50-m point-intercept transects following Pratchett et al. (2011b), recording the microhabitat category underlying 100 evenly spaced points along every transect. Four replicate transects were sampled in each of two different habitats (reef flat and reef slope) at each site.

Microhabitat selection was assessed using Manly's standardised selection ratio (B), which estimates the relative probability that a particular microhabitat type would be selected assuming that all microhabitats were equally available (Manly et al. 2010). Selection ratios were calculated for five major microhabitat types; i) *Acropora* on sand, ii) dead coral on sand, iii) *Acropora* on carbonate pavement, iv) dead coral on carbonate pavement, and v) sand or rubble without any structural microhabitat. There were many other distinct microhabitat types that were used infrequently, including macroalgae, or non-*Acropora* corals, but these were pooled into a single category, termed "other". Selection ratios were calculated separately for each location (North Keppel, Clam Bay, Halfway Island, Middle Island, Mial Island and Humpy Island) and then averaged across locations.



## 7.1 Effects of temperature and ocean acidification on larval survival

### 7.1.1 Larval growth and survival during the endogenous nutrition phase

Survival of larval coral trout was significantly reduced at increased temperatures above 28°C over the endogenous nutrition phase (Figures 7.1.1.1 and 7.1.1.2). The endogenous nutrition phase is the period between the developing embryo and first feeding on live prey items (exogenous feeding). Unlike the exogenous feeding larvae, the yolk-feeding larvae are energetically closed systems, in which energy utilisation is partitioned mainly between energy invested in tissue formation and energy invested in respiration. The survival of larvae at 600 degree-hours and after endogenous nutrition phase was significantly higher ( $P<0.05$ ) at 28°C compared to increased temperatures and remained comparatively similar (approximately 40%) for both trial periods. While 30°C and 32°C treatments had similarly lower survival rates at 600 degree-hours (Figure 7.1.1.1), higher mortality rates at 32°C after the endogenous nutrition phase resulted in significantly different ( $P<0.05$ ) survival across all treatments (Figure 7.1.1.2).

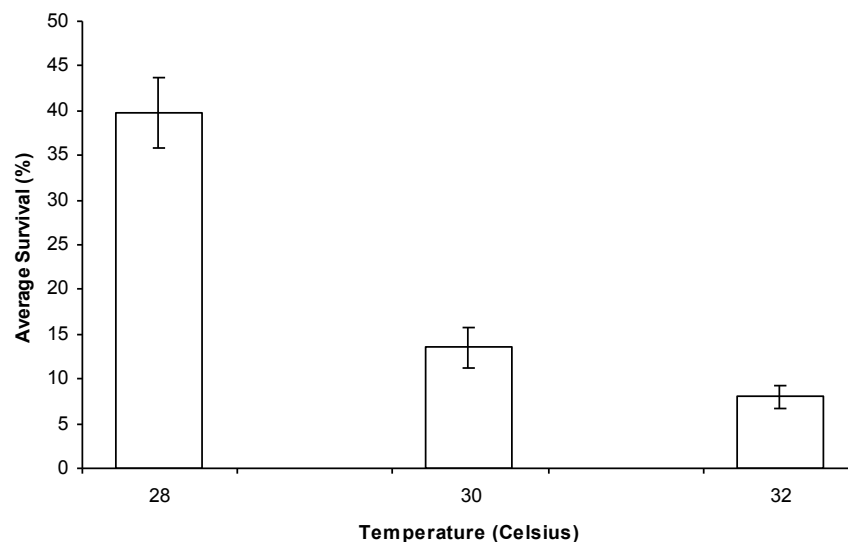


Figure 7.1.1.1. Survival rate (%) of coral trout larvae after 600 degree-hours of endogenous nutrition phase at different temperatures.



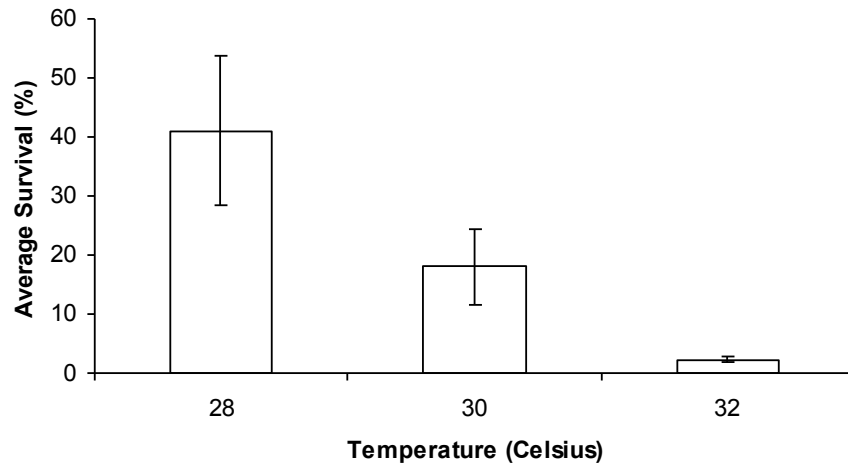


Figure 7.1.1.2. Survival rate (%) of coral trout larvae after endogenous nutrition phase at different temperatures.

Increased temperature resulted in significantly different ( $P < 0.05$ ) yolk sac volumes at hatching (Time=0 in Figure 7.1.1.3), while not significantly affecting larval size at hatching (Time=0 in Figure 7.1.1.4). The reduction in initial yolk reserves and increased rate of development during egg incubation are indicative of increased metabolic rate due to higher temperatures. Similarly, larger larval size at 28°C by the end of the endogenous phase indicates greater energy availability for somatic growth, while increased temperatures required yolk sac reserves to be used to maintain a higher metabolic rate.

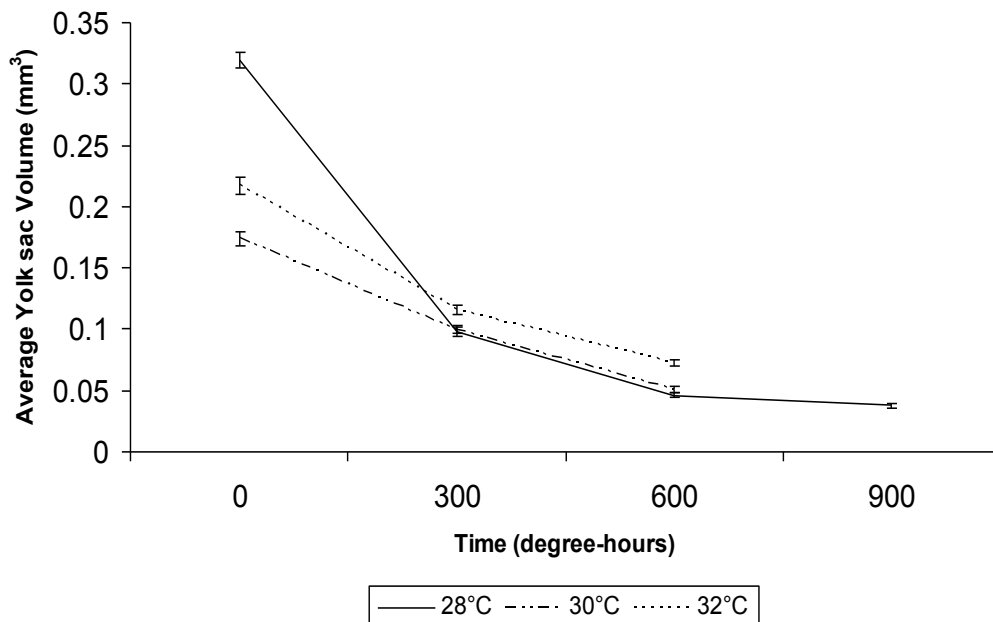


Figure 7.1.1.3. Average yolk sac volume of developing coral trout larvae during the endogenous nutrition phase at different temperatures.



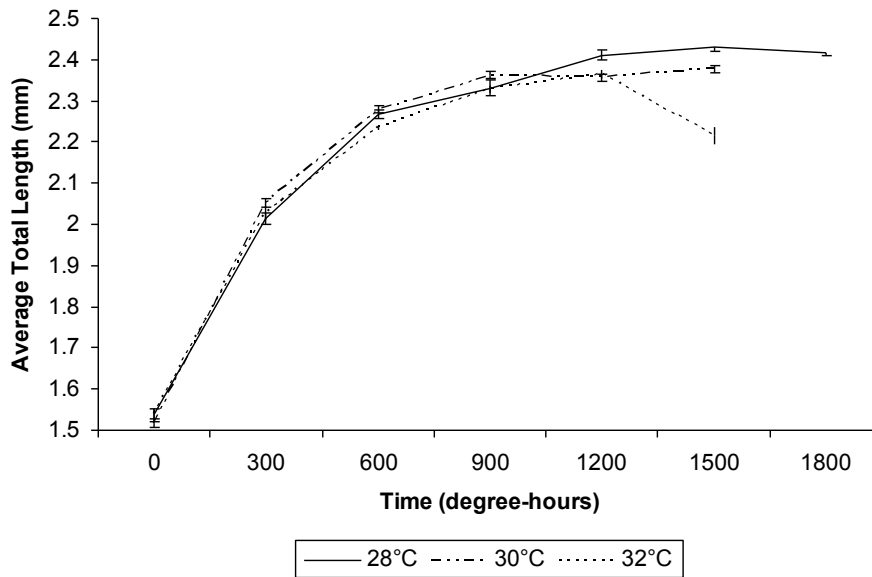


Figure 7.1.1.4. Average total length of developing coral trout larvae during the endogenous nutrition phase at different temperatures.

The higher mortality with increased temperature is likely due to starvation once yolk reserves, principally energy, are expended. Uniform sequential developmental changes occur in serranid larvae during the endogenous nutrition phase. Comparatively *Plectropomus spp* have a smaller total length and yolk volume to many *Epinephelus spp*, and under culture conditions are more susceptible to mortalities associated with starvation. Organogenesis, the rapid development of a digestive system, during the endogenous nutrition is most significant investment in tissue formation, as the requirement for initiating exogenous nutrition is critical. Even under optimal condition for larval development (27°C to 28°C) through the endogenous phase, mortalities result due to starvation if first feeding does not occur within several hours of larval mouth opening. Further potential for reduction of cohorts to survive is likely under these high temperatures, given larvae which develop through exogenous feeding are: significantly smaller; potentially weaker with lower reserves; and have a greater restriction on potential prey items to feed on due to smaller mouth gape.

Marked effects of moderate temperature (e.g, just 2°C above ambient) on early-life history stages of *P. leopardus* do not bode well given project temperature increases of in excess of 4°C by the end of this century (e.g., New et al. 2011). Unless there is acclimation or adaptation to these higher temperatures, reproductive success could decline to <30% of current levels by 2100. However, temperature is an important cue for timing of spawning among the family Serranidae, including *P. leopardus* (e.g., Samoilys 1997). Samoilys (1997)



suggested that *P. leopardus* begin spawning at both absolute temperatures (e.g., >24°C) and rapid increases in temperatures, such that coral trout are likely to respond to ocean warming by spawning occurring earlier in the year. In this way, very early life-stages are unlikely to ever be exposed to devastating temperatures above 32°C, but ongoing research is needed to assess how late-stage larvae and post-settlement juveniles might cope with extreme temperatures experienced during the height of summer.

### 7.1.2 Sperm activation, fertilisation and egg development

Sperm motility and energy content show rapid decreases after activation in marine fish, therefore progressive forward movement needed to reach the egg surface is limited to a critical period after activation. The strip spawning method ensures optimal conditions to achieve fertilisation, so trials were conducted on the effects of increased temperature on sperm activation levels (Figure 7.1.2.1). A ranking of 3 (less energetic head and tail movement; most with forward motion) and above is considered optimal to achieve fertilisation. At increased temperatures, both 30°C and 32°C, sperm motility decreased more rapidly compared to 28°C. The time for sperm motility remaining above minimal optimal activation level was significantly ( $P<0.05$ ) reduced at higher temperatures. While still activated at 30°C and 32°C after 180s and 300s, reduced sperm motility may result in lower fertilisation rates compared to 28°C.

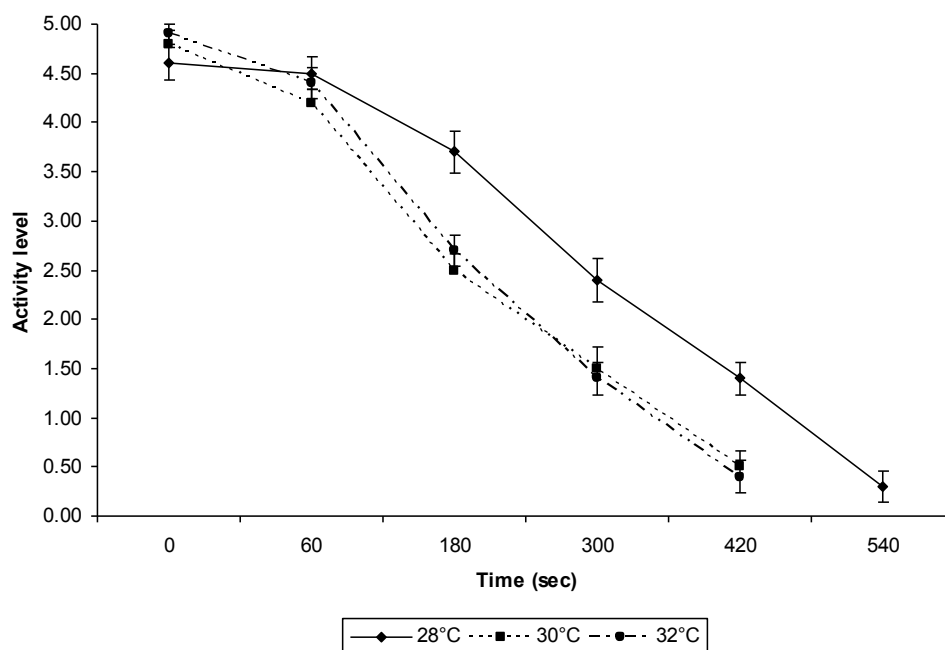


Figure 7.1.2.1. Activity levels of coral trout sperm after incubation and activation at different temperatures.



Initial investigation into the effects of increased temperatures on the rate of embryonic development, showed a significant decrease in developmental time with increased temperature. While at 28°C and 30°C hatch rates were similar (92±2% and 95±3.2% respectively), hatch rates at 32°C (66±8%) were significantly lower ( $P<0.05$ ) and the gastrula stage failed completion at 34°C. In addition, the development of fertilised eggs at 32°C and above showed irregularities in cellular growth, such as differences in cellular size during morula and blastula stages, individual cell death in cellular mass during early developmental stages, and asymmetric margins in blastula and gastrula stages.

The synergistic effects of temperature and pH had no significant effect on fertilisation of coral trout eggs between regimes. While the range of fertilisation rates varied between trials (72%±3.2 to 93.8%±5.1), this was a result of egg quality. Activation of sperm motility in most marine fish, with some exceptions, is controlled by osmotic pressure. Coral trout sperm was successfully activated by seawater in all temperature/pH regimes and no significant effect of decreased pH and increased temperature could be detected on fertilisation rates. However, egg development over time was significantly increased at decreased pH and increased temperature. At 28°C / pH 8.1 hatch rates were similar to 30°C / pH 7.8 (87.8±4.4% and 81.8±7.2%), while hatch rates at 32°C / pH 7.6 (78.3±8.2%) were significantly reduced ( $P<0.05$ ). Abnormal development and increased mortality rate of eggs and larvae were likely a function of temperature increases rather than decreasing pH, as the differences in mortality rates between treatments were similar to those found investigating temperature effects only.

## ***7.2 Effects of ocean acidification on larval development and olfactory discrimination***

The response of juveniles to predator odour differed among CO<sub>2</sub> treatments (Logistic regression:  $P<0.001$ ), but not between the days of testing ( $P=1.00$ ). Juveniles reared in control and 550 µatm CO<sub>2</sub> exhibited a strong avoidance of predator odour, spending less than 10% of their time in the water stream containing the odour. In contrast, all of the juveniles reared at 1000 µatm CO<sub>2</sub> exhibited a strong attraction to predator odour, spending over 90% of their time in the water stream containing the odour. For the juveniles reared at 750 µatm CO<sub>2</sub>, approximately half exhibited a strong avoidance of the predator odour, whereas the other half exhibited a strong attraction to the odour, as has been observed in previous experiments at similar CO<sub>2</sub> levels (Munday et al. 2010). As a result, the average time spent in the water stream containing the predator odour was approximately 47% among fish in this treatment,



however there was a large variation around the mean. There was no difference in the percent time spent in the predator odour when fish were tested at day 28 compared with day 4.

Activity levels and behaviour differed among treatments (ANOVA:  $P < 0.001$  for all tests), with the most marked difference occurring in the highest CO<sub>2</sub> treatment. Juvenile coral trout from the control and 550  $\mu\text{atm}$  CO<sub>2</sub> treatments spent over 75% of their time in the shelter during the behavioural trial and there was no difference in shelter use between these two treatments. In contrast, juveniles from the 750  $\mu\text{atm}$  CO<sub>2</sub> treatment spent approximately only 40% of their time in the shelter and those from the 1000  $\mu\text{atm}$  CO<sub>2</sub> treatment spent less than 10% of their time in the shelter. Activity level was even more markedly different between treatments. Juveniles from the control and 550  $\mu\text{atm}$  CO<sub>2</sub> treatments had very low activity levels, as expected given their preference to remain in shelter. Juveniles from the 750  $\mu\text{atm}$  CO<sub>2</sub> treatment were approximately 10 times more active than controls (45 versus 3.6 lines crossed 10 min<sup>-1</sup>) and juveniles from the 1000  $\mu\text{atm}$  CO<sub>2</sub> treatment were nearly 90 times more active than the lower CO<sub>2</sub> treatments (321 versus 3.6 lines crossed 10 min<sup>-1</sup>). Juveniles from the highest CO<sub>2</sub> treatment moved around the tank continuously and did not remain in shelter for more than a few seconds at a time.

Recent studies have shown that the behaviour and sensory responses of small, territorial reef fishes are altered by permanent exposure to elevated CO<sub>2</sub> for more than a few days. Our results demonstrate that the behaviour and olfactory responses of juvenile coral trout are equally sensitive to the effects of high CO<sub>2</sub> as other reef fish. In fact, the effects on activity levels and risk perception appear to be substantially greater in juvenile coral trout than in other species tested to date. Munday et al. (2010) found that the total distance moved by juvenile damselfishes increased 30% and distance ventured from shelter increased 60-80% following exposure to 850  $\mu\text{atm}$  CO<sub>2</sub> for 4 days. Juvenile coral trout reared for 28 days at a similar level of CO<sub>2</sub> were nearly 90 times more active than controls and ventured 6-7 times as far from shelter. Most notably, coral trout became highly active, moving throughout the test arena and rarely retreating to shelter for more than a few seconds. They also emerged from shelter much sooner after being startled, indicating they were less risk averse than fish reared under current-day conditions.

Although coral trout are one of the most abundant large predators on coral reefs, they are themselves highly vulnerable to predation during early life stages, including from adult





conspecifics (Leis and Carson-Ewart 1999). Juvenile coral trout are naturally highly cryptic, usually remaining hidden in shelter (Leis and Carson-Ewart 1999; Wen et al. 2013). Our results suggest they will become more active and bolder as CO<sub>2</sub> levels increase, substantially increasing their risk of mortality from adult piscivores. Increased juvenile mortality could significantly reduce the number of juveniles that ultimately recruit to adult populations, thereby having a negative effect on adult abundances.

### ***7.3 Effects of body size on aerobic scope***

The principal measure of physiological performance used in the experiments was aerobic scope (the difference between the minimum and maximum metabolic rate, measured based on oxygen consumption). A key finding of this work is that aerobic scope in coral trout is strongly size dependent, where body size has a strong negative effect on absolute aerobic scope (Figure 7.3.1). Juvenile *P. leopardus* have a much greater aerobic capacity than adults. It has been suggested previously for temperate salmon that aerobic scope is greatest amongst juvenile fishes and declines with increasing size of adults and during very early development (e.g., Pörtner and Farrell 2008), but this has never been tested for a large tropical marine species. Our results show aerobic scope to be highest in juvenile fish of 0.025-0.100kg (up to 9.5 mg/min/kg), dramatically dropping to less than 50% in adults with a body mass of approximately 0.5kg (4.5 mg/min/kg) and continuing to decline at a lesser rate with increasing body size to 2.5kg (2.5 mg/min/kg) (Figure 7.3.1). Contrary to our expectations and our findings to longer term exposure to 33°C (see section 7.4 and 7.5), aerobic scope did not seem to be affected by an acute (1-week) exposure to 33°C and followed a very similar decline with body mass to the ambient control temperature (28.5°C) (Figure 7.3.1)

This difference in aerobic scope with body mass may reflect the different aerobic challenges faced by coral trout at different points in their ontogeny. Specifically, juvenile coral trout must be capable of high aerobic performance to evade predators and spend considerable energy on growth. Adult *P. leopardus* on the other hand are one of the top predators on the reef with a relatively sessile existence.



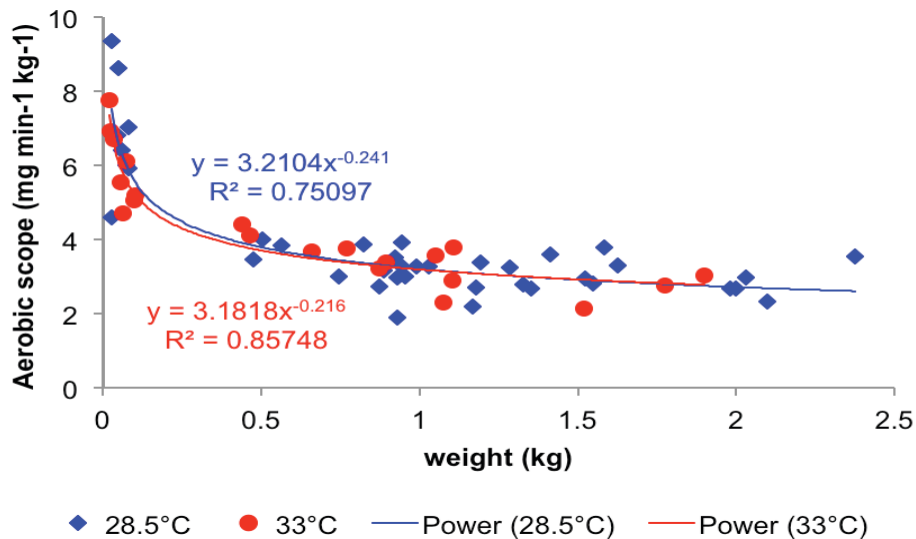


Figure 7.3.1. Relationship between aerobic scope ( $\text{mg min}^{-1} \text{kg}^{-1}$ ) and *P. leopardus* body mass (kg) at 28.5°C and 33°C.

At ambient temperature (28.5°C), minimum and maximum metabolic rates were significantly higher in juvenile than in adult coral trout (Figure 7.3.2a), which was also translated into significantly higher absolute aerobic scope (Figure 7.3.2b). Resting metabolic rate increased at 33°C in both juveniles and adults compared to 28.5°C, whereas maximum metabolic rate only increased in adults and remained constant in juveniles. Despite the constant maximum metabolic rate in juveniles across temperatures, the decline in aerobic scope was not significant. Adult aerobic scope remained the same during the acute exposure to 33°C at Lizard Island (Figure 7.3.2).

At this stage it is unclear whether an increased aerobic capacity in juvenile *P. leopardus* translates into higher thermal tolerance. Whilst juveniles have a greater aerobic capacity than adults, they were unable to increase their maximum metabolic rate during the acute exposure to 33°C. The increasing metabolic demands at higher temperatures are likely to come at a cost of less available energy for other critical processes, such as evading predators or growth. Further research is clearly needed to gain a better understanding of the thermal sensitivities at different ontogenetic stages, and how the present results compare to a longer acclimation to elevated temperatures.



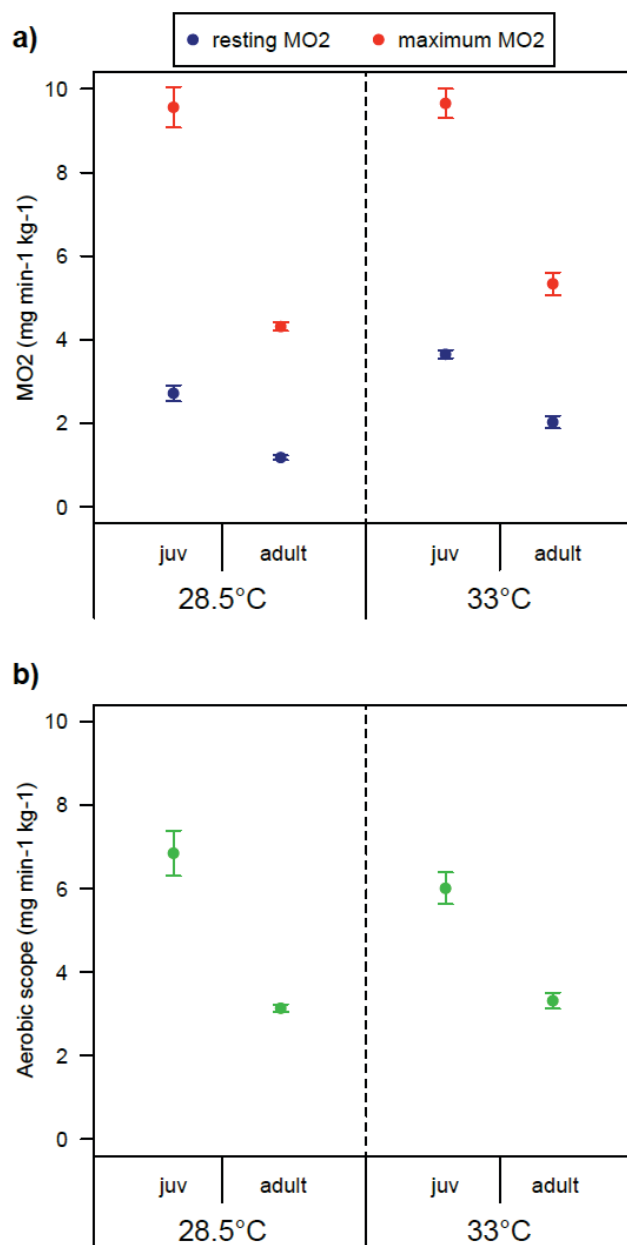


Figure 7.3.2. Comparison of Q10-corrected a) minimum and maximum metabolic rates, and b) aerobic scope (mg min<sup>-1</sup> kg<sup>-1</sup>) between juveniles and adults at 28.5°C and acute exposure to 33°C for 1 week.

#### 7.4 Effects of temperature on metabolic rate and aerobic scope

Temperature strongly affects the metabolism of ectotherms and resting metabolic rate is generally expected to double with every acute 10°C temperature increase (Farrell 2009). Similarly, our results showed that resting metabolic rate in *P. leopardus* doubled from 24°C (0.773 mg/min/kg ± 0.031) to 33°C (1.538 mg/min/kg ± 0.055) (Figure 7.4.1 a). As a consequence, coral trout need to spend twice as much energy on just maintaining basic body function at the higher temperatures compared to 24°C. *P. leopardus* is able to compensate for this increase in resting metabolic rate by increasing their maximum metabolic rate up to 30°C.



However, at 33°C maximum metabolic rate decreases quite dramatically, reducing their aerobic scope (Figure 7.4.1 b). The ability to compensate for the increasing energy demand to maintain basic body functions by increasing their maximum metabolic rate up to 30°C suggests that the fish are able to spend similar levels of energy on other activities such as digestion, foraging, reproduction etc. At 33°C, this capacity is drastically reduced, which is likely to impair on activities such as reproduction, growth and motility.

Although aerobic scope in this study is maximal at 30°C, it is unlikely to be indicative of the optimal temperature for coral trout. Our data show very clearly that the combined stress of higher temperatures (30°C, and even more so at 33°C) and strenuous physical activity are often lethal in coral trout, although the underlying causes are unclear at this stage (Figure 7.4.2).

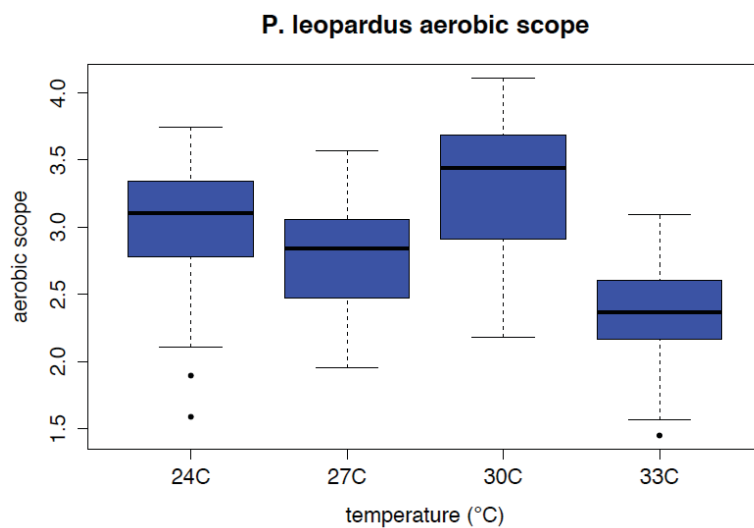
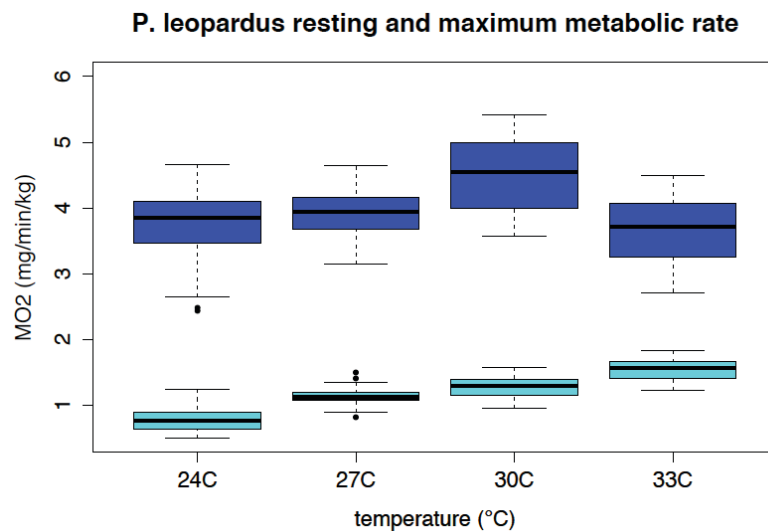


Figure 7.4.1 Box and whisker blots showing median, 25% quartiles and 95% confidence intervals for a) Resting (light blue) and maximum (dark blue) metabolic rate and b) aerobic scope for *P. leopardus* at 24°C, 27°C, 30°C and 33°C. Data is pooled across all fishes from both northern and southern populations.

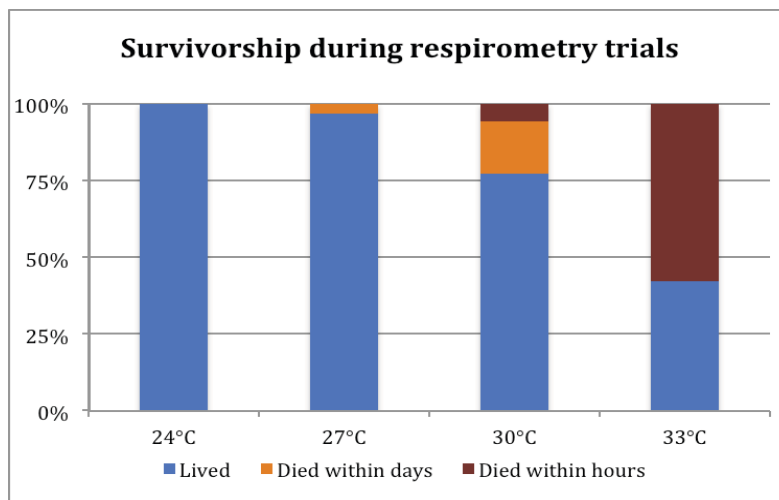


Figure 7.4.2 Proportional mortality of fishes from each of the four temperature treatments during respirometry trials. Mortality occurred within hours (orange) to days (brown) of being placed into the respirometers for 24 hours, after fish were chased for 3 minutes and air exposed for 1 minute.

Table 7.4.1 Percent survivorship in respirometry trials (mortality occurring within hours and days combined) at 24°C, 27°C, 30°C and 33°C. Swim performance scores (SP) ranked from 1 to 5.5, with 1 being extremely poor, 5.5 being extremely good. The numbers in brackets represent the sample size.

| SP  | 24°C      | 27°C     | 30°C      | 33°C     |
|-----|-----------|----------|-----------|----------|
| 1   | 100% (5)  | 100% (1) | 100% (2)  |          |
| 1.5 | 100% (16) | 100% (7) | 100% (10) | 100% (7) |
| 2   | 100% (2)  | 100% (1) | 100% (2)  | 100% (3) |
| 2.5 | 100% (4)  | 100% (2) | 100% (5)  |          |
| 3   | 100% (1)  | 100% (3) | 100% (3)  | 100% (1) |
| 3.5 | 100% (1)  | 100% (3) |           | 0% (1)   |
| 4   | 100% (5)  | 100% (4) | 60% (5)   | 0% (2)   |
| 4.5 | 100% (3)  | 100% (6) | 25% (8)   | 0% (9)   |
| 5   | 100% (2)  | 100% (4) |           | 0% (3)   |
| 5.5 |           | 0% (1)   |           |          |

### 7.5 Comparisons of thermal sensitivity among widely separated populations

Contrary to expectations, we found no apparent difference in the thermal sensitivities of coral trout from reefs around Princess Charlotte Bay (northern population) versus reefs in the Swains and Capricorn Bunkers (southern population). Comparisons of minimum and maximum metabolic rates and aerobic scope (Figure 7.5.1) at the 4 different treatment



temperatures showed no difference between coral trout sourced from the northern GBR versus the southern GBR. This suggests that these coral trout populations have not acclimated to their local environment and as a result are likely to have a very limited capacity to acclimate to increasing temperatures due to climate change. Although not presented here, limited differences between populations were also apparent based on the range of other metrics considered (blood concentrations of glucose and lactose), though these clearly responded to increasing temperatures.

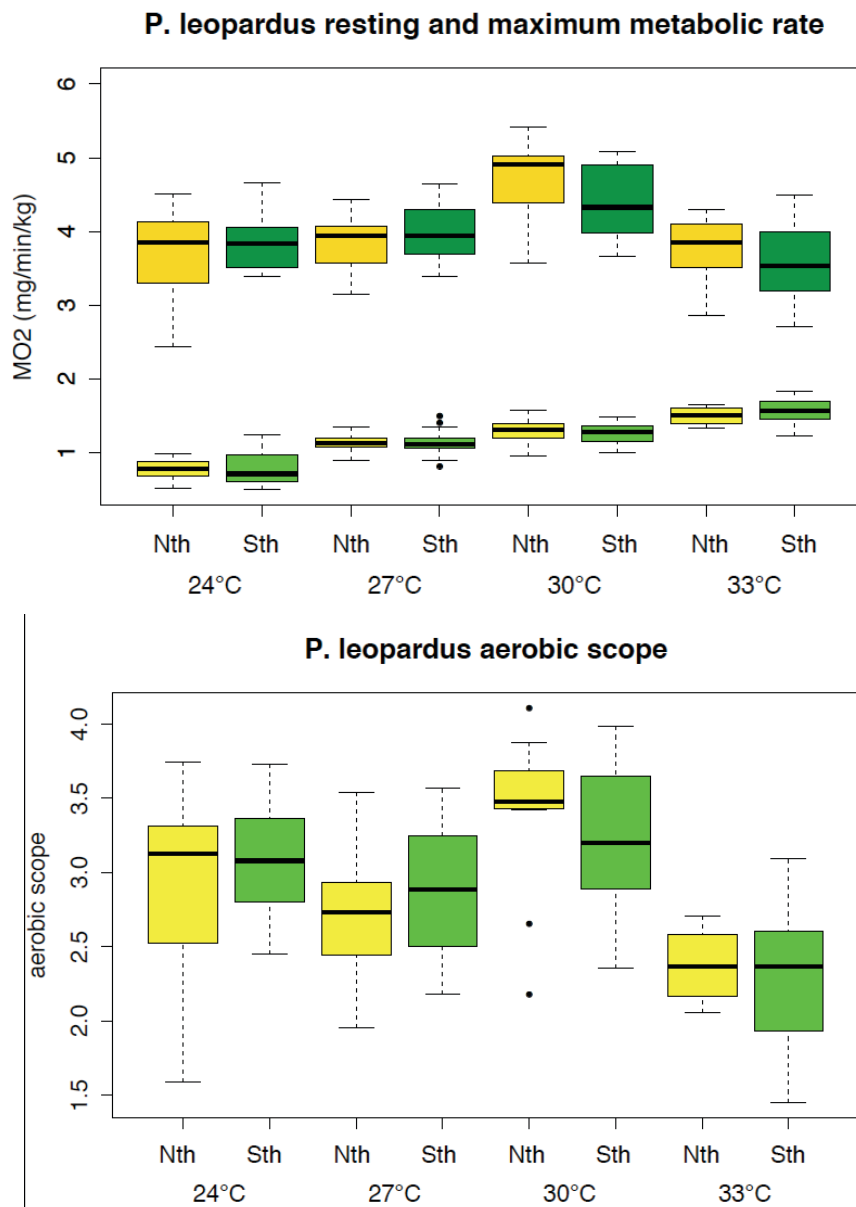


Figure 7.5.1 a) Resting and maximum metabolic rate and b) aerobic scope for *P. leopardus* at 24°C, 27°C, 30°C and 33°C from the northern GBR (yellow) and southern GBR (green).

An possible explanation for these findings is that coral trout populations on the GBR are one homogenous well-mixed genetic stock, and therefore strong inter-connectivity (and associated



genetic exchange) among populations at the latitudinal extremes of the GBR, obscures any trans-generational acclimation to local environmental conditions. Accordingly, van Herwerden et al. (2009) found that the entire stock of *P. leopardus* from northern and southern locations on the GBR were a single homogenous population, but distinct from populations in Western Australia, New Caledonia and Taiwan. However, this study of population structure was based exclusively on a mitochondrial marker (D-loop), known to be relatively conserved over ecological timeframes. Moreover, ecological studies of connectivity, based on patterns of recruitment tend to find that coral trout settle mainly on their natal reefs that would limit connectivity (Harrison et al. 2012) especially at the scale of the latitudinal extent of the GBR. Therefore, genetic samples (fin clips) taken from all fishes used in this study are currently being screened for 11 newly developed microsatellite markers (Harrison et al. 2012), which are expected to show strong differentiation between northern and southern populations. If so, ongoing research is required to understand the physiological basis for limited acclimation capacity within these fishes, while further planning will be needed to potentially reduce reliance on these species within the reef-based commercial fisheries sector.

If upper thermal tolerances are fixed, it is likely that fishes in the northern section of the GBR will be exposed to deleterious effects of high temperatures much sooner than those in the southern sector, in which case we may expect to see a southern contraction of the species throughout the course of this century. However, results from tank-based experiments do not account for increased capacity of adult coral trout to actively (e.g., through specific behaviours) minimise exposure to extreme temperatures in the field. For example, it may be that coral trout adapt to increasing sea surface temperature simply by moving to deeper waters, such that limited tolerance of thermal extremes may influence the depth distribution (and catchability) of coral trout, rather than their distribution and abundance.

### ***Hsp70***

During stress, heat shock proteins and their respective genes are upregulated. Our results show that *Hsp70* gene expression was significantly higher in the 33°C heat-stressed fish compared to fish exposed to 24°C in both Northern (1.88 fold) and Southern (2.63 fold) populations. This indicates that in both populations the 33°C treatment was more stressful than the 24°C treatment.



There was no significant difference in *Hsp70* expression between populations at either temperature treatment. Although insignificant, the Southern population showed a slight, 0.75 fold increase in *Hsp70* expression compared to the Northern fish at the 33°C heat-stress treatment. This may indicate that the Northern fish were coping fractionally better at this temperature. However, metabolic data indicates that at this temperature there was no difference in performance between populations.

The difference in *Hsp70* levels between the temperature treatments was lower than anticipated (Figure 7.5.2). Due to the high mortality at 33°C, it may be that the animal is too stressed and cellular processes are already declining. In a thermal tolerance threshold model proposed by Pörtner (2001), *Hsps* likely shift the molecular denaturation temperature to more extreme temperatures. Therefore, at 33°C, the fish from both locations may have exceeded this molecular denaturation, thus negating the requirement for extensive *Hsp* production. At lower heat stress conditions (27 or 30°C) the fish may have higher *Hsp70* expression than both the 24°C and 33°C treatments as the fish are stressed and yet have not reached the molecular denaturation stage. In addition, we may see a greater difference in population responses at these elevated temperatures.

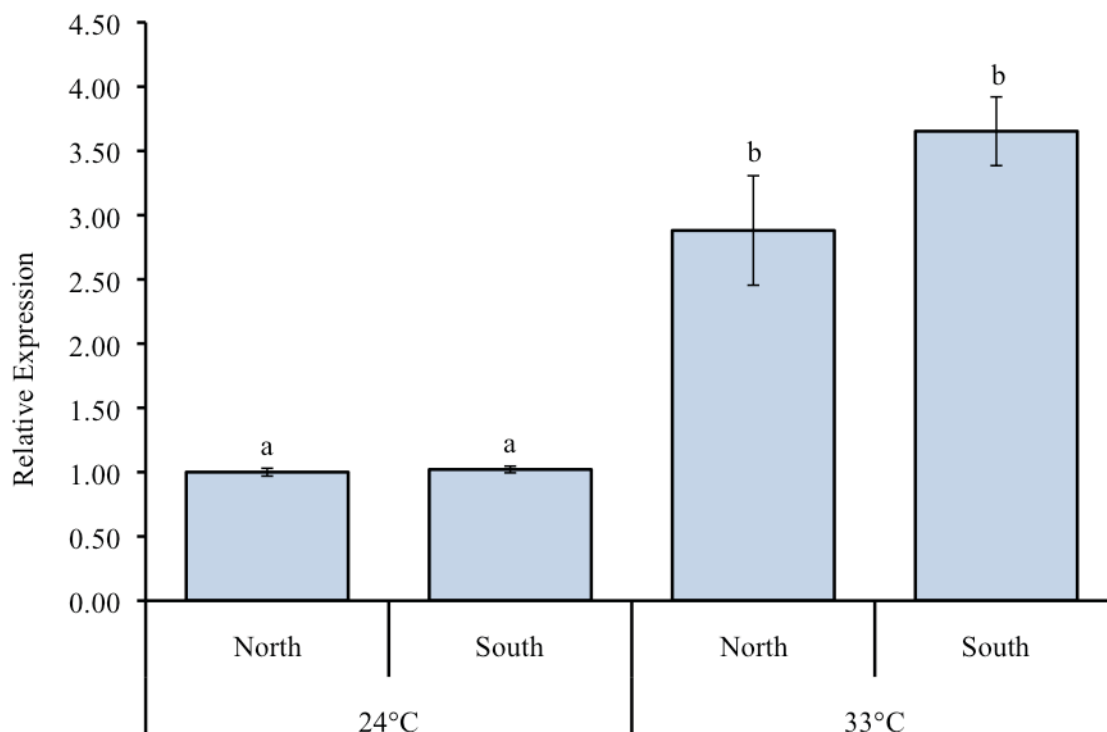


Figure 7.5.2. Relative expression of *Hsp70* for northern and southern *P. leopardus* populations kept for 8 weeks at 24°C and 33°C





## ***LdhB***

*LdhB* is linked to sustained aerobic activity in fish and we, therefore, would expect an increase in *LdhB* expression with increased aerobic demand at higher temperatures. In our study, *LdhB* expression showed high variation among individuals within each treatment. While there were significant higher (1.01 fold increase) expression of *LdhB* at 33°C compared to 24°C for the Northern population, but not for the Southern population (0.66 fold increase), there was no difference in expression of *LdhB* at 33°C between fishes from the Southern or Northern population. These results are however, very preliminary, and a large number of genes are currently being screened for their applicability in assessing thermal tolerance across disparate populations of *P. leopardus* and other reef fish. Analyses of the intermediate temperature treatments used in our experiments (27°C and 30°C) may also clarify whether fish at 33°C have already reached a state of denaturation.

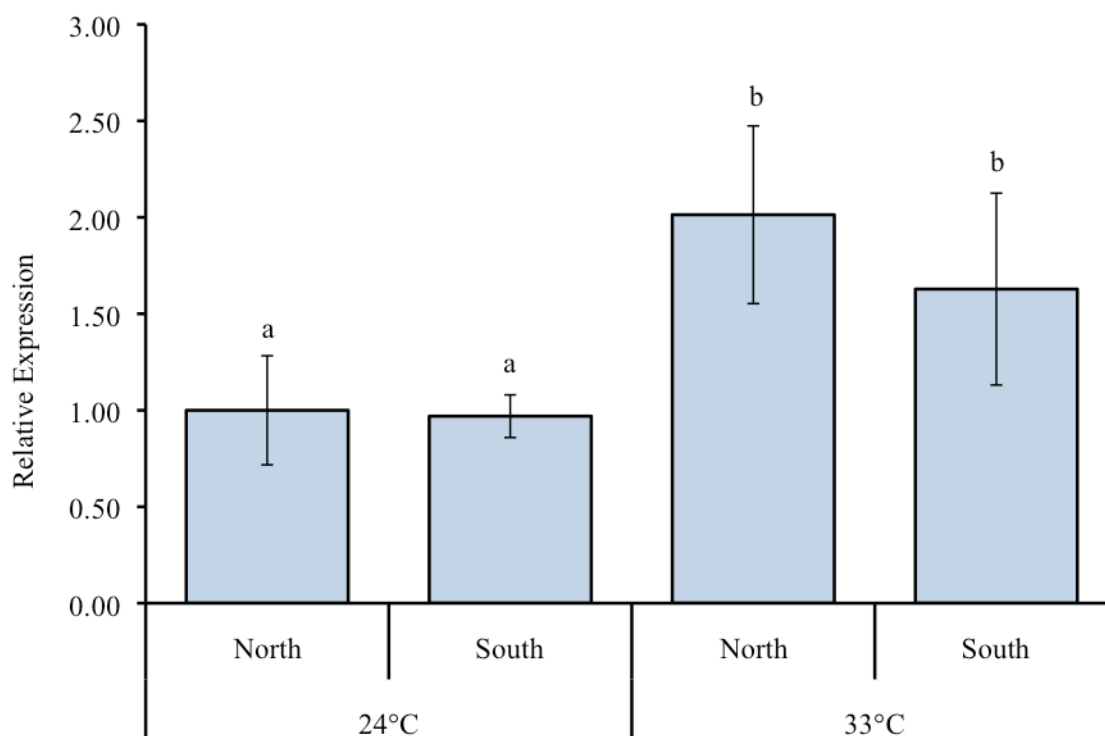


Figure 7.5.3. Relative expression of *LdhB* for northern and southern *P. leopardus* populations kept for 8 weeks at 24°C and 33°C

## **7.6 Temperature effects on daily food intake**

Despite apparent increases in the resting metabolic rates of *P. leopardus* over the range of temperatures used in these experiments (Figure 7.5.1a), we did not observe a corresponding



increase in meal size, but feeding frequency did increase. Average meal sizes ranged from  $4.8 \pm 0.3\%$  of body-weight at  $24^{\circ}\text{C}$  to  $5.68 \pm 0.3\%$  at  $33^{\circ}\text{C}$  (mean  $\pm$  S.E.), but did not change significantly across temperature treatments ( $F_{3,117} = 1.48$ ,  $p = 0.22$ , Figure 7.6.1). Elevated temperatures caused a significant increase in feeding frequency ( $F_{3,117}=17.79$ ,  $p < 0.01$ , Figure 7.6.2). On average, individuals ate every  $2.4 \pm 0.2$  days at  $33^{\circ}\text{C}$ , compared to every  $2.5 \pm 0.2$  days at  $30^{\circ}\text{C}$ , every  $2.7 \pm 0.3$  days at  $27^{\circ}\text{C}$ , and every  $3.8 \pm 0.3$  days at  $24^{\circ}\text{C}$  (mean  $\pm$  S.E.). Overall food intake increased significantly with increasing temperatures ( $F_{3,123} = 11.0$ ,  $p < 0.01$ ), with no detectable difference between populations ( $F_{1,123} = 0.1$ ,  $p = 0.80$ , Figures 3a,b). The greatest daily food intake was found at  $33^{\circ}\text{C}$ ,  $30^{\circ}\text{C}$  and  $27^{\circ}\text{C}$ , while food intake at  $24^{\circ}\text{C}$  was significantly reduced (Figure 3a, Table 1). Individuals ate  $2.6 \pm 0.1\%$  of their body-weight each day at  $33^{\circ}\text{C}$ , compared to  $2.3 \pm 0.2\%$  at  $30^{\circ}\text{C}$ ,  $2.2 \pm 0.2\%$  at  $27^{\circ}\text{C}$ , and just  $1.4 \pm 0.1\%$  at  $24^{\circ}\text{C}$  (mean  $\pm$  S.E.).

Between populations, there was a significant difference in meal-size ( $F_{1,117} = 5.06$ ,  $p = 0.03$ ), but no detectable difference between in feeding frequency ( $F_{1,117} = 0.09$ ,  $p = 0.76$ , Figure 2b). Individuals in the northern population ate  $5.6 \pm 0.2\%$  of body-weight per meal, compared to  $5.0 \pm 0.2\%$  in the southern population (mean  $\pm$  S.E.,  $n_{\text{North}} = 61$ ,  $n_{\text{South}} = 67$ , Figure 1b). At  $33^{\circ}\text{C}$ , overall food intake was significantly higher for the northern population, suggesting that there may be some capacity for fishes from northern waters to compensate for increasing metabolic demands associated with global warming, relative to fishes from the southern GBR.



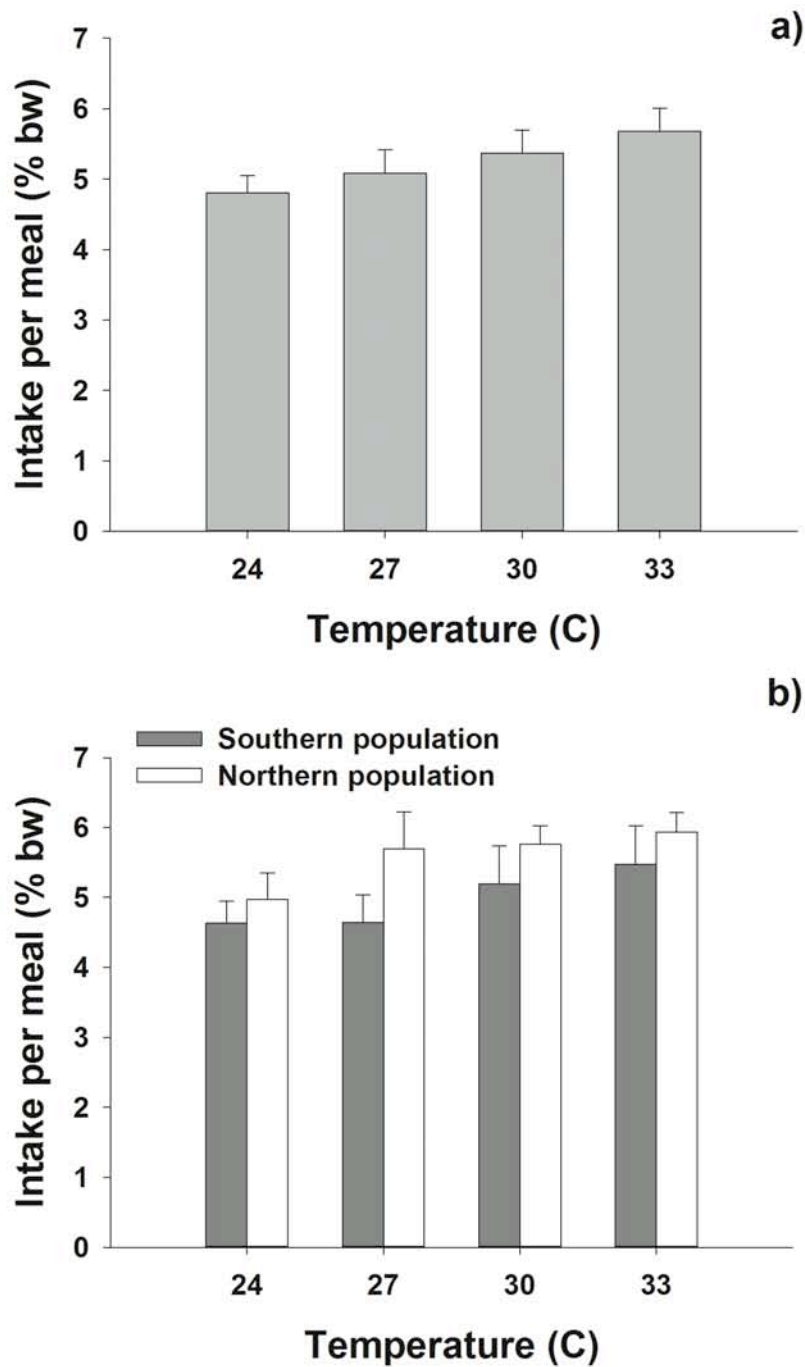


Figure 7.6.1. Average meal size of coral trout (*Plectropomus leopardus*) across four temperature treatments. Values are in % body-weight and error bars are standard error of the mean. Significant differences are shown above each column. Top graph **a)** shows the average meal size at each temperature pooled across all individuals. Bottom graph **b)** shows the average meal size of each population at each temperature treatment.



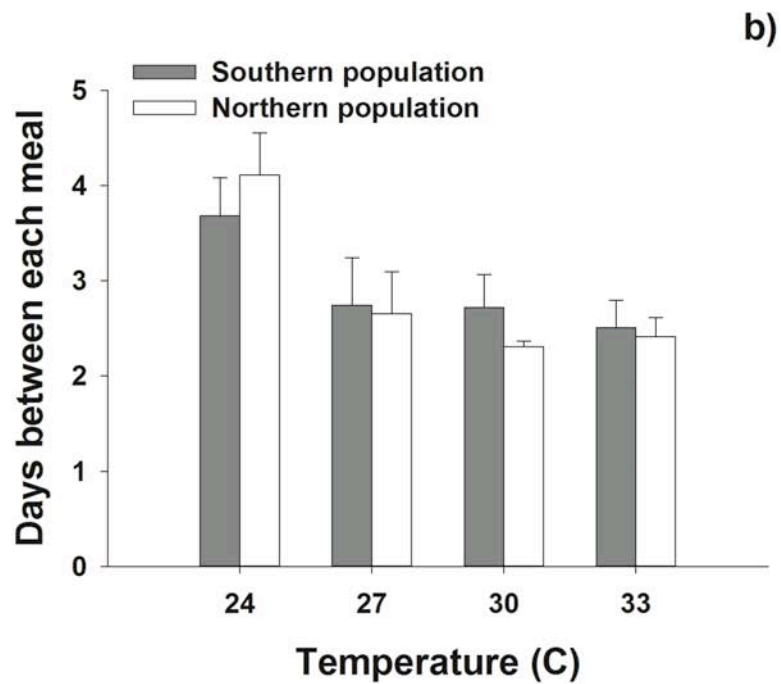
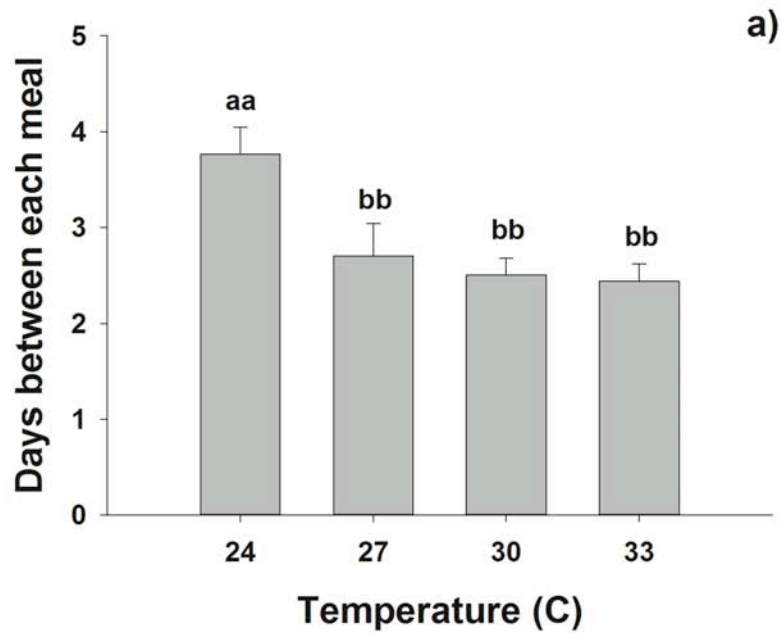


Figure 7.6.2. Feeding frequency of coral trout (*Plectropomus leopardus*) across four temperature treatments. Values are in “days” and error bars are standard error of the mean. Significant differences are shown above each column. Top graph **a**) shows the average number of days between each meal at each temperature pooled across all individuals. Bottom graph **b**) shows the average number of days between each meal for each population at each temperature treatment.



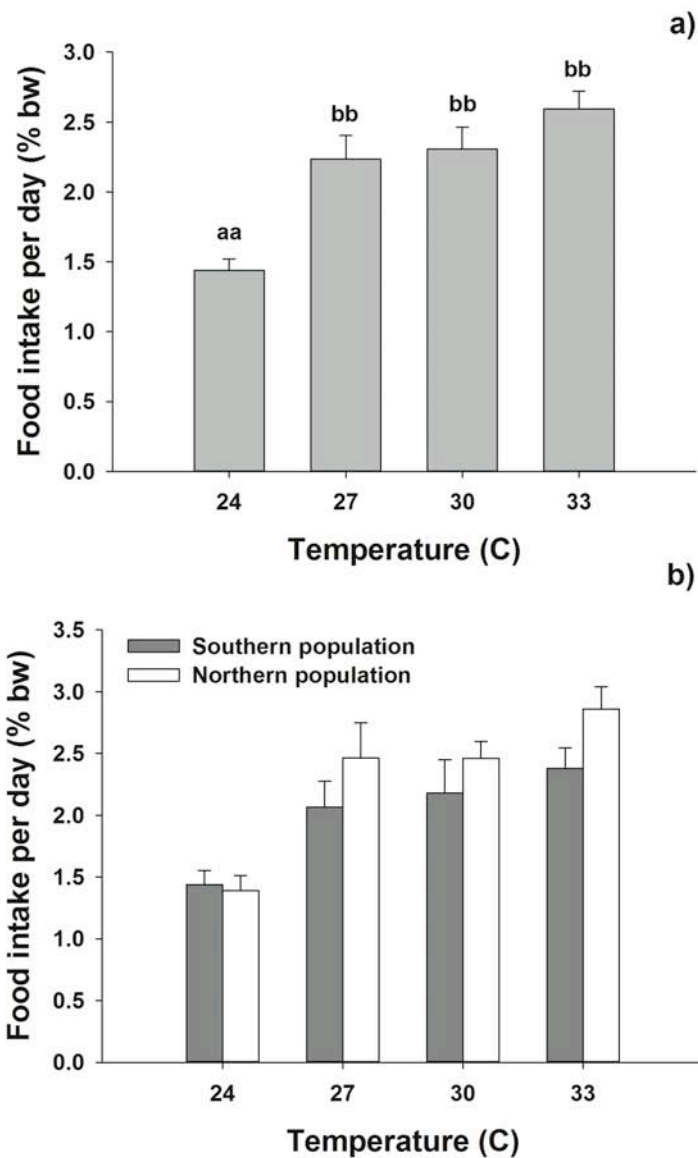


Figure 7.6.3. Average daily food intake of coral trout (*Plectropomus leopardus*) across four temperature treatments. Values are in % body-weight and error bars are standard error of the mean. Significant differences are shown above each column. Top graph **a)** shows the average daily food intake at each temperature pooled across all individuals. Bottom graph **b)** shows the average daily food intake of each population at each temperature treatment.

Table 7.6.1. Differences in daily food intake between four temperature treatments for coral trout (*Plectropomus leopardus*). Significant differences are marked in bold.

| Post-hoc Tukey HSD |              |       |       |
|--------------------|--------------|-------|-------|
| Temperature (°C)   | 24           | 27    | 30    |
| 27                 | <b>0.001</b> |       |       |
| 30                 | <b>0.000</b> | 0.999 |       |
| 33                 | <b>0.000</b> | 0.949 | 0.901 |



## 7.7 Effects of temperature on swimming and activity patterns of coral trout

Spontaneous swimming speeds changed significantly across temperatures ( $F_{3,257} = 23.4$ ,  $p < 0.01$ , Figure 7.7.1, Table 7.7.1). The lowest swimming speeds were recorded at the highest temperature (33°C), while the fastest swimming speeds were recorded at 27°C (Figure 1a). On average, swimming speeds were  $13.8 \pm 0.7 \text{ cms}^{-1}$  at 33°C, compared to  $17.3 \pm 0.8 \text{ cms}^{-1}$  at 30°C,  $23.4 \pm 0.8 \text{ cms}^{-1}$  at 27°C, and  $20.5 \pm 0.7 \text{ cms}^{-1}$  at 24°C ( $n_{33} = 86$ ,  $n_{30} = 75$ ,  $n_{27} = 80$ ,  $n_{24} = 130$ , mean  $\pm$  S.E.). There was a significant interaction between temperature treatment and population swimming speeds ( $F_{6,257} = 2.7$ ,  $p = 0.01$ ), however population swimming speeds did not differ within individual temperatures (Figure 7.7.1, Table 7.7.1). There was also a significant interaction between holding tank and population swimming speeds ( $F_{2,257} = 4.6$ ,  $p = 0.01$ ), however population swimming speeds did not differ within or across holding tanks (Table 7.7.2).

The proportion of time individuals spent resting motionless on the bottom increased significantly with increasing temperatures ( $F_{3,1137} = 23.2$ ,  $p < 0.01$ , Figure 2a). On average, individuals spent  $19.8 \pm 0.3\%$  of the time resting at 33°C, compared to  $13.4 \pm 0.2\%$  at 30°C,  $10.5 \pm 0.3\%$  at 27°C, and  $9.3 \pm 0.3\%$  at 24°C (mean  $\pm$  S.E.). There was a significant interaction between treatment temperature and holding tank ( $F_{3,1337} = 15.7$ ,  $p < 0.01$ ), with significant differences in time spent resting between holding tanks at 33°C ( $z = 8.0$ ,  $p < 0.01$ ; Figure 2b).

Table 7.7.1. Post-hoc analysis of the interaction between ambient temperature and the swimming speeds of two populations of coral trout (*Plectropomus leopardus*). The labels (e.g. 27N-24N) denote the temperature and the population (northern or southern). Significant differences are marked in bold.

| GLMM Tukey (temperature x population) |         |              |
|---------------------------------------|---------|--------------|
| Comparison                            | Z value | P value      |
| 27N – 24N                             | 0.35    | 0.760        |
| 30N – 24N                             | 2.73    | <b>0.017</b> |
| 33N – 24N                             | 3.64    | <b>0.001</b> |
| 30N – 27N                             | 1.92    | 0.094        |
| 33N – 27N                             | 2.51    | <b>0.028</b> |
| 33N – 30N                             | 0.57    | 0.640        |
| 27S – 24S                             | 1.98    | 0.085        |
| 30S – 24S                             | 1.97    | 0.085        |
| 33S – 24S                             | 2.59    | <b>0.023</b> |
| 30S – 27S                             | 3.66    | <b>0.001</b> |
| 33S – 27S                             | 4.23    | <b>0.000</b> |
| 33S – 30S                             | 0.43    | 0.716        |
| 24S – 24N                             | 1.60    | 0.171        |
| 27S – 27N                             | 1.43    | 0.226        |
| 30S – 30N                             | 0.18    | 0.870        |



|           |      |       |
|-----------|------|-------|
| 33S – 33N | 0.03 | 0.978 |
|-----------|------|-------|

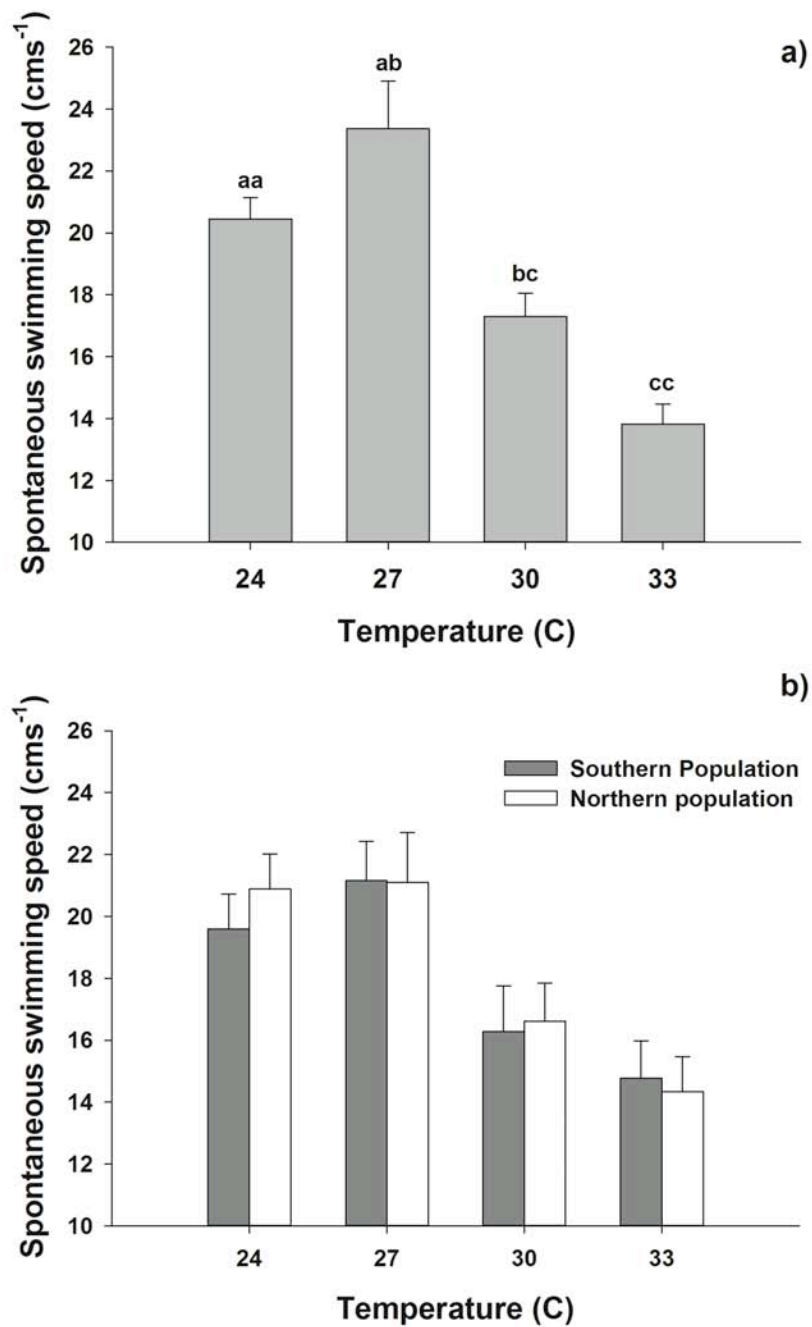


Figure 7.7.1. Spontaneous swimming speeds of two populations of coral trout (*Plectropomus leopardus*) across four different temperatures. Swimming speeds are in cm per second ( $\pm$ S.E.). Top graph **a)** shows average swimming speeds pooled across populations with significant differences between temperatures marked. Bottom graph **b)** shows average swimming speeds within each population.



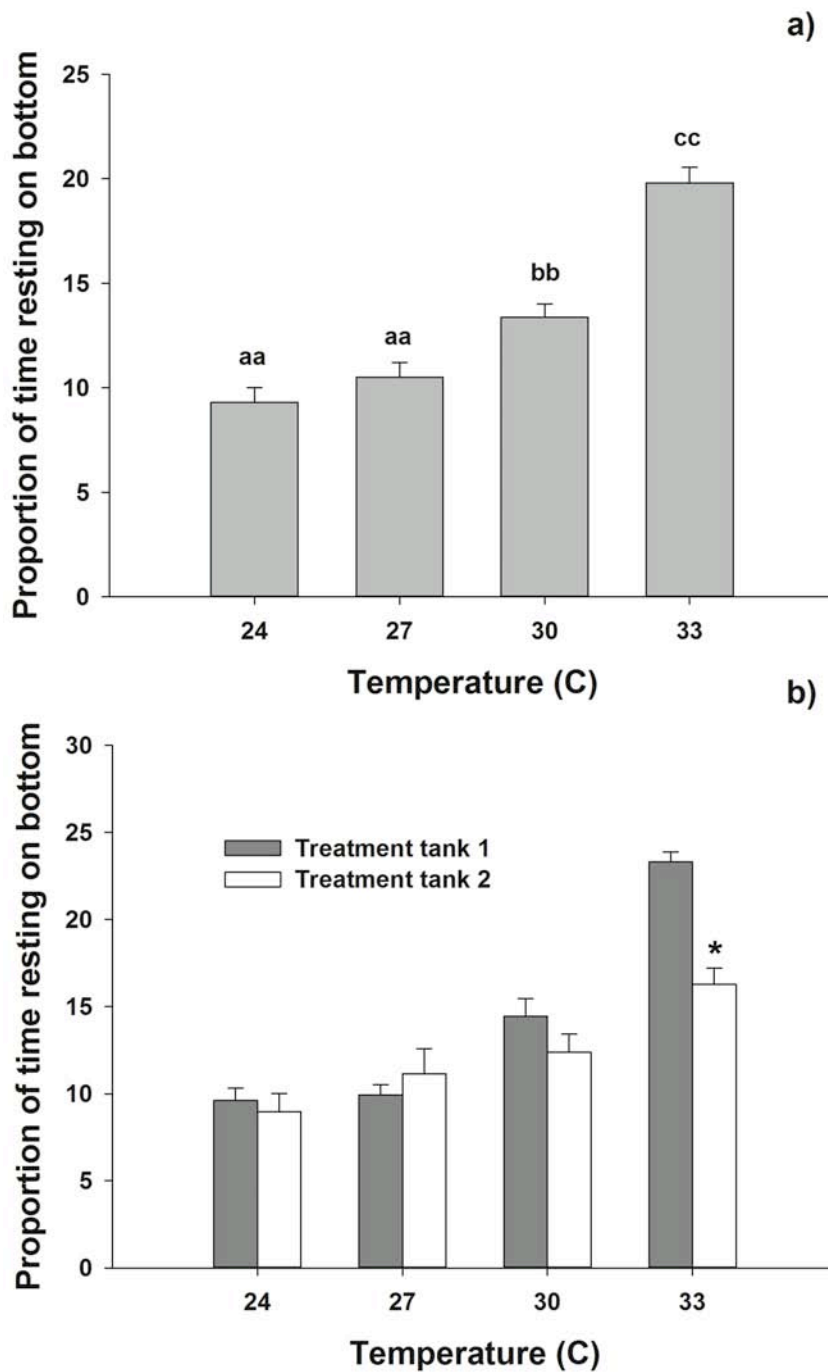


Figure 7.7.2. The proportion of time coral trout (*Plectropomus leopardus*) spends resting on the bottom across four ambient temperatures. Top graph **a)** shows average resting patterns pooled across treatment tanks with significant differences marked. Bottom graph **b)** shows resting patterns within each treatment tank. An asterisk marks significant differences between treatment tanks within each temperature.





Table 7.7.2. Post-hoc analysis of the interaction between treatment tank and the swimming speeds of two populations of coral trout (*Plectropomus leopardus*). The labels (e.g. 1S-1N) denote the treatment tank and the population (Northern or Southern).

| GLMM Tukey (treatment tank x population) |         |         |
|--|---------|---------|
| Comparison                               | Z value | P value |
| 1S – 1N                                  | 1.60    | 0.200   |
| 2S – 2N                                  | 0.89    | 0.466   |
| 2N – 1N                                  | 0.15    | 0.941   |
| 2S – 1S                                  | 1.68    | 0.200   |

### 7.8 Microhabitat preferences and reliance on corals

Patterns of microhabitat use were documented for a total of 212 juvenile coral trout. Approximately 60% of all fishes (127/ 212) were found associated with *Acropora* corals situated on loose substrates (e.g., sand), even though this specific microhabitat accounted for only 12.8% of benthic cover. By comparison, 41 fishes were found associated with macroalgae and non-*Acropora* corals, which covered for 43.8% of the substrate. The selection for *Acropora* on sand was very pronounced and also very consistent ( $B > 0.5$ ) across locations. Most notably, selection for *Acropora* on sand was 7 times that of *Acropora* on carbonate pavement and twice that of dead branching corals on sand (Figure 7.8.1).



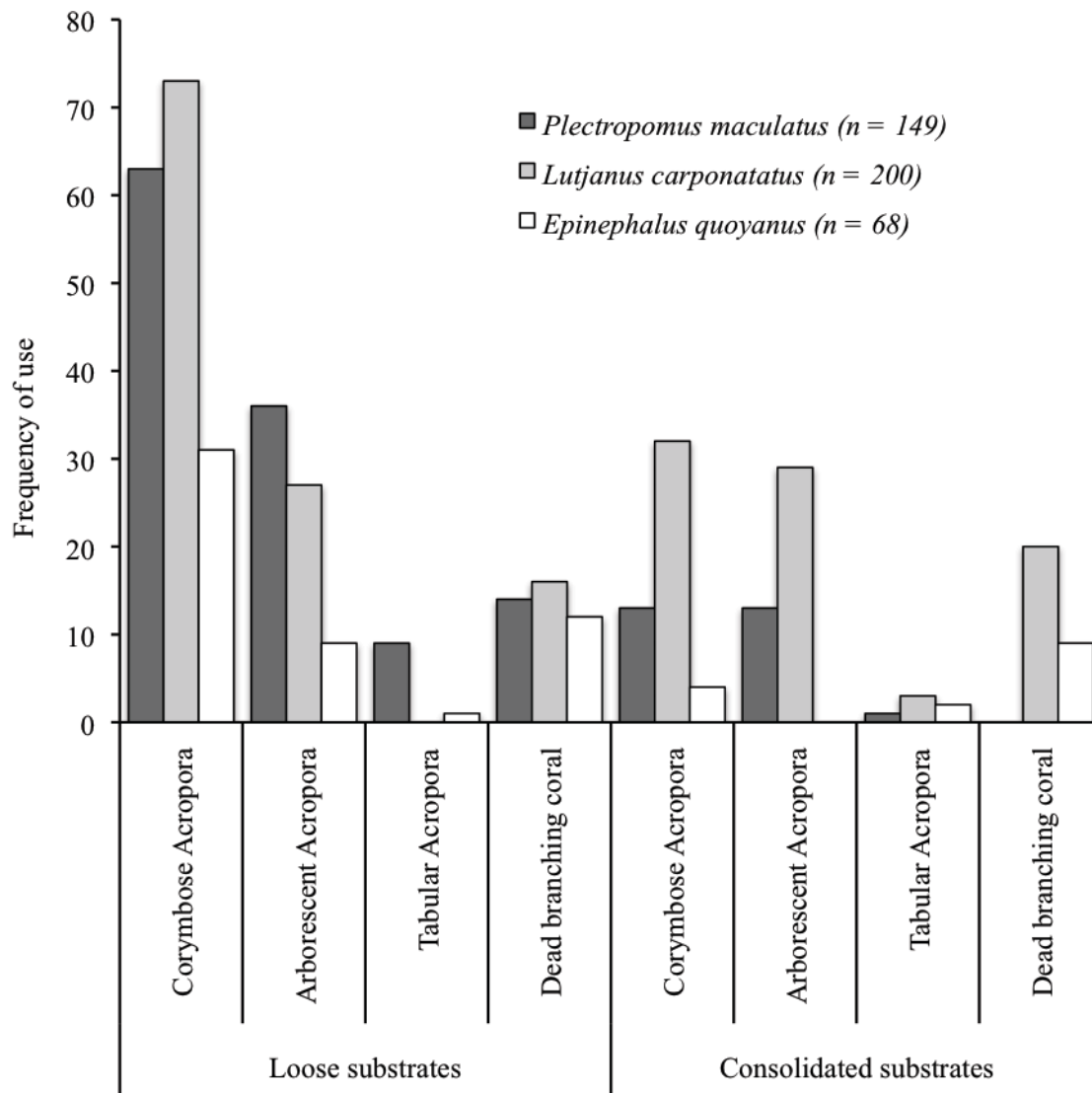


Figure 7.8.1. Frequency of use of different compound microhabitats by recruits (young of the year) for three carnivorous fishes (including *Plectropomus maculatus*) in the Keppel Islands, southern Great Barrier Reef.

This study revealed strong association with specific microhabitats for *P. maculatus*, as well as two other piscivorous fishes, *Lutjanus carponotatus* and *Epinephelus quoyanus*. Large predatory fishes form a significant part of the global catch in commercial, recreational and subsistence reef fisheries (Worm et al. 2005, Pauly 2008), and a large proportion of such species are overexploited (Myers and Worm 2003, 2005). The ecological and life history characteristics of predatory fishes, including low natural abundance, long life-span, slow growth and low recruitment rates are clearly factors that contribute to their susceptibility to overfishing (e.g., Musick 1999). However, degradation of reef habitats and declines in the availability of specific microhabitats are increasingly putting added pressure on larger predatory reef fishes (Graham et al. 2007, Wilson et al. 2010b; Pratchett et al. 2011).



The extent to which species may be able to adapt to changes in resource availability and habitat structure depends on their ecological versatility. Highly specialised species, such as coral feeding butterflyfish, are obligately dependent on specific types of corals and loss of these corals will inevitably lead to localised extinctions (Pratchett et al. 2008). However, fishes that use a wide range of different resources or are capable of switching their patterns of resource use may be able to withstand extensive coral loss (Pratchett et al. 2004). While all predatory fishes considered in this study used specific microhabitats disproportionately to their availability, the range of microhabitats used by each species of fish was very extensive. There were, for example, a small number of fishes from all life stages across all three species that were found living among macroalgae or in open areas of loose substrates with no major structural habitat. Variation in growth and survivorship of fishes associated with preferred versus non-preferred habitats needs to be quantified in order to assess the long-term consequences of coral loss and habitat degradation. However, it is very likely that declines in availability of specific microhabitats (especially those used by recruits) may explain declines in the abundance of predatory fishes following extensive coral loss (Graham et al. 2007; Russ et al. 2008). Notably, this research was conducted on an inshore reef, where the dominant coral trout species was *P. maculatus*. Similar research is still required to assess microhabitat requirements (especially at settlement) for *P. leopardus*, which is the major target species of commercial reef line fisheries.



## 8.0 BENEFITS AND ADOPTION

There has been significant recent research into effects of climate change on coral reef fishes, either documenting the changes in abundance or fitness of fishes following climate-related disturbances (reviewed by Wilson et al. 2006; Pratchett et al. 2008), or experimentally assessing direct effects of increasing temperature (e.g., Nilsson et al. 2009; Gardiner et al. 2010; Johansen and Jones 2011; Donelson et al. 2011) or ocean acidification (e.g., Munday et al. 2009) on individual, and mostly small bodied reef fishes. While there remain many crucial knowledge gaps (Wilson et al. 2010) this body of research points to three distinct effects of climate change on reef fishes, which will vary greatly in the timing of expected occurrence. First and foremost, climate change indirectly affects coral reef fishes by exacerbating declines in the quality and quantity of benthic habitats. These effects of climate change are already very apparent. In 1998, for example, climate induced coral bleaching caused extensive (45%) coral loss across the Indian Ocean, leading to declines in diversity and abundance of coral reef fishes (Graham et al. 2006). Secondly, projected increases in the temperature of shallow ocean waters will have direct effects on the physiology and performance of reef fishes. Many reef fishes appear to be living very close to their optimal temperatures, such that even small increases in temperature could have significant ramifications. Mostly however, small increases in temperature will have sub-lethal effects (e.g., changes in movement patterns, feeding, growth and reproduction) and these effects may be offset by distributional shifts, as well as behavioural or physiological acclimation (Donelson et al. 2011). The third and final effect of climate change on reef fishes relates to direct effects of increasing CO<sub>2</sub> concentration, and associated declines in ocean pH (ocean acidification). Elevated levels of CO<sub>2</sub> will impose physiological costs on fishes similar to increases in temperature (Munday et al. 2012). Of greatest concern however, are observed changes in the sensory discrimination and behaviour of fishes subject to elevated CO<sub>2</sub> (Munday et al. 2009, 2010). Still, significant effects of ocean acidification are only apparent at levels of pCO<sub>2</sub> above 700, which is not expected to occur until the end of the century.

Results from this study represent the first explicit tests on the effects of climate change on a large, commercially important reef fish species. As for other smaller reef fishes, coral trout *Plectropomus* spp. are clearly sensitive to i) climate-induced habitat degradation (specifically, the loss of complex corals used at settlement), ii) direct effects of temperature on reproduction, growth, development and metabolic costs, and iii) direct effects of ocean



acidification, especially in relation to olfactory discrimination. These findings indicate the critical importance of minimising ongoing greenhouse gas emissions as a critical step towards minimising extreme effects of global climate change on both ecological and economic benefits arising from corals reefs. Beyond that, this research shows increasing temperatures are likely to have a significant effect on the timing of spawning activities. In order to limit exposure of early life-stages (e.g., newly fertilised eggs) to devastating effects of extreme summer temperatures there is likely to be strong selective pressure for spawning to occur earlier in the year (see section 7.1). If so, this will require explicit changes to current temporary closures intended to protect spawning populations at the start of the austral summer. Also, current data suggests that the effects of climate change on coral trout (*Plectropomus* spp.) will be most pronounced in the northern Great Barrier Reef. Importantly, we found little or no differences in the thermal sensitivities of coral trout from northern versus southern populations. Rather, there was an absolute temperature threshold (approximately 30°C), above which, the individual condition and fitness of fishes was compromised. Given absolute temperatures are already highest in the northern Great Barrier Reef (e.g., Hughes et al. 2012), negative effects of high temperatures on *Plectropomus* spp. are likely to be first and worst felt in these northern populations. If so, northern-most populations of *Plectropomus* may require greater protection from fisheries, explicitly recognising differential effects of climate change along the length of the GBR. This provides additional incentive for implementing differential management plans within different latitudinal sectors, which is already being considered by the Queensland Department of Agriculture, Fisheries and Forestry, owing to price differentials along the length of the GBR. For commercial fishermen the ability to move between latitudinal sectors is considered critical for enhancing adaptive capacity to localised disturbances, such as cyclones (Tobin et al. 2010), and so any further restrictions to fishing effort that are applied disproportionately in the northern GBR must also consider alternative adaptation strategies for fishers in the event of further localised disturbances.

Adaptation policy, planning, and action are key steps towards identifying, assessing, implementing, and monitoring responses to disturbance, in this case climate change (Pecl et al. 2009). Impact assessments typically measure and project the risk and opportunity associated with climate change trends (e.g., Hoegh-Guldberg et al. 2007, Munday et al. 2008, Pratchett et al. 2008). As outlined above, climate change effects on coral trout include changes in distribution, abundance, growth, and behaviour, which may significantly influence availability, catchability, and predictability of catches for diverse fisheries associated with this



resource (e.g., commercial fisheries, recreational fisheries, indigenous fisheries, and charter fisheries). Vulnerability and risk assessments are thus required to allow scientists, managers and industry to consider what such outcomes, individually and in combination, might mean for ecosystems and people, and to identify appropriate adaptation responses (e.g., Johnson and Marshall 2007). However, to-date, few decision-support tools support nuanced, place-based appraisal of adaptation options to evaluate their potential to effectively and equitably deliver positive outcomes for social and ecological systems (but see Pecl et al. 2009, Bell et al. 2011). Prior research into adaptation and adaptive capacity has highlighted the importance of context and scale in determining adaptation outcomes. Impacts, responses, and outcomes are typically location specific, depending on how global climate change manifests at regional and local scales and on the vulnerability of the regional, national, and local contexts to these changes (e.g., Allison et al. 2009). Similarly, while resource-dependent sectors are particularly vulnerable to changing environmental conditions, this is mediated by their location, their dependency on particular resources and environments, and the aggregate capacity of operators to cope with change. For instance, adaptation options available to the fishing sectors based in the northern GBR may differ compared with those in the south. Within regions, individual operators will exhibit differing capacities for adaptation, depending on, for instance, their perception of risk, their capacity to plan, learn and change, their proximity to thresholds of coping, and their level of interest in adapting to change (Marshall and Marshall 2007). Thus, one-size-fits-all adaptation solutions will rarely suit everyone and adaptation planning needs to account for context and scale.

The adaptive capacity of different fisheries sectors on the GBR is generally highest amongst recreational and charter sectors, and lowest for commercial fisheries (Tobin et al. 2010). It is imperative therefore, that increased research be undertaken to directly assess adaptation options within the commercial fisheries sector, whereby any changes in the productivity, abundance or profitability of wild fisheries for coral trout (and especially *P. leopardus*) will have significant impacts within this industry. Alternative adaptation options that must be considered include those that i) reduce exposure (e.g, increase efficiencies within fisheries practices), ii) reduce sensitivity (e.g., diversify catch and product), or iii) enhance adaptive capacity (e.g., diversify livelihoods and economic opportunities). Given the likely sensitivity of coral trout to increasing temperature, all possible adaptation options must be considered. A key limitation however, is that we are whether alternative fishery species (e.g., red throat emperor) are equally sensitive to projected changes in environmental conditions.



## 9.0 FURTHER DEVELOPMENT

Results of our preliminary research suggest that there is limited acclimation to local temperature regimes apparent among wild populations of coral trout. This is based on the seeming lack of any differences in the thermal tolerances/ sensitivities of *P. leopardus* from northern versus southern populations, the next step is to test whether the same is true for other large carnivorous reef fishes. This is important because it establishes the generality of our detailed findings for *P. leopardus*, and if there are alternative species that are expected to be more tolerant of increasing temperatures, then these species may be important in sustaining ongoing commercial fisheries. We compared the aerobic scope of a total of 11 different species (including *P. leopardus*) caught opportunistically on reefs around Lizard Island (Figure 9.1). These preliminary data suggest that fishes from the family Lutjanidae (*L. russelli* and *L. carponotatus*) had significantly higher aerobic scope. This suggests, but does not prove, that the Lutjanidae could be much more tolerant of increasing temperatures, but this will need to be confirmed using experimental tests of changes in physiology and behaviour across a range of temperatures (as was conducted for *P. leopardus*). Unfortunately, we were not able to catch sufficient numbers of other *Plectropomus* species to test if all species (e.g., *P. laevis* and *P. maculatus*) have aerobic scope equivalent to that of *P. leopardus*, but this remains an important research question.

Further tests of interspecific differences in tolerances of increasing temperatures were conducted based on estimates of critical thermal maxima (CT<sub>max</sub>), defined as the non-lethal limit to dynamic exposure, assumed to approximate the maximum temperature that fishes can withstand short-term (Elliott and Elliott 1995). CT<sub>max</sub> has been criticised in the past as a method for distinguishing thermal tolerances of fishes because no clear difference could be found between CT<sub>max</sub> of fishes from tropical versus temperate environments. However, it is increasingly apparent that CT<sub>max</sub> varies within and among species, and may be used to infer relative (rather than absolute) thermal tolerances. It is also still unclear to what extent the CT<sub>max</sub> correlates with optimum temperature, which is more important for the distribution and abundance of fishes.



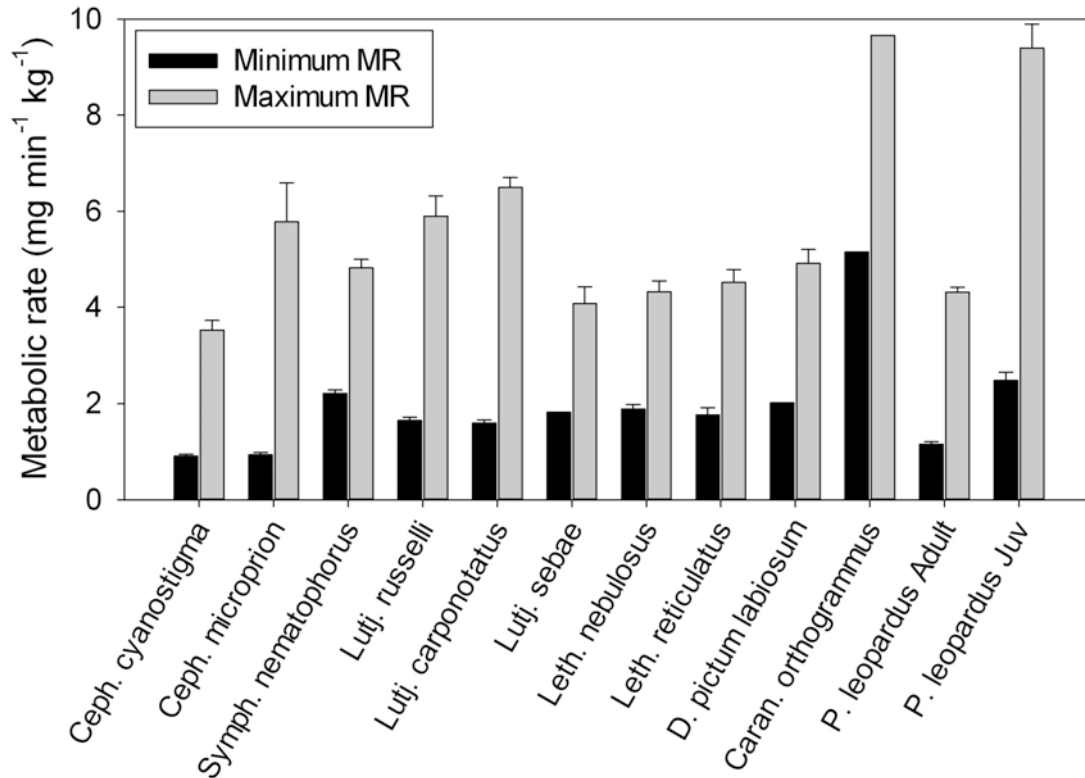


Figure 9.1 Inter-specific differences in the minimum and maximum rate of oxygen consumption (measured by placing fishes into respirometers for 24 hours after a chase for 3 minutes). Data was collected at Lizard Island.

In these experiments, temperature was increased manually by 0.5°C every 5 minutes to ensure a constant rate and is the equivalent of 0.1°C/minute or 6°C/hour. Between 1 and 23 individuals were tested for each of 21 different species, and CT<sub>max</sub> varied very little among individuals within species (Figure 9.2). These data indicate (as suggested previously) that *Plectropomus* and *Lethrinus* species (only 1 species of *Lethrinus* tested though) have the lowest capacity to withstand direct exposure to increasing temperature. Based on CT<sub>max</sub>, we found that *P. laevis* and *P. maculatus* were very similar to *P. leopardus*, but inter-specific differences in thermal sensitivities need to be much more carefully studied within this group. The species with greatest CT<sub>max</sub> were in general the Lutjanidae, while Serranidae exhibited marked interspecific differences in CT<sub>max</sub> (Figure 9.2).





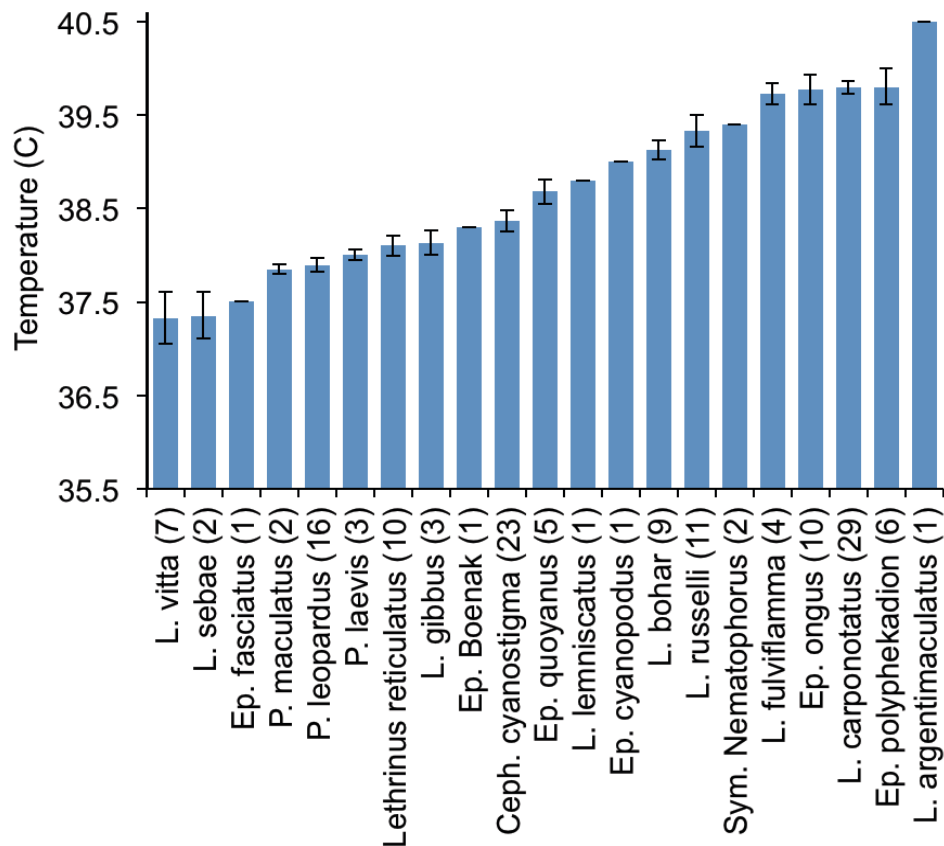


Figure 9.2 Inter-specific differences in CTmax for fishes caught around Lizard Island, northern Great Barrier Reef. Numbers in brackets indicate the number of fishes tested for each species. Where >1 individual per species was tested, the mean ( $\pm$  SE) temperature at which individuals lost equilibrium is displayed.

Testing the generality of our recent findings (on the sensitivity of *Plectropomus* spp. to climate change) amongst other commercial fisheries is a key priority for future research. However, it is also clear that we do not fully understand the differential effects of climate change (e.g., habitat degradation, increasing temperature and ocean acidification) on all aspects of the biology and at different stages in the life-history of *Plectropomus* spp. In particular, we are unclear if, or how, coral trout may adapt to changing environmental conditions. If for example, the timing of spawning is linked to local temperatures, then *P. leopardus* will probably spawn earlier in warmer years and thereby limit exposure of newly fertilised eggs to temperatures  $>30^{\circ}\text{C}$  (see section 7.1.1). It is likely however, that late stage larvae will still be exposed to extreme temperatures at the height of summer, and we have not as yet tested how these 30+ day old fishes will cope with temperatures  $>30^{\circ}\text{C}$ . Post-settlement fishes may behaviourally adapt to limit exposure to extreme temperatures, either going into deeper water or minimising feeding and movement (section 7.7), which is nonetheless



important because it will lead to lower catches, and may require changes in standard fisheries practices in order to maintain productivity and profitability within the commercial sector.



## 10.0 PLANNED OUTCOMES

Global climate change is an important emerging threat to the productivity of marine fisheries (Bell et al. 2013) and there is widespread recognition that fisheries plans must increasingly consider the effects of climate change. Focus areas for Australia's National Climate Change and Fisheries Action Plan 2009-2012 are to "improve understanding and awareness of climate change impacts on fisheries", and "facilitate ongoing assessment and monitoring of climate change impacts at suitable scales". To this end, the current project has provided important new information on the potential effects of climate change on coral trout (*Plectropomus* spp.), which support significant commercial and recreational fishery activities. Most notably, this project identifies specific thresholds (e.g., absolute temperatures) for important effects on reproduction, growth, development, and survivorship. This provides an explicit focus for ongoing environmental monitoring throughout the known distribution of these species (mainly, *P. leopardus*) to assess if and when individual thresholds are likely to be reached. Many of the major direct effects of climate change on coral reef fishes (e.g., declines in settlement success due to ocean acidification) are not likely to be felt for several years or decades, but potential effects must be considered as soon as possible in order to maximise potential adaptation options for both the fishes and fisheries.



## 11.0 CONCLUSIONS

Results of this research clearly indicate that coral trout (mainly, *P. leopardus*) are vulnerable to global climate change. *Plectropomus* spp. will be affected by climate change in three main ways; i) increasing temperature of shallow tropical waters will begin to exceed optimal temperatures, leading to declines in growth and development, and potentially causing increased disease prevalence, ii) declining pH will interfere with sensory abilities and modify behaviour, potentially reducing juvenile survival, and iii) increasing loss of live coral habitats used at settlement will reduce the abundance and survival of new recruits. It is likely therefore, that ongoing climate change will significantly undermine the sustainability of current reef-based fisheries, providing a strong imperative for new improved management of these stocks. As such, we advocate for immediate consideration of different adaptation options that could be used to reduce exposure, reduce sensitivity, and/ or enhance adaptive capacity within each of four distinct fisheries sectors; i) commercial, ii) recreational, iii) charter, and iv) indigenous fisheries. While some adaptation options, such as increased stewardship, will have beneficial effects across all fisheries sectors, most adaptation options will have disproportionate benefits for specific sectors. Future management structures need to account for the effects of climate change of fisheries stocks, but selection of optimal adaptation options requires careful consideration of ecological, economic and societal priorities.



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## 13.0 APPENDICES

### 13.1 Intellectual property

The research described in this report is for the public domain. The report and resulting manuscripts are intended for wide dissemination and promotion. All data and statistics presented conform to confidentiality arrangements.

### 13.2 Staff

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