COPPER TOXICITY AND HOMEOSTASIS IN *RAJA ERINACEA AND MYOXCHEPHALUS OCTODECEMSPINOSUS*

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Preliminary data suggested that elasmobranch and teleost fish differs with respect to ambient copper tolerance. The objective of this New Pilot Project study at Mount Desert Island were to compare copper-induced physiological disturbances and copper homeostasis in an elasmobranch and a teleost fish.

The clear nosed skate (*Raja erinacea*) an elasmobranch, was exposed to 0.4 and 2.0 μM copper in MDIBL seawater at 17 ± 1°C. Fish were sampled after 0, 1, 3 and 7 days of exposure. The intent was to perform parallel experiments on a teleost fish, sculpin *(Myoxocephalus octodecemspinosus)*, exposing both fish to the same conditions. Due to shortage of fish, however, only a 0 and 7 day sample from a 2.0 μM exposure was obtained for the comparison. All fish were sacrificed by exposure to 0.2 g MS-222 l^{-1} after which blood samples were obtained via a heparinized syringe. After hematocrit determination, plasma was obtained by centrifugation for subsequent analytical work. Two gill arches from each fish were freeze-clamped and stored at -80°C for later isolation of RNA, for determination of Na/K-ATPase activity and copper concentration. One additional gill arch was fixed for imuno-histological analysis while another was stored for specific protein analysis. From both species, liver, kidney, muscle, brain and intestine were sampled for later analysis of copper concentrations. In addition, the rectal gland was obtained from the clear nosed skate. Due to the role of the intestine in teleost osmoregulation and possibly in copper uptake via drinking, both anterior, mid and posterior segments of the sculpin intestine were sampled not only for metal analysis but also for Na/K-ATPase activity.

The aim of the present study was to induce mild disturbance in copper homeostasis and only minor additional physiological effects. Based on the parameters analyzed so far, both aspects were succesful. While the sculpin exhibits no copper-induced physiological disturbance after exposure to 2.0 μM copper, the skate exhibits greatly elevated plasma ammonia concentrations during exposure to both 0.4 and 2.0 μM copper. For both species, plasma Na^+, Cl^- and Mg^{2+} demonstrated no copper-induced osmoregulatory disturbance. Na/K-ATPase activity in the osmoregulatory tissues, sculpin intestine and skate rectal gland, and the gill of both species, still awaits analysis.

Branchial copper concentrations were elevated in response to copper exposure in both species (Fig. 1). While the branchial copper concentration in the sculpin increased approximately 3-fold, the branchial copper concentration in the more sensitive skate increased by more than 40-fold. These differences in branchial copper accumulation did not result in differences in plasma copper concentrations between the two species; Both exhibited minor increases in response to copper exposure (Fig. 1). The minor effect of copper exposure on plasma copper status was expected because plasma copper in fish in general is well regulated. The liver of at least teleost fish is the major copper homeostatic organ. Hepatic copper concentrations are generally high compared to other tissues and tend to reflect copper exposure history. Hepatic copper concentrations in the non-exposed skate are comparable to what have been reported for most teleost fish. Interestingly, the hepatic copper concentrations in skates exposed to the lowest concentration did not increase (Fig. 2) despite greatly elevated branchial copper concentrations in the same animals. In contrast, both skates and sculpins exposed to the highest copper concentration exhibited increased hepatic copper levels. Another interesting observation is the very low hepatic copper concentration in the sculpin. The levels are generally one order of magnitude lower than what is reported for most other teleost fish and two orders of magnitude lower than values reported for adult rainbow trout. The copper accumulation pattern in the sculpin also differed from other teleost fish by exhibiting highly elevated renal copper concentrations. The intestine of the skate and the anterior segment of the sculpin intestine exhibited a slightly elevated copper concentration, but copper concentrations in other tissues were not affected by copper exposure.

The copper concentration in any cell or tissue is a sum of uptake and elimination. The teleost sculpin and the elasmobranch skate clearly differ with respect to copper homeostasis. When exposed to the same ambient copper concentration, the skate clearly has a greater difference between copper uptake from the water into the gill cells and subsequent copper export to the blood than the sculpin resulting in a much greater branchial copper concentration. The difference in branchial copper accumulation reflects the difference in sensitivity to copper exposure. The sculpin exhibited no physiological disturbances (aside from copper accumulation) whereas the skate exhibited elevated plasma ammonia which could have arisen from impaired ammonia excretion across the gill, possibly caused by the copper accumulation.

The skate clearly has a much greater whole-body copper uptake than the sculpin, evident from the much greater increase in copper concentrations in the liver and the kidney of the skate compared to the sculpin (note that the hepatosomatic index of elasmobranchs generally is much higher than in teleosts). This suggests that the relative big difference between copper uptake into gill cells and transport from the gill cells to the blood in skates resulting in the high branchial copper accumulation is associated with very efficient uptake from the water rather than low transport from the gill to the blood.

The present study revealed great differences in copper metabolism between an elasmobranch and a teleost fish and demonstrates well the potential of fish as vertebrate models for copper homeostasis. The difference in branchial copper metabolism between the two species of the present study, and the different hepatic copper concentrations in teleost fish in general spans two orders of magnitude offering great comparative value. For example, the hepatic copper concentrations in sculpin of the present study would result in copper deficiency symptoms in rainbow trout whereas normal hepatic copper levels of rainbow trout would be fatal to sculpins. These piscine species have the potential to serve as important models of both Menke's and Wilson's disease in man where genetic defects in the Cu-ATPase cause copper deficiency and excess, respectively.

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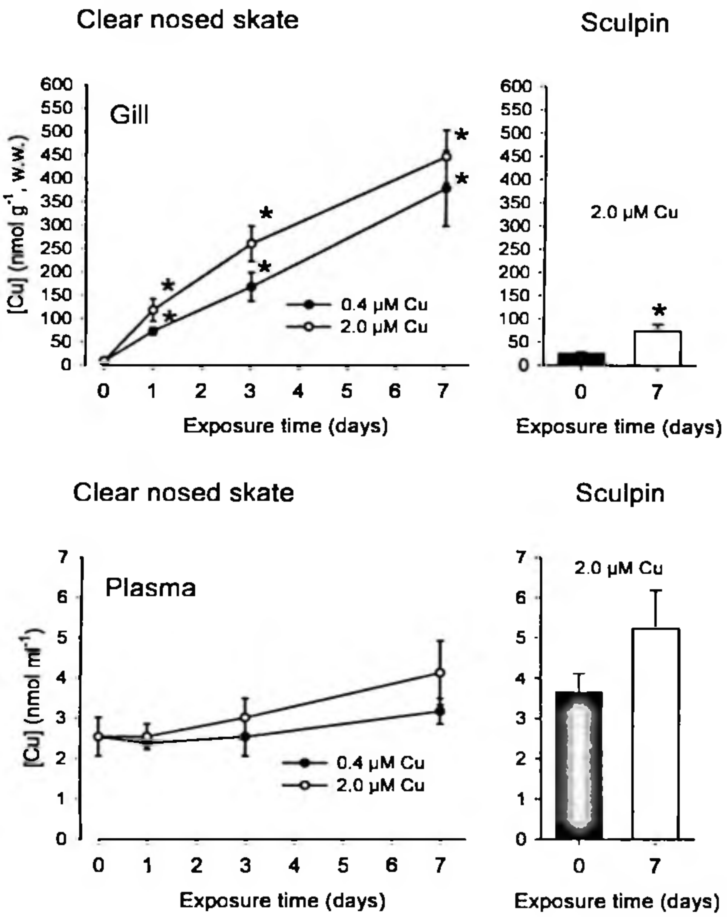

Fig. 1. Copper concentration in the gills (top panel) and plasma (bottom panel) of the clear nosed skate (line graphs) and the sculpin (bar graphs) during/after seven days of exposure to 0.4 and 2.0 μM copper for the skate and 2.0 μM for the sculpin. * Indicates significant difference from control.

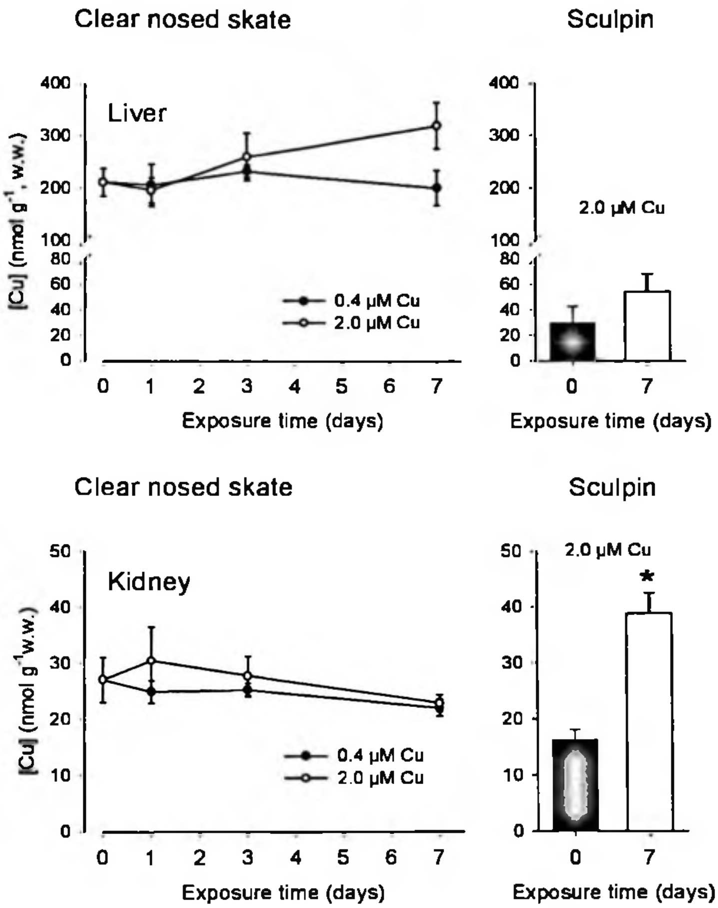

Fig. 2. Copper concentration in the liver (top panel) and kidney (bottom panel). Other details as in Fig. 1.