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# Alterations in gill structure in tropical reef fishes as a result of elevated temperatures

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ABSTRACT

Tropical regions are expected to be some of the most affected by rising sea surface temperatures (SSTs) because seasonal temperature variations are minimal. As temperatures rise, less oxygen dissolves in water, but metabolic requirements of fish and thus, the demand for effective oxygen uptake, increase. Gill remodelling is an acclimation strategy well documented in freshwater cyprinids experiencing large seasonal variations in temperature and oxygen as well as an amphibious killifish upon air exposure. However, no study has investigated whether tropical reef fishes remodel their gills to allow for increased oxygen demands at elevated temperatures. We tested for gill remodelling in five coral reef species (*Acanthochromis polyacanthus*, *Chromis atripectoralis*, *Pomacentrus moluccensis*, *Dascyllus melanurus* and *Cheilodipterus quinquelineatus*) from populations in northern Papua New Guinea (2° 35.765′ S; 150° 46.193′ E). Fishes were acclimated for 12–14 days to 29 and 31 °C (representing their seasonal range) and 33 and 34 °C to account for end-of-century predicted temperatures. We measured lamellar perimeter, cross-sectional area, base thickness, and length for five filaments on the 2nd gill arches and qualitatively assessed 3rd gill arches via scanning electron microscopy (SEM). All species exhibited significant differences in the quantitative measurements made on the lamellae, but no consistent trends with temperature were observed. SEM only revealed alterations in gill morphology in *P. moluccensis*. The overall lack of changes in gill morphology with increasing temperature suggests that these near-equatorial reef fishes may fail to maintain adequate $O_2$ uptake under future climate scenarios unless other adaptive mechanisms are employed.



## 1. Introduction

Average sea surface temperatures (SSTs) are projected to be 2–3 °C higher at the end of the century as a result of rising greenhouse gases (Meehl et al., 2007; Poloczanska et al., 2007; Munday et al., 2009). Evidence suggests that tropical species will be especially vulnerable (more so than temperate species) to such temperature rises as a result of having evolved in a relatively more stable thermal environment (Tewksbury et al., 2008). Furthermore, tropical marine species are predicted to be more vulnerable to warming temperatures than tropical terrestrial species because the former live in environments that may already encompass their full thermal tolerance range, while the latter tend to occupy a narrower range of temperatures than their tolerance limits (Sunday et al., 2012). Therefore, understanding how tropical marine fishes cope with and potentially acclimate to shifting environmental conditions is paramount in determining the impact of climate change on their populations (Munday et al., 2012).

The body temperature of ectotherms closely tracks environmental temperatures, and therefore ectotherms, such as fishes, may be particularly affected by global warming if physiological mechanisms cannot keep pace with rising temperatures. Higher temperatures can result in an increased $O_2$ demand and therefore increase basic maintenance costs for the organism (Clarke, 2003). Increased maintenance costs reduce the total scope for aerobic performance, resulting in less energy to allocate to key performance traits such as reproduction, growth, immune function, and swimming upon which long-term species survival relies (Pörtner and Farrell, 2008; Johansen and Jones, 2011).

In fishes, the gill is the primary site of $O_2$ uptake and consequently a key organ where adjustments to maintain $O_2$ uptake and the scope for aerobic metabolic performance at elevated temperatures may be expected (Venkatesh, 2003; Evans et al., 2005). When metabolic needs increase as a function of temperature (Brett, 1971; Pörtner and Knust, 2007) and less $O_2$ is available, remodelling the gill to increase

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the functional surface area could aid in maintaining $O_2$ uptake necessary to support metabolism. Temperature-mediated gill remodelling has been well studied in a few temperate species such as the crucian carp (*Carassius carassius*) and goldfish (*Carassius auratus*) (Sollid et al., 2005; Sollid and Nilsson, 2006; Nilsson, 2007; Mitrovic et al., 2009). Under cooler water temperatures (typically < 15 °C), both *C. carassius* and *C. auratus* produce a cell mass that fills the space between adjacent gill lamellae, termed the interlamellar cell mass (ILCM) (Nilsson et al., 2012). The ILCM is shed when water temperatures increase in order to increase respiratory surface area (Sollid et al., 2003; Bradshaw et al., 2012). The proliferation and discarding of the ILCM effectively decrease or increase the surface area available for gas exchange (e.g. $O_2$, $CO_2$), but a trade-off may come in the form of ion regulation because increases in gill surface area simultaneously increase the energy needed to counteract ion and water fluxes over the respiratory surface (Mitrovic and Perry, 2009).

In addition to *Carassius* spp., gill remodelling has been observed in several temperate freshwater species over wide temperature ranges (Tuurala et al., 1998; Matey et al., 2008), as well as in mangrove killifish in response to air exposure (Ong et al., 2007; Turko et al., 2012). However, few studies have been conducted to determine if tropical marine fishes also exhibit temperature-induced gill remodelling. The paucity of studies may be because many populations inhabit very narrow temperature ranges and would not require a high degree of plasticity in their respiratory surfaces. Nevertheless, gill remodelling may be an important mechanism for maintaining the performance of tropical marine fishes under future climate change scenarios. Thus, the aim of this study was to determine if tropical reef fishes display morphological alterations to their gills upon exposure to temperatures predicted to occur in shallow water coral reefs by the end of the century due to global warming. Populations that live close to the equator off northern Papua New Guinea (2° 35.765′ S; 150° 46.193′ E) were specifically chosen because of the very narrow seasonal temperature range they experience (~3 °C) (Rummer et al., 2014a).

## 2. Materials and methods

### 2.1. Fish collection and gill sampling

Four species of damselfish (Pomacentridae) (mean mass ± SD; mean standard length ± SD), *Acanthochromis polyacanthus* (4.43 ± 1.31 g; 47.8 ± 5.5 mm), *Chromis atripectoralis* (2.62 ± 0.65 g; 43.7 ± 5.1 mm), *Pomacentrus moluccensis* (2.94 ± 0.97 g; 40.4 ± 3.8 mm), and *Dascyllus melanurus* (3.02 ± 0.87 g; 39.5 ± 3.8 mm), and one species of cardinalfish (Apogonidae), *Cheilodipterus quinquelineatus* (3.31 ± 0.83 g; 54.4 ± 5.1 mm) were collected using a barrier net or hand nets and clove oil anaesthetic (Munday and Wilson, 1997) near Nago Island, New Ireland Province, Papua New Guinea. Fishes were transported to the laboratory at the National Fisheries College Nago Island Mariculture and Research Facility (NIMRF) where they were maintained in aquaria supplied with flow-through seawater. Upon resuming normal feeding behaviour, fishes (10–12 individuals per species) were held at one of four temperatures (29, 31, 33, and 34 °C) ± 0.2 °C for 12–14 d. After 2 d at 34 °C exposure, all *A. polyacanthus* individuals stopped eating, and 100% mortality was recorded within 7 d. All other species maintained their feeding behaviour over the acclimation period. The two lowest temperatures, 29 °C and 31 °C, represent the current seasonal temperature range for this location, while 33 °C and 34 °C represent the 2–3 °C increase predicted by end-of-century climate change scenarios (Meehl et al., 2007; Poloczanska et al., 2007). After 12–14 d at experimental temperatures (except *A. polyacanthus* held at 34 °C), all fishes were euthanized via cranial concussion, and the 2nd and 3rd gill arches were removed from the left side of each fish and preserved in Karnovsky's fixative (Boyd et al., 1980). All animal holding and experimental protocols complied with James Cook University ethics regulations (permit: A1722).

### 2.2. Histology

The 2nd gill arches were removed from the fixative, and prepared for histological sampling as described by Handy et al. (2002). Briefly, samples were dehydrated through a series of graded ethanol (EtOH) concentrations (Shandon Southern Duplex Processor BS5), embedded in paraffin wax blocks (Shandon Histocentre 3, Thermo Electron Corporation), sectioned (5 μm) using a microtome (Microm HM 325), and stained using Mayer's haematoxylin and eosin stain. Digital photographs (Olympus DP12 Microscope Digital Camera System) were taken of each section for analysis as described below.

### 2.3. Light microscopy image processing

Histological images were analysed using ImageJ 1.46r (Wayne Rasband, National Institutes of Health, USA). In each section, 5 filaments were chosen from immediately posterior to the apex of the arch. The length of each filament was measured and the lamellae along one side of it were counted. At 3 designated positions (sites) on each filament, i.e. at 25%, 50% and 75% of the full filament length (Fig. 1) (Hughes, 1984), 3 lamellae were traced and their perimeter, cross-sectional area, base thickness, and lamellar length were measured using the ImageJ software. After preliminary investigation, sectioning error was more likely to occur at the extreme ends of the filament (25% and 75%), and therefore only measurements from the 50% location along each filament were utilised in analyses. This has also been suggested by Hughes (1984). Perimeter of the lamellae was used as the primary proxy for surface area.

### 2.4. Scanning electron microscopy

The 3rd gill arches from at least 3 individuals per species from each temperature group were removed from the fixative, placed in individual vials, and washed via agitation three times with 0.1 M phosphate buffer. The buffer was then replaced with 50, 60, 70, 80, 90, and two replications of 100% ethanol (EtOH) for 15 min each. Then, the gills were immersed in a 1:1 solution of EtOH to hexamethyldisilazane (HMDS) (Nation, 1983) and finally, 3 replicates of 100% HMDS. Samples

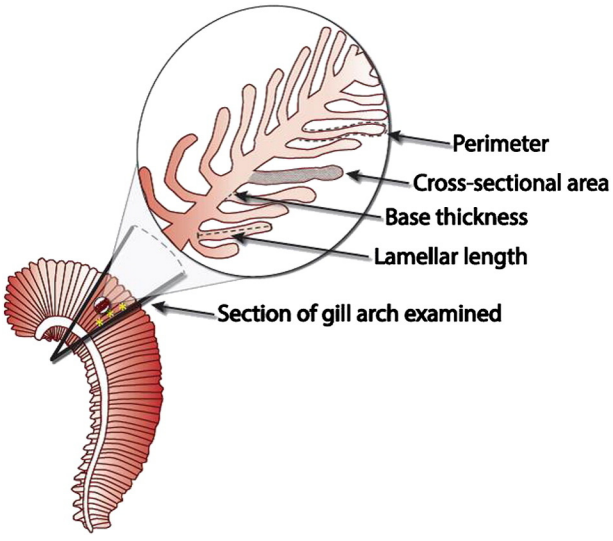


**Fig. 1.** Schematic depicting the location on the branchial arch of the 5 filaments selected for analysis as well as how measurements were taken for lamellar perimeter (dotted line around a lamella), cross-sectional area (shaded area within a lamella), base thickness (dotted line at the base of a lamella), and lamellar length (dotted bar along one side of a lamella). The yellow asterisks denote the sites along the filament where measurements were taken. The asterisk closest to the base of the filament is the 25% site followed by the 50% and 75% sites furthest along the filament. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

remained in each solution for 15 min with the exception of the third bath of 100% HMDS, where the HMDS was allowed to evaporate overnight. Gill samples were mounted on aluminium stubs and then gold coated at the Advanced Analytical Centre (AAC) at James Cook University. Micrographs were taken for each sample using a scanning electron microscope (SEM) (JEOL 5410 LV) at 2 different magnifications (10 and 50 μm scale bars) to achieve a gross overview and close up of the morphology of the gill arch. Images were analysed visually and qualitatively used to supplement information derived from light microscopy.

### 2.5. Statistical analyses

All values are reported as means ± S.E.M. by species and treatment temperature. A linear mixed effects (LME) model was conducted to test the effect temperature had on each parameter within a species. Three nested levels of variability were included as random effects: individual, section within individual, and filament within section. Temperature was the fixed effect in all analyses. In LME models the mean value for each treatment is tested against the control value, therefore, comparisons are reported for the relevant temperate treatments (31, 33, 34 °C) compared to the control (29 °C). The number of measurements for each parameter is shown in Table 1. All statistical analyses were conducted using S-Plus 8.0 (Insightful Corporation, Seattle, WA, USA).

## 3. Results

### 3.1. Light microscopy

The lamellar perimeter of filaments examined from each species varied among treatment temperatures, but the pattern of change differed markedly between the five species examined (Fig. 2). Consequently, there was no clear correlation between treatment temperature and lamellar perimeter. For *A. polyacanthus*, lamellar perimeter was significantly greater compared to control (29 °C) only at 33 °C (AIC = 5246.56; $F_{df=2}$ = 6.49884, $p < 0.005$, Fig. 2). Whereas, for *C. atripectoralis*, lamellar perimeter was significantly greater in all treatment temperatures (AIC = 5251.62; $F_{df=3}$ = 59.5328, $p < 0.0001$, Fig. 2) with the greatest perimeter at 33 °C. In contrast, the lamellar perimeter of *P. moluccensis* gill filaments was significantly reduced at 31 and 33 °C compared to 29 °C, whereas no significant difference could be detected between measurements at 29 and 34 °C (AIC = 4256.54; $F_{df=3}$ = 26.9969, $p < 0.0001$, Fig. 2). Lamellar perimeter of gill filaments examined from *D. melanurus* acclimated to 33 °C was, on average, 17% less than in individuals from 29, 31, or 34 °C (AIC = 9004.64; $F_{df=3}$ = 61.5869, $p < 0.0001$, Fig. 2). Finally, *C. quinquelineatus* exhibited significantly higher lamellar perimeters at 31 and 34 °C, while lamellar perimeter at 33 °C was significantly lower than at 29 °C (AIC = 7946.43; $F_{df=3}$ = 20.2925, $p < 0.0001$, Fig. 2).

Varied trends were observed in the other gill parameters measured as well (cross-sectional area, base thickness, and lamellar length; Table 1). In the lamellae examined from *A. polyacanthus* and *C. atripectoralis*, cross-sectional area and lamellar length resembled the changes measured for lamellar perimeter, whereas base length in both species only varied slightly. In *A. polyacanthus*, values significantly increased at 33 °C compared to 29 °C for cross-sectional area (AIC = 6611.45; $F_{df=2}$ =5.63, $p = 0.0038$, Table 1) and lamellar length (AIC = 4333.14; $F_{df=2}$ = 9.37, $p < 0.0001$, Table 1), whereas base length of the lamellae exhibited a steady increase, with 31 and 33 °C being significantly different than the control (AIC = 2611.79; $F_{df=2}$ = 9.84, $p < 0.0001$, Table 1). In *C. atripectoralis*, cross-sectional area (AIC = 6686.86; $F_{df=3}$ = 57.17, $p < 0.0001$, Table 1) and lamellar length (AIC =4238.29; $F_{df=3}$ = 272.38, $p < 0.0001$, Table 1) exhibited significant differences at all three treatment temperatures with a peak occurring at 33 °C. Base length in *C. atripectoralis* was the only parameter to have no significant differences detected between temperatures (Table 1). For *P. moluccensis* and *D. melanurus*, lamellar length, but not cross-sectional area or base length, resembled perimeter trends. Significant differences in lamellar length were detected at 33 °C and 31 °C in *P. moluccensis* (AIC = 3587.45; $F_{df=3}$ = 36.38, $p < 0.0001$, Table 1) and at 33 °C in *D. melanurus* (AIC = 7496.61; $F_{df=3}$ = 64.03, $p < 0.0001$ Table 1). Cross-sectional area in the lamellae of *P. moluccensis* significantly decreased at 33 °C compared to 29 °C (AIC = 5402.42; $F_{df=3}$ = 14.79, $p < 0.0001$, Table 1) and base length values were significantly higher at 31 and 34 °C (AIC = 2187.33; $F_{df=3}$ = 4.31, $p = 0.0053$, Table 1). In. *D. melanurus*, cross-sectional area decreased significantly at 31 and 33 °C and was significantly higher at 34 °C (AIC = 11792.66; $F_{df=3}$ = 22.76, $p < 0.0001$, Table 1), whereas, base length was significantly lower

**Table 1**
Mean values (±SEM) for the gill parameters (cross-sectional area, base thickness, and lamellar length) of all the species studied at each temperature. Significant differences of the treatment temperatures (31, 33, 34 °C) compared to the control temperature (29 °C) are demarcated with an (*). All parameters exhibited significant differences with the exception of base length for *C. atripectoralis*. The number of measurements for all parameters for each species at each temperature is stated in column 'n'. N/A — no data are available for *A. polyacanthus* at 34 °C due to 100% mortality at that temperature.

| Species | n | Temperature (°C) | Cross-sectional area (μm) | Base length (μm) | Lamellar length (μm) |
|---|---|---|---|---|---|
| *A. polyacanthus* | 203 | 29 | 365.1 ± 11.0 | 9.8 ± 0.2 | 52.4 ± 1.4 |
| | 129 | 31 | 318.0 ± 12.0 | 9.0 ± 0.3* | 44.9 ± 1.4 |
| | 192 | 33 | 387.3 ± 12.7* | 10.6 ± 0.3* | 55.5 ± 1.5* |
| | N/A | 34 | N/A | N/A | N/A |
| *C. atripectoralis* | 103 | 29 | 223.8 ± 9.1 | 10.1 ± 0.4 | 41.9 ± 1.4 |
| | 103 | 31 | 356.6 ± 22.1* | 9.8 ± 0.5 | 62.3 ± 2.0* |
| | 176 | 33 | 422.4 ± 15.3* | 9.9 ± 0.3 | 82.5 ± 1.2* |
| | 135 | 34 | 325.7 ± 12.0* | 10.2 ± 0.3 | 46.6 ± 1.0* |
| *P. moluccensis* | 129 | 29 | 288.6 ± 9.8 | 7.5 ± 0.3 | 57.5 ± 1.6 |
| | 157 | 31 | 310.8 ± 11.3 | 8.7 ± 0.3* | 48.5 ± 1.3* |
| | 134 | 33 | 231.0 ± 9.7* | 8.5 ± 0.3 | 38.3 ± 1.2* |
| | 21 | 34 | 351.6 ± 40.0 | 10.6 ± 0.7* | 47.3 ± 3.7 |
| *D. melanurus* | 234 | 29 | 304.9 ± 9.3 | 9.4 ± 0.3 | 44.7 ± 1.1 |
| | 134 | 31 | 290.9 ± 10.3* | 7.9 ± 0.3* | 48.8 ± 1.7 |
| | 257 | 33 | 288.2 ± 8.8* | 10.1 ± 0.2* | 34.4 ± 0.7* |
| | 297 | 34 | 366.5 ± 9.1* | 9.8 ± 0.2 | 47.3 ± 1.0 |
| *C.quinquelineatus* | 29 | 471.0 ± 22.6 | 14.8 ± 0.5 | 64.1 ± 2.3 | |
| 104 | 256 | 31 | 511.9 ± 13.6 | 11.7 ± 0.3* | 77.9 ± 1.6* |
| | 195 | 33 | 412.9 ± 12.5* | 12.5 ± 0.3* | 60.0 ± 1.7 |
| | 204 | 34 | 532.4 ± 12.5* | 11.3 ± 0.2* | 75.7 ± 1.4* |

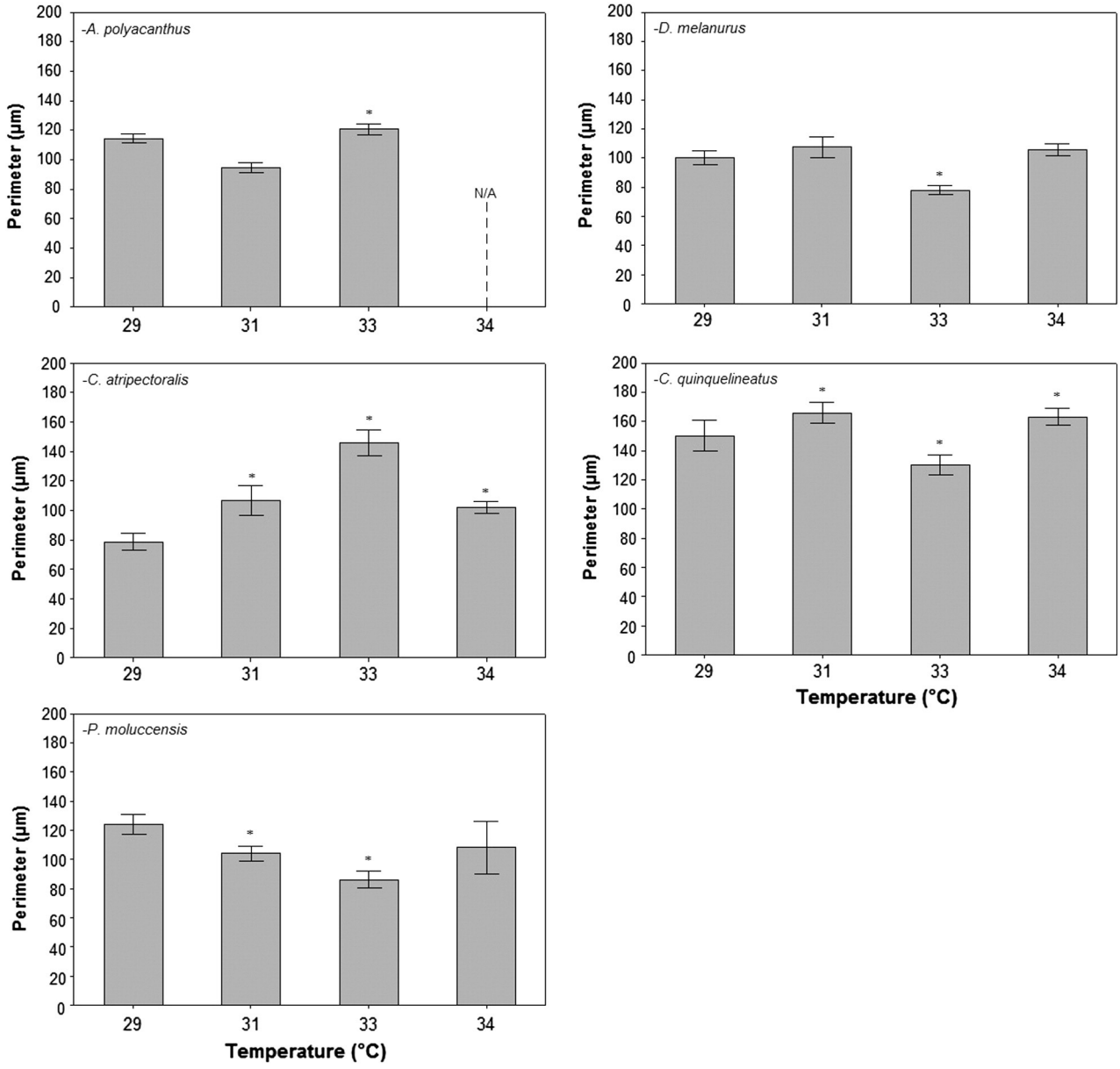


**Fig. 2.** Mean values (±SEM) for the average lamellar perimeter of five filaments for the four damselfish and one cardinalfish species investigated at each of four treatment temperatures. Significant differences compared with the control (29 °C) are shown with an (*).

at 31 °C and higher at 33 °C compared to the control (AIC = 4846.97; $F_{df\ =\ 3} = 26.41$, $p < 0.0001$, Table 1). The cardinalfish, *C. quinquelineatus*, was the only species for which none of the other parameters closely resembled the perimeter. Cross-sectional area was significantly lower at 33 °C and higher at 34 °C (AIC = 9993.69; $F_{df\ =\ 3} = 17.11$, $p < 0.0001$, Table 1). The base length values were significantly lower at all treatment temperatures (AIC = 4276.83; $F_{df\ =\ 3} = 12.91$, $p < 0.0001$, Table 1), whereas the lamellar length was significantly higher at 31 and 34 °C compared to the control (AIC =6778.21; $F_{df\ =\ 3} = 30.12$, $p < 0.0001$, Table 1).

The number of lamellae per mm of filament was similar between all temperatures within each species (mean ± S.E.M. across all temperatures; *A. polyacanthus* 28 ± 0.4, *C. atripectoralis* 32 ± 0.6, *D. melanurus* 34 ± 0.3, and *C. quinquelineatus* 29 ± 0.3) with the exception of *P. moluccensis*, which exhibited a decreased number of lamellae at the highest treatment temperature (33 ± 0.5 for 29, 31, and 33 °C compared to 21 ± 0.3 at 34 °C).

### 3.2. Scanning electron microscopy

Four of the five species exhibited no discernible differences in the appearance of the lamellae between temperatures and no sign of an ILCM (interlamellar cell mass), as shown for *C. atripectoralis* (Fig. 3). The micrographs, however, did provide visual evidence to support the quantified decrease in lamellar perimeter and cross-sectional area observed via light microscopy for *P. moluccensis* with increasing temperatures. In particular, the lamellae of *P. moluccensis* acclimated to 29 °C were protruding away from the filaments, similar to what was observed in the other species examined (Fig. 4A). However, the lamellae examined from *P. moluccensis* acclimated to 34 °C were noticeably lacking, with the disappearance of lamellae commencing at approximately 50% of the distance along the filament until a prominent decrease in protruding lamellae was observed next to the branchial arch (Fig. 4B). The phenomenon is clearly depicted in a comparison of light microscopy images of *P. moluccensis* filaments at 29 and 34 °C (Fig. 5).

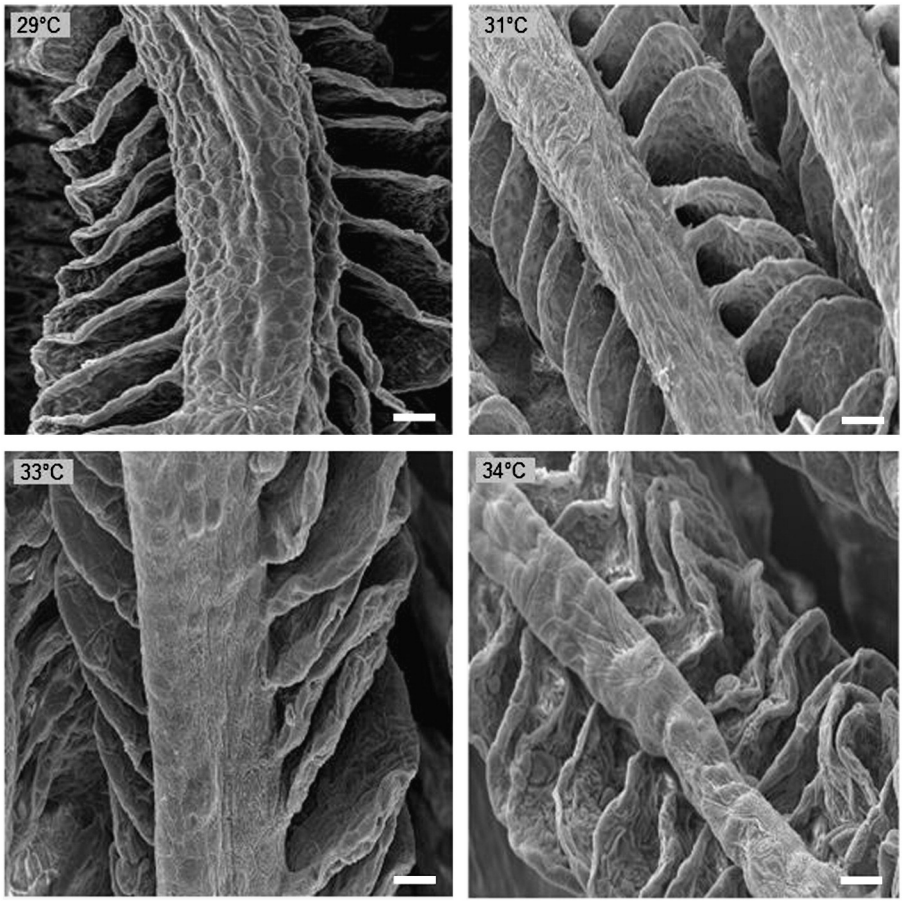


**Fig. 3.** Scanning electron micrographs of *C. atripectoralis* gills from individual fish acclimated to 29, 31, 33, and 34 °C. At all four temperatures, the lamellae are protruding from the filament with no sign of degradation. Scale bars are 10 μm. F, filament; L, lamellae.

## 4. Discussion

The five coral reef fishes investigated in this study exhibited quantifiable alterations of the dimensions of their gills upon 12–14 d exposure to elevated temperatures, but the patterns differed markedly among species and with no consistent trend with increasing temperature in four of the five species. *C. atripectoralis* exhibited a stepwise increase in all parameters except for base length up to 33 °C before falling at the highest temperature. However, visual observations via SEM images do not show noticeable morphological differences between the temperatures. Therefore, the patterns of change, in conjunction with the general lack of changes observed in SEM images, are inconsistent with morphological gill remodelling that we understand from studies on temperate species to aid in gas exchange with rising temperature. The differences in gill dimensions may, instead, be explained by physiological alterations, such as changes in perfusion patterns. Indeed, both freshwater and marine fishes have been noted to perform cutaneous gas exchange to enhance $O_2$ uptake (reviewed in Feder and Burggren, 1985). For instance,

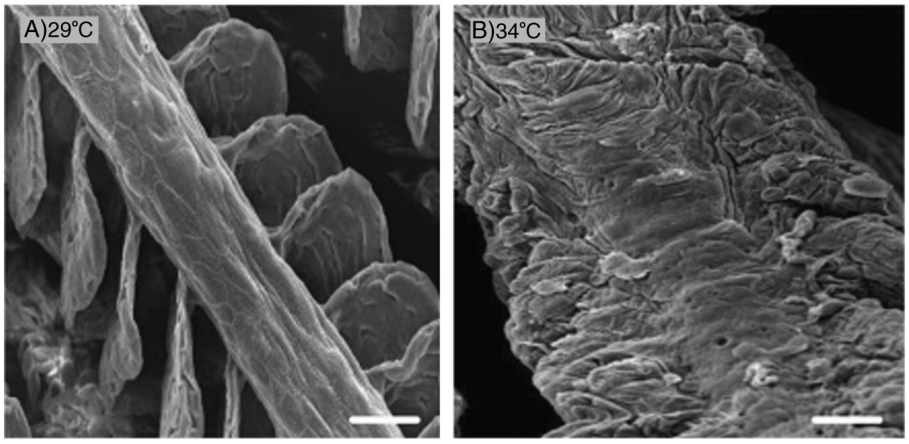


**Fig. 4.** Scanning electron micrographs of gills from *P. moluccensis* acclimated to 29 °C (A) and 34 °C (B) at the base of the filament adjacent to the branchial arch. At 29 °C, lamellae appear healthy and protrude from the filament. At 34 °C, the lamellae at the base of the filament are noticeably lacking when compared to gills from fish acclimated to 29 °C. Scale bars are 10 μm. F, filament; L, lamellae.

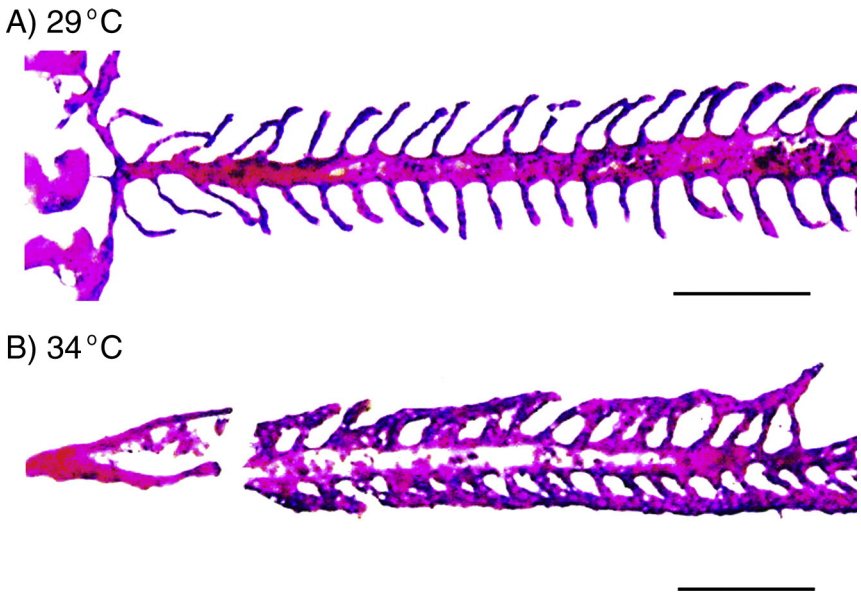


**Fig. 5.** Light microscopy images of *P. moluccensis* acclimated to 29 °C (A) where the lamellae appear healthy and protruding and 34 °C (B) where the lamellae seem to be fusing at the tips resulting in the regressed appearance of Fig. 4. Scale bars are 100 μm.

in a recent study, glass catfish (*Kryptopterus bicirrhis*) were found to have red blood cells in their secondary vascular system during active swimming at the surface during periods of aquatic hypoxia as well as immediately following exercise (Rummer et al., 2014b). Therefore, aquatic surface respiration and alterations to cutaneous gas exchange cannot be completely ruled out as a supplement to branchial respiration for the tropical reef fishes. Indeed, we found no obvious trends in the changes in gill morphology at the elevated temperatures, suggesting that the gills of these near-equatorial populations of coral reef fishes may fail to maintain adequate $O_2$ uptake as SST continue to increase under future climate scenarios if other adaptive mechanisms are not employed.

### 4.1. Morphological remodelling vs. physiological adjustments

The near-equatorial marine fishes from this study did not exhibit the same degree of morphological changes in their gills or linear increases in gill surface area with increasing temperature that has been previously described in some temperate species. Rather, the fishes we examined exhibited less predictable and subtle changes in total gill surface area with increasing temperatures. With the exception of *P. moluccensis*, we could not detect substantial alterations to the external gill morphology using scanning electron micrographs alone as has been well documented in temperate species like *C. carassius* and *C. auratus* (Evans et al., 2005; Sollid et al., 2005). However, changes in perimeter of the lamellae were quantifiable, using stained gill sections and light microscopy. We did not detect an ILCM at any temperature, and therefore the differences observed in perimeter with temperature suggest that tropical reef fishes may cope with elevated temperatures differently than temperate species. Because the current average temperatures experienced by the near-equatorial populations are already high, the presence of an ILCM could hinder $O_2$ uptake, and thus tropical species may not be adding or losing cells to morphologically alter gill surface area, but rather employing other physiological mechanisms to enhance gill performance.

Some fishes compensate for low environmental $O_2$ conditions by increasing heart stroke volume (Randall, 1982). This is thought to increase the functional respiratory surface area by increasing the intralamellar blood pressure and thereby the number of fully perfused lamellae (lamellar swelling), i.e. both opening more of the vascular space in each perfused lamella and opening previously closed lamellae (Farrell et al., 1979; Farrell et al., 1980; Taylor and Barrett, 1985). In addition, the increased stroke volume may be combined with a reduced heart rate (hypoxic bradycardia) resulting in increased retention time of blood within the gills (Randall and Daxboeck, 1984). Because the fishes used in this study were sampled and gills preserved within moments of cranial concussion, it is likely that the state of lamellar swelling and/or recruitment would be preserved as well. Thus, the alterations we observed, such as the minor, yet significant changes in lamellar length and base thickness, may be a result of short-term physiological adjustments rather than morphological changes relying on cell proliferation or apoptosis.

If the significant changes in lamellar dimensions recorded in this study are primarily a result of increased lamellar perfusion by blood, this may be why the changes were not consistently detected via SEM micrographs or to the extent of what has been detected in temperate species that employ an ILCM when remodelling the gills. Tropical reef fishes may currently live in warm waters where a larger gill surface area may already be needed to maintain adequate $O_2$ uptake. Tropical species have higher metabolic rates than similarly sized temperate species, and so tropical fishes tend to reach smaller maximum sizes (reviewed in Pauly, 1998). One way to achieve a larger gill surface area to support a high metabolism – especially if space is limited due to fish size – is to have a high number of lamellae per millimetre of filament (Hughes, 1966). Highly active pelagic species such as the common mackerel (*Scomber scombrus*), the common dolphinfish (*Coryphaena hippurus*), and the Atlantic menhaden (*Brevoortia tyrannus*) have a high number of lamellae per millimetre of filament ranging from 26 to 31, and this is thought to allow high $O_2$ uptake during activity (Table 1 in Gray, 1954). In contrast, less active species such as black sea bass (*Centropristis striatus*), sheepshead (*Archosargus probatocephalus*), and scup (*Stenotomus chrysops*) possess fewer lamellae per filament, ranging from 21 to 26 (Table 1 in Gray, 1954). The tropical reef species we investigated may not be considered highly active compared to fast swimming pelagic fishes, but they are small and live in warm waters, and therefore have higher mass specific oxygen consumption rates when compared to larger, temperate fishes. Indeed, our results show that they do possess a high number of lamellae per millimetre of filament (28–34), which

could be a newly understood characteristic of small, tropical species. Therefore, at the current environmental conditions these species may already have the highest lamellar density and size that can be achieved due to geometrical constraints. Further enlarging lamellae could be detrimental, that is, filling in the interlamellar water channels and effectively decreasing the size of the "sieve".

Marine species, in general, must also protect against a constant influx of ions from the surrounding hypertonic aquatic environment, which can be exacerbated by an increased gill surface area and result in higher energy costs to expel the unwanted ions (Nilsson, 2007). Therefore, tropical, marine fishes may not be able to afford morphologically enlarged respiratory surfaces in the manner we understand from past studies on temperate, freshwater fishes (Sollid et al., 2005; Sollid and Nilsson, 2006; Nilsson, 2007; Mitrovic and Perry, 2009; Turko et al., 2012) and amphibious killifish (Turko et al., 2012) because of the cost associated with ion influx (osmo-respiratory compromise) (Nilsson, 1986). Tropical marine fishes, instead, may resort to a more plastic physiological response such as increasing perfusion and recruiting lamellae. The species used in this study have a wide latitudinal distribution, with the exception of *D. melanurus* (equatorial distribution). Morphological differences may be easier to perceive through comparisons with different populations from higher latitudes such as Heron Island, where a wider temperature range is experienced (Gardiner et al., 2010).

Nevertheless, the lemon damsel, *P. moluccensis*, did exhibit striking changes in gill morphology that were evident via SEM images, and the response was clearly different from the other species examined. At 34 °C, *P. moluccensis* gills were noticeably lacking in protruding lamellae at the base of the filaments (Fig. 4). The mechanism behind the morphological change was not specifically explored in this study. The relationship between aerobic performance and changes in gill surface area may not be direct. In a previous study by Gardiner et al. (2010), *P. moluccensis* maintained aerobic metabolic scope between 29 and 33 °C, and data from a parallel study by Rummer et al. (2014a) suggest that aerobic metabolic scope was maintained between 29 and 34 °C. Interestingly, Rummer et al. (2014a) found that *P. moluccensis* exhibited a high degree of temperature sensitivity ($Q_{10} = 7.2$ between 29 and 34 °C) in terms of resting $O_2$ consumption rates. This may be related to the overall decrease of respiratory surface area observed via the parameters measured in this study.

### 4.2. Ecological implications

Equatorial populations of coral reef fishes may have a limited scope for temperature-mediated gill remodelling, even if – or perhaps because – those temperature increases are only 2–3 °C above ambient conditions. Consequently, the ability to meet the metabolic demands of the organism may be compromised, and important life history traits such as growth, reproduction, and predator/prey relationships in a warmer world may fail to be maintained if other pathways are not employed to maintain gas exchange. The temperature at which maximum $O_2$ consumption rates peak and aerobic metabolic scope is the greatest should, in theory, correspond to when fishes exhibit the greatest gill surface area. However, such a trend for the five coral reef fish species investigated here was not apparent when compared to previous measurements of oxygen consumption rates on the same fish (Rummer et al., 2014a). Our findings suggest that the observed changes in lamellar perimeter do not occur in an adaptive manner and, as a result, the species may fail to maintain aerobic metabolic scope at elevated temperatures expected to occur due to global warming. Other studies have determined the thermal reaction norms for aerobic metabolic performance in damselfish and cardinalfish populations from higher latitudes (Lizard and Heron Islands) where greater variations in seasonal temperatures are experienced (Nilsson et al., 2009; Gardiner et al., 2010). Lizard Island populations exhibited lower aerobic scopes at higher temperatures than their Heron Island higher latitude counterparts, suggesting that thermal history and seasonal variations in temperature may be important in determining which populations are most susceptible to future increases in ocean temperatures. Investigating gill morphology in response to temperature in fish populations from higher latitudes – where seasonal temperature ranges are much larger – could possibly reveal a level of gill remodelling similar to that of temperate species (Nilsson et al., 2012).

### 4.3. Future research

Future studies are needed to more closely link the changes in gill morphology or lamellar recruitment/perfusion and changes in aerobic performance observed in tropical fish species to identify the mechanisms responsible for enhancing or sustaining metabolic oxygen demands under elevated temperatures. Because developmental acclimation and transgenerational acclimation have been found to positively impact aerobic metabolic performance in at least one coral reef fish species (Donelson et al., 2011; Donelson et al., 2012), it may be that rearing temperatures or thermal history influences gill modifications later in life or for future generations. As mentioned earlier, latitudinal comparisons could aid in determining whether populations that experience a wider range of seasonal variation in temperature in the higher latitudes are more capable of morphological gill remodelling than their lower latitude conspecifics. Finally, ocean warming will not occur in isolation; climate change models predict decreases in ocean pH due to elevated $CO_2$, increases in areas and instances of hypoxia, and increases in storm surge and turbidity, all of which could also prompt alterations in gill morphology to maintain gas exchange and ion/osmotic balance and should be considered in future studies.

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