nature climate change

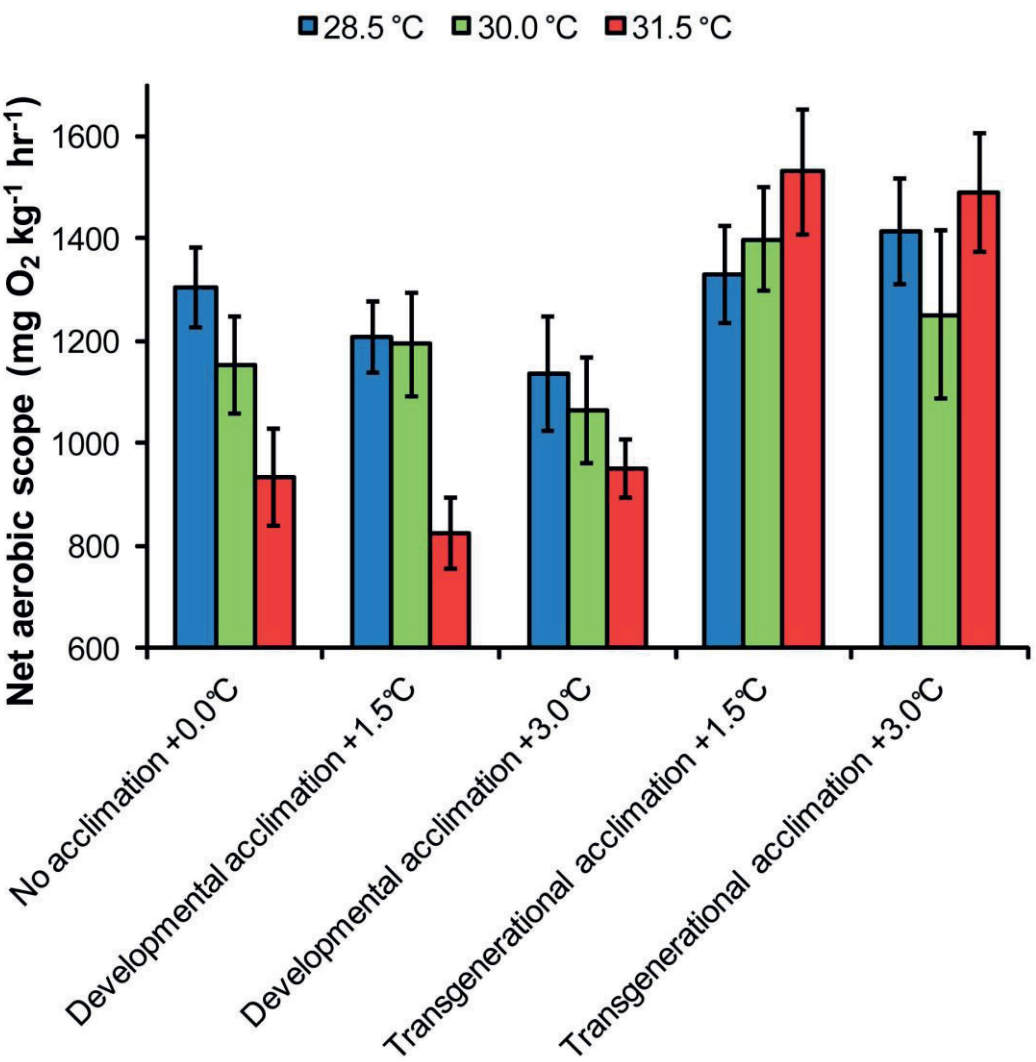
SUPPLEMENTARY INFORMATION

DOI: 10.1038/NCLIMATE1323

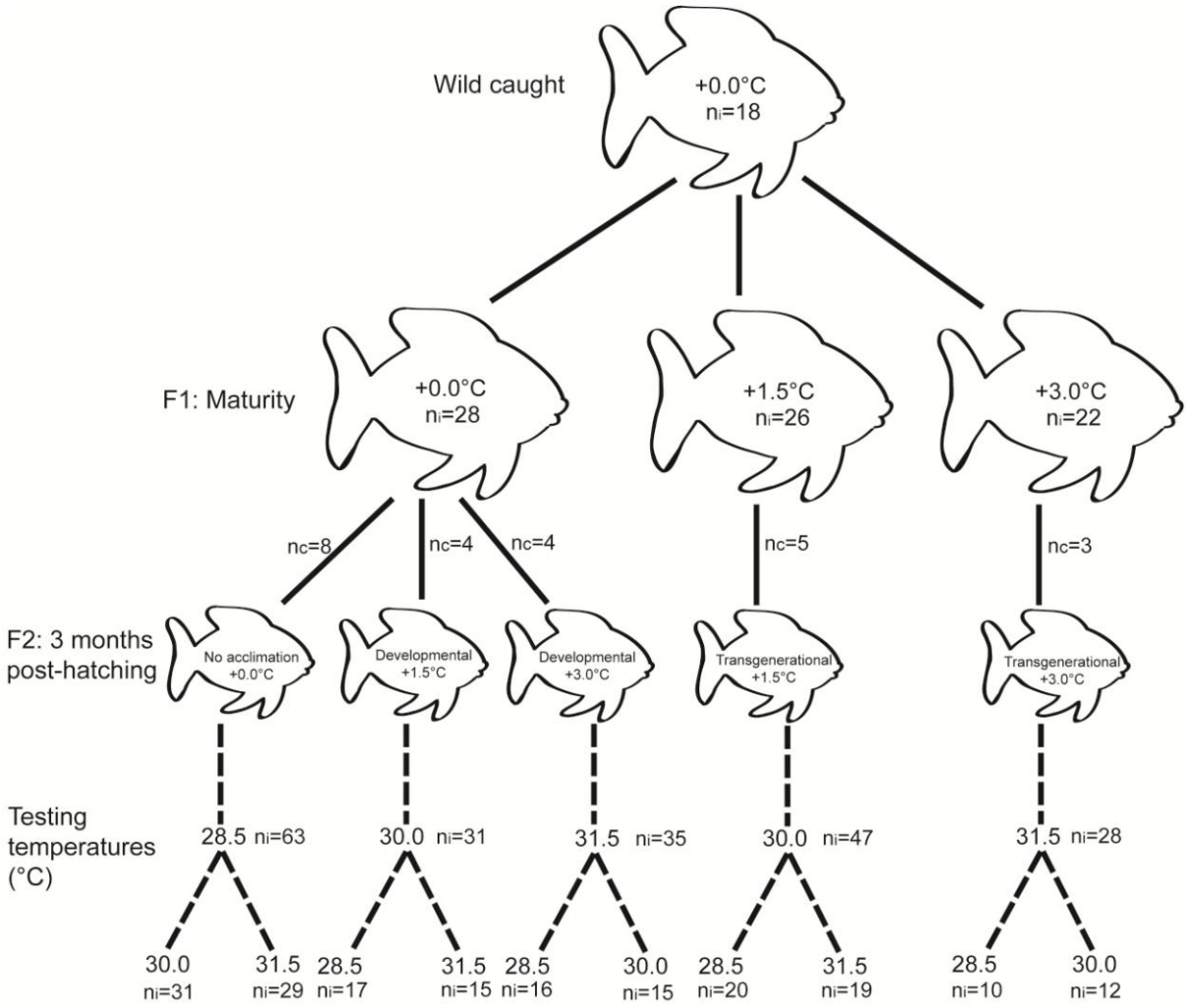
Rapid transgenerational acclimation of a tropical reef fish to climate change

By: J. M. Donelson, P. L. Munday, M.I. McCormick and C. R. Pitcher

Supplementary Figures



Supplementary Figure 1 | Net aerobic performance of acclimation groups across all summer treatment temperatures. Net aerobic scope of fish across the three summer average temperatures depending on acclimation treatments (mean ± s.e.m.).



Supplementary Figure 2 | Experimental design tree. Experimental design where fish icons represent acclimation treatments and temperature regimes that subjects were raised in from hatching until maturation (2 years F1) or 3 months (F2). F2 offspring were first tested in their acclimation treatment summer average temperature before being tested in one of the other two treatment temperatures. Numbers inside fish represent total numbers of individuals in a treatment (n) and numbers outside represent numbers of clutches (n_c) or individuals (n_i) tested.

Supplementary Tables

Supplementary Table 1 | Statistical results of differences in metabolic attributes of F2 acclimation treatment groups at 3 testing temperatures. Factorial ANOVA comparison of resting metabolic rate (RMR), maximum metabolic rate (MMR), factorial and net aerobic scope (log_{10} transformed) of F2 fish across testing temperatures for all acclimation treatments. Significant p values are indicated in bold.

	RMR		MMR		Factorial scope		Net scope	
Testing temperature	$F_{2,344}$=15.3	**p<0.001**	$F_{2,316}$=0.00	p=0.97	$F_{2,290}$=12.05	**p<0.001**	$F_{2,290}$=2.00	p=0.137
Acclimation treatment	$F_{2,344}$=7.2	**p<0.001**	$F_{2,316}$=2.4	p=0.05	$F_{2,290}$=17.25	**p<0.001**	$F_{2,290}$=6.66	**p<0.001**
Testing temperature* Acclimation treatment	$F_{2,344}$=1.1	p=0.392	$F_{2,316}$=2.1	**p=0.036**	$F_{2,290}$=2.11	**p=0.035**	$F_{2,290}$=2.88	**p=0.004**

Supplementary Table 2 | Statistical results of differences in metabolic attributes between fish from wild grandparent pair #41 and all other grandparent pairs. Factorial ANOVA comparison of resting metabolic rate (RMR), maximum metabolic rate (MMR), factorial and net aerobic scope (log_{10} transformed) between offspring of F0 wild pair #41 and all F0 pairs (Parental ID). The full-factorial ANOVA design was tested, but only the combinations involving parental ID are shown below.

	RMR		MMR		Factorial scope		Net scope	
Parental ID	$F_{1,286}$=3.2	p=0.075	$F_{1,262}$=0.1	p=0.767	$F_{1,238}$=1.538	p=0.216	$F_{1,238}$=0.04	p=0.839
Testing temperature* Parental ID	$F_{2,286}$=0.3	p=0.758	$F_{2,262}$=0.4	p=0.671	$F_{2,238}$=0.935	p=0.415	$F_{2,238}$=0.33	p=0.717
Acclimation treatment* Parental ID	$F_{3,286}$=0.06	p=0.598	$F_{3,262}$=0.8	p=0.503	$F_{3,238}$=0.953	p=0.696	$F_{3,238}$=0.75	p=0.523
Testing temperature* Acclimation treatment* Parental ID	$F_{6,286}$=0.08	p=0.597	$F_{6,262}$=1.1	p=0.371	$F_{6,238}$=1.546	p=0.163	$F_{6,238}$=1.22	p=0.297

Supplementary Methods

Study Species and Climate Change Predictions

The coral reef damselfish *Acanthochromis polyacanthus* is a widespread Indo Pacific species (15°N–26°S and 116°E–169°E). Fish were collected from the Palm Island region (18° 37' S, 146° 30' E) of the central Great Barrier Reef, which experiences a mean temperature range of 23.2 °C to 28.5 °C. Average sea surface temperatures in the Great Barrier Reef, Australia, are predicted to increase up to 3 °C by 2100 due to global warming[33,34]. Temperature increases of this magnitude during summer are known to have negative effects on metabolic rate, reproduction, growth and physical condition of *A. polyacanthus*[35-37]. The longevity of *A. polyacanthus* in the collection region is approximately 9 years[38].

Multigenerational Rearing and Experimental Design

Nine established pairs of *A. polyacanthus* were collected from July to August 2007 and maintained in 60 l aquariums inside an environmentally-controlled facility at James Cook University, Townsville, Australia. Pairs were maintained at the mean present-day ocean temperature for the collection location and provided with the average food consumed by wild pairs[35]. During the austral summer 2007-2008, breeding bouts from 8 F0 pairs were used for the current study. Offspring from these pairs were kept with their parents for 30 days post-hatching. At this time F1 individuals from each clutch were divided into 3 groups for rearing in 3 seasonally-cycling temperatures regimes; splitting clutches in this way ensured that each experimental treatment contained similar genetic diversity and allowed investigation of possible genetic effects on acclimation ability. One treatment group was kept at the present-day average temperature cycle at the collection location (+0.0 °C), while

the other two groups were gradually adjusted to, and reared at, two higher temperature treatments: either +1.5 °C or +3.0 °C (Supplementary Fig. 2). Temperature was kept within ±0.2 °C of the desired treatment mean. Temperatures at the collection location have naturally fluctuated between 0.2-2.5 °C in a single day, but on average vary only 0.45 °C daily (JCU/AIMS weather station 1999-2008). Sibling fish were kept in groups of 6 in 40 l aquaria for 1 year after hatching, at which time density was reduced to pairs (see [19] for more details).

During the austral summer 2009-2010 nesting sites of F1 pairs were checked daily at 09:00 for the presence of eggs. Following the observation of a clutch, tanks were checked again daily at 11:00 for the presence of hatched offspring. Directly after hatching groups of 30-40 F2 individuals were removed from the parents and reared in 25 l aquaria under temperature treatments (Supplementary Fig. 2) until 3 months post-hatching when metabolic testing occurred. Offspring from +0.0 °C fish (4 clutches) were separated into 3 groups, of which one group was kept at the natal temperature and two groups were gradually adjusted to the two higher temperature treatments (+1.5 °C and +3.0 °C). Offspring from +1.5 °C (5 clutches) and +3.0 °C (3 clutches) F1 pairs remained at their natal temperature until testing.

At the conclusion of the breeding season (April 2010) all mature F1 fish were euthanised and measurements of standard length (to nearest 0.01 mm), body wet weight (to 0.01 g) and liver weight (to 0.001 g) were taken. Hepatosomatic index was calculated as liver weight as a percentage of the total body weight.

Metabolism Methods

Resting metabolic rate (RMR) and maximum metabolic rate (MMR) were measured directly in mg O_2 kg^{-1} h^{-1}, using a closed respirometer[18,19], which allowed the

calculation of factorial (MMR/RMR) and net aerobic scope (MMR-RMR). For RMR measurements, each fish was allowed to acclimatize in the respirometer (a 350 ml Perspex cylinder with 68 mm inner diameter) for 1 h with a constant water flow. Following acclimatization the chamber was sealed and oxygen concentrations were monitored with an oxygen electrode (WTW OXI 340i or OXI 3310, Germany) for 30 min. Oxygen concentrations remained above 70 % of air saturation during all respirometry. Fish were given at least 2 hours rest before measuring MMR. For measurement of MMR the chamber was placed upright so the chamber could be opened at the bottom for addition of the fish and created a circular swimming area[39]. Water current was created by a 60 mm magnetic stirring bar inside the sealed chamber and the current speed was set to the maximum aerobic swimming speed of the fish. Oxygen level in the water was measured for 5 to 10min during which oxygen decline was stable (see [39] for further details). For both trials the respirometer was submerged in a temperature-controlled aquarium to maintain a stable temperature. Subsequently, the wet weight of each fish was measured to the nearest mg.

Fish ranged in size from 182 mg to 538 mg in all treatments and were starved for 24 hours prior to testing to remove any effects of digestion on oxygen consumption. Testing temperatures were 28.5 °C, 30.0 °C and 31.5 °C, corresponding to the summer averages of each of the rearing treatment groups (+0.0 °C, +1.5 °C and +3.0 °C respectively). Fish were initially tested at their rearing temperature treatment and then given 1 to 2 days of rest. All tested fish were then divided equally in two groups and gradually adjusted to one of the other two treatment temperatures over a 2 to 3 hour period. Fish were maintained at the new temperature for 5 to 6 days before metabolic attributes were tested again at the new temperatures. All experiments were conducted within a temperature controlled room

and during respirometry temperatures did not vary more than ±0.2 °C from the intended temperature.

Statistical Analysis

RMR, MMR, factorial and net aerobic scope were analysed by factorial ANOVA with both testing temperature and rearing treatment as fixed factors. A $\log_{10}$ transformation was used to satisfy ANOVA assumptions. Fisher's LSD post-hoc tests were used to compare treatment means following ANOVA. The same procedure was used to evaluate the response in RMR, MMR and aerobic scope of offspring from grandparent pair #41 (which was found to be selcted for at +3.0 °C) compared to all other grandparents, however transgenerational +3.0 °C group was not included as all groups tested originally were from grandparent pair #41. The effect of rearing treatment on the hepato-somatic index, weight and standard length of F1 offspring was tested with two separate one-factor ANOVAs. Mann-Whitney U tests were used to compare hepato-somatic index of breeding and non-breeding fish within each rearing treatment.

Supplementary References

33. Lough, J. in *Climate Change and the Great Barrier Reef: A Vulnerability Assessment*, Johnson, J. E., Marshall, P. A., Eds. (Great Barrier Reef Marine Park Authority, Townsville, 2007) pp.15-50.
34. Meehl, G. A., *et al.,* in *Climate Change 2007: the Physical Science Basis*, Solomon, S. *et al.*, Eds (Cambridge University Press, Cambridge and New York, 2007) pp. 686–688.

35. Donelson, J. M., Munday, P. L., McCormick, M. I., Pankhurst, N. W. & Pankhurst, P. M. Effects of elevated water temperature and food availability on the reproductive performance of a coral reef fish. *Mar. Ecol. Prog. Ser.* **401,** 233-245 (2010).
36. Hilder, M. L., Pankhurst, N. W. Evidence that temperature change cues reproductive development in the spiny damselfish, *Acanthochromis polyacanthus*. *Environ. Biol. Fishes* **66,** 187-196 (2003).
37. Munday, P. L., Kingsford, M. J., O'Callaghan, M. Donelson, J. M. Elevated temperature restricts growth potential of the coral reef fish. *Acanthochromis polyacanthus*. *Coral Reefs* **27,** 927–931 (2008).
38. Kingsford, M. J., Hughes, J. M. Patterns of growth, mortality, and size of the tropical damselfish *Acanthochromis polyacanthus* across the continental shelf of the Great Barrier Reef. *Fish. Bul.* **103,** 561-573 (2005).
39. Nilsson, G. E., Östlund-Nilsson, S., Penfold, R., Grutter A. S. From record performance to hypoxia tolerance: respiratory transition in damselfish larvae settling on a coral reef. *Proc. R. Soc. London Ser. B* **274,** 79-85 (2007).