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Metal concentrations and metallothionein-like protein levels in deep-sea fishes captured near hydrothermal vents in the Mid-Atlantic Ridge off Azores

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ABSTRACT

The knowledge of metal contamination in deep-sea fishes living in the surroundings of hydrothermal vents is very scarce, along with the detoxification mechanisms that allow them to live near one of the most metal contaminated marine environments. Six deep-sea fish species, although not vent endemic were collected near three Mid-Atlantic Ridge (MAR) hydrothermal vents (Menez Gwen, Lucky Strike and Rainbow) and the gills, muscle and liver were selected for this study due to their importance in metal metabolism and storage. The concentrations of seven metals (Ag, Cd, Cr, Cu, Fe, Mn, and Ni) and a metal-related biomarker (metallothionein-like proteins–MTL) were assessed. Major differences in metal accumulation among fish species are related to their feeding habits and vent site of their capture. The liver and gills are in general the most important tissues for metal accumulation compared to the muscle, but tissue partitioning is very dependent on the fish species considered. Compared to other deep-sea fishes, fish capture in the vicinity of hydrothermal vents accumulates higher amounts of metals in general. However, MTL levels are not considerably different from what is found in commercial coastal fishes, and is poorly correlated with metal concentrations in the tissues. Therefore, MTL may not constitute one major detoxification system for deep-sea species living in the vicinity of three important MAR vent sites.

1. Introduction

The deep-sea is the largest habitat on earth and it is still largely unexplored, as well as its associated faunal community, including deep-sea fishes. With the development of both manned submersibles and remotely operated vehicles, scientists had the opportunity to observe fish species unknown at more superficial waters. Unfortunately, catching these fishes is difficult and many studies rely on images captured by submersible cameras. Special attention has been given to fish species living at hydrothermal vents and cold-seeps, either "vent-endemic" species that live inside the active vent fields or species pertaining to the bathyal environment but recorded from the close proximity to the vents or cold-seeps. An exhaustive review made by Biscoito et al. (2002) accounted for approximately 21 fish species living inside hydrothermal vent sites, the so-called "vent-endemic" species and 90 bathyal fish species inhabiting the periphery of these deep-sea systems in the Atlantic, Pacific and Indian Oceans. Among the latter, the species studied in the present work have been observed regularly less than 10 m from the active sites (*Hydrolagus pallidus* and *Synaphobranchus* spp.) or within a 300 m radius (*Deania hystricosa, Etmopterus princeps, Antimora rostrata* and *Mora moro*), during the dives made by the authors to the concerned hydrothermal vent fields.

As for other hydrothermal vent species, fishes that venture to these environments must also withstand harsh environmental conditions even though for more limited periods of time, including total darkness, extreme cold, great hydrostatic pressure, low oxygen levels and metal-enriched waters. Due to chemical interactions between the magma source and the surrounding sea water, the hydrothermal vent fluids have high concentrations of metal compounds such as Ag, Cd, Cr, Cu, Fe, Hg, Mn, Ni and Zn (Charlou et al., 2000, 2002; Douville et al., 2002). To cope with their "harmful" environment, hydrothermal vent species like other marine organisms have developed efficient mechanisms against the toxicity of metals including their intracellular sequestration as non-toxic granules, or the production of metallothioneins and antioxidants (Cosson and Vivier, 1997; Bebianno et al., 2005). Metallothioneins (MT) are low molecular weight, cysteine-rich proteins without any aromatic or heterocyclic amino acids or histidine (Nordberg, 1998). The involvement of MT in the regulation of essential metals metabolism, in heavy metal detoxification and in protection against oxidative stress

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was clearly demonstrated (Cosson et al., 1991; Bauman et al., 1993; Viarengo et al., 2000; Baird et al., 2006).

Metal absorption in fish is carried out via two main uptake routes: gills surface (water exposure) and digestive tract (diet exposure) (Ptashynski et al., 2002). Gills are one of the most critical organs in terms of metal toxicity in fish (Wood, 2001), as they are in direct contact with the water and are a site for uptake of metals (Bury et al., 2003). In fish, disruption of ion transport across the gills is a major cause of toxicity (Wood, 2001). After being absorbed by the gills, metals are further transferred via blood to other target organs, such as the liver and kidney. The liver has a high metal accumulating capacity and is one of the most specialised tissues for metal metabolism, performing important and complex biological functions like energy metabolism that are essential for survival (Bernet et al., 1999).

On the other hand, fish muscle is not an active tissue in accumulating metals with the exception of organic mercury (Uysal et al., 2008). However, since this is normally the edible portion for human consumption, many studies only report metal levels in muscle tissue especially in fish from polluted regions or farmed fish in order to evaluate if the acceptable levels for human health are not exceeded. Therefore, this study focused on the metal body burden and tissue partitioning in the gills, liver and muscle of several vent-related deep-sea fishes.

The knowledge of metal levels in deep-sea fishes living near hydrothermal vents is scarce. Martins et al. (2006) working with 8 fish species (*Hydrolagus pallidus*, *Hydrolagus affinis*, *Synaphobranchus kaupii*, *Epigonus telescopus*, *Mora moro*, *Antimora rostrata*, *Deania calceus* and *Etmopterus princeps*), occurring in the vicinity of the MAR vent sites (Menez Gwen, Lucky Strike and Rainbow), reported the levels of total mercury and methyl mercury. Other studies account for the metal content for other deep-sea fishes captured in the North Atlantic continental slope (Cronin et al., 1998), the continental slope of the Rockall Trough, west of Scotland (Mormede and Davies, 2001a,b) and top predator pelagic fish from Mozambique channel and Reunion Island in the western Indian Ocean (Kojadinovic et al., 2007). However, the majority of studies about metal contamination refer to commercial fish species, such as the work on top predator pelagic fish from Mozambique channel and Reunion Island in the western Indian Ocean (Kojadinovic et al., 2007), due to its direct impact in human health. Therefore, the main purpose of this study was to determine the concentration of metals (Ag, Cd, Cr, Cu, Fe, Mn, Ni) that are present in three tissues (gills, muscle and liver) of three Chondrichthyes (*Deania hystricosa, Etmopterus princeps* and *Hydrolagus pallidus*) and three Osteichthyes (*Antimora rostrata, Mora moro* and *Synaphobranchus* spp.) captured near hydrothermal vents at MAR and to assess the response of metallothionein-like proteins (MTL) in these species.

2. Materials and methods

2.1. Fish species

2.1.1. Chondrichthyes

Deania hystricosa (Garman, 1906) (rough longnose dogfish) is a squaliform shark belonging to the family Centrophoridae. In the East Atlantic it is known from the insular slopes of the Canaries, Madeira and Azores, including the Mid-Atlantic Ridge, Namibia and South Africa. In the Pacific Ocean it occurs in Japan and possibly New Zealand (Compagno et al., 2005; Freitas et al., in press). This little known shark is probably benthic or epibenthic, from 470 to 1300 m (Compagno et al., 2005). There is no data on its feeding habits.

Etmopterus princeps (Collett, 1904) (great lantern shark) is a blackish-brown squaliform shark belonging to the family Etmopteridae. It is known for sure from the Atlantic Ocean, from Greenland to Senegal and from Nova Scotia to New Jersey (Compagno et al., 2005). It is also found on the Mid-Atlantic Ridge (Hareide et al., 1997; Martins et al., 2006). Its presence in the western Pacific is uncertain (Compagno et al., 2005). Its bathymetric distribution ranges between 350 and 4500 m (Compagno et al., 2005). The diet consists of a wide variety of organisms dominated by teleosts, cephalopods and crustaceans (Cortés, 1999). Both shark species have been observed during several dives passing by within a 300 m radius of the active chimneys, *D. hystricosa* at Menez Gwen and *E. princeps* at the three studied sites.

Hydrolagus pallidus (Hardy and Stehmann, 1990) (shortnose chimaera or pallid chimaera) is a large white to pale gray ratfish belonging to the family Chimaeridae. As in all other members of this family, the skin is smooth without scales. It is known only from a few locations in the Northeast Atlantic, including the Azores and the Mid-Atlantic Ridge, at depths between 1200 and 2075 m (Hardy and Stehmann, 1990; Saldanha and Biscoito, 1997). Its diet consists mainly of small fishes and invertebrates including mussels from hydrothermal vents (Marques and Porteiro, 2000; Martins et al., 2006). This species is one of the most commonly observed during dives at Lucky Strike and in more than one occasion it was seen by the authors as close as 10 m from an active chimney.

2.1.2. Osteichthyes

Antimora rostrata (Günther, 1878) (blue antimora) is a cod-like fish of the family Moridae found on the continental shelf of all oceans except the North Pacific north of 10°N. It is a bathypelagic species ranging from 350 to 3277 m. Its diet is varied with a preference for bottom-living invertebrates (Cohen et al., 1990; Martins et al., 2006). This species has been observed a few times at Rainbow, well off the active site, but within a 300 m radius.

Mora moro (Risso, 1810) (common mora) also belonging to the family Moridae, is found in the upper continental slopes worldwide in temperate seas. It is a bathypelagic species that lives at depths between 300 and 2500 m. The diet consists of a wide variety of preys including fishes, crustaceans, molluscs and other invertebrates as well as food of terrestrial origin (Cohen et al., 1990; Martins et al., 2006). In almost all dives done by the authors in this field specimens from this species have been observed at the borderline of the active field.

Synaphobranchus spp. (cutthroat eel) are dark-gray anguilliform fishes of the family Synaphobranchidae found on the continental slopes near the upper limit of abyssal zone in Eastern, Western and Northwest Atlantic, Indo-West Pacific and Indian Ocean. Their wide bathymetric distribution ranges between 120 and 4800 m (Sulak and Shcherbachev, 1997). When the specimens used in the present paper were collected, they were all identified as *S. kaupii* (Johnson, 1862), the only species of this genus recorded at that time from the area. However, as Almeida and Biscoito (2007) stated, there are two other sympatric species, *S. affinis* (Günther, 1877) and *S. brevidorsalis* (Günther, 1877) in these vents. As pointed out by these authors, *S. brevidorsalis* is easily distinguishable from the other two species, which in turn are sometimes very difficult to separate. As the specimens used in the present analyses were not preserved, they may either belong to *S. kaupii* or to *S. affinis*, therefore the reason to consider here as *Synaphobranchus* spp. *S. kaupii* is a scavenger feeding mainly on small crustaceans (decapods and amphipods), but also fishes and cephalopods (Uiblein et al., 2002; Martins et al., 2006). *Synaphobranchus* spp. have been observed in all dives made at Menez Gwen and Lucky Strike, in some cases well within the active fields, less than 50 cm above the seafloor, either on bare rocks or over mussel beds.