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Hypoxia in paradise: widespread hypoxia tolerance in coral reef fishes

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Using respirometry, we examined the hypoxia tolerance of 31 teleost fish species (seven families) inhabiting coral reefs at a 2–5 m depth in the lagoon at Lizard Island (Great Barrier Reef, Australia). All fishes studied maintained their rate of oxygen consumption down to relatively severe hypoxia (20–30% air saturation). Indeed, most fishes appeared unaffected by hypoxia until the oxygen level fell below 10% of air saturation. This, hitherto unrecognized, hypoxia tolerance among coral reef fishes could reflect adaptations to nocturnal hypoxia in tide pools. It may also be needed to enable fishes to reside deep within branching coral at night to avoid predation. Widespread hypoxia tolerance in a habitat with such an extreme biodiversity as coral reefs indicate that there is a wealth of hypoxia related adaptations to be discovered in reef fishes.

Keywords: predator avoidance; damselfish; cardinalfish; coral reefs; hypoxia; anoxia

1. INTRODUCTION

Coral reefs are not generally thought of as hypoxic habitats, and coral reef fishes are not known for their hypoxia tolerance. However, the data presented here show that hypoxia tolerance is a widespread phenomenon among coral reef teleosts. While examining the respiratory consequences of mouthbrooding in two species of cardinalfish (*Apogon leptacanthus* and *Apogon fragilis*) at Lizard Island, on the Northern portion of the Great Barrier Reef, we found that these fishes display a critical oxygen (O_2) concentration ($[O_2]_{crit}$) just below 20% of air saturation (G. E. Nilsson and S. Östlund-Nilsson, unpublished data). $[O_2]_{crit}$ is the O_2 level below which the fish is unable to maintain a resting rate of O_2 consumption (VO_2) that is independent of the ambient $[O_2]$ (Beamish 1964). Thus, at an $[O_2]$ below the $[O_2]_{crit}$, the fish starts relying on anaerobic metabolism.

An $[O_2]_{crit}$ below 20% was unexpectedly low for fishes living in a tropical sea habitat. First, severe hypoxia had never, to our knowledge, been reported in this habitat, and our own measurements of water $[O_2]$ where the fishes were caught showed levels near 100% of air saturation. Second, maintaining O_2 uptake in hypoxia is quite an achievement in sea water at such a high temperature (30 °C) due to the combined effects of a low solubility of O_2 in warm sea water, and the high rate of O_2 consumption of a small fish at such a high temperature. As in other animals, VO_2 of fishes increases with body temperature (also after acclimatization) and decreases with body mass (Schmidt-Nielsen 1997), which in the case of the cardinalfish was 1–2 g.

There seems to be no comparable measurements of hypoxia tolerance in tropical sea fishes. However, a comparison can be made with tropical freshwater fishes for which there are data available. Some African cichlid species, including tilapia (*Oreochromis niloticus*) are renowned for their hypoxia tolerance. These cichlids have a $[O_2]_{crit}$ of *ca.* 20% of air saturation at 25 °C—very similar to what we saw in the reef fishes. However, the cichlids are relatively large fishes adapted to tropical freshwater habitats that are known to regularly become severely hypoxic (Verheyen *et al.* 1994; Chapman *et al.* 1995). Similarly, several hypoxia-tolerant fishes inhabit the Amazon basin—an area where hypoxia is often encountered (Val *et al.* 1998).

In cardinalfish, the males carry the fertilized eggs in their mouth (Thresher 1984). Thus, our first hypothesis was that the low $[O_2]_{crit}$ measured in the cardinalfish reflected an adaptation for mouthbrooding, as it would allow the fish to strip more O_2 from the water when faced with a reduced ability to ventilate the gills. To test this hypothesis, we decided to measure $[O_2]_{crit}$ in several species of cardinalfish (all mouthbrooders) as well as in several other fish species (non-mouthbrooders) in the same habitat. The habitat was reefs that were 2–5 m deep with a high abundance of branching coral (figure 1), situated in the lagoon outside the Lizard Island Research Station (LIRS). We could dismiss our initial hypothesis as it soon became clear that hypoxia tolerance is a general phenomenon among teleosts in this habitat.

2. MATERIAL AND METHODS

All experiments were carried out in February to March and October to November 2002 at LIRS (www.lizardisland.net.au) on the Great Barrier Reef, Australia. The fishes were caught using SCUBA diving in the lagoon at LIRS, over branching coral at a depth of 2–5 m (figure 1). They were caught with a hand-net after lightly anaesthetizing them with clove oil.

The fishes were kept in shaded outdoor aquaria with a continuous supply of water pumped in directly from the ocean (28–31 °C) for at least 48 h before measurements. The water O_2 level varied between 80–105% of air saturation. They were fed daily with mysid shrimps until satiation, but were starved for 24 h before any experiments. All experiments were carried out in shaded daylight (10.00–18.00).

Closed respirometry was carried out essentially as described by Nilsson (1992, 1996). In closed respirometry, a fish is placed in a sealed container and the falling water O_2 level is recorded continuously. The respirometer was custom made out of a Perspex cylinder (inner Ø of 80 mm) with a plunger that allowed us to adjust the chamber volume (150–1200 ml) according to the size of the fish, so that each experiment took *ca.* 3–5 h. The O_2 level in the respirometer was monitored using a galvanometric O_2 electrode (OXI 340i, WTW, Germany) equipped with a small magnetic propeller driven by a magnetic stirrer placed outside the chamber. The chamber was submerged in a flow-through aquarium to maintain a stable temperature (30 ± 1 °C). The data were recorded with a Powerlab 4/20 using the program CHART v. 4.0 (both from AD Instruments). Before each experiment we let water run through the respirometer to allow the fish to acclimatize for at least 2 h. All fishes included in this study settled down rapidly and remained virtually motionless during respirometry. The experiments were terminated when $[O_2]_{crit}$ had been reached, or later, when the fish showed the first signs of agitation or a problem with maintaining equilibrium. VO_2, in mg O_2 h^{-1} kg^{-1} (fish wet weight), was plotted against water $[O_2]$, measured in per cent of air saturation. The $[O_2]_{crit}$ was determined by fitting two linear regression lines to the curve, one for the normoxic (O_2 independent) part of the curve and one for the steeply falling hypoxic part (illustrated in figure 2). The point where these lines crossed was taken as the $[O_2]_{crit}$. All fishes recovered rapidly after the experiments

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Figure 1. Branching coral at the reef near LIRS, a habitat where all fish examined were found to display a considerable hypoxia tolerance. Depth of 3 m. (Photograph: G. E. Nilsson.)

and were subsequently released at the site of capture. The experiments followed University of Queensland ethical guidelines.

$[O_2]$ is given as a percentage of air saturation which can easily be converted to O_2 partial pressure (100% = 151 mm Hg) or weight based concentration (100% = 6.0 mg O_2 l^{-1} at 30 °C in sea water).

3. RESULTS

The $[O_2]_{crit}$ of the 112 fish studied, representing 31 fish species from seven families, varied between 13% and 34% of air saturation (table 1; illustrated in figure 2). Most of the fishes did not show any signs of agitation or loss of balance until the O_2 level fell below 10% of air saturation (the $[O_2]_{out}$ value in table 1), indicating high anaerobic capacities. Some fishes (gobies and blennies) even tolerated O_2 levels below 3% of air saturation. This hypoxia tolerance was combined with high metabolic rates. Most of the fishes studied weighed less than 10 g and had a resting VO_2 in normoxia of 200–500 mg O_2 kg^{-1} h^{-1} (table 1), which is several times higher than that of fishes in cold temperate water.

4. DISCUSSION

Being hypoxia tolerant has its drawbacks. It demands a haemoglobin with a high O_2 affinity, which means that the

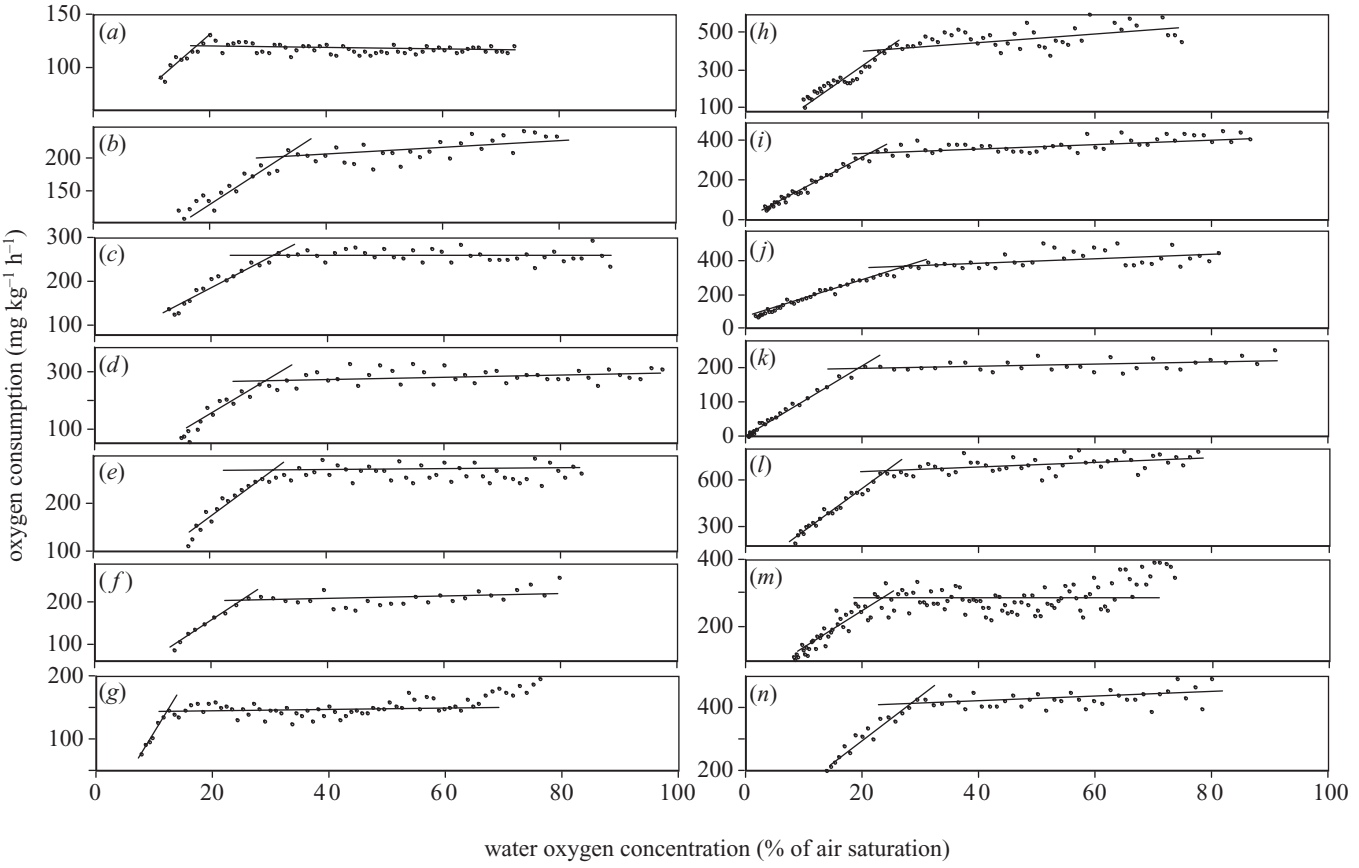

Figure 2. (*a–n*) Representative graphs showing VO_2 versus $[O_2]$ in 14 fish species from the Lizard Island Lagoon. (*a*) *Sphaeramia nematoptera*: weight 7.3 g; $[O_2]_{crit}$ 17%. (*b*) *Archamia fucata*: weight 5.8 g; $[O_2]_{crit}$ 34%. (*c*) *Cheilodipterus quinquelineatus*: weight 1.8 g; $[O_2]_{crit}$ 31%. (*d*) *Apogon compressus*: weight 5.4 g; $[O_2]_{crit}$ 27%. (*e*) *Apogon cyanosoma*: weight 2.2 g; $[O_2]_{crit}$ 30%. (*f*) *Apogon exostigma*: weight 3.7 g; $[O_2]_{crit}$ 26%. (*g*) *Apogon leptacanthus*: weight 2.4 g; $[O_2]_{crit}$ 12%. (*h*) *Paramonacanthus japonicus*: weight 1.7 g; $[O_2]_{crit}$ 23%. (*i*) *Amblygobius phalaena*: weight 2.4 g; $[O_2]_{crit}$ 21%. (*j*) *Asteropteryx semipunctatus*: weight 1.4 g; $[O_2]_{crit}$ 26%. (*k*) *Atrosalarias fuscus*: weight 5.2 g; $[O_2]_{crit}$ 19%. (*l*) *Chromis viridis*: weight 1.5 g; $[O_2]_{crit}$ 24%. (*m*) *Dascyllus aruanus*: weight 3.7 g; $[O_2]_{crit}$ 23%. (*n*) *Scolopsis bilineata*: weight 1.9 g; $[O_2]_{crit}$ 28%. The $[O_2]_{crit}$ is the intercept of the two regression lines.

Table 1. Hypoxia tolerance at 30 °C of fishes in the lagoon at LIRS, Great Barrier Reef.
(Normoxic VO_2, rate of O_2 consumption at a water $[O_2]$ of more than 70% air saturation; $[O_2]_{crit}$, critical $[O_2]$: below this level VO_2 starts falling and is no longer independent of ambient $[O_2]$; $[O_2]_{out}$, $[O_2]$ at which the fish showed signs of agitation or balance problems, at which point it was taken out of the respirometer and allowed to recover. Values for three or more fish are means ± s.d. Taxonomy follows Randall *et al.* (1997).)

family and species	*n*	weight (g)	normoxic VO_2 (mg kg^{-1} h^{-1})	$[O_2]_{crit}$ (% air saturation)	$[O_2]_{out}$ (% air saturation)
cardinalfish (Apogonidae)					
Apogon compressus	4	7.0 ± 1.2	179 ± 67	19 ± 5	6.7 ± 1.9
Apogon cyanosoma	1	2.2	259	30	
Apogon doederleini	1	4.4	288	31	
Apogon exostigma	1	3.7	218	26	11.4
Apogon fragilis	14	1.9 ± 0.1	255 ± 17	17 ± 1	7.2 ± 1.0
Apogon leptacanthus	14	1.5 ± 0.1	239 ± 19	19 ± 1	7.0 ± 1.2
Archamia fucata	1	5.8	225	34	
Cheilodipterus quinquelineatus	2	1.8–7.4	244–263	23–31	7.2–11.1
Sphaeramia nematoptera	1	7.3	131	17	10.0
damselfish (Pomacentridae)					
Acanthochromis polyacanthus	1	15.4	197	26	6.5
Chromis atripectoralis	5	8.4 ± 2.5	358 ± 84	22 ± 2	8.8 ± 0.8
Chromis viridis	6	2.5 ± 1.1	555 ± 108	23 ± 1.2	7.4 ± 0.9
Chrysiptera flavipinnis	1	2.4	384	30	12.0
Dascyllus aruanus	3	4.1 ± 1.3	306 ± 37	19 ± 0	5.9 ± 0.6
Neoglyphidodon melas	6	32.1 ± 8.8	216 ± 32	25 ± 2	5.6 ± 0.7
Neoglyphidodon nigroris	6	14.9 ± 2.4	162 ± 21	22 ± 3	8.9 ± 1.5
Neopomacentrus azysron	1	3.2	493	32	
Pomacentrus ambionensis	4	12.6 ± 1.7	201 ± 11	22 ± 4	7.1 ± 2.0
Pomacentrus bankanensis	1	7.8	237	19	
Pomacentrus coelestis	6	7.8 ± 3.0	387 ± 85	22 ± 4	9.3 ± 1.0
Pomacentrus lepidogenys	5	3.1 ± 0.6	516 ± 73	31 ± 2	12.5 ± 1.1
Pomacentrus moluccensis	4	5.2 ± 4.0	397 ± 85	25 ± 3	10.4 ± 1.9
Pomacentrus philippinus	2	2.2–6.9	320–348	26–33	9.3–10.5
gobies (Gobiidae)					
Amblygobius phalaena	1	2.4	333	21	2.8
Asteropteryx semipunctatus	1	1.4	403	26	1.4
Gobiodon histrio	10	1.2 ± 0.2	248 ± 31	18 ± 1	2.8 ± 0.5
blennies (Blennidae)					
Atrosalarias fuscus	3	7.3 ± 1.9	208 ± 34	18 ± 2	1.6 ± 07
Atrosalarias fuscus juvinile	1	0.29	552	13	1.5
filefish (Monacanthidae)					
Paramonacanthus japonicus	1	1.7	486	23	9.5
breams (Nemipteridae)					
Scolopsis bilineata juvenile	1	1.9	375	28	12.8
wrasses (Labridae)					
Halichoeres melanurus	1	1.8	394	25	6.8
Labroides dimidiatus juvenile	3	0.56 ± 0.09	736 ± 35	24 ± 5	7.8 ± 0.9

rate by which O_2 can be downloaded to the tissue is reduced. Moreover, a high ability to strip O_2 from the water demands a large respiratory surface area, which at the same time means increased ion fluxes that have to be counteracted by costly ion pumping (Schmidt-Nielsen 1997). Exposing the internal milieu to the ambient water through a large respiratory surface area will also mean increased contact with pathogens and toxic substances.

This poses the obvious question: what is the selection pressure that makes virtually all fishes in this coral reef habitat display a $[O_2]_{crit}$ that is much lower than the O_2 levels that they, at a first glance, can be expected to encounter? We would like to suggest two possible reasons for this. The first is related to the fact that the same species may also occur on more shallow reefs that periodically become cut off from the surrounding ocean during low tides. When this happens at night, the respiration of the coral and associated organisms can make the enclosed water hypoxic. At Heron Island, further south on the Great Barrier Reef, water O_2 has been found to fall to 30% of air saturation on the reef platform when this becomes cut off from the ocean during nocturnal low tides (Kinsey & Kinsey 1966). The Heron Island reef platform is inhabited by the epaulette shark (*Hemiscyllium ocellatum*), which is the only coral reef vertebrate that has previously been found to be hypoxia tolerant (Routley *et al.* 2002). From temperate regions, there are several examples of hypoxia-tolerant fishes inhabiting tide pools (Martin 1995). Shallow parts of the reef around Lizard Island can get partially air exposed, during exceptionally low tides, with the resultant formation of tide pools. We have hitherto not had the opportunity to measure O_2 levels in such tide pools at night, or examine the composition of the fish fauna that may remain there.

A second possible explanation for the hypoxia tolerance displayed by at least some of the species examined at Lizard Island is that they move deep into the branching coral to feed or hide from predators. If they do this at night, as many night divers reportedly have seen, they may enter into a microhabitat that becomes hypoxic due to coral respiration. Coral tissue is known to become hypoxic at night (Jones & Hoegh-Guldberg 2001). In coral taken into the laboratory at Lizard Island, we have found that water [O_2] between coral branches may fall below 20% of air saturation at night (G. E. Nilsson and S. Östlund Nilsson, unpublished data).

Lizard Island is far from any major human settlements (270 km north of Cairns) so hypoxia related to pollution can be excluded.

Coral reefs have the highest biodiversity of any marine habitat. If hypoxia and hypoxia tolerance is common in this habitat, as suggested by the present results, this could mean that there is an exceptional wealth of hypoxia adaptations waiting to be explored in this ecosystem. So far, most hypoxia-tolerant vertebrates where physiological adaptations have been studied in detail, which include North American freshwater turtles, carps and goldfish (Lutz *et al.* 2003), are animals that have evolved their hypoxia tolerance to allow overwintering in hypoxic habitats at temperatures close to 0 °C. The water temperature of coral reefs (*ca.* 30 °C), is not far from the body temperature of homeothermic vertebrates such as mammals. Therefore, studying the mechanisms that coral reef fishes have evolved to allow hypoxic survival could be of particular relevance for biomedical hypoxia and ischemia research. Of course, such studies are also needed for a more complete understanding of the coral reef ecosystem and its inhabitants.

Hypoxia and hypoxia tolerance have been well documented in temperate aquatic waters and in tropical freshwater habitats. For reasons that are not immediately obvious, very few scientists appear to have set out to study hypoxia in the paradisiacal setting of a coral reef. The present study is the first, to our knowledge, to suggest that hypoxia tolerance is widespread, maybe even ubiquitous, among teleost fishes intimately associated with coral. Indirectly, this indicates that hypoxia in coral reef habitats is a much more common phenomena than generally thought.

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