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Copper acclimation in juvenile fathead minnows: Is a cycle of branchial damage and repair necessary?★

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Abstract

Fathead minnows exposed to sublethal Cu concentrations may undergo branchial damage followed by repair, and may also develop enhanced Cu tolerance. The primary objective of this study was to determine whether the cycle of damage and repair was necessary for the development of enhanced Cu tolerance. Inferences regarding damage and repair were made from changes in the whole body Na⁺ of juvenile (0.5 g) fathead minnows (*Pimephales promelas*). Two experiments were conducted in which juvenile minnows were exposed to various sublethal Cu concentrations for 16 d. In the first experiment, fish were exposed to one of four different Cu concentrations (0, 73, 118, 189 µg/L Cu) then challenged with an 8-d exposure at 302 µg Cu/L. Only the fish exposed to the highest Cu dose experienced enhanced Cu tolerance relative to the other three doses. In the second experiment, whole body Na⁺ was monitored in fish exposed to one of five different Cu exposures (0, 70, 127, 202, 289 µg/L Cu). Fish exposed to 70 µg/L Cu did not experience a significant decline in whole body Na⁺ at any point during the 16-d exposure period. The reduction in whole body Na⁺ was short lived and moderate (17%) in the fish exposed to 127 µg/L Cu, but more severe (>30%) and longer lasting in the fish exposed to 202 µg/L Cu. At 289 µg/L Cu, the fish experienced irreversible reductions in whole body Na⁺ and ultimately died. When taken together, results from these two experiments suggest that enhanced tolerance will only develop in fathead minnows that have experienced a pronounced, relatively long-term cycle of branchial damage and repair.

Keywords

Copper; Enhanced copper tolerance; Acclimation; Branchial damage and repair; Whole-body Na⁺

1. Introduction

Several factors influence the development of metal acclimation in fish. Metal acclimation has been defined as the ability to tolerate typically lethal metal exposures while maintaining normal physiological function (McDonald and Wood, 1993). Development of the acclimatory

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response has been shown to be dependant upon exposure time (Marr et al., 1995; Sellin et al., 2005) and exposure concentration (Dixon and Sprague, 1981; Benson and Birge, 1985; Taylor et al., 2000; Hansen et al., 2002). This study further investigates the relationship between metal concentration and the development of enhanced tolerance specifically focusing on the development of enhanced tolerance in juvenile fathead minnows exposed to different exposure doses of Cu.

When fish are exposed to a sublethal dose of Cu, one possible outcome can be branchial damage followed by subsequent repair. The branchial damage can include: a reduction in Na^+ influx (Laurén and McDonald, 1985, 1987a) a stimulation of Na^+ efflux (Laurén and McDonald, 1985) and an inhibition of branchial Na^+-K^+ ATPase activity (Stagg and Shuttleworth, 1982; Laurén and McDonald, 1987b; Pelgrom et al., 1995; Kolok et al., 2002). These branchial changes can lead to an increase in net Na^+ loss (Croke and McDonald, 2002; Taylor et al., 2004), which can culminate in a significantly reduced concentration of whole-body Na^+ (Laurén and McDonald, 1987a; McGeer et al., 2000; Kolok et al., 2002). The damage phase is often followed by a repair phase during which Na^+ influx rates are restored (Laurén and McDonald, 1987a), efflux rates are reduced (Laurén and McDonald, 1987a), and Na^+-K^+ ATPase specific activity are adjusted levels that are consistent with (Laurén and McDonald, 1987b) or in excess of (McGeer et al., 2000) pre-exposure levels. As a consequence of these acclimatory changes, there can be a recovery of whole-body Na^+ to pre-exposure levels (Laurén and McDonald, 1987a; McGeer et al., 2000).

The damage and repair cycle detailed above appears to be necessary for the development of enhanced metal tolerance. Acclimation is thought to occur when the metal exposure is severe enough to cause gill damage, allowing for repair of damaged tissues and processes, but not severe enough to result in mortality (Booth et al., 1988; McDonald and Wood, 1993). Interestingly, to our knowledge, the dependence of enhanced metal tolerance on a cycle of branchial damage and repair has never been directly tested.

In this study, enhanced Cu tolerance was inferred from two quantifiable endpoints: mortality and whole-body Na^+ concentration. Reductions in mortality have been used previously to infer enhanced Cu tolerance in rainbow trout (Dixon and Sprague, 1981; Taylor et al., 2000; Hansen et al., 2002) and fathead minnows (Sellin et al., 2005). Likewise, the ability of fish to maintain physiologically normal levels of whole-body Na^+ has also been used to infer enhanced Cu tolerance in rainbow trout and fathead minnows (Laurén and McDonald, 1987a; Kolok et al., 2002; Croke and McDonald, 2002). Using these two endpoints, the primary objective of this study was to determine whether a significant damage and repair cycle is necessary for the development of enhanced Cu tolerance.

2. Methods

2.1. Fish maintenance

The fish used in this study were produced by the University of Nebraska at Omaha fathead minnow colony (Peake et al., 2004). For the two experiments discussed below, the fish were produced by a minimum of nine parental pairs. The offspring from each brood stock pair were evenly divided among the various treatment groups. After hatch, fish were raised for 2 months to an average mass of 0.5 g prior to being used in subsequent experiments. Minnows were maintained in aerated, dechlorinated tap water at room temperature (23 °C) and were kept under a photoperiod of 16:8 h light:dark. Minnows were fed to satiation daily using a high protein flake food (Aquatic Ecosystems, Apopka, FL, USA), and were fed during all experiments. Static renewal of water in the aquaria occurred daily. Renewal water had the following water quality (mean $\pm$ 1 S.E.): pH, 7.87 ± 0.17 ; conductivity, 573 ± 6.1 μS ; hardness, 166 ± 1.16 (expressed as mg/L CaCO_3); alkalinity, 99 ± 1 (expressed as mg/L CaCO_3).

2.2. Experimental design

Two experiments were conducted investigating the role of exposure concentration on the development of acclimation for Cu in juvenile fathead minnows. For each experiment, 500 fish were equally divided among the treatment groups in duplicate 45-L aquaria. Data from the duplicate treatments were pooled for statistical analysis.

In both experiments, fish were acclimated to aquaria either containing 0 µg/L Cu or to a geometric progression of nominal Cu doses (93, 150, 237, 375 µg/L Cu; log dose = 1.97, 2.17, 2.37 and 2.57, respectively) for 16 d. The exposure doses were chosen to bracket 150 µg/L Cu, an exposure dose where enhanced tolerance had previously been demonstrated (Sellin et al., 2005). In addition to these groups of fish, an additional control group was also included in the experiment.

The first experiment was a 16-d exposure (pre-exposure) that was immediately followed by an 8 d exposure to 375 µg/L Cu (Cu challenge). Prior to, and immediately after the Cu challenge, five fish were sampled from each duplicate tank for whole-body Na⁺ analysis. Dead fish were counted and removed from the 375 µg/L exposure tanks at 3, 6, and 12 h, and at 1, 2, 4, and 8 d. In this experiment, an additional group of fish (controls) was maintained in clean water throughout the pre-exposure and Cu challenges. These fish experienced no mortality throughout the experiment, and were sampled for whole body Na⁺.

In the second experiment, a fifth 16-d exposure dose (375 µg/L) was included. Eight fish (four from each of the duplicate exposure tanks) were sampled at 0, 3, 6, and 12 h, and at 1, 2, 4, 8, and 16 d intervals during the exposure regime. These fish were killed then analyzed for whole-body Na⁺.

2.3. Cu analysis

During the Cu exposures and the Cu challenge, a water exchange (>30%) was conducted daily by static renewal. A stock solution of CuSO₄ was used to manually dose all of the experimental tanks. Water samples (125 ml) were collected daily from each experimental aquarium. Each water sample was acidified with nitric acid to a pH of <2.0 and stored at 4 °C. Actual Cu concentrations were determined by flame atomic absorption spectrometry according to previously established methods (US EPA, 1983).

2.4. Whole-body Na⁺

Upon sampling, minnows were sacrificed using MS-222 (0.3 g/L), weighed, and placed in a 30 ml glass culture tube and digested in 5 ml concentrated nitric acid (Grippio and Dunson, 1991). The digested fish were then placed in a test tube heater and heated at approximately 100 °C until the solution had reduced by approximately 75%, then allowed to cool. Five millilitres of sulfuric acid was then added and the sample was brought back up to >100 °C until all fat and organic material was disassociated and the sample became clear. The sample was then diluted with nanopure water to 50 ml. Following dilution, a 0.5 ml samples was then removed from each fish sample and diluted in 9.5 ml of potassium chloride buffer solution (2200 ppm), then analyzed with a flame atomic absorption spectrometer.

2.5. Statistical analyses

All statistical analysis was performed using the statistical program JMP v. 5.0.1. Statistical significance throughout the experiments was based on $\alpha = 0.05$.

2.5.1. Experiment 1—Survival-time analysis was used to test the hypotheses regarding differences in survival between treatment groups (Dixon and Newman, 1991). The times at which individuals died during the lethal challenge were recorded (time-to-death). Minnows

alive at the end of the exposures were recorded as right-censored. During each experiment, all of the minnows in the control group were alive and therefore recorded as right-censored. Due to the lack of mortality in the control groups, they were not included in the survival-time analysis.

To analyze for differences in survival between groups, the time-to-death data were fit to distribution models (Weibull, log-normal or exponential) describing the relationship between survival and time of exposure to Cu. The various model distributions were compared, and the one with the greatest log-likelihood value was taken to be the model of best fit. The models included one covariate, treatment group. The effects of the covariate on survival time were tested by likelihood ratio tests, in which log-likelihoods of the model with and without the covariate were computed and the results were compared against a chi-square distribution. For each of the pre-exposure doses, differences in whole-body Na^+ were compared between fish collected immediately before and immediately after the Cu challenge using *t*-tests.

2.5.2. Experiment 2—Comparisons were made across time periods (0, 3, 6, 12, h and 1, 2, 4, 8, and 16 d) using time zero as a control. The data was statistically analyzed for each treatment with ANOVA followed by multiple pair-wise comparison tests (Tukey-Kramer HSD). The only multiple comparison test results discussed were those between time zero and the other time periods.

3. Results

3.1. Cu concentrations

The Cu concentrations in the controls and 0 $\mu\text{g/L}$ groups in both experiments were below detection limits (6 $\mu\text{g/L}$ Cu). The actual Cu concentrations in experiment 1 during the sublethal exposures averaged 73 $\mu\text{g/L}$ for the 93 $\mu\text{g/L}$ dose (78% of nominal), 118 $\mu\text{g/L}$ for the 150 $\mu\text{g/L}$ dose (78.9% of nominal), and 189 $\mu\text{g/L}$ for the 237 $\mu\text{g/L}$ dose (80% of nominal). The actual Cu concentrations in the Cu challenges ranged from 296 to 306 $\mu\text{g/L}$ Cu with an overall average of 302 $\mu\text{g/L}$ Cu. The actual concentrations in the aquaria during experiment 2 averaged 70 $\mu\text{g/L}$ for the 93 $\mu\text{g/L}$ dose (76% of nominal), 127 $\mu\text{g/L}$ for the 150 $\mu\text{g/L}$ dose (85% of nominal), 202 $\mu\text{g/L}$ for the 237 $\mu\text{g/L}$ dose (85% of nominal), and 289 $\mu\text{g/L}$ Cu for the 375 $\mu\text{g/L}$ dose (77% of nominal). Exposure doses throughout the results and discussion sections of this manuscript will focus on actual rather than nominal Cu concentrations.

3.2. Experiment 1

When exposed to the Cu challenge, significant differences were found in survivorship of the minnows among the fish from different pre-exposure Cu concentrations Cu ($\chi^2 = 28.5$, $P < 0.0001$; Fig. 1). Mortality of the fish pre-exposed to 0, 73, and 118 $\mu\text{g/L}$ concentrations of Cu was significantly greater than that found for the fish pre-exposed to 189 $\mu\text{g/L}$ ($P < 0.0029$, $P < 0.0001$, and $P < 0.0001$ respectively for each pair-wise comparison).

After the Cu challenge, the whole body Na^+ of surviving fish was compared to the whole body Na^+ before the Cu challenge. Fish from all groups experienced a significant reduction in whole body Na^+ , with the exception of the fish acclimated to 189 $\mu\text{g/L}$ Cu (Table 1). The percent reduction in whole body Na^+ exceeded 30% in the fish pre-exposed to 73 $\mu\text{g/L}$ Cu, but was only 13.8% in the fish pre-exposed to 189 $\mu\text{g/L}$ Cu. Furthermore, when the Cu challenged groups were compared to the pre-exposure controls (49.3 $\mu\text{mol/g}$ Na^+) all groups of fish experienced significant reductions in whole body Na^+ , with the exception of the fish pre-exposed to 189 $\mu\text{g/L}$ Cu (Table 1).

3.3. Experiment 2

With the exception of the 289 µg/L exposure group, mortality was minimal during experiment 2. The 0, 70, and 127 µg/L groups experienced no mortality, while 4 individuals died in the 202 µg/L group. In contrast, fish exposed to 289 µg/L experienced pronounced mortality as all but 3 individuals died during this exposure.

Significant differences in whole body Na⁺ occurred at various time intervals during the all of the exposure doses (ANOVA, $P < 0.002$ in all cases). Multiple comparisons between the time 0 group and all other time intervals, revealed significant differences in the 127, 202 and 289 exposure groups but not in the control or 70 µg/L groups. In the 127 µg/L group, significant reductions (relative to time zero) in whole body Na⁺ were found on day 2 (17% reduction) and on day 16 (22% reduction). In the 202 µg/L group, the mean whole-body Na⁺ at 2 and 4 d were significantly reduced relative to time zero (20 and 36% reduction, respectively). In the 289 µg/L Cu group, the mean whole body Na⁺ concentrations at 4 and 8 d were significantly reduced relative to time zero (38 and 36% reduction respectively).

4. Discussion

The experiments in this study were conducted to determine the range of Cu concentrations that elicit enhanced Cu tolerance in juvenile fathead minnows, and to determine if a significant damage phase obligatorily occurred in the fish that developed enhanced tolerance to Cu, but did not occur at the other Cu exposure doses. The results of this study clearly show that the ability to develop enhanced Cu tolerance is related to Cu dose in a very predictable, dose dependant manner (Table 2). Juvenile fathead minnows exposed to 0, 73, Cu for 16 d did not undergo any significant changes in whole body Na⁺ nor did they develop enhanced tolerance. At 118–127 µg/L Cu, the minnows experienced moderate damage (as exemplified by a 20% reduction in whole body Na⁺), moderate repair, but no enhanced Cu tolerance. Between 189 and 202 µg/L Cu, the fish experienced a significant damage phase followed by significant repair, which led to an enhanced Cu tolerance. At 289 µg/L, the fish underwent irreversible damage that was ultimately lethal.

4.1. The 118–127 µg/L Cu acclimation dose

The exposure dose of 118–127 µg/L Cu is of particular interest as the minnows experienced the cost of acclimation while reaping only minor benefit. Minnows at this dose, undergo a damage phase, as is evident from the whole body Na⁺ data from day 2 (Fig. 2). However this damage phase is not of the duration or magnitude of that which occurs when the fish are exposed to the 202 µg/L Cu, a dose at which the minnows develop enhanced Cu tolerance. The fish acclimated to 118–127 µg/L Cu must have undergone some degree of branchial repair, as the significant reduction in whole body Na⁺ is no longer evident by day 4. The repair however is not sufficient enough to prevent these fish from experiencing a significant reduction in whole body Na⁺ on day 16, nor does it convey enhanced survivorship or an enhanced ability to maintain whole body Na⁺ during a subsequent Cu challenge. Thus, it can be concluded that these fish have experienced significant branchial damage during the damage phase of acclimation while only reaping short-term benefits.

When the results from Sellin et al. (2005) are considered along with the results from the current study, the unique nature of the 113–127 µg/L Cu dose becomes more apparent. In that study, fathead minnows of the same age and size used in this study were acclimated to 113 µg/L Cu for 16 d, then were exposed to 333 µg/L Cu for 8 d. Following the copper challenge, the whole body Na⁺ of the surviving fish was dramatically reduced relative to unexposed controls. However, the acclimated minnows in Sellin et al. (2005), unlike the ones in the current study, had significantly less mortality during the Cu challenge. At doses from 113 to 127 µg/L Cu,

fathead minnows are at the lower edge of the window of acclimation. While these fish experience branchial damage, the subsequent repair to ionoregulatory function is not complete. Under a potentially lethal Cu challenge, the partial repair that had occurred during acclimation may (Sellin et al., 2005) or may not (this study) result in enhanced survivorship.

4.2. Whole body Na⁺ post Cu challenge

It is interesting that the results from post Cu challenge whole body Na⁺ did not directly match those related to post Cu challenge survivorship. Specifically, the fish acclimated to 189 µg/L Cu experienced enhanced survivorship during the Cu challenge, but were unable to maintain whole body Na⁺ relative to pre-Cu challenge individuals. The resulting reduction in whole body Na⁺ was less than 14%, and the pre-exposure fish had the highest mean value of any of the groups. Therefore it is questionable whether or not the observed significant reduction in whole body Na⁺ reflected a true alteration in net branchial Na⁺ flux. Indeed, when whole body Na⁺ of the post-challenge fish was compared to that of the pre-Cu challenge controls (Table 1), the significant difference for the fish acclimated to 189 µg/L Cu was no longer evident. Nevertheless, the precedent for enhanced survivorship during a Cu challenge without maintenance of whole body Na⁺ has already been established in the literature (Sellin et al., 2005). Changes in whole body Na⁺ appears to be a more sensitive indicator of post-acclimation exposure to Cu than whole-animal survivorship.

4.3. Branchial damage, repair and enhanced Cu tolerance

The experiments conducted for this project support the necessity of branchial damage followed by repair for the development of enhanced Cu tolerance. Results from this study also support the fact that minor damage (as occurred during the 16-d exposure to 118 µg/L Cu) was not sufficient enough to cause a long-lasting cellular repair that lead to enhanced Cu tolerance. Considering that the acclimatory response may involve hypertrophy and hyperplasia of chloride cells, as well as an increased expression of Na⁺-K⁺ ATPase (for a review see McDonald and Wood, 1993), it is clear that there is a major anabolic component to acclimation. Presumably, the acclimatory response is not engaged until there has been enough cellular damage to the gill to induce these anabolic processes. Results from this project support the fact that initiation of anabolism is only initiated after a relatively long and severe damage phase.

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References

- Benson WH, Birge WJ. Heavy metal tolerance and metallothionein induction in fathead minnows: results from field and laboratory investigations. *Environ. Toxicol. Chem* 1985;4:209–217.
- Booth CE, McDonald DG, Simons BP, Wood CM. Effects of aluminum and low pH on net ion fluxes and ion balance in the brook trout (*Salvelinus fontinalis*). *Can. J. Fish. Aquat. Sci* 1988;45:1563–1574.
- Croke SJ, McDonald DG. The further development of ionoregulatory measures as biomarkers of sensitivity and effect in fish species. *Environ. Toxicol. Chem* 2002;21:1683–1691. [PubMed: 12152770]
- Dixon GD, Sprague JB. Acclimation to copper by Rainbow Trout (*Salmo gairdneri*)—A modifying factor in toxicity. *Can. J. Fish. Aquat. Sci* 1981;38:880–888.

- Dixon, PM.; Newman, MC. Analyzing toxicity data using statistical models for time-to-death: an introduction.. In: Newman, MC.; McIntosh, AW., editors. Metal Ecotoxicology Concepts and Applications. Lewis; Chelsea, MI, USA: 1991. p. 207-242.
- Grippio RS, Dunson WA. Use of whole body sodium loss from the fathead minnow (*Pimephales promelas*) as an indicator of acid and metal toxicity. Arch. Environ. Contam. Toxicol 1991;21:289–296. [PubMed: 1958081]
- Hansen JA, Lipton J, Welsh PG, Morris J, Cacela D, Suedkamp MJ. Relationship between duration, tissue residues, growth, and mortality in rainbow trout (*Oncorhynchus mykiss*) juveniles sub-chronically exposed to copper. Aquat. Toxicol 2002;58:175–188. [PubMed: 12007873]
- Kolok AS, Hartman MM, Sershan J. The physiology of copper tolerance in fathead minnows: insight from an intraspecific, correlative analysis. Environ. Toxicol. Chem 2002;21:1730–1735. [PubMed: 12152776]
- Laurén DJ, McDonald DG. Effects of copper on branchial ionoregulation in the rainbow trout, *Salmo gairdneri* Richardson. J. Comp. Physiol. B 1985;155:635–644.
- Laurén DJ, McDonald DG. Acclimation to copper by rainbow trout, *Salmo gairdneri*: physiology. Can. J. Fish. Aquat. Sci 1987a;44:99–104.
- Laurén DJ, McDonald DG. Acclimation to copper by rainbow trout, *Salmo gairdneri*: biochemistry. Can. J. Fish. Aquat. Sci 1987b;44:105–111.
- Marr JCA, Bergman HL, Lipton J, Hogstrand C. Differences in relative sensitivity of naïve and metals-acclimated brown and rainbow trout exposed to metals representative of the Clark Fork River, Montana. Can. J. Fish. Aquat. Sci 1995;52:2016–2030.
- McDonald, DG.; Wood, CM. Branchial mechanisms of acclimation to metals in freshwater fish.. In: Rankin, JC.; Jensen, FB., editors. Fish Eco-physiology. Chapman and Hall; London, UK: 1993. p. 297-321.
- McGeer JC, Szebedinszky C, McDonald DG, Wood CM. Effects of chronic sublethal exposure to waterborne Cu, Cd, or Zn in rainbow trout. 1: Iono-regulatory disturbance and metabolic costs. Aquat. Toxicol 2000;50:231–243. [PubMed: 10958957]
- Peake EB, Locke JC, Tierney LL, Kolok AS. Copper tolerance in fathead minnows: II. Maternal transfer. Environ. Toxicol. Chem 2004;23:208–211. [PubMed: 14768887]
- Pelgrom SMGJ, Lock RAC, Balm PHM, Wendelaar Bonga SE. Integrated physiological response of tilapia, *Oreochromis mossambicus*, to sublethal copper exposure. Aquat. Toxicol 1995;32:303–320.
- Sellin MK, Tate-Boldt EK, Kolok AS. Acclimation to Cu in fat-head minnows: does age influence the response? Aquat. Toxicol 2005;74:96–107.
- Stagg RM, Shuttleworth TJ. The effects of copper on ionic regulation by the gills of the seawater-adapted flounder (*Platichthys flesus* L.). J. Comp. Physiol 1982;149:83–90.
- Taylor LN, McGeer JC, Wood CM, McDonald DG. Physiological effects of chronic copper exposure to rainbow trout (*Oncorhynchus mykiss*) in hard and soft water: evaluation of chronic indicators. Environ. Toxicol. Chem 2000;19:2298–2308.
- Taylor LN, McFarlane WJ, Pyle GG, Couture P, McDonald DG. Use of performance indicators in evaluating chronic metal exposure in wild yellow perch (*Perca flavescens*). Aquat. Toxicol 2004;67:371–385. [PubMed: 15084413]
- U.S. Environmental Protection Agency. Methods for chemical analysis of water and wastes. Cincinnati, OH: 1983. EPA/600/4-79/20

Fig. 1. Cumulative survival of juvenile fathead minnows during an 8 d lethal exposure to 302 µg/L Cu. Fish were pre-exposed to 0, 73, 118, and 189 µg/L Cu for 16 d. $N = 100$ at the beginning of the lethal exposure.

Fig. 2. Whole-body Na⁺ of juvenile fathead minnows exposed to 0, 70, 127, 202, and 289 µg/L of Cu for 16 d. Asterisks indicate significant difference from time zero. Error bars indicate ± 1 standard error. The dashed line in each graph represents the mean whole-body Na⁺ over 16-d of the 0 µg/L Cu treatment group.

Table 1Whole-body Na⁺ (mean $\pm$ 1 S.E.) of juvenile fathead minnows following an 8 d exposure to 302 $\mu\text{g/L}$ Cu

Pre-exposure ($\mu\text{g/L}$)	Whole-body Na ⁺ (pre Cu challenge) ($\mu\text{mol/g}$)	Whole-body Na ⁺ (post Cu challenge) ($\mu\text{mol/g}$)	Percent reduction
Control	49.3 $\pm$ 2.2	45.1 $\pm$ 1.1	8.6
0	49.0 $\pm$ 1.6	41.7 $\pm$ 2.2* ⁺	14.8
73	52.1 $\pm$ 2.8	35.2 $\pm$ 2.2* ⁺	32.5
118	50.3 $\pm$ 2.3	40.5 $\pm$ 2.5* ⁺	19.6
189	54.9 $\pm$ 1.4	47.4 $\pm$ 2.8*	13.8

Fish were pre-exposed to 0, 73, 118, and 189 $\mu\text{g/L}$ of Cu for 16 d. Control group = fish unexposed to the Cu challenge. Sample size = 10 in all cases. An (*) designates significant differences between pre-challenged and post-challenged fish, an + designates significant differences between the pre Cu challenge controls (49.3 $\pm$ 2.2) and the post-challenged fish.

Table 2
A tabular summary of the results from the experiments 1 and 2, along with the results from Sellin et al. (2005) (as designated with an *)

Acclimation dose (µg/L)	16 d acclimation			Cu challenge		Conclusion
	Damage	Repair	Day 16-maintained whole body Na+	Enhanced survivorship	Day 8-maintained whole body Na+	
70–73	–	–	+	–	–	No damage, no repair
113–127	+	+	–	–/+*	–/+*	Damage, unsustainable repair
189–202	+	+	+	+	–	Damage, repair, enhanced Cu tolerance
289	+	–	–			Irreparable damage