

Methylmercury causes reduced GPx activity in brain of adult zebrafish females, and interrupts motoneuron axon growth in zebrafish embryos after maternal transfer.

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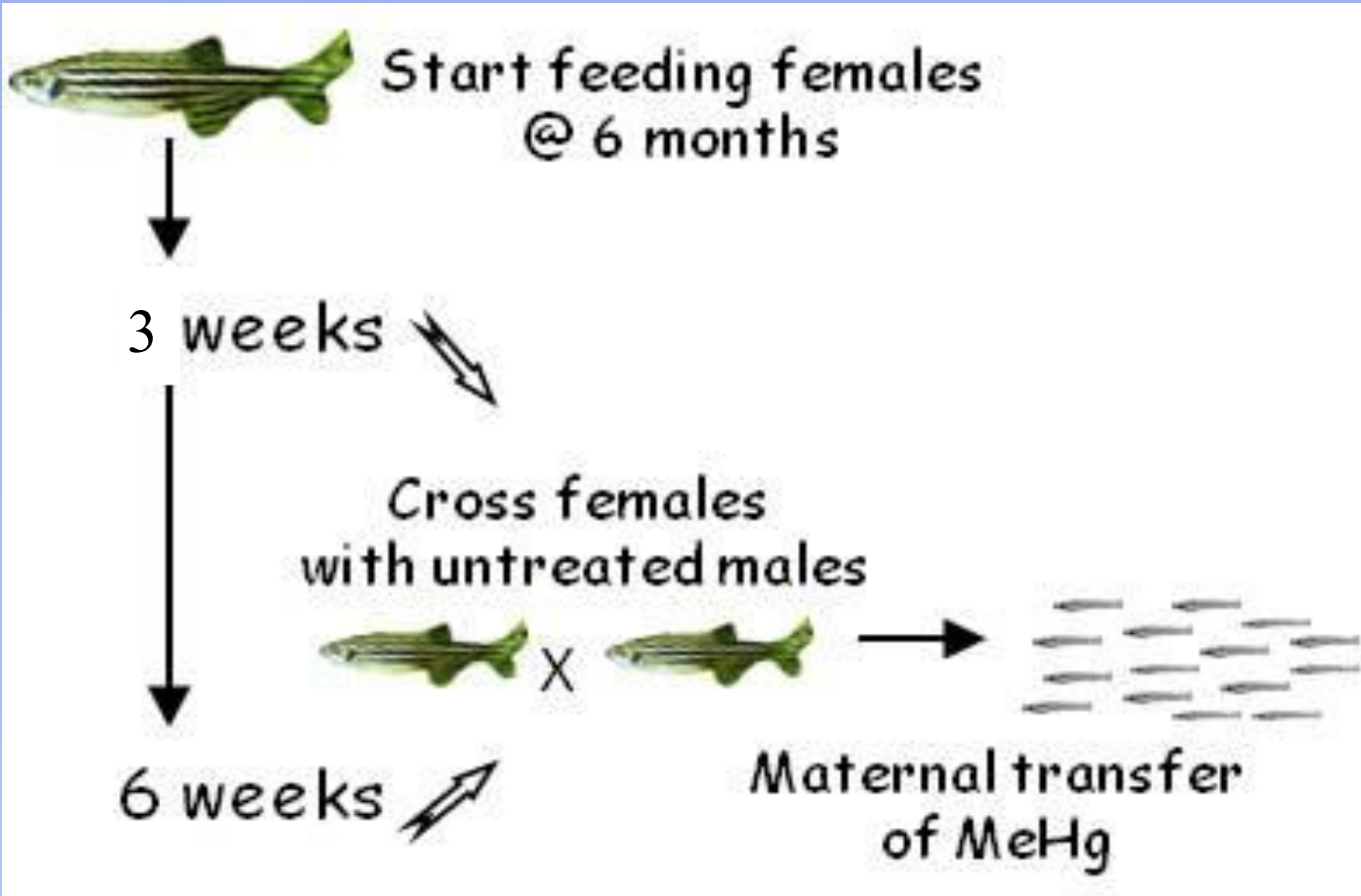
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Introduction

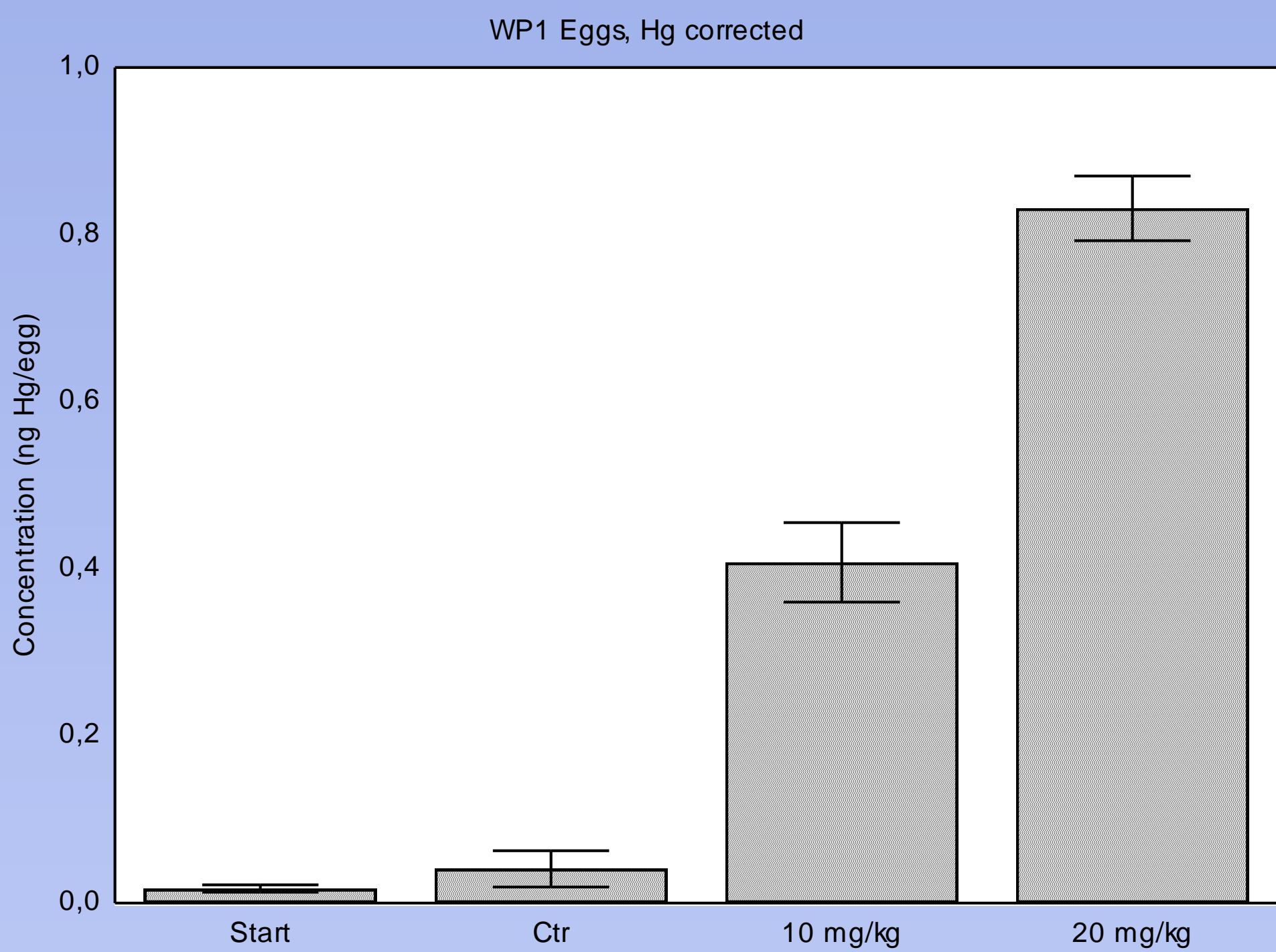
Methylmercury (MeHg) is an environmental contaminant that accumulates in the seafood chain and represents a risk to human health. Many of the common food contaminants, including MeHg, have incomplete neurotoxicity profiles which results in a large need for efficient model systems for neurotoxicity assessment. Early life stages are particularly vulnerable to contaminant exposure, and maternal transfer of MeHg is frequently associated with neurobehavioral deficits in progeny like altered motoric and cognitive functions. Understanding the underlying mechanisms of MeHg toxicity is important for future risk assessment. We have investigated neurotoxic effects from dietary MeHg in adult zebrafish, and in zebrafish embryos after maternal transfer of MeHg. In pooled samples of brain of adult female zebrafish exposed to dietary MeHg, we found total glutathione (GSH) concentrations and glutathione-S-transferase (GST) total specific activity to be unaltered, however, the specific activity of glutathione peroxidase (GPx) was significantly decreased. In zebrafish embryos, we identified disturbance of trunk motoneuron axon growth after maternal transfer of MeHg. Further embryo analyses have shown changes in mRNA expression levels of genes from the axon guidance protein families Ephrin and Semaphorin. Previous studies have indicated that selenium can neutralize the damaging effects of MeHg. We found that by co-exposure to dietary selenium, the observed damaging effects from MeHg on motoneuron axon growth could be reduced.

Experimental outline



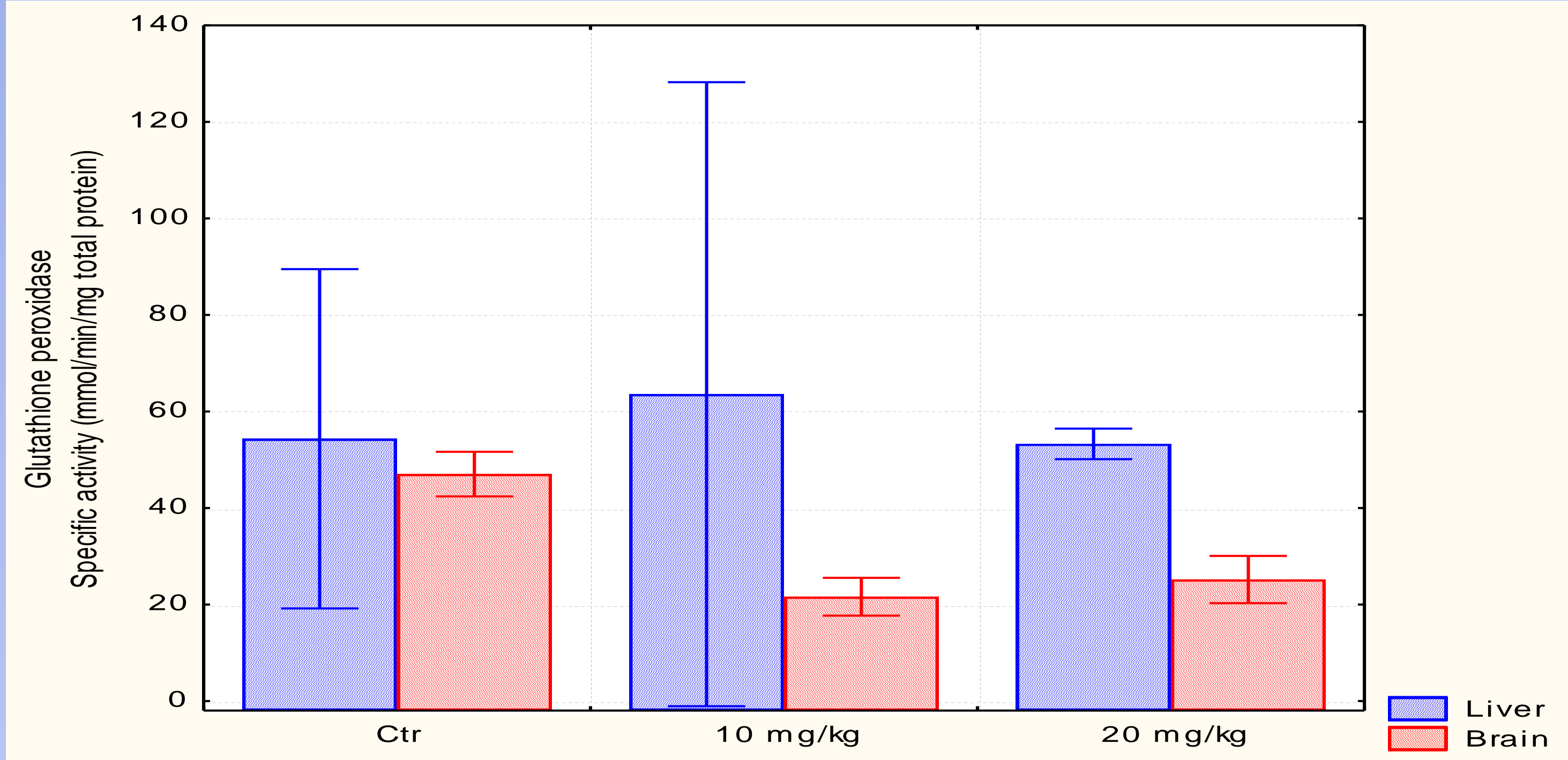
Quadruplicate groups of 25 female zebrafish were exposed to dietary MeHg at nominal concentrations of 0, 10 and 20 mg Hg/kg and/or dietary selenium at nominal concentrations of 0 and 5 mg Se/kg. Methylmercury was added to a commercial zebrafish diet as methylmercury-cysteine while selenium was added as selenomethionine. Exposed females were crossed against unexposed males at 3 and 6 weeks. After spawning, eggs were collected (sub-samples of 100 eggs) and the females were sacrificed.

Maternal transfer of dietary MeHg in zebrafish embryo



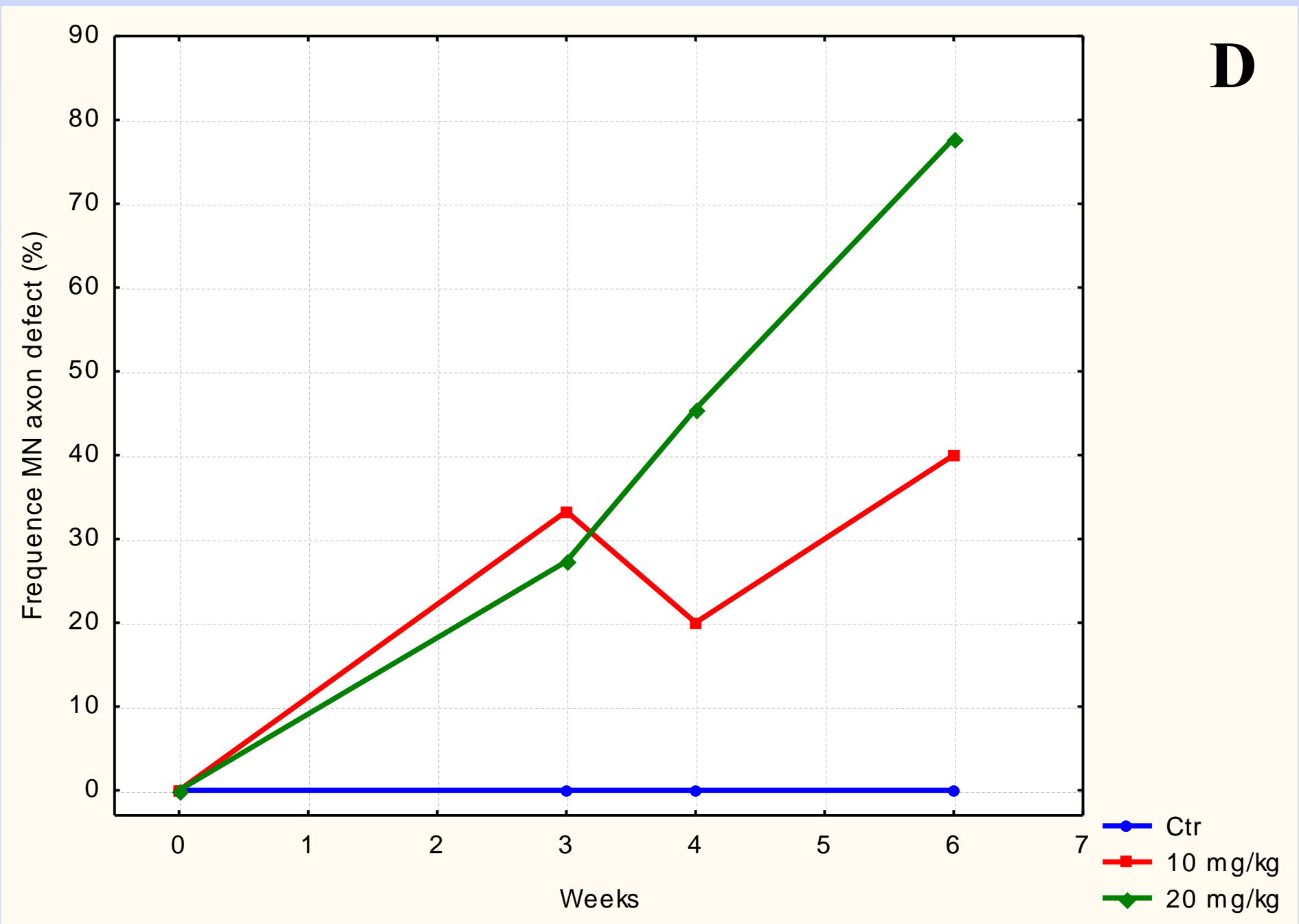
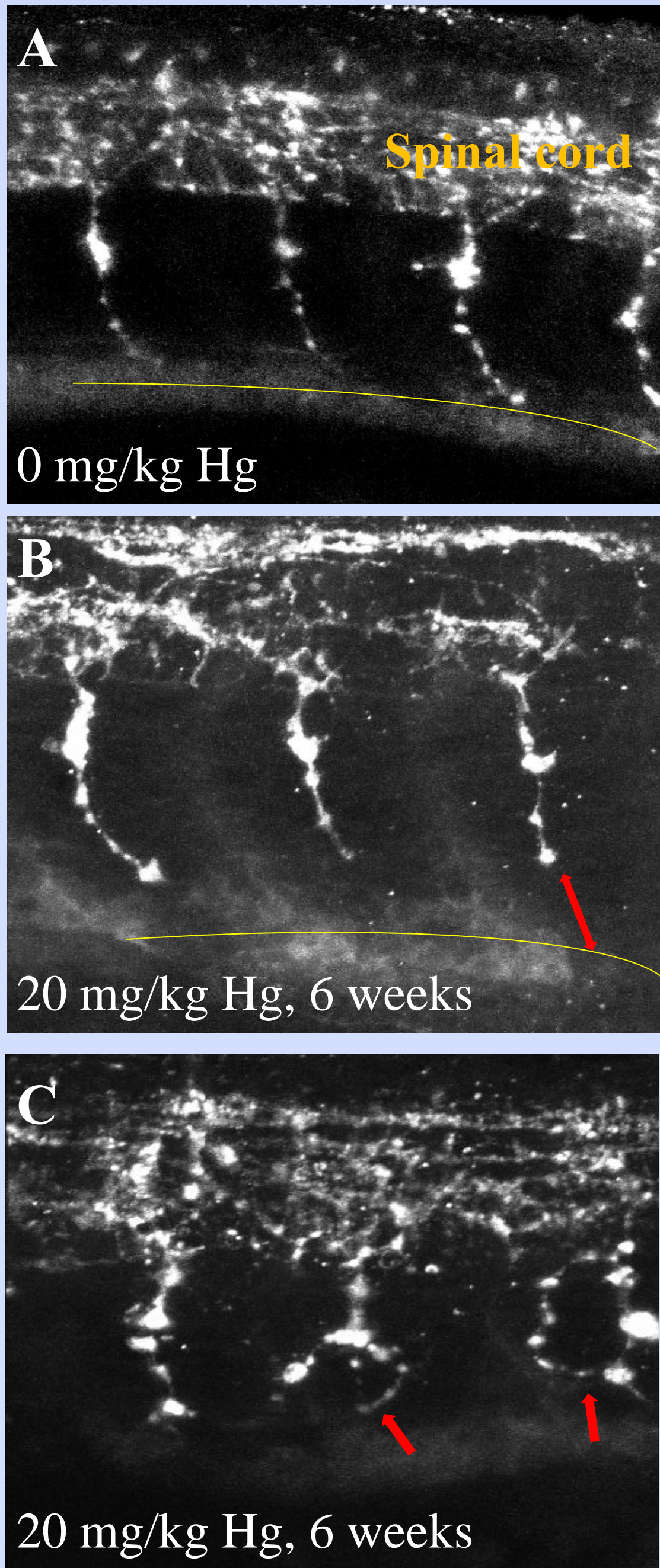
Batches of 100 eggs from crosses of female fish fed 0, 10 and 20 mg Hg/kg for 6 weeks were analyzed for their mercury content using a direct mercury analyzer (DMA-80). Amount of maternally transferred mercury was dose dependent, with the highest levels found in eggs from females fed the highest dietary level (20 mg Hg/kg). Amounts are given as ng Hg/egg.

MeHg reduces GPx enzyme activity in zebrafish brain



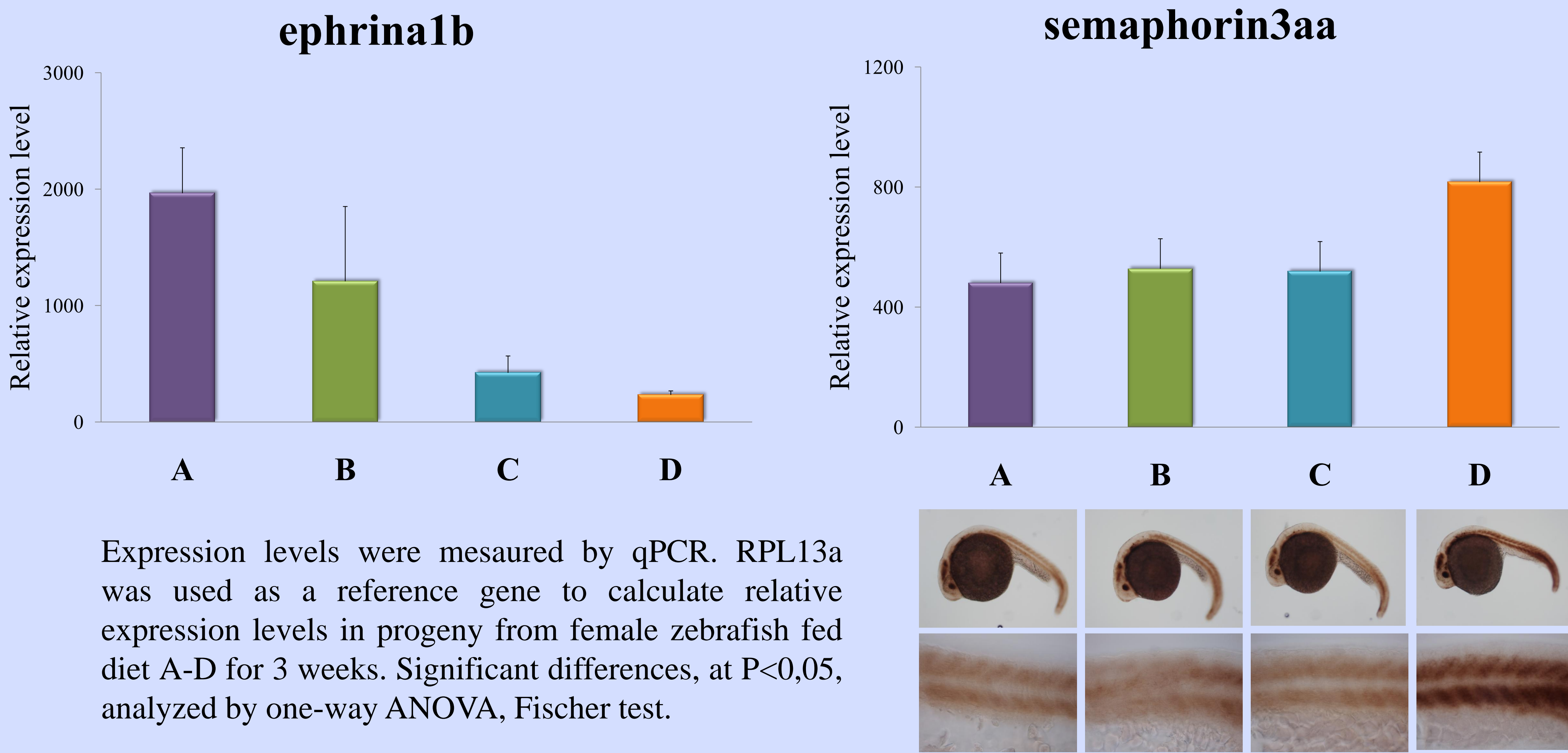
Total specific activity of glutathione peroxidase (GPx) was measured in pooled samples of brain and liver (n = 4) of female zebrafish exposed to three levels (0, 10 and 20 mg Hg/kg) of dietary MeHg for 6 weeks. The specific activity of GPx was similar in liver and brain of fish fed the control diet; the activity in liver was not affected by dietary treatment, while in brain the specific activity of GPx decreased in samples of fish fed the two mercury diets. Levels of GSH found in liver and brain were not affected by dietary treatment (data not shown). The GST activity was higher in liver than in brain, but the activity was not affected by dietary treatment in either organ (data not shown).

Maternal transfer of MeHg leads to disturbance in spinal cord motoneuron axon growth in zebrafish embryos



Spinal cord primary motoneurons were visualized by immunohistochemistry (Znp-1 antibody) on batches of 10 eggs from each diet. We observed a significant disturbance in caudal primary CaP axon growth in embryos from females fed the 10 and 20 mg Hg/kg diets (D). Their axons projected to the branching point, but then growth ceased, leading to a shortened axon, or in other cases led to abnormal branching and misguided axon growth compared to unexposed control (red arrows, yellow line, A-C).

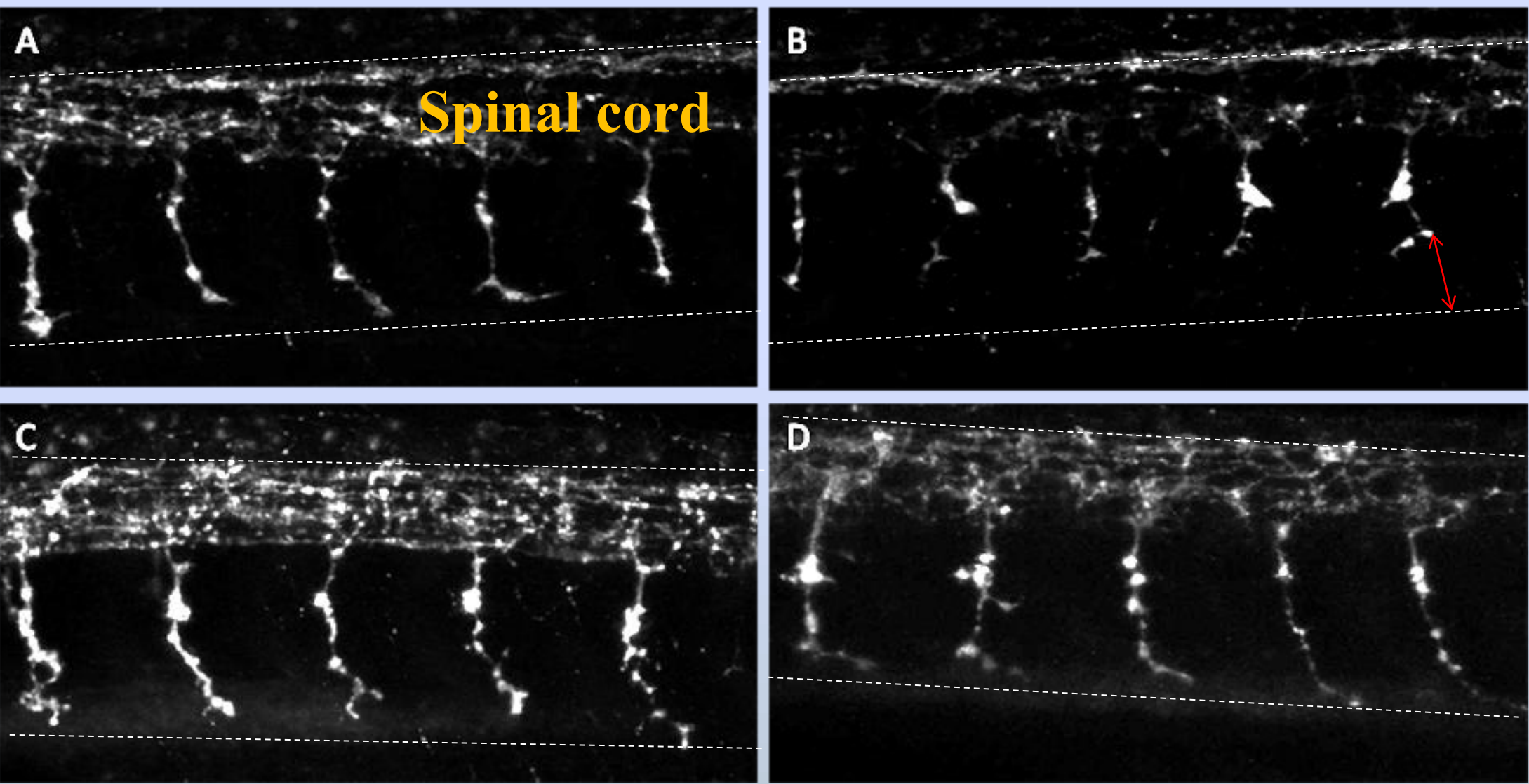
Maternal transfer of MeHg and selenium affects expression levels of ephrina1b and semaphorin3aa in developing muscles in zebrafish embryos



Expression levels were measured by qPCR. RPL13a was used as a reference gene to calculate relative expression levels in progeny from female zebrafish fed diet A-D for 3 weeks. Significant differences, at P<0,05, analyzed by one-way ANOVA, Fischer test.

In situ hybridising for semaphorin3aa was done with a RNA-DIG-labelled probe in zebrafish embryos at 24 hpf for diets A-D: lateral view - whole mount (8x) and trunc muscles (20x). Diets: A; control, B; 10 mg Hg/kg, C; 5 mg Se/kg, D; 5 mg Se/kg + 10 mg Hg/kg.

MeHg induced disturbance in spinal cord motoneuron axon growth can be inhibited by dietary selenium



Diett	Hg (ng/embryo)	Se (ng/embryo)
A	0.002	0.07
B	0.28	0.08
C	0.01	0.14
D	0.25	0.23

Spinal cord primary motoneurons were visualized by immunohistochemistry (Znp-1 antibody) on batches of 20 24 hpf embryos from female zebrafish fed diets (3 weeks) containing: A; control, B; 10 mg Hg/kg, C; 5 mg Se/kg, D; 5 mg Se/kg + 10 mg Hg/kg. The disturbance in caudal primary CaP axon growth in embryos from MeHg enriched diet (B) could be alleviated by introducing dietary selenium (D). Diet enriched with selenium alone did not affect axon growth (C).

Concluding remarks

- Dietary MeHg and selenium are transferred from female zebrafish to progeny.
- The specific activity of GPx is decreased in brain of fish fed diets enriched with MeHg for 6 weeks.
- Maternally transferred MeHg affects growth of spinal cord primary motoneurons that innervate trunk muscles, and can be reduced by dietary selenium.
- Maternally transferred MeHg and selenium affects expression of axon guidance proteins ephrina1b and semaphorin3aa in developing trunk muscles.
- Interaction of MeHg and selenium seems to potentiate effects on gene regulation compared to individual effects from either MeHg or selenium.