Ockham's razor gone blunt: coenzyme Q adaptation and redox balance in tropical reef fishes

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The ubiquitous coenzyme Q (CoQ) is a powerful antioxidant defence against cellular oxidative damage. In fishes, differences in the isoprenoid length of CoQ and its associated antioxidant efficacy have been proposed as an adaptation to different thermal environments. Here, we examine this broad contention by a comparison of the CoQ composition and its redox status in a range of coral reef fishes. Contrary to expectations, most species possessed CoQ₈ and their hepatic redox balance was mostly found in the reduced form. These elevated concentrations of the ubiquinol antioxidant are indicative of a high level of protection required against oxidative stress. We propose that, in contrast to the current paradigm, CoQ variation in coral reef fishes is not a generalized adaptation to thermal conditions, but reflects species-specific ecological habits and physiological constraints associated with oxygen demand.

Keywords: coenzyme Q; fishes; redox balance; temperature

1. INTRODUCTION

In the course of evolution, aerobic organisms had to develop sophisticated antioxidant defence systems to counteract the side effects of life in the presence of oxygen. Indeed, highly damaging reactive oxygen species (ROS) are continuously generated during normal metabolism and, unless removed, cause a state of unbalanced tissue oxidation (i.e. oxidative stress), leading to DNA damage and physiological dysfunction (Finkel & Holbrook 2000). Generally, antioxidant defences are very efficient in controlling ROS production and eliminating these unwanted reactants from different cellular compartments. Nevertheless, severe environmental conditions or exposure to pollutants can accelerate ROS production and concomitantly exhaust or suppress the antioxidant defences of an organism, with serious consequences for its physiological state and survival (Cheung *et al.* 2001; Abele & Puntarulo 2004).

Coenzyme Q (CoQ), also known as ubiquinone because of its ubiquitous presence in all aerobic organisms, is recognized as a front-line antioxidant defence against ROS production (Bentinger *et al.* 2007), and its redox status is often a useful marker of oxidative stress (Yamamoto & Yamashita 1997, 2001; Yamamoto 2005). CoQ exists as a metabolic ubiquinol/ubiquinone redox couple, and its reduced form (ubiquinol [CoQH₂]) is a powerful lipid-phase antioxidant that not only prevents lipid peroxidation in biological membranes and lipoproteins, but also regenerates other important antioxidants such as vitamin E (Navas *et al.* 2007). All CoQ molecules are composed of a quinone ring attached to an isoprenyl side chain and the length of this side chain determines the homologous form of the molecule (1–13 units). While the predominant form in humans and many vertebrate species examined is CoQ₁₀, the homologous CoQ composed of nine (instead of 10) isoprenoid units in the side chain is also common, and is found primarily in rodents (Battino *et al.* 1990) and Antarctic fishes (Giardina *et al.* 1997). Differences in the length of the side chains have been shown to reflect the antioxidant efficacy of CoQ against oxidative damage, and ubiquinols with a short isoprenoid chain have been shown to be more potent inhibitors of lipid peroxidation than longer chain ones (Kagan *et al.* 1990; Niki 2001). Indeed, Giardina *et al.* (1997) found that CoQ₁₀ commonly observed in temperate fishes was replaced by CoQ₉ in Antarctic fishes, which tend to be more vulnerable than their temperate counterparts to the elevated rates of ROS formation, and hence may require a more efficient control of cellular oxidation. Given the difference in crystallization temperature between CoQ₁₀ and CoQ₉ (Giardina *et al.* 1997), this finding was suggested to represent an adaptation of fishes to ensure the adequate functioning of this antioxidant defence system within cellular membranes across different thermal environments.

Despite the appealing ecological and evolutionary implications of this theory, its validity is questionable since the contention was based solely on the comparison of two Antarctic and one temperate fish species. If the length of the side chain relates to the thermal CoQ adaptation in the redox metabolism of fishes as suggested, then tropical species may be expected to have CoQ with 10 or more isoprenoid units. To examine the hypothesis that variation in CoQ homologous forms between fish species is linked to physiological adaptations to temperature, we assessed the isoprenoid length of CoQ homologous forms in tropical reef fishes and also its redox status as a possible marker of oxidative stress. Since there is a general trend for decreased amounts of unsaturated fatty acids, and hence relatively lower susceptibility to lipid peroxidation in cellular membranes (Viarengo *et al.* 1998) at higher growth temperatures (Hazel 1995), we also hypothesized that coral reef fishes would be less susceptible than their polar and temperate counterparts to oxidative stress. As the aerobic capacity of fishes varies greatly with lifestyle, we further expected to find differences in CoQ redox status among families in relation to their ecological habits.

2. MATERIAL AND METHODS

A total of 182 adult individuals from six ecologically distinct tropical reef fish families, Apogonidae (1 spp.), Clupeidae (1 spp.), Gobidae (3 spp.), Labridae (1 spp.), Pomacentridae (10 spp.), and Scombridae (1 spp.) were collected during the summer period from the Lizard Island lagoon, Great Barrier Reef, Australia. Fishes were collected using fence and hand nets and were transported to the laboratory at Lizard Island Research Station, where individuals were immediately placed in 3141 round plastic aquaria supplied with a constant flow of sea water. To avoid inflicting undue stress to the animals, aquaria were held outdoors to ensure that temperature ($28 \pm 0.4^\circ\text{C}$), salinity (34 ± 0.1 ppt) and light regimes remained identical to the natural environment. All fishes were killed within 3–6 h from the time of collection, and their livers were immediately removed and preserved in liquid nitrogen. All liver samples were then stored at -80°C pending extraction for CoQ analysis.

To measure the CoQ redox status (determined as the proportion of CoQH_2 (ubiquinol) to total CoQ (ubiquinol+ubiquinone)), each liver sample (mean weight equal to 0.1 ± 0.04 g) was homogenized and extracted with 1 ml of 1:1 isopropanol-ethyl acetate solution. To achieve maximum efficiency of extraction of CoQ and CoQH_2 , the mixture was vortexed vigorously for 30 s and centrifuged at $14\,000g$ for 3 min at 10°C . From the supernatant, a 25 μl aliquot was injected immediately onto a high-performance liquid chromatography (HPLC) system for simultaneous measurement of liver ubiquinol and ubiquinone concentrations. The HPLC system was equipped with a guard column (ODS(2), 30×4.6 mm), an analytical column (Phenomenex Phenosphere ODS(2), 5μ , 250×4.6 mm), followed by an online reduction column (Irica RC-10) and an electrochemical detector (Shiseido Nanospace SI-2) operating at +600 mV. The mobile phase consisted of 50 mM sodium perchlorate in methanol/isopropanol delivered at a flow rate of 1.0 ml min^{-1} . CoQ components were quantified using the peak area response of a CoQ_{10} external standard.

3. RESULTS

Surprisingly, the majority of coral reef fishes examined in this study were characterized by the presence of CoQ_8 (table 1). Out of 17 species examined, CoQ_{10} was found only in 3 species, the goby *Amblygobius phalaena* and the congeneric pomacentrids *Amphiprion akindynos* and *Amphiprion melanopus*. Predictably, there were no variations of CoQ composition within individual species.

CoQ redox balance was significantly different among fish families (Kruskal–Wallis test: $p < 0.001$; figure 1). The percentages of ubiquinol (% CoQH_2) in the total CoQ pool recorded in the livers of Labridae, Pomacentridae and Scombridae indicated that liver CoQ present in these families is mostly (more than 90%) in its reduced form (figure 1). By contrast, the Clupeidae and Gobidae had the lowest % CoQH_2 redox balance, where approximately 20–25 per cent of the total liver CoQ existed in its oxidized form.

Although the CoQ redox status varied significantly even among congeneric species (e.g. pomacentrids; Kruskal–Wallis test: $p < 0.001$), such differences were consistently independent of the isoprenoid length of the CoQ present in the liver of the fish families examined (Mann–Whitney U -test: $p = 0.31$, Pomacentridae; $p = 0.53$, Gobidae). For example, while both *Amphiprion* spp. possessed the homologous Q_{10} , only the redox status in *A. akindynos* was significantly different from the redox status of CoQ_8 found in other pomacentrids (Mann–Whitney U -test: $p < 0.02$, *A. akindynos*; $p = 0.25$, *A. melanopus*). Similarly, we found no difference in the % CoQH_2 redox balance among the three gobid species, despite the occurrence of different CoQ homologous (Kruskal–Wallis test: $p = 0.44$).

Table 1. Natural occurrence of CoQ isoforms among coral reef fishes.

Family	genus	species	n	CoQ isoforms
Apogonidae	<i>Apogon</i>	<i>cyanosoma</i>	21	CoQ_8
Clupeidae	<i>Spratelloides</i>	<i>delicatulus</i>	8	CoQ_8
Gobidae	<i>Amblygobius</i>	<i>phalaena</i>	7	CoQ_{10}
		<i>rainfordi</i>	5	CoQ_8
		<i>eviota</i>	24	CoQ_8
		<i>sigillata</i>	23	CoQ_8
Labridae	<i>Thalassoma</i>	<i>lunare</i>	23	CoQ_8
Pomacentridae	<i>Acanthochromis</i>	<i>polyacanthus</i>	5	CoQ_8
		<i>akindynos</i>	5	CoQ_{10}
		<i>melanopus</i>	4	CoQ_{10}
		<i>viridis</i>	23	CoQ_8
		<i>rex</i>	4	CoQ_8
		<i>rollandi</i>	5	CoQ_8
		<i>amboinensis</i>	21	CoQ_8
		<i>molluccensis</i>	19	CoQ_8
		<i>wardi</i>	5	CoQ_8
		<i>nigricans</i>	2	CoQ_8
		<i>bicarimatus</i>	1	CoQ_8

Figure 1. CoQ redox status across coral reef fish families. Means $\pm$ 95% CI. Average ranks for all possible pairs of fish families were compared to determine significant differences in CoQ redox status. Letters indicate significant differences based on multiple *post hoc* comparisons of average ranks (i.e. fish families with no significantly different CoQ redox status are indicated by the same letter).

4. DISCUSSION

Our initial hypothesis that coral reef fishes would have CoQ with 10 or more isoprenoid units in the side chain for inhabitation in warm tropical waters can be dismissed outright. Most coral reef fishes have the homologous CoQ with eight isoprenoid units, thus far considered to be characteristic of protozoans, yeasts and bacteria. While the majority of species examined had CoQ_8 , the occurrence of CoQ_{10} in some of them indicates that variation among CoQ homologous forms of fishes is not specifically associated with thermal adaptation; instead, it may have evolved in response to the temperature range experienced rather than mean temperature. In fact, polar and coral reef fishes alike inhabit relatively stable temperature environments compared with temperate fishes, and hence are equally expected to exhibit an enhanced metabolic sensitivity towards thermal anomalies.

Interestingly, the only three species having CoQ₁₀ rather than CoQ₈ have particular lifestyles that distinguish them from all the other study species. For example, *Amb. phalaena* differs from its congeneric *Amblygobius rainfordi* (and more generally from the other species examined), in that it resides in a sand burrow for protection. Similarly, both *Amphiprion* spp. live in anemones, a unique characteristic separating them from both the other pomacentrids and all other species examined here. These three species' feeding requirements are easily met within their immediate surroundings (planktivores and benthic micro-carnivores) and they display virtually no pelagic activity. Such behavioural habits result in significantly reduced physiological swimming demands, otherwise required of life in the water currents of coral reef habitats (Johansen *et al.* 2008). In fact, sustained swimming in warm waters is generally associated with higher heart rates, demands an elevated oxygen supply and ultimately requires high oxidative ATP production (Guderley 2004). Moreover, as oxygen solubility in warm sea water is low, meeting a higher oxygen demand can be challenging at higher temperatures, and failure to supply adequate oxygen, particularly to tissues with high rates of oxygen usage such as the liver, leads to hypoxia and paradoxically increased mitochondrial ROS production as part of the cellular stress response (Kassahn *et al.* 2007). By having CoQ₈ rather than CoQ₁₀, thus ensuring more efficient control of ROS production to lessen oxidative damage (cf. Niki 2001), coral reef fishes may be able to counteract the potential effects of hypoxia in their tissues (potentially through some trade-offs in biochemical adaptation), while maintaining a very active lifestyle in tropical waters. This idea is further supported by our results on redox status differences observed at both the family and the species level. In fact, most pomacentrids and species such as the labrid *Thalassoma lunare* and the scombrid *Grammatorcynus bicarinatus*, which are all characterized by enhanced swimming capacity befitting an energy-demanding life in strong current flow, appear endowed with a much greater CoQ ratio of the ubiquinol antioxidant in their liver (where circulatory lipids are transported from to be oxidized and fuel swimming muscles, Weber & Haman 1996) compared with relatively more sedentary species with limited swimming capacity. Accordingly, the natural variation in CoQ composition and redox balance observed among coral reef fishes appears to reflect differences in ecological habits and physiological demands of individual species, while remaining independent of the phylogenetic relationships among species.

By examining the natural occurrence of CoQ of different isoprenoid length and its redox status in a wide range of coral reef fish species, we are able to shed further light on the metabolic adaptations of marine organisms. Indeed, our results suggest that the variation in CoQ composition in fishes is not a simple adaptation to specific thermal conditions as previously suggested in temperate fishes (Giardina *et al.* 1997), but better represent an adaptation to environmental oxygen availability in the context of different species-specific ecological demands. As such,

both polar and tropical fishes are expected to experience elevated oxidative stress, even though the underlying mechanisms may be quite different (i.e. excessive oxygen demand at high temperatures or insufficient aerobic capacity of mitochondria at low temperatures; Pörtner 2001).

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