

Hypoxia tolerance and air-breathing ability correlate with habitat preference in coral-dwelling fishes

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Received: 20 July 2006 / Accepted: 19 October 2006 / Published online: 22 February 2007
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Abstract Hypoxia tolerance and air-breathing occur in a range of freshwater, estuarine and intertidal fishes. Here it is shown for the first time that coral reef fishes from the genera *Gobiodon*, *Paragobiodon* and *Caracanthus*, which all have an obligate association with living coral, also exhibit hypoxia tolerance and a well-developed air-breathing capacity. All nine species maintained adequate respiration in water at oxygen concentrations down to 15–25% air saturation. This hypoxia tolerance is probably needed when the oxygen levels in the coral habitat drops sharply at night. Air-breathing abilities of the species correlated with habitat association, being greatest (equaling oxygen uptake in water) in species that occupy corals extending into shallow water, where they may become air exposed during extreme low tides. Air-breathing was less well-developed or absent in species inhabiting corals from deeper waters. Loss of scales and a network of subcutaneous capillaries appear to be key adaptations allowing cutaneous respiration in air. While hypoxia tolerance may be an ancestral trait in these fishes, air-breathing is

likely to be a more recent adaptation exemplifying convergent evolution in the unrelated genera *Gobiodon* and *Caracanthus* in response to coral-dwelling lifestyles.

Keywords Coral reef · Hypoxia · Air-breathing · *Caracanthus* · *Gobiodon* · *Paragobiodon*

Introduction

Aquatic animals living in low oxygen environments, or that inhabit boundary regions between terrestrial and aquatic existence, often exhibit particular adaptations that enable them to sustain their metabolic functions. For example, freshwater fishes that live in rivers or lakes that experience extreme fluctuations in dissolved oxygen concentrations often show a considerable tolerance to hypoxia (e.g., Val et al. 1998), and some species even have air-breathing capabilities (Martin 1995; Graham 1997; Helfman et al. 1997). In contrast, most marine environments appear to provide their inhabitants with high and less variable oxygen levels, but this does not apply to tropical estuaries and tidal areas, where mudskippers and sculpins that live in the boundary between water and air have evolved mechanisms for gas exchange across their skin or the buccal chamber (Martin 1995, 1996). Coral reefs are not usually considered to be oxygen limited environments. However, it has recently been shown that coral reef fish (teleosts and at least one elasmobranch) can tolerate strikingly low oxygen concentrations (Nilsson and Östlund-Nilsson 2004; Nilsson and Renshaw 2004). In general, reef teleosts are able to maintain a steady rate of oxygen uptake down to levels of about 15–30% of

Communicated by Biology Editor M.I. McCormick.

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air saturation, and can survive at even lower oxygen levels for several hours (Nilsson and Östlund-Nilsson 2004, 2006). This ability to cope with hypoxia is thought to be an adaptation to nocturnal hypoxia experienced within the reef matrix, where many reef fishes spend the night sheltering from predators.

On coral reefs a particularly high degree of hypoxia tolerance has been observed in two species of coral-dwelling goby, *Gobiodon histrio* and *G. erythrospilus*, both of which have an obligate association with living corals (Nilsson et al. 2004). This capacity to tolerate very low oxygen levels is consistent with measurements showing dramatic nocturnal falls in the water oxygen levels among the branches of the coral colonies that these fish inhabit (Nilsson et al. 2004). These two gobies were also found to have the ability to breathe air, which is consistent with field observations that some of the coral colonies they inhabit become air exposed for up to 4 h during extreme low tides. These results suggest that the oxygen environment experienced in different reef habitats plays a major role in shaping the respiratory functions of coral reef fishes.

Gobies of the genera *Gobiodon* and *Paragobiodon*, as well as the unrelated but ecologically similar velvetfishes of the genus *Caracanthus*, are all obligate coral dwellers that spend most of their lives among the branches of coral colonies (Randall et al. 1997). However, they vary in their preference for coral species, water depth and reluctance to leave their host coral. In particular, some species inhabit coral colonies that may become air exposed for several hours at low tide, whereas others live in coral colonies that never become air exposed. This variation in habitat use provides an ideal opportunity to examine the relationship between habitat choice and respiratory performance in reef fishes. Comparing respiratory characteristics between closely related species from different habitats, and between distantly related species that use similar habitats, will help establish if hypoxia tolerance and air-breathing capacities are ancestral traits or are more recent adaptations to the niches that these fishes occupy.

Closed respirometry was used to compare hypoxia tolerance and air-breathing capacity among seven species of *Gobiodon* (Gobiidae), *Paragobiodon xanthosomus* (Gobiidae), and *Caracanthus unipinna* (Scorpaenidae) at Lizard Island on the Great Barrier Reef, Australia. Detailed analysis of habitat use of these species (e.g., Lassig 1976; Munday et al. 1997; Wong et al. 2005) combined with many years of field observations by one of the authors (Munday) allowed us to categorize each species with regard to habitat choice (coral preference and depth range), as outlined in Table 1. *C. unipinna* and four of the *Gobiodon* species

(*G. axillaris*, *G. erythrospilus*, *G. histrio* and *G. unicolor*) inhabit coral species with distributions that extend into very shallow water, where they may become air exposed at low tide. In contrast, two of the *Gobiodon* species (*G. acicularis* and *G. ceramensis*) and *P. xanthosomus* occupy coral species that mostly occur below the lowest water level. Some colonies of *Seriatorpora hystrix*, which is inhabited by both *G. ceramensis* and *P. xanthosomus*, occur in very shallow water; however, these shallow water colonies usually exhibit extensive partial mortality caused by exposure during low tides and appear to be marginal habitats for coral-dwelling fishes. Natural social groups of *G. ceramensis* and *P. xanthosomus* mostly occupy the larger, undamaged colonies of *S. hystrix* below the lowest water levels (P. L. Munday and M. Wong, personal observation). Finally, *G. okinawae* differs from all the other species by often moving between coral colonies and spending most of its time on top of coral colonies rather than deep among the coral branches. The prediction was made that all species would be hypoxia tolerant, because all are obligate coral dwellers and, thus, likely to be exposed to low oxygen levels among the branches of their host corals at night. In contrast, it was predicted that air-breathing capacity would differ among the species, depending on their depth distributions, or their propensity to move from their host coral colony. Species of fish inhabiting corals with distributions extending into shallow water where they will become air exposed at low tide are more likely to exhibit air-breathing capacity than species of fish inhabiting corals that rarely become air exposed, or species of fish that can move from their host coral to avoid becoming air exposed.

Materials and methods

Study species

The nomenclature for *Gobiodon* follows Munday et al. (1999), with the addition that *G. erythrospilus* and *G. histrio* are now confirmed to be separate species (Munday et al. 2004). *P. xanthosomus* and *C. unipinna* are shown in Randall et al. (1997) and the phylogenetic classification of the genus *Caracanthus* is reviewed in Shinohara and Imamura (2005). Fish were collected from the reef at Lizard Island on the Great Barrier Reef (14°40'S 145°28'E) by temporarily anaesthetising them with a dilute solution of clove oil. Anaesthetized fish were carefully removed from their home coral, placed in plastic bags full of fresh seawater and transported to the laboratory where they were transferred to shaded out-door aquaria with a continuous supply of

Table 1 Habitat preference and air-breathing capacity of coral-dwelling fishes at Lizard Island, Great Barrier Reef

Family/species	Preferred corals	Relative risk of air exposure	Mobility	Air-breathing capacity
Gobiidae				
<i>Gobiodon axillaris</i>	<i>Acropora</i> spp.	High	Rarely leaves coral	Good
<i>G. erythrosphilus</i>	<i>Acropora</i> spp.	High	Rarely leaves coral	Good
<i>G. histrio</i>	<i>Acropora</i> spp.	High	Rarely leaves coral	Good
<i>G. unicolor</i>	<i>Acropora</i> spp.	High	Rarely leaves coral	Good
<i>G. acicularis</i>	<i>Echinopora horrida</i> <i>E. mammiformis</i> <i>Hydnophora rigida</i>	Low	Rarely leaves coral	Poor
<i>G. ceramensis</i>	<i>Stylophora pistillata</i> <i>Seriatopora hystrix</i>	Low	Rarely leaves coral	Poor
<i>G. okinawae</i>	Various Acroporids	High, but may leave	Often leaves coral	Poor
Data on air-breathing capacity is from the present study. Other information is from Munday et al. (1997, 1999) or from unpublished field observations by the authors	<i>Paragobiodon xanthosomus</i>	Low	Rarely leaves coral	None
Scorpaenidae				
<i>Caracanthus unipinna</i>	<i>Acropora</i> spp. <i>Pocillopora</i> spp.	High	Rarely leaves coral	Good

water pumped directly from the ocean (27–29°C). Water O₂ level varied between 86–101% of air saturation. Fish were acclimatised to laboratory condition for at least 3 days before use. They were fed daily with mysid shrimps ad libitum, but were fasted for 24 h before use in experiments, which were conducted outdoors in shaded daylight (0900–1800 hours).

Fish respirometry

Closed respirometry was used to determine hypoxia tolerance and air-breathing abilities as described in Nilsson (1996) and Nilsson et al. (2004). In short, the test animal was placed in a sealed container and the rate at which the water O₂ level declines was measured continuously using an O₂ electrode (a microprocessor controlled Oximeter 340i from WTW in Weilheim, Germany) and recorded with a Powerlab 4/20 (AD Instruments in Bella Vista, Australia) connected to a Dell laptop computer, using the program Chart 5.0 (AD Instruments). Oxygen concentrations are given as percentage of air saturation. Hundred percent equals a pO₂ of 20.1 kPa (151 mmHg), or 6.0 mg O₂ l⁻¹ under the present conditions.

Respirometry in water

The closed respirometer used for water breathing was custom-made out of a Perspex cylinder (inner Ø=80 mm) with a variable volume of 150–250 ml, as previously described (Nilsson et al. 2004). The cylinder was submerged in a flow-through aquarium to maintain it at ocean temperature (27–29°C). To ensure circulation in

the chamber and over the electrode, a small magnetic propeller was attached to the tip of the O₂ electrode. The propeller was driven by a magnetic stirrer placed outside the aquarium. Being sedentary species, all fish quickly settled to the bottom of the chamber and remained virtually motionless during respirometry. Each experiment took approximately 6–9 h, and was terminated when the fish began to show signs of agitation or difficulty maintaining equilibrium. The oxygen concentration in the water at this point was recorded, and denoted [O₂]_{out}. All fish recovered within a few minutes of being transferred to aerated water.

The O₂ consumption (MO₂), in mg O₂ h⁻¹ kg⁻¹ fish wet weight, was plotted against water O₂ concentration ([O₂]), given as percentage of air saturation. The critical O₂ concentration ([O₂]_{crit}) is the concentration below which the fish is unable to maintain a resting MO₂ that is independent of the ambient [O₂] (Prosser and Brown 1961). This was determined by fitting two linear regression lines to the curve, one for the normoxic, O₂ independent, part of the curve, and one for the steeply falling hypoxic part. The point where these lines crossed was taken as the [O₂]_{crit}. The mean MO₂ between 70 and 100% of air saturation was considered to be the normoxic MO₂. The microbial background respiration was measured daily and was negligible provided the respirometer walls were kept clean.

Respirometry in air

For measuring MO₂ in air, the same set up was used, but the tip of the O₂ electrode (without propeller) was put into a 28 ml air filled chamber (submerged in the

aquarium). The chamber was a flat bottom glass tube with an inner diameter of 23 mm and a tissue paper wetted with 300 μ l of water was placed in the bottom of tube to provide a humid environment. The partial pressure of O₂ in the chamber never fell by more than 10% (from 151 to 136 mmHg) during the measurements.

Route of oxygen uptake in air

Species that exhibited an ability to breathe air were examined closely to identify the route of oxygen uptake. In the previous study on *G. histrio* and *G. erythrospilus* (Nilsson et al. 2004), it was observed that when provided with a water droplet, the fish often pointed its mouth into the droplet and showed continuous ventilatory movements of the opercula, indicating that it may be circulating water through the mouth and over the gills. This suggested to us that oxygen uptake may occur through recirculation of a water droplet over the gills. Alternatively, oxygen uptake may occur across the skin in a humid environment, as has been found in some fishes (Graham 1997). To examine the route of oxygen uptake in air the species that showed the best air-breathing capacities were allowed to respire in either (1) a glass chamber provided with a droplet (300 μ l) of seawater or (2) in the same glass chamber provided with a wetted paper (300 μ l water) at the bottom of the chamber. Thus, in the latter situation the fish was without access to liquid water.

The skin surface of all species was visually inspected using a stereo dissecting microscope (Olympus SZX9). In addition, some individuals of *G. erythrospilus* and *G. histrio* were sacrificed and dissected to determine if

there were any vascularized areas in the buccal cavity or a vascularized swimbladder that could aid in air-breathing.

Statistics

Differences in respiratory capacity among species was examined using one-way ANOVA followed by Tukey–Kramer multiple comparisons test (InStat 2.01 for Macintosh). Student's *t* test was used for simple pairwise comparisons. All reported values are means \pm SD.

Results

Respiration in water

From the respirometric measurements in water, three variables were determined: normoxic MO₂, [O₂]_{crit} (below which the resting rate of O₂ uptake could no longer be maintained) and [O₂]_{out} (the O₂ level where the fish became agitated or lost equilibrium) (Table 2). All species exhibited a very high level of hypoxia tolerance, with [O₂]_{crit} values varying between 15 and 25% of air saturation. *G. ceramensis* had the lowest [O₂]_{crit} (14.7% of air saturation), although it was only significantly lower than the [O₂]_{crit} of the two species with the highest values (*G. unicolor* and *P. xanthosomus*). Two species (*G. ceramensis* and *G. unicolor*) appeared to have particularly well-developed anaerobic capacities, as indicated by [O₂]_{out} values below 1% of air saturation, which were significantly lower than the values of the other species ([O₂]_{out} between 2.4 and 5.7%).

Table 2 Respiratory characteristics in water (Means \pm SD)

Family/species	Number	Weight (mg)	MO ₂ (mg kg ⁻¹ h ⁻¹)	[O ₂] _{crit} (% of air saturation)	[O ₂] _{out} (% of air saturation)
Gobididae					
<i>G. axillaris</i>	6	938 \pm 247	250 \pm 26	18.9 \pm 1.5	3.3 \pm 0.5
<i>G. erythrospilus</i>	11	565 \pm 103	260 \pm 58	17.5 \pm 3.2	2.9 \pm 1.1
<i>G. histrio</i>	5	533 \pm 82	263 \pm 83	19.3 \pm 6.5	3.0 \pm 1.4
<i>G. unicolor</i>	7	1713 \pm 712	302 \pm 58	25.0 \pm 5.8	0.9 \pm 0.7 ^a
<i>G. acicularis</i>	6	824 \pm 239	188 \pm 28	19.6 \pm 3.2	2.8 \pm 0.9
<i>G. ceramensis</i>	6	902 \pm 274	230 \pm 35	14.7 \pm 2.1 ^b	0.4 \pm 0.5 ^c
<i>G. okinawae</i>	11	718 \pm 323	298 \pm 72	21.8 \pm 8.2	5.3 \pm 1.6 ^d
<i>P. xanthosomus</i>	6	689 \pm 181	266 \pm 42	24.3 \pm 5.2	5.7 \pm 1.3 ^e
Scorpaenidae					
<i>C. unipinna</i>	5	1789 \pm 902	238 \pm 58	22.5 \pm 2.6	2.4 \pm 0.6

^a Significantly lower than all others except *G. ceramensis*

^b Significantly lower than *G. unicolor* and *Paragobiodon*

^c Significantly lower than all others except *G. unicolor*

^d Significantly higher than all others except *Paragobiodon*

^e Significantly higher than all others except *G. okinawae*

Respiration in air

Excellent air-breathing ability was initially shown by all the *Gobiodon* species and by *Caracanthus* (Fig. 1a, b). However, there were clear differences in the ability of different species to maintain air-breathing over time. *G. axillaris*, *G. erythrospilus*, *G. histrio* and *G. unicolor* maintained a relatively steady rate of MO_2 in air for 4 h (the duration of the experiment, Fig. 1a), although in *G. erythrospilus* and *G. unicolor*, air- MO_2 fell below the resting MO_2 in water during the last 3 h ($P < 0.05$). By contrast, *G. acicularis*, *G. ceramensis* and *G. okinawae* could only breathe air for about an hour, and all had to be removed from the air chamber during the second hour as their MO_2 plummeted (Fig. 1b). *C. unipinna* exhibited an air-breathing ability similar to the best *Gobiodon* air-breathers, maintaining a MO_2 in air that did not fall significantly below its resting MO_2 in water during the 4 h long experiment (Fig. 1a). *P. xanthosomus*, on the other hand, showed no detectable ability to remove oxygen from the air in the respirometer (Fig. 1b).

Route of oxygen uptake in air

Contrary to the initial expectations, the four best air-breathing *Gobiodon* species (*G. axillaris*, *G. erythrospilus*, *G. histrio*, *G. unicolor*) all exhibited a significantly higher rate of oxygen uptake in the air chamber when they had no access to liquid water (Fig. 2; $P < 0.01$ in all four cases). Indeed, the presence of a water droplet in the chamber reduced the rate of oxygen uptake by nearly 50%, whereas in humid air all four species achieved rates of oxygen uptake that were not significantly different from their resting oxygen uptake in water (Fig. 2). Microscopic examination of the skin revealed that *Gobiodon* species and *Caracanthus* have a scale-less skin with a network of subcutaneous capillaries (Fig. 3a, b) that are likely to participate in cutaneous respiration. Honeycomb shaped toxin glands (Munday et al. 2003; Schubert et al. 2003) were also readily observed in the skin of *Gobiodon* species. By contrast, *P. xanthosomus*, which lacked any ability for oxygen uptake in air has a skin that is fully covered with scales (Fig. 3c). Dissection of *G. erythrospilus* and *G. histrio* did not reveal any highly vascularized areas in the buccal cavity or a vascularized swimbladder that could constitute major routes for oxygen uptake.

Discussion

All the coral-dwelling fishes studied here showed a high level of hypoxia tolerance, even better than the

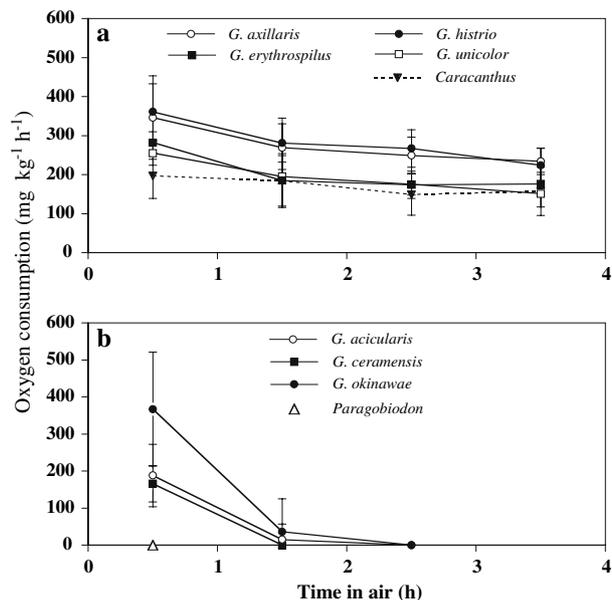


Fig. 1 Oxygen consumption in a humid air chamber (without liquid water) for **a** species with distributions extending into shallow water where they occupy corals that become air exposed (*Gobiodon axillaris*, *G. erythrospilus*, *G. histrio*, *G. unicolor*, *Caracanthus unipinna*) and **b** species with distributions that do not extend into shallow water where they may become air exposed (*G. acicularis*, *G. ceramensis*, *Paragobiodon xanthosomus*) or are mobile (*G. okinawae*). Values shown are means \pm SD over 1 h period from 5–7 individuals

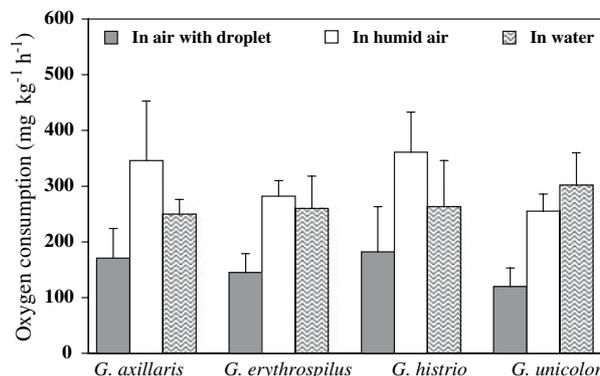


Fig. 2 Oxygen consumption in air (with and without a droplet of water) and in normoxic water in the four species of *Gobiodon* (*G. axillaris*, *G. erythrospilus*, *G. histrio*, *G. unicolor*) exhibiting the best air-breathing capabilities

considerable levels displayed by a range of other coral reef fishes. Twenty seven species of coral reef fish belonging to the families Apogonidae, Labridae, Monacanthidae, Nemipteridae, and Pomacentridae showed an average $[\text{O}_2]_{\text{crit}}$ of $24.6 \pm 4.7\%$ and an average $[\text{O}_2]_{\text{out}}$ of $8.7 \pm 2.1\%$ (Nilsson and Östlund-Nilsson 2004). The same measures for the nine species of coral dwellers considered here were $20.4 \pm 3.3\%$ for $[\text{O}_2]_{\text{crit}}$ and $3.0 \pm 1.7\%$ for $[\text{O}_2]_{\text{out}}$ (Student's *t* test: $P < 0.02$ and $P < 0.0001$, respectively). These differences suggest

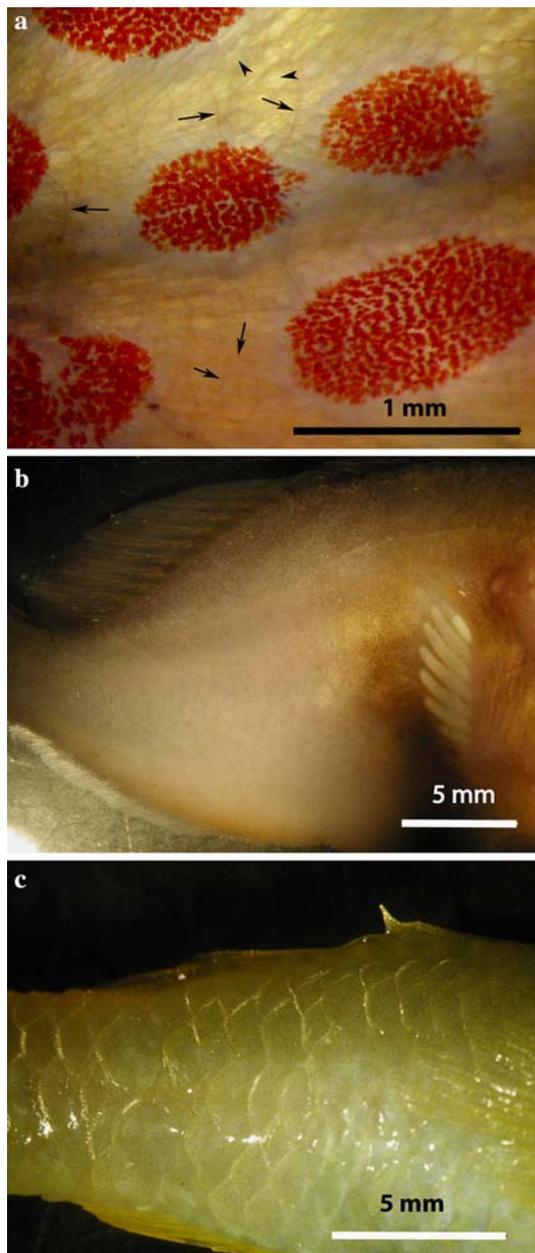


Fig. 3 Micrographs of the skin on the mid lateral side of the body of **a** *G. erythrospilus*, **b** *C. unipinna* and **c** *P. xanthosomus*. In *G. erythrospilus*, a network of capillaries in the skin (shown by arrows) overlay the honeycombed network of toxin glands (arrow heads) and are best visible in areas void of red pigmentation. Note that both *G. erythrospilus* and *C. unipinna* lack scales, whereas the skin of *P. xanthosomus* is covered in scales. Micrographs taken with an Olympus Camedia 4040 digital camera connected to an Olympus SZX9 stereomicroscope

that an obligate association with living coral demands a particularly well-developed tolerance of hypoxia. However, there is also a phylogenetic component to these differences, because previous studies and additional measurements made in connection with the present study indicate that other reef associated gobies (Gobiidae),

which are not obligate coral dwellers, also have relatively low $[O_2]_{crit}$ and $[O_2]_{out}$ (Table 3). A similar pattern applies to other scorpion fishes (Scorpaenidae) (Table 3). This suggests that a physiological heritage has allowed a high degree of hypoxia tolerance to evolve and may have assisted both gobiids and scorpaenids to acquire a coral-dwelling life style.

Tolerance to hypoxia and air exposure in obligate coral-dwelling fishes appear to be important adaptations to their highly specialized life style. As shown by Nilsson et al. (2004), oxygen levels in coral colonies can potentially become very low at night (<20% of air saturation). Hypoxia tolerance should benefit coral-dwelling fishes because it would enable them to remain within the shelter of their host coral at night, even if oxygen saturation drops sharply. In addition, some coral colonies in shallow areas become air exposed for several hours during the lowest tides. Among the coral-dwelling species examined here, those with the best air-breathing capacities (*G. axillaris*, *G. erythrospilus*, *G. histrio*, *G. unicolor*, and *Caracanthus*) inhabit coral species whose distributions extend into very shallow water, where prolonged air exposure during low tides could occur. Indeed, these species of fish can be observed nestled among the branches of their host corals when they are fully exposed to the atmosphere during the lowest tides of the year at Lizard Island.

Only a relatively small fraction of the coral colonies inhabited by the good air-breathers (*G. axillaris*, *G. erythrospilus*, *G. histrio*, *G. unicolor*, and *Caracanthus*) are likely to become air exposed at low tide. These are the colonies at the top end of each coral species depth distribution. Nevertheless, air-breathing capacity might still be expected to evolve in the species of fish that inhabit these corals because individual fish probably have little choice about whether they occupy a coral colony in very shallow water, or one in deeper water. Coral colonies are a limited resource for coral-dwelling fishes and there is intense competition for vacant space (Munday et al. 2001; Hobbs and Munday 2004; Munday 2004). The ability to breathe air would be highly advantageous because an individual might have no other option but to occupy a coral in shallow water, where it is likely to be air exposed at some stage of its life. On Lizard Island, coral colonies are only air exposed during the lowest tides, which occur about 30 times a year, and then only for a few hours each time. Apparently, even these relatively rare occurrences provide strong selection for air-breathing abilities. In general, other examples of air-breathers among fishes appear to involve species that are exposed to air much more frequently or for much longer periods (Graham 1997).

Table 3 Respiratory characteristics of some non-coral dwelling gobiids and scorpaenids (Means \pm SD)

Family/species	Number	Weight (mg)	MO ₂ (mg kg ⁻¹ h ⁻¹)	[O ₂] _{crit} (% of air saturation)	[O ₂] _{out} (% of air saturation)
Gobiidae					
<i>Asteropteryx semipunctata</i> ^a	1	1400	403	26	1.4
<i>Amblyogobius phalaena</i> ^a	1	2400	333	21	2.8
<i>Koumansetta rainfordi</i>	5	651 \pm 606	637 \pm 413	20.9 \pm 4.8	7.3 \pm 2.1
Scorpaenidae					
<i>Sebastapistes cyanostigma</i>	1	1442	228	26.6	2.9
<i>Parascorpaena mossambica</i>	1	319	193	17.5	3.0

^a Data from Nilsson and Östlund-Nilsson (2004)

In contrast to the good air-breathers, *G. acicularis*, *G. ceramensis* and *G. okinawae* had limited ability to breathe air and *P. xanthosomus* had no air-breathing capacity. These species either occupy coral colonies in deeper water where air exposure is unlikely to occur or will be of short duration (*G. acicularis*, *G. ceramensis* and *P. xanthosomus*) or are less tightly bound to their coral home (*G. okinawae*) and could temporarily move away from an air exposed coral colony. The absence of good air-breathing capacity in these two different genera indicates that either: (1) air-breathing is a derived condition in species of *Gobiodon* and has not been selected for among species that are unlikely to become air exposed, or (2) air-breathing evolved early in the diversification of *Gobiodon* species, but is a costly trait that has been selected against in species where it is no longer needed as a result of shifts in habitat use, or the evolution of more mobile behaviour. Phylogenetic analyses will be useful in distinguishing between the alternatives.

The results indicate that the main route for O₂ uptake in air was through the skin. Thus, contrary to the initial expectations, the presence of a small amount of liquid water did not increase the air-breathing capacity of any *Gobiodon* species. In fact, it reduced the rate of oxygen uptake by nearly 50%. Observing the animal in the chamber provided a plausible explanation for this result. In general, the fish pointed its nose into the water droplet and rested one side of its body against the glass wall. Capillary forces caused water from the droplet to fill the space between the body and the glass. This effectively blocked oxygen exchange between air in the chamber and that half of the body surface facing the glass. From this observation it can be concluded that *Gobiodon* species take up oxygen in air through cutaneous respiration across most of their body surface, and that circulating a droplet of water over the gills is not a major route of oxygen uptake during air exposure. This conclusion was confirmed by the observation that *G. unicolor* maintained oxygen uptake in air, even though it often stopped its ventilatory movements.

Many fishes, including gobies, have previously been shown to utilize cutaneous respiration in water and air (Graham 1976, 1997; Liem 1981; Martin 1995). It is likely that having a scale-less and well-vascularized skin, as observed in the *Gobiodon* species and *Caracanthus*, is a prerequisite for effective cutaneous respiration. This was emphasized by the inability of *Paragobiodon*, which possess a scaled skin, to breathe air. However, other routes of oxygen uptake in air that could explain the present results cannot be excluded. For example, the presence of water drawn into the oral cavity could stop the fish from using air in the buccal cavity for oxygen uptake. The Australian desert goby *Chlamydogobius eremius* uses a buccal air bubble for respiration and reduces its opercular movements (Thompson and Withers 2002). Also, the regulation of blood flow partitioning between the gills and skin could be altered when a drop of water is present over the gills (Piiper 1982).

The results suggested that a scale-less body per se does not result in good cutaneous air-breathing abilities since the more deep living *Gobiodon* species were unable to maintain air-breathing for an extended period. This result is paralleled by a study on some relatively scale-less species of sculpins (Cottidae) on the US West coast, showing that the ability to breathe air for an extended period correlated with the depth of the habitat, i.e., the likeliness of being air exposed (Martin 1996). Thus, in addition to being scale-less, the fish probably need additional adaptations to become a good cutaneous air-breather.

A scale-less and highly vascularized skin most likely has certain disadvantages. For example, it might leave the fish vulnerable to cutaneous parasites. *Gobiodon* species possess numerous skin glands that excrete potent toxins. Although it has been suggested that these toxins function as a predation deterrent (Schubert et al. 2003), they may also serve to deter parasites from their otherwise unprotected and highly vascularised skin (Munday et al. 2003). The absence of scales

provides opportunities for both the effective deployment of toxin glands and the capacity to breathe air. It seems likely that the loss of scales and the acquisition of toxin glands and air-breathing capacity might have evolved in concert with each other.

To conclude, all the obligate coral-dwelling fishes examined here showed a high degree of hypoxia tolerance, being able to maintain a steady resting rate of oxygen consumption down to an oxygen level of about 20% of air saturation, and tolerating oxygen levels as low as 3% or less. This would enable them to stay in their coral shelters at night, when this microhabitat can potentially become severely hypoxic. In addition, *Caracanthus* and some *Gobiodon* species were found to be excellent air-breathers, a trait that allows them to survive hours of air-exposure during low tides. Air-breathing in fish has previously only been reported from freshwater, coastal mud flats, rocky shores and estuarine habitats (Graham 1997), and these are the first known cases of air-breathing in fish intimately associated with living coral. However, the air-breathing capacities among species showed striking differences that correlated with their choice of coral habitat. Those fishes that live in coral colonies that may become air exposed were found to be excellent air-breathers, using their skin to take up oxygen at the same rate in air as in water for several hours. In contrast, the species that prefer corals usually occurring in deeper water were either unable to breathe air or were only capable of air-breathing for a short period of time. The unrelated genera *Gobiodon* and *Caracanthus* have apparently evolved a similar capacity for air-breathing by acquiring a scale-less skin. It can be concluded that air-breathing has evolved at least twice in coral-dwelling fishes, probably relatively recently. The excellent hypoxia tolerance of coral-dwelling gobies and velvetfishes, on the other hand, may largely depend on ancestral traits, as indicated by the occurrence of a similar hypoxia tolerance in other gobiids and scorpaenids.

Acknowledgments We thank the personnel of Lizard Island Research Station for assistance. The study was financed by the Research Council of Norway and the Australian Research Council.

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