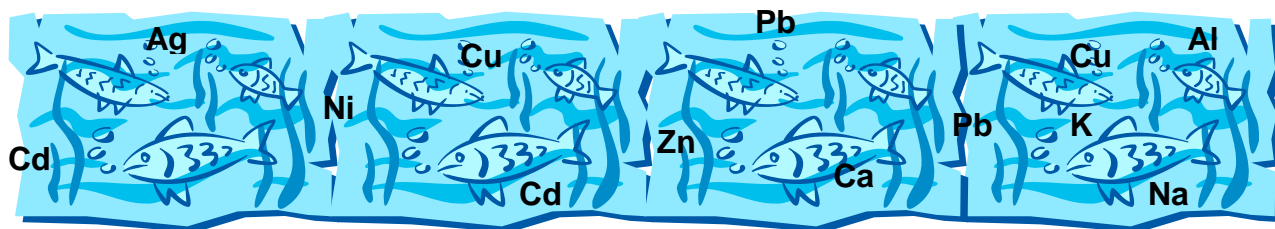


NSERC – Industry Project on Metal Bioavailability Research Newsletter



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Wilfrid Laurier & McMaster University

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NEWS

Good day to one and all. It's been a few months since the last newsletter, so we're due an update. The plan is to produce this newsletter a little more frequently, maybe a few times a year, which will make it more of a bite-size morsel of scientific goodness, rather than a whole hearty meal.

The aim of this newsletter is to report on people and the projects they are working on. The star of this newsletter is **Margaret Tellis**, who will be sharing some recent work from her M. Sc.

Comings and goings (Nov. 2011 – May 2012):

Rachael Diamond has just (April) finished her M. Sc. Thesis entitled "Characterizing Dissolved Organic Matter and Cu^{2+} , Zn^{2+} , Ni^{2+} , and Pb^{2+} Binding in Salt Water and Implications for Toxicity". She is now working as an analytical chemist at Germiphene in Brantford ON. **Jocelyn Schaefer** and **Amanda Johnston** have completed their 4th year Honours Theses, "Determination of Free Lead in Seawater using an Ion-Selective Electrode" and "Copper Complexation to Sodium Channel Blocking Drugs Furosemide and Amiloride: A Spectroscopic Study", respectively. Amanda is now doing an M. Sc. in Biochemistry at the University of Toronto.

Scott Smith has asked me to put a call out there to see if any potential M. Sc. and Ph. D. students are willing to continue Amanda's and Jocelyn's stellar work.

In November **Jessie Cunningham** completed her M. Sc. Thesis, "The effect of

chronic Cd exposure on repeat swim performance and recovery in rainbow trout, brown trout and lake whitefish". Two more M. Sc. students have graduated from the McGeer lab since then: **Gurki Malhi** (Chronic toxicity of titanium dioxide nanoparticles to *Hyaella azteca*) in December and **Nish Pais** (Studies on waterborne Cd exposure to *Lymnaea stagnalis*, effect of water chemistry and the development of a novel tissue residue approach" in January.

At McMaster University **Derek Alsop** has completed a PDF in **Chris Wood's** lab where he studied "Interactions of waterborne and dietary Pb in rainbow trout". He is currently working on P450 enzymes in zebrafish as a PDF with **Dr. Joanna Wilson**, also at McMaster. **Usman Yousaf** has completed a 4th year Honours Thesis that involved "Examining the influence of dissolved organic matter (DOM) on sodium transport in *Daphnia magna*: whole body sodium (Na^+) content and unidirectional flux". In **Grant McClelland's** lab, **Victoria Ransberry** is now 1 year into her M. Sc. project entitled "Oxidative stress response of hypoxia and Cu-exposed killifish", and **Daniel Li** has completed his 4th year Honours project on "The effects of acute hypoxia and copper exposure on gill morphometry and gene expression in the common killifish". In addition, **Narina Jabari** just finished a 4 month research project on "Physiological effects of copper and hypoxia exposure in the common Killifish as measured through oxygen-dependent gene expression". She is

currently working at UBC with **Dolph Schluter** for the summer before returning to

Grant's lab to complete a 4th year honours project.

New Funding:

- **Grant McClelland** successfully acquired one of the last NSERC RTI grants for “Imaging system for environmental physiology research” (\$49,265).
- **Jim McGeer** and **Scott Smith** have received funding from Environment Canada to study toxicity and geochemistry of lanthanide elements (\$30,000 per year for 5 years).
- **Jim McGeer, Steve Kokelj** (and 10 other people) have A NWT Cumulative Impact Monitoring Program grant entitled “A Watershed Approach to Monitoring Cumulative Impacts of Landscape Change” (\$540,000 over 5 years).
- **Greg Pyle** (Lakehead University), **Patrice Couture** (INRS), and **Chris Wood** were awarded a 1-year Environment Canada-Metals in the Environment grant to study metal mixture effects on fish. The award is potentially renewable for up to 5 years.
- **Chris Wood** was awarded a 5-year NSERC Discovery grant, 2011-2015, “Transport and metabolism in fish”.

Construction has also begun on the **Centre for Cold Regions and Water Science** slated to open in March 2013 (architect's rendering pictured right). The building will have a gross floor area of about 14,000 square feet and will cost about \$6.2 million, with another \$5.6 million budgeted for equipment. The two-storey facility is being funded, in part, by the Canada Foundation for Innovation and the Southern Ontario Water Consortium (SOWC), which is made up of seven universities, including plus private sector companies, municipalities, and not-for-profit organizations.



Upcoming Conferences and Meetings:

Rochester Meeting – 31st May, 2012:

A meeting of academics, students and project partners associated with Scott Smith's NSERC CRD “marine & estuarine BLM” project. Morning session primarily focuses on Cu, whereas the afternoon session is more Zn and Ni orientated.

Vale Meeting – 6th June, 2012:

The ‘2nd Vale Base Metals Sustainability Research Symposium’ is also taking place June 6th, Vale Canada's offices at 200 Bay St., Toronto

Woodstock 2012 (SEB Animal Biology Symposium 2012) and the Society for Experimental Biology (SEB) Annual Meeting – 23rd June to 2nd July, 2012:

“Woodstock 2012” is a tribute to Prof. Chris Wood and takes place at Abbazia di Spineto, an 11th century monastery located in the heart of Tuscany, Italy. Straight afterwards, SEB is taking place in Salzburg so a number of people are making the 2 events as one. Here are a few of the presentations being given from our group:

- **Bianchini, A., Jorge, M. B., Nogueira, L. S., Lauer, M. M., Tellis, M. S., Klinck, J., Nadella, S. and Wood, C. M.** Calcium uptake in marine invertebrate larvae: Effects of metals and dissolved organic matter. Woodstock 2012, 23rd June-27th June 2012. Tuscany, Italy.
- **Blewett, T. and Wood, C. M.** Mechanisms of nickel toxicity using oxidative stress endpoints in the euryhaline teleost *Fundulus heteroclitus*. Woodstock 2012, 23rd June-27th June 2012. Tuscany, Italy.
- **Blewett, T., Robertson, L., MacLatchy, D. and Wood, C. M.** Effect of Metabolic Rate on EE2 Uptake in *Fundulus heteroclitus*. Society of Experimental Biology Annual Meeting 29th June-2nd July, 2012. Salzburg, Austria.
- **Niyogi, S., Nadella, S. and Wood, C. M.** Interactive effects of waterborne metals in binary mixtures on short-term metal and ion uptake in rainbow trout (*Oncorhynchus mykiss*). Woodstock 2012, 23rd June-27th June 2012. Tuscany, Italy.
- **Zimmer, A., Barcarolli, I., Wood, C. M. and Bianchini, A.** Mechanisms of copper-induced inhibition of ammonia excretion and sodium uptake in fish. Society of Experimental Biology Annual Meeting, 29th June-2nd July, 2012. Salzburg, Austria.



The 33rd Annual SETAC North America Meeting takes place at Long Beach, California, from 11th – 15th November, 2012:

There is a session dedicated to ‘**Fate and Effects of Metals: Marine Concerns**’, chaired by the good Dr. Smith. Although some platform presentations have already been confirmed, more platform and poster submissions are still needed. Interested individuals are encouraged to submit to the session (deadline for submissions is June 1st). Here are the session details:

Track: Terrestrial or Wildlife Toxicology and Ecology.

Session: Fate and Effects of Metals: Marine Concerns.

“Metal bioavailability approaches, such as the Biotic Ligand Model, have been successful in establishing Ambient Water Quality Criteria for freshwater environments. Using bioavailability methodologies, appropriate and protective regulatory criteria can be established in a site-specific manner. The majority of water on this planet is not fresh water though. The chemistry and biology of both metals and organisms change as salinity increases. This session will focus on research relevant to assessing the utility of metal bioavailability frameworks for regulatory purposes at elevated salinities (estuarine to full seawater). It is estimated that 70% of the world's population live in coastal environments and metal impacts in saltwater environments are potentially significant, with sources from municipal/industrial effluent, building material runoff, plumbing, anti-fouling paints and sometimes directly from mines and auxiliary facilities operating in coastal areas. To date, saltwater environments have not received as much research attention as freshwater environments and this session aims to pull together experts in the field and summarize the state of the art in understanding saltwater metal bioavailability. Relevant topics include basic saltwater-specific bioavailability research, physiological mechanisms, chemistry (speciation), role of organic matter, salinity as well as toxicity test species to expand the species sensitivity distribution in estuarine and marine environments.”

Other presentations (Nov. 2011 – May 2012):

- **Blewett, T., Wood, C. M.** (2012) Mechanisms of nickel toxicity using oxidative stress endpoints in the euryhaline teleost *Fundulus heteroclitus*. Canadian Society of Zoology. Sackville, NB.
- **Chan, K., McGeer, J. C.** (2012). The effects of Ca²⁺, Mg²⁺, and NOM quantity and quality on Ni toxicity to *Hyalella azteca*. 3rd Annual WLU-UW World Water Day Graduate Student Symposium, Waterloo, ON.
- **Cooper, C. A., Tait, T., McGeer, J. C., Smith, D. S.** (2012). Influence of salinity and DOC on acute Cu toxicity to the rotifer *Brachionus plicatilis*. Gananoque Conference of Environmental Science and Engineering. Gananoque, ON.
- **Cooper, C. A., Tait, T., McGeer, J. C., Smith, D. S.** (2012). Influence of salinity and DOC on acute Cu toxicity to the rotifer *Brachionus plicatilis*. Canadian Society of Zoology. Sackville, NB.
- **Costa, E.-J., McGeer, J. C.** (2012). Nano-silver toxicity in *Daphnia pulex*: determining the role of ionic silver. 3rd Annual WLU-UW World Water Day Graduate Student Symposium, Waterloo, ON.
- **Cunningham, J., McGeer, J. C.** (2012). The effects of cadmium exposure and recovery time on repeat swimming performance in rainbow trout (*Oncorhynchus mykiss*). Gananoque Conference of Environmental Science and Engineering. Gananoque, ON.
- **Cunningham, J., Nasir, R., McGeer, J. C.** (2012). Influence of salinity and DOC on acute Cu toxicity to the euryhaline shrimp *Mysidopsis bahia*. Canadian Society of Zoology. Sackville, NB.
- **Diamond, R., Smith, D. S.** (2012). Characterizing Dissolved Organic Matter and Cu²⁺, Zn²⁺, Ni²⁺, and Pb²⁺ Binding in Salt Water and Implications for Toxicity. Gananoque Conference of Environmental Science and Engineering. Gananoque, ON.
- **Ellis, J., McGeer, J. C.** (2012). The effect of chronic nickel exposure on the sustained swimming ability of rainbow trout (*Oncorhynchus mykiss*). 3rd Annual WLU-UW World Water Day Graduate Student Symposium, Waterloo, ON.

- **Ellis, J., McGeer, J. C.** (2012). The effect of chronic nickel exposure on bioaccumulation and swim performance in rainbow trout (*Oncorhynchus mykiss*). Gananoque Conference of Environmental Science and Engineering. Gananoque, ON.
- **Johnston, A., Smith, D. S.** (2012). Copper Complexation to Sodium Channel Blocking Drugs Furosemide and Amiloride: A Spectroscopic Study. Gananoque Conference of Environmental Science and Engineering. Gananoque, ON.
- **Livingstone, K., McGeer, J. C.** (2012). Effect of natural organic matter quantity and quality on the acute toxicity of Cu to the aquatic amphipod *Hyalella azteca*. 3rd Annual WLU-UW World Water Day Graduate Student Symposium, Waterloo, ON.
- **Livingstone, K., McGeer, J. C.** (2012). Physiochemical and biological characterization of the protective effects of natural organic matter source. Gananoque Conference of Environmental Science and Engineering. Gananoque, ON.
- **Nasir, R., Cunningham, J., McGeer, J. C.** (2012). The effects of salinity on the acute copper toxicity to the mysid shrimp (*Mysidopsis bahia*). Canadian Society of Zoology. Sackville, NB.
- **Ransberry, V. E., McClelland, G. B.** (2012). Effects of waterborne copper and hypoxia on the oxidative stress response and gene expression in killifish (*Fundulus heteroclitus*). Canadian Society of Zoology. Sackville, NB.
- **Ransberry, V. E., McClelland, G. B.** (2012). Effects of waterborne copper and hypoxia on the oxidative stress response in killifish (*Fundulus heteroclitus*). Annual Comparative Physiology and Biochemistry Workshop, Elmhirst's Resort. Keene, ON.
- **Tait, T., Smith, D.S.** (2012). Application of cupric ion selective electrode in salt water. 3rd Annual WLU-UW World Water Day Graduate Student Symposium, Waterloo, ON.

The following peer reviewed papers and book chapters were published by the Metals Bioavailability Group (Nov. 2011 – May 2012):

- **Al-Reasi, H. A., Smith, D. S., Wood, C. M.** (2012). Evaluating the ameliorative effect of natural dissolved organic matter (DOM) quality on copper toxicity to *Daphnia magna*: Improving the BLM. Ecotoxicology 21: 524-537.
- **Blewett, T., MacLatchy, D., Wood, C. M.** (2012). The effects of temperature and salinity on 17- α -ethynylestradiol uptake and its relationship to oxygen consumption in the model euryhaline teleost (*Fundulus heteroclitus*). Aquat. Tox. [IN PRESS].
- **Khan, F. R., Keller, W., Yan, N. D., Welsh, P. G., Wood, C. M., McGeer, J. C.** (2012). Application of biotic ligand and toxic unit modelling approaches to predict improvements in zooplankton species richness in smelter-damaged lakes near Sudbury, Ontario. Environ. Sci. Technol. 46: 1641-1649.
- **Loro, V. L., Jorge, M. B., Rios da Silva, K., Wood C. M.** (2012). Oxidative stress parameters and antioxidant response to sublethal waterborne zinc in a euryhaline teleost *Fundulus heteroclitus*: protective effects of salinity. Aquat. Tox. 111: 187-193.
- **McGeer, J. C., Niyogi, S., Smith, D. S.** (2011). Cadmium. In Homeostasis and Toxicology of Non-Essential Metals, C. Wood, Ed., vol. 31B. Academic Press 126-168.
- **McGeer, J. C., Smith, D. S., Brix, K. V., Adams, W. J.** (2012). Speciation of metals and its application in biotic ligand modelling to understand effects in aquatic biota. In Encyclopedia of Sustainability Science and Technology, G. Bird, Ed. Springer, [IN PRESS].

- Ng, T. Y. T., Pais, N. M., Dhaliwal, T., Wood, C. M. (2012). Use of whole-body and subcellular Cu residues of *Lumbriculus variegatus* to predict waterborne Cu toxicity to both *L. variegatus* and *Chironomus riparius* in fresh water. *Chemosphere* 87: 1208-1214.
- Wood, C. M., Al-Reasi, H., Smith, D. S. (2011). The two faces of DOC. *Aquat. Tox.* 105: 3-8.

Research Highlight:

Mechanistic Analysis of the Chronic Toxic Effects of Pb, Zn, Cu and Ni on the Embryonic and Larval Stages of Development in Sea Urchins

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Introduction

Across the globe, metals enter marine ecosystems through various anthropogenic and natural modes. At high concentrations metals, including Pb, Zn, Cu and Ni are known to be extremely toxic to aquatic organisms, reducing their general survival as well as hampering larval development.

While extensive research exists on the effects of Pb, Zn, Cu and Ni in freshwater environments (e.g. Wang, 1987; Naimo, 1995), there is limited research on the effects of these metals in marine and estuarine environments (Supanopas et al., 2005; Franca et al., 2005; Kobayashi and Okamura, 2004; Radenac et al., 2001). There is therefore a degree of uncertainty surrounding current marine environmental guidelines and more research needs to be performed in this area.

An important factor in reducing uncertainty surrounding environmental regulations is to account for the influence of assimilation and metabolism of contaminants by aquatic organisms. Sea urchin embryonic bioassays in particular have been historically utilized as a means of monitoring water quality (Kobayashi, 1971). For the purposes of the current research, the rapidly developing sea urchin is an ideal test organism as it allows us to study the effects of these metals on different cellular processes as they appear in the developing embryo. This is an effective way of pinpointing mechanisms of toxicity. Mechanisms of toxicity, such as sub-lethal

effects on molecular and biochemical processes, are important factors to consider when developing policy. They shed light on more sensitive endpoints, which can be detected earlier and at lower concentrations than traditional endpoints such as mortality and inhibited growth.

The objective of the current research was to elucidate the mechanisms of toxicity of Pb and Zn periodically over the first 96 hours of development of the purple sea urchin (*Stroglyocentrotus purpuratus*). Our research focused specifically on Ca homeostasis as it was hypothesized that it would be a target of metal toxicity considering the antagonistic relationship of metals with Ca uptake in freshwater animals (e.g. Rogers et al., 2004; Spry and Wood, 1985). To test this hypothesis, a variety of endpoints of Ca homeostasis were analyzed including unidirectional Ca uptake in acute and chronic Pb, Zn, Cu and Ni exposures, and Ca accumulation and Ca ATPase activity in chronic Pb, Zn, Ni and Cu exposures. Regulation of Na, K, and Mg concentrations was also monitored in Pb and Zn exposed larvae. Through our research we hope to learn more about the different effects of the metals on each stage of early development.

Results and Discussion

Analysis of various biomarkers over embryonic development determined that Pb, Zn, Cu and Ni render their toxic action, at

least in part, through ionoregulatory disruption. Toxic effects of these metals were particularly detrimental to Ca homeostasis.

Inhibition of Ca uptake over 96 hours of development

Ca uptake by the developing sea urchin showed an interesting pattern over 96 h development depending on the differing Ca requirements of each developmental stage. There were no direct competitive effects of Pb, Zn, Cu and Ni on Ca uptake, evident from a lack of Ca uptake inhibition in larvae acutely exposed to a range of metals, including much higher concentrations than used in the chronic tests, at each stage of development (Acute exposure series - Pb: 75, 150 and 300 µg/L; Zn: 150, 300 and 600 µg/L; Cu: 10, 20 and 40 µg/L and Ni: 75, 150 and 300 µg/L - data not shown).

However, in larvae chronically exposed to Pb (54 µg/L), Zn (140 µg/L), Cu (6 µg/L) and Ni (47 µg/L), Ca uptake inhibition was observed at several time points over 96 h of development (Figure 1 a, b, c and d). Interestingly, these chronically exposed larvae mainly displayed inhibition of Ca uptake during those developmental stages when Ca uptake rates were highest. This could indicate that the Ca channels were compromised and were unable to meet the increased Ca demands during these stages (Figure 1a, b, c and d).

All metals in the chronic exposures caused greatest inhibition of Ca uptake at the gastrulation stage. This stage of development is known to be an especially critical and vulnerable landmark in development. On completion of this developmental stage, three germ layers (ectoderm, mesoderm and endoderm) and a rudimentary gut are formed and skeletogenesis is initiated. Understandably, abnormalities at this phase often result in complications in later development of the skeleton (Yaroslavtseva and Sergeeva, 2002).

One reason why the gastrulation stage might display such sensitivity to metal toxicity is that the maternal reserves of metallothioneins are depleted by this time. Metallothioneins are a group of low molecular weight proteins, which protect organisms by virtue of their high affinity for metals. Their affinity for metals is a result of their cysteine rich content (Warnau et al., 1996). A decrease in available metallothioneins at gastrulation might leave the embryos vulnerable until an increase in de novo synthesis of these proteins is initiated (Warnau et al., 1996). Newly synthesized metallothioneins may also be more effective in their protective role against metals, as they have not been previously exposed to metals as the maternal metallothioneins might have been. Evidence for this is seen in a return to normal of Ca levels in Pb, Cu and Ni exposed larvae in stages after gastrulation (Figure 2b, c and d) as well as a return to normal of Ca ATPase levels in Cu and Ni exposed larvae as discussed later (data not shown).

Calcium was the only ion out of the four nutritive ions measured that increased in whole body concentration consistently over the 96 h development period under control conditions (Figure 2). This underscores the importance of measuring Ca uptake, as it is obvious that Ca has a significant role in sea urchin larval development. A disruption of Ca homeostasis therefore significantly hinders normal sea urchin larval development and is an important mechanism of toxicity.

Calcium uptake rates in control larvae over time corresponded with whole body Ca accumulation over time. However, there was a latent period in between the increase in Ca uptake rate and the increase in whole body Ca content as the ion presumably required time to accumulate. Similarly, there was a latent period in between inhibition of Ca uptake by metal exposure and lower levels of Ca accumulated in the metal exposed larvae (Figures 1 and 2).

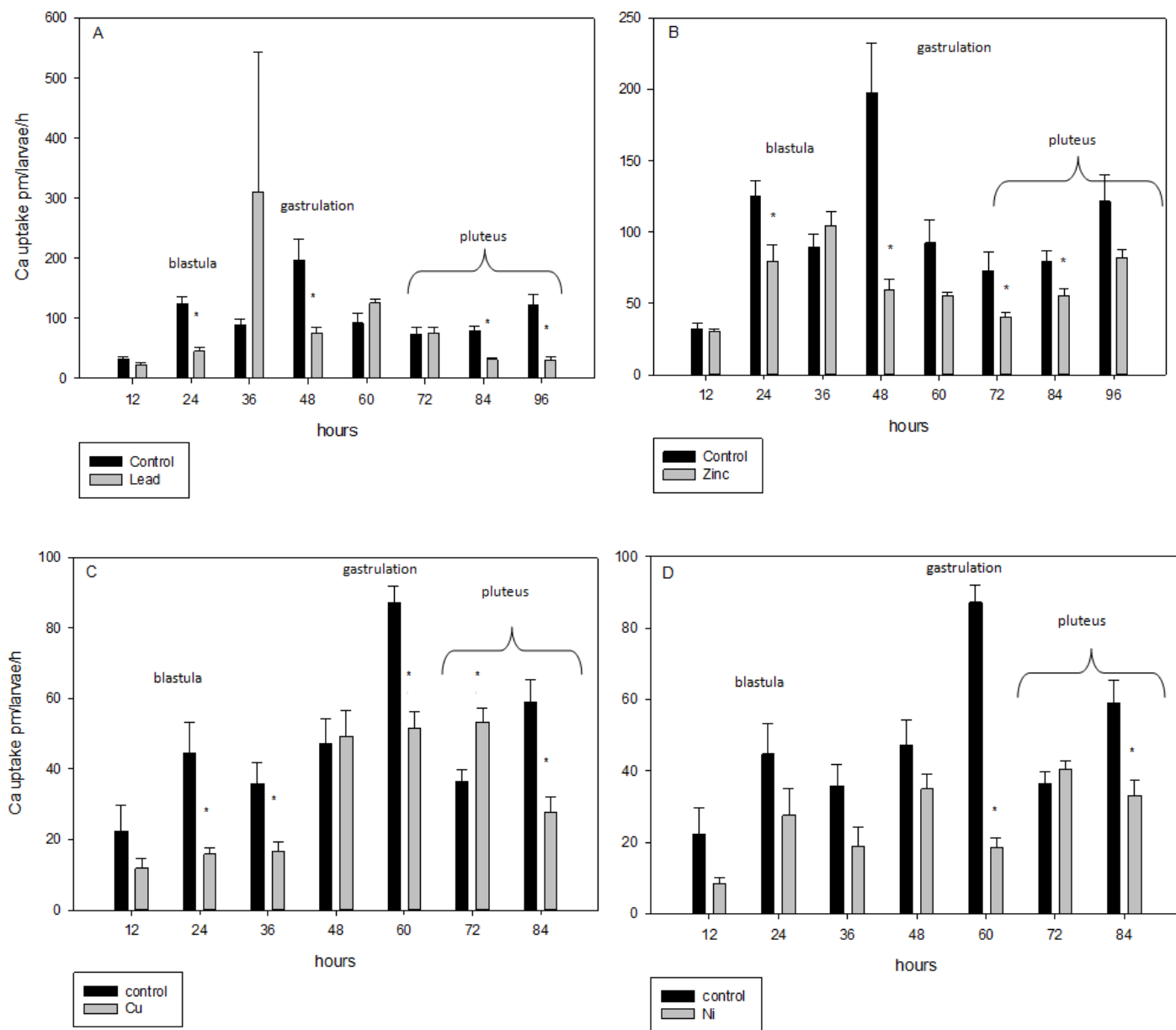


Figure 1 Unidirectional Ca uptake rates, measured every 12 hours over the first 84 or 96 hours of development in A) chronically Pb exposed larvae (54 µg/L); B) chronically Zn (140 µg/L) exposed larvae; C) chronically Cu (6 µg/L) exposed larvae; D) chronically Ni (47 µg/L) exposed larvae. An asterisk (*) indicates a significant difference from control levels as determined with a Student's t-test ($P < 0.05$). $N = 6$; values are means \pm SEM.

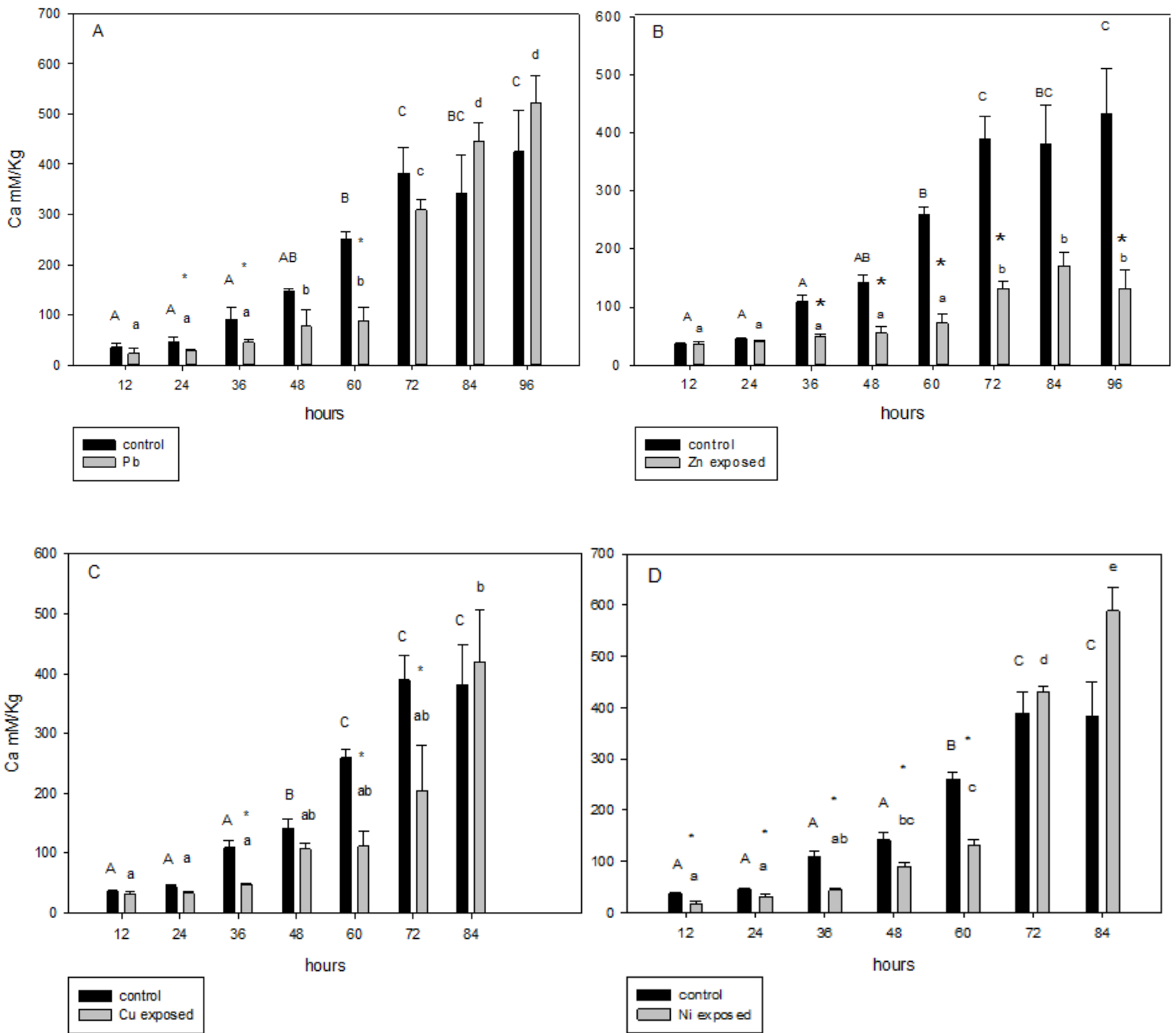


Figure 2. Whole body Ca levels in larvae exposed to A) Pb (54 µg/L); B) Zn (140 µg/L); C) Cu (6 µg/L) and D) Ni (47 µg/L) over 84 or 96 h of development. An asterisk (*) indicates a significant difference from control levels at the same time point as determined with a Student's t-test (P<0.05). Values with different letters are significantly different as determined by an ANOVA followed by Fisher LSD post hoc. Letters of different cases indicate comparisons within treatments; upper case letters represent comparison to controls and lower case letters represent comparison between treatments. N = 5; values are means ± SEM.

Our current research suggests inhibition of Ca^{2+} ATPase activity as a possible mechanism of toxicity of chronic Zn, Cu and Ni exposure. Ca^{2+} ATPase activity was significantly lower than in controls at various time points over development in Zn, Cu and Ni exposed larvae (data not shown). Pb exposed larvae however showed an upregulation of Ca^{2+} ATPase at 48 h and 60 h (data not shown). This could have contributed to the return to normal of Ca uptake rates at 60 and 72 h (Figure 1a) and normal Ca levels at 48 h and after 72 h (Figure 2a).

What is apparent from the Ca uptake flux experiments and the Ca concentration measurements in larvae over development is that larvae possess some capacity to recover from metal stress. This is because Ca uptake rates and Ca concentrations periodically

return to normal at various time points over the metal exposure even though the larvae have evidently suffered toxic injury from metal stress. For example, by the end of the experimental period Pb, Cu and Ni exposed larvae had regained normal calcium levels (Figure 2a, c and d). Ca ATPase levels also returned to normal in Cu and Ni exposed larvae during the later stages of development (data not shown). This leads us to believe that toxic action of the metal might occur during the early hours of development, after which the larvae undergo some damage repair. However, this does not exclude the fact that irreversible damage to development might be occurring during early key developmental

stages, which might not be apparent through measuring only Ca levels at 72 hours of development – i.e. other key metabolic processes may be disturbed.

Conclusion

Our findings suggest that toxicity of Pb, Zn, Cu and Ni stems, at least in part, from a disruption of Ca homeostasis during early development of embryos and larvae. However, the acute metal challenge experiments demonstrated that this was not by a direct competition of Pb or Zn for the Ca uptake mechanism. Interestingly, the larvae display some capacity for recovery from toxicant stress as is evident from the return to normalcy of Ca uptake rates, Ca accumulation and Ca ATPase activity periodically over development. Although there is some damage repair evident, other toxic effects may still be apparent from measuring biological endpoints not analyzed in the present study. Additionally, compensatory mechanisms employed to combat metal toxicity may also have effects on the health of urchins later in their lives.

Toxic effects of metal exposure seen at earlier time points during development were sometimes not apparent at 72 h of development, the endpoint of standard toxicity tests used routinely in environmental monitoring. We propose measuring endpoints of toxicity periodically over early development as a more effective way of studying the toxic stress of contaminants.

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An amoeba with a French Horn: @.

And one with chopsticks: \./