The effects of winter cold on acute and chronic cadmium bioaccumulation and toxicity in the banded killifish *(Fundulus diaphanus*).

by

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ABSTRACT

Metals such as cadmium (Cd) are important contaminants in fish. Fish experience seasonal temperature fluctuations that can potentially affect their exposure and sensitivity to metals. However, temperature effects are overlooked in ecotoxicology studies, especially for cold temperatures. I investigated the effects of cold on Cd bioaccumulation and toxicity in a freshwater fish, the banded killifish (*Fundulus diaphanus)*. Killifish were gradually acclimated to either 4°C or 14°C over an 11-week period, then exposed to 0, 0.5 or 5 µg Cd L^{-1} for 28 d at both temperatures. At day 2, 5 and 28, I measured Cd bioaccumulation and markers of oxidative and ionoregulation stress. Cadmium accumulation increased over time, and was typically lower in cold-acclimated fish. In agreement with the higher Cd bioaccumulation, Cd toxicity was generally higher in warm-acclimated fish. There was little evidence that cold and warm-acclimated fish displayed differences in their sensitivity to Cd. Overall, our study suggests that cold does not exacerbate the effects of Cd on banded killifish.

DEDICATION

To Rex, my puppy and friend for over a decade, who I lost while away doing this thesis. I could always count on you to come say goodnight after late nights and comfort me during stressful times. I still cannot believe that you are truly gone but I am so thankful for the time I had with you. Thank you for all those years of joy and love.

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Table of Contents

List of Tables

List of Figures

Symbols and Abbreviations

α.....Significance level

AIC.....Akaike Information Criterion

AICc.....Akaike Information Criterion corrected for sample size

ANOVA.....Analysis of variance

CAT.....Catalase

CRW.....Certified reference water

deCl.....Dechlorinated

df.....Degrees of freedom

DOC.....Dissolved organic carbon

ε.....Extinction coefficient

FCF.....Fulton condition factor

HSI.....Hepatosomatic index

ICP-OES.....Inductively coupled plasma - optical emission spectrometry

MDA.....Malondialdehyde

n.....Sample size

PC.....Protein carbonyls

ROS.....Reactive oxygen species

rpm.....Rotations per minute

SEM.....Standard error

UV.....Ultraviolet

wt.....Weight

1. INTRODUCTION

1.1. Cadmium in the freshwater environment

Cadmium (Cd) is a trace element that is introduced into the biosphere either naturally or through anthropogenic sources (Cullen and Maldonado, 2013; McGeer et al., 2012). Anthropogenic release of Cd occurs mainly indirectly as a result of metal mining and fossil fuel combustion (Cullen and Maldonado, 2013). Cadmium is typically found at low concentrations in freshwater systems and is rarely measured at dissolved concentrations >0.5µg L^{-1} in Canada (Environment and Climate Change Canada, 2021). In fact, the typical Cd concentration range in uncontaminated freshwater systems is between 0.01 and 0.1 µg L^{-1} (Bjerregaard and Andersen, 2007). However, although uncommon, elevated levels (e.g. up to 1000µg L^{-1}) can occur in polluted freshwater systems, such as those impacted by mining (Bjerregaard and Andersen, 2007; Boyle and Jonasson, 1979). Cadmium is considered to be a non-essential element in vertebrates (McGeer et al., 2012). It is amongst the most toxic trace elements to aquatic organisms (Kolarova and Napiórkowski, 2021) and is designated as a priority substance in Canada, due to its potential to cause harmful effects (Health Canada, 2004). Indeed, Cd can be toxic to aquatic organisms at relatively low concentrations and short exposure periods, especially for salmonids (Cusimano et al., 1986; Franklin et al., 2005; Hansen et al., 2002). As such, the chronic water quality guideline to protect aquatic life has been set as low as 0.04 µg L^{-1} (for water hardness< 17 mg L^{-1}) in Canada (Canadian Council of Ministers of the Environment, 2014).

1.2. Cadmium toxicity to freshwater fish

Fish can take up Cd from the water and from their diet, with waterborne exposure being the most studied and the better described route (Harrison and Klaverkamp, 1989; Kraemer et al., 2006; McGeer et al., 2012; Szebedinszky et al., 2001). Internalization of waterborne Cd occurs mainly through the gills, where Cd uptake has been well characterized (Franklin et al., 2005; Harrison and Klaverkamp, 1989; McGeer et al., 2012; Thomann et al., 1997; Verbost et al., 1987; Verbost et al., 1988). Cadmium is believed to enter the gill epithelium in the form of free Cd^{2+} ions, through ion transporters used by its chemical analogue calcium (Ca^{2+}) (e.g. via voltage-insensitive epithelial Ca^{2+} channel (ECaC)), since, as a non-essential metal, there are no Cd-specific transporters (Galvez et al., 2007; McGeer et al., 2012; Playle et al., 1993; Verbost et al., 1989). Once Cd enters the organism at the gills, it can then be distributed to other tissues via the circulatory system (Chowdhury et al., 2003; Thomann et al., 1997; Verbost et al., 1989). In fish, the main sites of chronic Cd accumulation are the liver and kidneys, with the kidneys being the eventual site of long-term Cd storage (Chowdhury et al., 2003; Chowdhury et al., 2005; Harrison and Klaverkamp, 1989).

Ion regulation is an important challenge for freshwater fish that must compete against the loss of ions to their more dilute environment (McRae et al., 2018; Moyes and Schulte, 2007). Ionoregulatory disturbances, in particular of Ca^{2+}, is considered to be the main mechanism of acute Cd toxicity (Hollis et al., 2000; McGeer et al., 2012). Indeed, Cd exposure can affect Ca^{2+} branchial transport by disrupting Ca^{2+} uptake at the apical and basolateral membranes of gill epithelial cells (Verbost et al., 1988; Verbost et al., 1989). Ultimately, these effects on Ca homeostasis can lead to plasma hypocalcaemia

and, eventually, death (Galvez et al., 2007; Roch and Maly, 1979). The homeostasis of other ions (e.g. Na^+ and K^+) may also be affected, depending on factors such as Cd exposure duration and concentration (Chowdhury et al., 2004; Dave and Kwong, 2020; Giles, 1984; McGeer et al., 2000; McGeer et al., 2012; McRae et al., 2018). The mechanisms of chronic Cd toxicity are less understood in comparison to acute toxicity mechanisms (Groh et al., 2015; McGeer et al., 2012). However, oxidative stress is among the most commonly reported effects. Oxidative stress is characterized as an imbalance between reactive oxygen species (ROS) and antioxidant processes (Livingstone, 2001; Sies and Jones, 2007). Reactive oxygen species, such as hydrogen peroxide, are a natural by-product of cellular metabolic processes and can damage biological molecules, such as lipids (causing lipid peroxidation) and proteins (causing protein carbonylation) (Livingstone, 2001; Lushchak, 2011; McGeer et al., 2012). Antioxidant processes within cells, such as antioxidant enzymes (e.g. catalase activity), help maintain ROS at a safe level (Cuypers et al., 2010; Livingstone, 2001; Lushchak, 2011). However, certain contaminants, such as Cd, can disrupt the balance between ROS and antioxidant processes thus causing oxidative stress (Cuypers et al., 2010; Livingstone, 2001; Lushchak, 2011; McGeer et al., 2012). More precisely, Cd has been shown to induce oxidative damage in various ways, such as by inhibiting antioxidant enzymes activities (e.g. catalase) (Wang et al., 2015) and by increasing production of ROS via the inhibition of the electron transport chain (Wang et al., 2004).

1.3. Effects of winter cold on Cd toxicity

Various factors influence Cd bioaccumulation and toxicity to aquatic organisms, such as metal exposure duration, water pH, hardness, and fish age. Temperature is a 'master' abiotic factor that profoundly influences the physiology of fish, whose body temperature is dependent on the temperature of their environment (Crockett and Londraville, 2006). Typically, higher temperatures increase metal toxicity, due to increased uptake and/or decrease tolerance due to thermal stress (Douben, 1989; Heugens et al., 2002; Philippe et al., 2018; Sokolova and Lannig, 2008). Thus, in contrast, a colder environmental temperature would typically result in a lower Cd bioaccumulation and toxicity (Heugens et al., 2002; Sokolova and Lannig, 2008). However, temperatures below a fish's optimum could also result in a higher Cd sensitivity (i.e., a higher toxic response for a given Cd bioaccumulation) (Hallare et al., 2005).

In temperate regions such as Canada, winter is a 3-5 month period that is characteristically cold, food-limited and can be considered an important natural stressor for many aquatic organisms (Shuter et al., 2012; Studd et al., 2021). Notably, limited energy stores during the winter (Studd et al., 2021) may not be sufficient for fish to effectively detoxify metals (e.g. via production of metallothionein) (Lemly, 1996), thus reducing its prospect to survive the winter months (Driedger et al., 2010; Lemly, 1993). Depletion of energy stores has been previously observed in juvenile bluegill (*Lepomis macrochirus*) exposed to dietary and waterborne selenium during simulated winter conditions (Lemly, 1993). Lemly (1993) proposed a Winter Stress Syndrome theory to describe the condition of severe energy depletion in response to combined exposures to a metabolic stressor (e.g. metals) and to winter conditions (Lemly, 1993; Lemly, 1996).

However, increased metal toxicity to fish in the winter was not consistently observed in rare field studies (Bennett and Janz, 2007a; Bennett and Janz, 2007b), where only one out of three fish species supported the concept of Winter Stress Syndrome possibly due to differences in their foraging habits (Bennett and Janz, 2007a). More recently, it has been proposed that cold temperatures could exacerbate Cd-driven lipid peroxidation in aquatic organisms, via changes in biological membrane composition (Fadhlaoui and Couture, 2016; Fadhlaoui et al., 2018). Indeed, cold temperatures induce changes in membrane phospholipid fatty acid composition, through homeoviscous adaptation, and tend to increase cell membrane polyunsaturated fatty acids (PUFA) (Fadhlaoui et al., 2018; Hazel and Eugene Williams, 1990; Sinensky, 1974). Membranes with higher unsaturation levels are more prone to lipid peroxidation (Fadhlaoui et al., 2018). Thus, colder temperatures could increase the risk of Cd-driven lipid peroxidation due to higher levels of PUFA (Fadhlaoui et al., 2018).

1.4. Objective, general methodology and hypotheses

Overall, winter-cold temperatures have been seldom studied in ecotoxicology studies, and metals remain regulated based on toxicity studies conducted at ambient lab temperatures (i.e., around 20°C) (Heugens et al., 2002). Yet, freshwater fish from temperate regions, such as in Canada, experience prolonged cold winter temperatures (Environment and Climate Change Canada, 2021; Sänger and Stoiber, 2001) that may affect their tolerance to metals. The objective of my MSc study was to investigate the effects of a cold temperature on acute and chronic Cd bioaccumulation and toxicity in the banded killifish (*Fundulus diaphanus*). This freshwater fish was selected because of its

broad geographic distribution in North America and its broad tolerance for various environmental factors including temperature, salinity and dissolved oxygen content (COSEWIC, 2014). Furthermore, the *Fundulus* genus is a model in various scientific fields, including ecotoxicology (Burnett et al., 2007).

Briefly, in the present study, I gradually cooled (~2°C/week) and then acclimated (6weeks) wild-caught banded killifish to 4°C or maintained fish at 14°C. I then exposed the fish to a sublethal Cd concentration (at either 0, 0.5 or 5 µg Cd L^{-1}) for 28 days at their respective acclimation temperature. At days 2, 5 and 28, I measured Cd bioaccumulation, fish condition as well as markers of oxidative and ionoregulation stress.

I hypothesized that, due to thermodynamic effects on biochemical rates, Cd bioaccumulation occurs quicker in warm-acclimated fish in comparison to cold-acclimated fish. Yet, I also hypothesized that cold-acclimated fish are more sensitive to Cd due to cold stress. Thus, I anticipated that for a similar Cd bioaccumulation, Cd toxicity would be greater in cold-acclimated than in warm-acclimated fish

2. METHODS

2.1. Fish capture and maintenance

Animal use was approved by the UNB Saint John Animal Care Committee following Canadian Council of Animal Care guidelines. Adult wild banded killifish, *Fundulus diaphanus* (3.60 ± 0.05g, n=288), were caught by beach seining in September 2019 in the Wolastoq (Saint John River) approximately 60km southeast of Fredericton (45°36'45.6"N 66°04'27.0"W) in New Brunswick. They were transported to the Canadian Rivers Institute at the University of New Brunswick (UNB, Saint John, NB) and kept in a ~288-L fiberglass aquarium supplied with flow-through dechlorinated (deCl) freshwater [pH = 6.93 ± 0.23; [Ca] = 3.84 ± 0.03 mg L^{-1}; [Na] = 4.04 ± 0.02 mg L^{-1}; [Mg] = 0.65 ± 0.01 mg L^{-1}; [K] = 0.25 ± 0.01 mg L^{-1}; [Cl^-] = 10.70 ± 0.26 mg L^{-1}; [Cd] <0.00001µg L^{-1}; [SO_4] = 3.00 mg L^{-1}; [DOC] (Dissolved Organic Carbon) = 1.5 mg L^{-1}; Alkalinity = 4.33 ± 0.58 mg L^{-1}; Hardness = 12.27 ± 0.06 mg L^{-1}, unpublished report for August 2017 obtained from RPC]. The fish were kept at ~15±1°C and under a 12D:12L light cycle. Fish were fed daily *ad libitum* with a mixture of commercial freshwater fish flakes (Omega one) and commercial fish pellets (Gemma 1.5, Skretting).

2.2. Fish temperature acclimation

Four months after capture, fish were divided into two semi-recirculating freshwater systems. Each system consisted of a ~110-L fiberglass aquarium with partial flow-through of deCl freshwater and a recirculating loop that mechanically and biologically filtered the water. Water temperature in each system was maintained at approximately 14°C using water chillers (± 0.6°C; DBM-250, 1/3 HP Arctica, JBJ Lighting). One group of fish was

then cooled to 4°C at a rate of approximately 2°C per week to simulate the natural beginning of winter (Lemly, 1993). The second group was kept at 14°C. As shown in Figure 1, fish were then maintained at their respective acclimation temperatures for 6 weeks before being used in the Cd experiment.

Note that all fish used in this study went through a 4°C acclimation process at least once. Indeed, almost 6 weeks after the fish reached their desired temperature and before we were able to start the Cd exposure, the university locked down because of the covid-19 pandemic. The 4°C-acclimated fish were gradually brought back to 14°C (at 0.55°C per day) to avoid keeping them at a sub-optimal temperature for an extended period of time. After a 3-month lockdown, the temperature acclimation process was started again, with the original 14°C-acclimation group becoming the new 4°C-acclimation group and the original 4°C-acclimation group becoming the 14°C-acclimation group. Thus, all experimental fish were similarly treated with respect to temperature exposures prior to the acclimation period.

2.3. Cadmium exposures

After the temperature acclimation period, fish from each temperature group (either 4 or 14°C) were exposed to waterborne Cd for up to 28 days (acclimation and exposure timeline illustrated in Figure 1). We tested three sublethal Cd exposure concentrations (0, 0.5 or 5µg L^{-1} Cd) at each acclimation temperature, resulting in a total of six treatment conditions, as shown in Figure 2. These Cd concentrations were selected based on a preliminary toxicity test used to determine an appropriate sublethal concentration range (see Appendix A1 – 28-day range-finding preliminary test). As detailed in Figure

2, each treatment condition was replicated four times using glass aquaria (50cm x 43cmx 28cm) containing 33.4L of flow-through test water with n=13-14 fish per aquarium (note: while the initial plan was to have exactly 14 fish per tank, we ran out of fish so that some tanks only had 13 fish). Aquaria were scattered within two temperature-controlled water baths (244cm x 122cm x 30cm) to maintain a constant temperature of either 4°C or 14°C using chillers (± 0.6°C, DBM-250, 1/3 HP Arctica, JBJ Lighting).

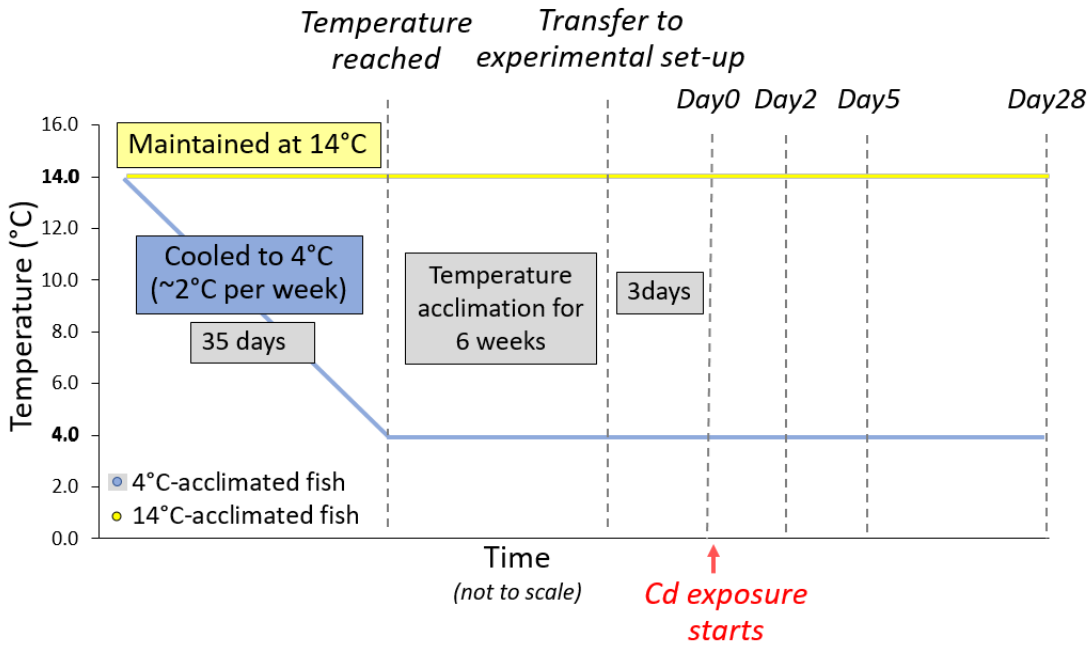

Figure 1. Timeline of the temperature acclimation period, the 3-day habituation to the experimental set-up, the beginning of the experimental Cd exposures and the fish sampling days (0, 2, 5 and 28) during the 28-day Cd exposure experiment.

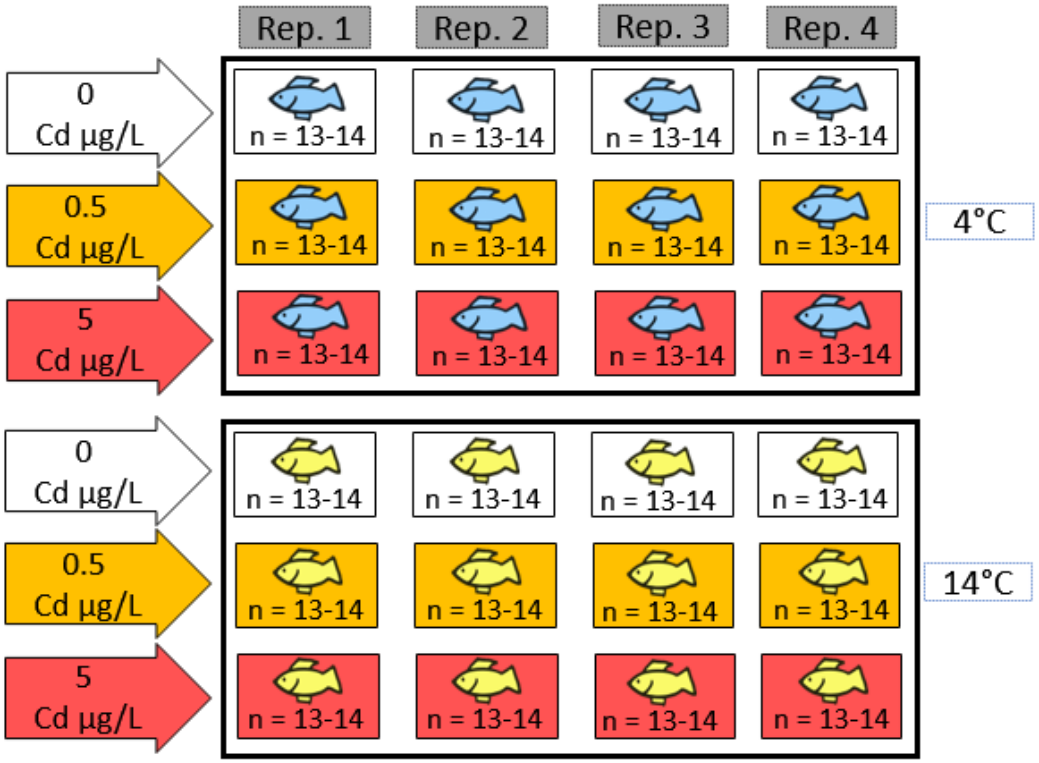

Figure 2. Schematic of the experimental set-up for the cadmium experiment. Fish (n=13-14) were held within individual aquaria (thin border) immersed in water baths (thick border) held at the two acclimation temperatures (4°C and 14°C). At each temperature, fish were exposed to either 0, 0.5 or 5 µg L^{-1} of Cd, with four replicate aquaria per treatment. Note that in the actual experiment, the position of each aquarium was scattered haphazardly within each water bath.

All test solutions were prepared in 220-L clear polyethylene drums, by spiking UNB deCl freshwater with a 1 g L^{-1} stock solution of Cd ($CdCl_2 \bullet 2.5H_2O$, ACS grade, Alfa Aesar). The solutions were prepared the day before use, to allow the various chemical species of Cd to reach thermodynamic equilibrium. They were then continuously pumped into the fish aquaria to obtain a 62.95 ± 0.46% daily renewal rate, using peristaltic pumps (Masterflex L/S) and tubing (Tygon, 2-stop #14 and Masterflex PharMed #14). Aquaria of both temperature regimes, within a Cd exposure group, were fed by the same drum. The incoming water (14.59 ± 0.11 mL/min) was brought to the target temperature by coiling the tubing in the respective water baths before the water reached the aquaria. Figure 3 shows a photograph of the experimental set-up.

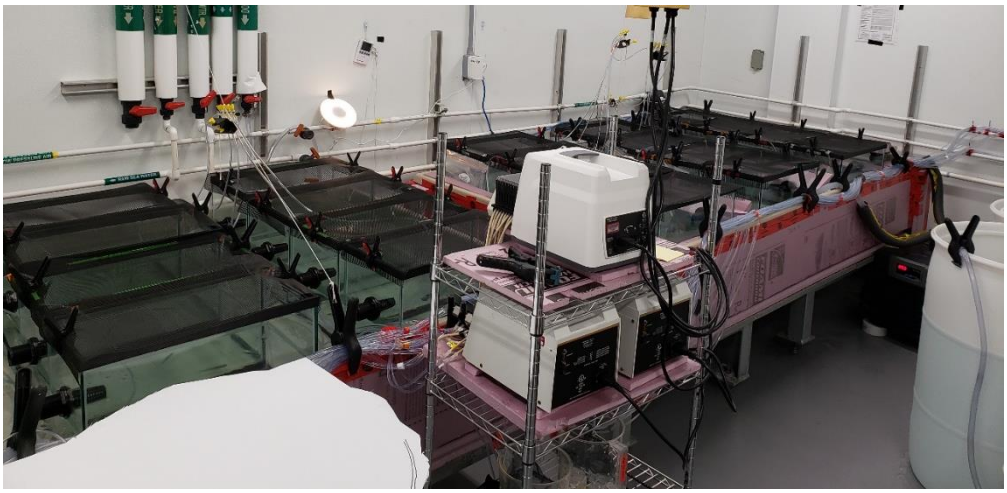

Figure 3. Photograph of the Cd exposure experimental set-up, showing the fish aquaria sitting in two large insulated 4°C or 14°C baths. Water temperature was controlled with chillers (at right of photograph, on floor) and test solutions were supplied from drums into each aquarium with peristaltic pumps (see middle of picture).

The beginning of the experiment was staggered on two consecutive days, by dividing the replicate aquaria for each treatment into two groups. This strategy allowed sufficient time to collect all the samples in a given sampling day. At the start of the experiment, fish from each temperature acclimation group (either 4 or 14°C) were sampled from their temperature acclimation systems and transferred to the cadmium exposure systems 3 days prior to the start of the cadmium exposure, to allow them to habituate to their new surroundings. In each temperature bath, this transfer was conducted one row of aquarium at a time (Figure 2), by collecting a subset of ~50 fish from their corresponding acclimation tank, and placing individual fish in the 4 consecutive aquaria in the same row, and starting again until each aquarium had 13 – 14 fish (Figure 2). Then, Cd exposures were started with an initial 30% water change in each aquarium to quickly reach targeted Cd concentrations. During the 28-day experiment, fish were fed daily *ad libitum* freshwater fish flakes (Omega One) and excess food was netted from the aquaria 0.5 to 1-h post-feeding. Water temperature and flow rate of test solutions were measured every day. After ensuring measurements were consistent among all the fish aquaria, a subset of aquaria was chosen for the daily checks: for each temperature scenario, the four corner aquaria and one randomly generated middle aquarium were chosen for daily temperature checks and a randomly generated row was selected for daily pump rate checks for which all aquaria within the row were measured. On days 0, 2, 5, 13, 21 and 28, water samples were filtered (< 0.45µm, polyethersulfone membrane syringe filter, Cytiva Whatman™) and stored in the fridge (4°C) until measurements of Cd, major ions (Ca, Mg, K and Na), and dissolved organic carbon (DOC) concentrations. Unfiltered water samples were also

collected to measure ammonia and pH. Finally, fish (n=3 per replicate aquarium) were sampled on days 0, 2, 5 and 28 of the experiment (Figure 3). These fish collections were conducted by dividing each aquarium into 4 quadrants (Q1, Q2, Q3, Q4) and randomly selecting the day 0 quadrant where fish were firstly collected, then moving up in order for the following collection days (e.g. Q2 for day 0, Q3 for day 2, Q4 for day 5, and Q1 for day 28). If fish were not in the selected quadrant (e.g. Q2) then the net was slowly moved to the next one (e.g. Q3). Fish were samples for measurements of fish condition, Cd tissue concentrations, plasma ion (Cd, Ca, Mg, K and Na) concentrations and oxidative stress markers. Fish were rinsed during 10 seconds in Cd-free water before euthanization in 0.5 g/L MS-222 (Syndel) buffered with sodium bicarbonate (Fisher Chemical). They were then quickly blot dried, weighed and measured for their total and standard length. The plasma of fish was then sampled by quickly cutting off their tail (behind the dorsal and anal fins), drawing blood from the caudal vein and centrifuging the blood in hematocrit tubes at 5000 rpm for 5 min (Thermo Scientific Sorvall Legend Micro 17 Microcentrifuge). Plasma was stored at -20°C until ion analyses. Fish liver and gills were quickly dissected, flash frozen in liquid nitrogen and stored at -80°C until oxidative stress assays and Cd analyses. The gut, kidneys and white muscles were then collected and stored at -20°C for Cd analyses.

2.4. Water physico-chemistry analyses

Water temperature was measured *in situ* using a temperature probe (Traceable Model 4378, ITM) and pH was measured in water samples using a benchtop pH Meter (A214, Thermo Scientific Orion Star). Following the measurement of day 0 pH samples,

30µL of 3M KCl was added to the day 2, 5, 13, 21 and 28 pH samples to speed up the readings. Ammonia checks were conducted using kits (API freshwater aquarium test kits). Concentrations of DOC were analyzed *via* Combustion/NDIR TOC analyzer (Shimadzu TOC-L). Cadmium and major cations (Ca, Na, Mg and K) concentrations were analyzed in water samples *via* inductively coupled plasma - optical emission spectrometry (ICP-OES; Thermo Scientific iCAP 6000 Series). Instrument signal drift was corrected by analyzing a blank and standard every 12 samples. The Cd, Ca, Na, Mg and K detection limits were 0.09 µg L^{-1}, 13.6 µg L^{-1}, 214 µg L^{-1}, 2.7 µg L^{-1} and 28.4 µg L^{-1}, respectively. Certified reference water (SCP Science, 140-025-031) was analyzed in each run, with a 100±1, 116±9.2, 106±0.4, 114±2.8 and 98 ±1.3 % recovery for Cd, Ca, Na, Mg and K, respectively.

2.5. Tissue cadmium and plasma ion analyses

Plasma samples that were <5µL or a deep red color (suggesting red blood cell contamination) were excluded from the analysis. Plasma samples were diluted in ultrapure water with 4% (v/v) HNO_3 (trace metal grade, Fisher Chemical™) prior to Cd analyses. The dilution factor varied depending on the volume of available plasma sample and ranged from 501x to 1668x. The remaining volume of plasma samples were further diluted with ultrapure water prior to cations analyses. The dilution factor varied depending on the volume of available plasma sample and ranged from 1668x to 9270x for cation analyses. Tissues were weighed then dried at 60°C for at least 3 days to obtain wet and dry weights (ME235P, Sartorius). Dried tissues were digested in concentrated HNO_3 (trace metal grade, Fisher Chemical™) at 60°C overnight and further digested by

adding concentrated H_2O_2 (Ultrapure for trace metal analysis, VWR) for approximately 24h at room temperature. The volumetric ratio of HNO_3 to H_2O_2 was 5:2 for all tissues. For each fish, the digestion volumes were 700 µL for white muscles and guts, 350 µL for liver and gills, and 70 µL for kidneys. The digestions were then diluted with ultrapure water (0.056 µS cm^{-1} at 25°C) to a final 4% HNO_3 (v/v) matrix. Ten certified reference material (TORT-2; National Research Council Canada) were also digested and analyzed.

Major cation concentrations (Ca, Mg, K and Na) were analyzed in the plasma samples by ICP-OES (Thermo Scientific iCAP 6000 Series). Instrument signal drift was corrected by analyzing a blank and standard every 12 samples. Certified reference water (SCP Science, 140-025-031) was included at the before running the samples for each analysis with a 95 ±1.1 %, 101±125 %,110 ±9.6 % and 96±2.5 % recovery for Ca, Na, Mg and K, respectively.

Cadmium concentrations were analyzed in plasma and tissue samples by inductively coupled plasma mass spectrometry (ICP-MS, Thermo Scientific™ iCAP™ RQ) at the Institut national de la recherche scientifique (INRS, Quebec City). ^{103}Rhodium was used as an internal standard. Blanks and a certified control (SCP Science, 900-Q30-100) were included approximately every 12 samples. Data was automatically corrected using a 10ppb ^{95}Molybdenum solution due to possible interferences with ^{95}Mo and ^{16}Oxygen. Data was then blank-corrected and normalized according to the certified control. Cadmium spikes were added to 3-6 samples per tissue to check for matrix interferences. We obtained an average spike recovery of 98.40 ± 4.5%, suggesting negligible interferences. All certified reference material digestions had

an average 99.5% Cd recovery, except for an obvious outlier (139% Cd recovery). The Cd detection limit was 0.0012µg L^{-1}.

2.6. Fulton condition factor and hepatosomatic index in fish

Fish condition was estimated using Fulton condition factor (FCF) and the hepatosomatic index (HSI), where FCF = ((weight in g)/(total length in $cm)^3$) x 100 (Nash et al., 2006) and HSI = (liver mass/fish mass) × 100.

2.7. Oxidative stress analyses

Livers and gills were ground on liquid nitrogen with a pestle and mortar, then divided into aliquots to analyze markers of oxidative stress (catalase activity) and damage (protein carbonyl and lipid peroxidation).

2.7.1. Catalase activity and protein carbonyl in livers and gills

Gill and liver tissues were homogenized by sonication in cold buffer (50mM potassium phosphate buffer, pH 7, containing 1mM EDTA). Livers were homogenized at a 1:20 dilution for aliquots between 40-80mg and a 1:60 dilution for aliquots <40mg. For gills, 1:25 dilution was used for tissue weights above the 12^{th} percentile and 1:75 for tissue weights below, to ensure adequate volume for each assay. Samples were sonicated at 50A for 10sec followed by 10sec rest, in an ice bath, three times (Qsonica Sonicator Q55, Fisher Scientific™). Homogenates were then centrifuged at 10,000*g* for 15min at 4°C. Supernatants were subsequently separated into two aliquots for either protein carbonyl assay or catalase assay, both of which were performed on the same day.

Catalase activity was determined by measuring the decomposition of 20mM hydrogen peroxide (H_2O_2) through changes in absorbance at 240nm over time ($\varepsilon = 43.6$ M^{-1} cm^{-1}) (Blewett et al., 2016; Claiborne, 1985; Ransberry et al., 2016) with a spectrophotometer (BioTek Epoch 2 Microplate Spectrophotometer, EPOCH2NS). Catalase activity was normalized by protein content and is reported as units (U = µmol min^{-1}) per milligram of protein (Claiborne, 1985; Ransberry et al., 2016). Protein content was performed using commercial kits (Pierce BCA Protein Assay Kit, Thermo Scientific, and Protein determination (BCA) kits, Cayman Chemicals), with Bovine Serum Albumin (BSA) standards prepared in 2mg/ml aliquots (Sigma) and measured at 562nm using the same spectrophotometer as above.

Protein carbonyl content was determined using a commercial kit (Protein Carbonyl Colorimetric Assay Kit; Cayman Chemicals). Nucleic acids can cause an overestimation of the carbonyl content (Reznick and Packer, 1994). Therefore, as per the kit's recommendation, streptomycin sulfate was added to the samples (using a 8-10% streptomycin stock in 50mM potassium phosphate buffer, pH 7.2) for a final ~1% (w/v) concentration. The samples were then centrifuged at 6,000*g* for 10min at 4°C and the resulting supernatant was used for protein carbonyl determination at 370nm with the same spectrophotometer as above. Protein carbonyl concentrations were normalized to protein content (reported as nmol mg^{-1} protein), which was determined at 280nm on the final pellet (Protein Carbonyl Colorimetric Assay Kit; Cayman Chemicals) obtained using a modified cuvette protocol to perform it in 96-well UV plates, using a 3:10 dilution.

2.7.2. Lipid peroxidation in livers

Due to limited tissue availability, lipid peroxidation was only analyzed in livers from the fish collected at days 0 and 28 of Cd exposure. Tissues were thawed on ice and homogenized in cold RIPA buffer (Thermo Scientific™) (25 mg tissue weight: 250µL buffer volume) using a handheld homogenizer at 12000 rpm for 40 sec (850 Homogenizer, Fisherbrand™). Tubes were then centrifuged at 1,600*g* for 10min at 4°C, then stored at -65°C to be assayed the following day.

Lipid peroxidation was determined by measuring thiobarbituric acid reactive substances following a modified version of the protocol described by a commercial assay (TBARS kit, Cayman Chemical). Absorbance was measured at 530nm (Varian Cary 50 Bio UV-Vis Spectrophotometer with Varian Cary 50MPR microplate reader). Protein concentration was measured in these samples on the same spectrophotometer, using a Bradford assay (Thermo Scientific™) with BSA as a standard.

2.8. Data presentation and statistical analyses

Statistical analyses were conducted using R (R Core Team, 2019) and Prism (v 9.2.0, GraphPad Software, Inc. San Diego, California, USA). Outliers were first detected in R using the very conservative median absolute deviation method and then removed (Leys et al., 2013). All data was tested for normality using D'Agostino-Pearson test and homoscedasticity of distribution in Prism 9.2.0.

We first assessed whether temperature affected Cd tissue accumulation at each Cd exposure time (0, 2, 5 and 28 days). To do this, at each exposure time, we analyzed Cd concentration in each tissue (mean ± standard error (SEM) of n = 2 – 3 fish) as a function of the Cd water concentration measured on that day, for both temperature

groups (4 vs 14°C). The plotted data consist of mean ± SEM of n =2-3 measured fish from a same aquarium. The 4 and the 14°C datasets were each fitted with linear models. We evaluated temperature effects on Cd bioaccumulation using extra sum-of-squares F tests comparing a shared temperature model with a separate temperature model (α = 0.05). Essentially, this analysis compared the full model (y ~ Temperature + Cd effect + interaction) *vs* the Cd effect only model (y ~ Cd effect). If assumptions of normality or homoscedasticity were not met for the F-tests, the data was also analyzed using Akaike Information Criterion (AIC) corrected for sample size (AICc) (e.g. Brix et al., 2017). AICc results are presented as the probability of no effect-models being correct. Cadmium accumulation in each tissue was then assessed by comparing the model slope(s) to zero using an extra sum-of-squares F test, and AICc if assumptions of normality or homoscedasticity were not met. Essentially, this analysis compared the Cd effect only model (y ~ Cd effect) *vs* a null model (y ~ 1).

Second, using the same statistical approach as described above, we assessed whether temperature affected fish condition, gill and liver oxidative stress and ion plasma levels across the range of Cd exposure concentration. To do this, we analyzed these biological parameters (mean ± standard error (SEM) of n = 2 – 3 fish) as a function of measured Cd water concentration with linear models. The plotted data consist of mean ± standard error (SEM) of n =2-3 measured fish from a same aquarium. Cadmium toxicity was evaluated from the slopes of the linear models, as described above for Cd bioaccumulation. Temperature effects on Cd toxicity were assessed by comparing a shared temperature model with a separate temperature model, as described above. This latter method allowed detecting temperature effects in control fish (Cd-unexposed fish)

when only a temperature effect was detected (i.e. no Cd effect across the Cd exposure concentration). When both significant Cd and temperature effects were detected, temperature effects on control fish were assessed using a Welch's t-test ($\alpha = 0.05$).

Finally, for the lipid peroxidation data collected in the control day 0 fish, we assessed temperature effects using a Welch's t-test ($\alpha = 0.05$).

The same statistical approach described above was used again to assess whether temperature affected the susceptibility of banded killifish to Cd, i.e., the level of toxic response observed for a given Cd tissue accumulation. For this purpose, the biological data were plotted and analyzed as a function of Cd concentration measured in the corresponding tissue. Here, since each fish had their own Cd tissue concentration, the analysis was conducted at the fish level rather than the aquarium level. Likewise, each plotted data point represented a single fish.

Data is described using gradual evidence language (Muff et al., 2022).

3. RESULTS

3.1. Water physico-chemistry

The 28-day average water physico-chemistry data are presented in table 1 and the full dataset is available in the Appendix. Data are presented as mean ± standard deviation. Over the 28-day experiment and across all conditions (n=144), we measured [DOC] = 2.0 ± 0.4 mg L^{-1}, pH = 7.1 ± 0.16, and [Ca] = 6990 ± 720 µg L^{-1}, [Na] = 11400 ± 310 µg L^{-1}, [Mg] = 603 ± 11 µg L^{-1} and [K] = 282 ± 13 µg L^{-1}. Note that water mean Ca concentrations increased by a factor of 1.3-fold (from 6400 to 8400 µg L^{-1}) over the 28 days of exposure ($p < 0.0001$).

The mean water temperature was 4.05 ± 0.23°C (n=154) and 14.2 ± 0.21°C (n=154) in the 4°C and 14ºC temperature-controlled aquaria, respectively. Note that, after 11.5 days of Cd exposure, a chiller power disruption resulted in the temperature inadvertently rising from 4 to 13°C during a ~22h period in the 4°C aquaria. The fish were returned to 4°C over the course of 5h, and the daily temperature check was performed. No mortalities or other effects of this transient temperature anomaly were observed.

The overall mean Cd concentrations at both temperatures were < 0.09 µg L^{-1} (detection limit) in the control treatments. For the Cd exposed groups, Cd concentrations were systematically lower in the 4°C aquaria than in the 14ºC aquaria (on average 1.5 and 1.3x lower for the 0.5µg L^{-1} and 5µg L^{-1} treatments, respectively). More precisely, for the low Cd treatment, the mean dissolved Cd concentration was 0.30 ± 0.09 µg L^{-1} (n=24) in the 4°C aquaria and 0.47 ± 0.05µg L^{-1} (n=24) in the 14ºC aquaria. For the high

Cd treatment, the mean dissolved Cd concentration was 3.5 ± 1.0 µg L^{-1} (n=24) in the 4°C aquaria and 4.7 ± 0.19 µg L^{-1} (n=24) in the 14ºC aquaria. We found that this dissimilarity was due to differences in the material of the airline tubing (flexible polyvinyl chloride vs silicone tubing) used to supply the Cd-spiked water to the 4 and 14°C aquaria. When setting up the experiment, all the 4°C aquaria except one aquarium at the high exposure group were fed by a tubing that differed from the 14°C group after running out of the one type of airline tubing. This issue is the reason why we analyzed our Cd bioaccumulation and toxicity datasets *via* regression analysis as a function of measured dissolved Cd concentrations, rather than *via* two-way ANOVA using nominal dissolved Cd concentrations.

Table 1. Mean water physico-chemistry over the 28 day exposure to control (0 µg L^{-1}), low (0.5 µg L^{-1}) and high (5 µg L^{-1}) Cd treatments at 4°C or 14°C. Data presented as mean ± standard deviation.

	Control (0 µg L^{-1})		**Low Cd (0.5 µg L^{-1})**		**High Cd (5 µg L^{-1})**	
	4°C	**14°C**	**4°C**	**14°C**	**4°C**	**14°C**
Temperature (°C)	4.1 ± 0.24 (71)	14.1 ± 0.17 (39)	4.1 ± 0.24 (68)	14.1 ± 0.20 (44)	3.9 ± 0.12 (15)	14.2 ± 0.22 (71)
pH	7.02 ± 0.14 (24)	6.97 ± 0.23 (24)	7.09 ± 0.16 (24)	7.07 ± 0.15 (24)	7.07 ± 0.14 (24)	7.07 ± 0.12 (24)
[DOC](mg L^{-1})	2.2 ± 0.7 (24)	2.0 ± 0.2 (24)	2.1 ± 0.5 (24)	2.0 ± 0.2 (24)	2.0 ± 0.4 (24)	2.0 ± 0.3 (24)
[Cd] (µg L^{-1})	<0.09 (24)	<0.09 (24)	0.30 ± 0.09 (24)	0.47 ± 0.05 (24)	3.51 ± 1.01 (24)	4.72 ± 0.19 (24)
[Ca] (µg L^{-1})	6990 ± 750 (24)	6970 ± 710(24)	7000 ± 670 (24)	7010 ± 740 (24)	7030 ± 790 (24)	6970 ± 700 (24)
[Na] (µg L^{-1})	11 390 ± 290 (24)	11400 ± 280(24)	11 430 ± 310 (24)	11 470 ± 340 (24)	11 420 ± 370 (24)	11 390 ± 260 (24)
[Mg] (µg L^{-1})	600 ± 10 (24)	610 ± 10(24)	600 ± 10 (24)	610 ± 10 (24)	600 ± 10 (24)	600 ± 10 (24)
[K] (µg L^{-1})	280 ± 10 (24)	290 ± 10 (24)	280 ± 20 (24)	290 ± 10 (24)	280 ± 10 (24)	280 ± 10 (24)

3.2. Tissue Cd concentrations

Cd concentrations in the gills, liver, kidneys, guts and white muscles of banded killifish are compiled in Table 2 and presented as a function of dissolved Cd concentration in Figure 4. Data are presented as mean ± SEM.

After only 2 days of Cd exposure, we observed a strong increase in Cd-gill levels with Cd water concentration, at both temperatures (AICc = <0.01%; $p<0.0001$) (Fig. 4A, B, C, Table 2). At days 2 and 5, there was very strong evidence (AICc = <0.01%; $p<0.0001$, for both days) that the 14°C fish had higher Cd-gill levels than the 4°C fish (3.6-fold and 2.9-fold difference at days 2 and 5 respectively). However, after 28 days of Cd exposure, gill bioaccumulation was no longer affected by temperature (AICc = 80%; $p = 0.44$).

A similar temperature effect was observed on Cd liver accumulation (Fig. 4D, E, F, Table 2). Indeed, while Cd-liver levels strongly increased with Cd-water levels in the 14°C fish after 5 and 28 days of Cd exposure ($p <0.0001$ and AICc <0.01%; $p<0.0001$, for day 5 and 28, respectively), there was no evidence of such accumulation in the 4°C fish throughout the 28-day experiment ($p = 0.19$ at day 5 and AICc = 40%; $p=0.085$ at day 28).

Likewise in the kidneys (Fig. 4G, H, I, Table 2), a strong temperature effect was observed after 28 days of Cd exposure (AICc < 0.01%; $p < 0.0001$), with the 14°C fish accumulating about 51-fold more Cd than the 4°C fish. On the other hand, there was no

evidence of a temperature effect on days 2 and 5, respectively (AICc = 91% and 45%; p = 0.96 and 0.096 for days 2 and 5, respectively). For these shorter exposure durations, while Cd bioaccumulation was observed, Cd kidney levels were only weakly predicted by Cd water concentration (R^2 = 0.1942 and 0.07073, for days 2 and 5, respectively).

Cadmium bioaccumulation in the guts (Fig. 4J, K, L, Table 2) was only observed after 28 days of Cd exposure, with a 28-fold greater accumulation observed at 14°C than at 4°C. Surprisingly in the 4°C-fish at day 2, there was moderate evidence of a slight Cd decrease (slope = -0.04, p= 0.014) in Cd-gut levels with increasing Cd water concentration, but these two parameters were only weakly correlated (R^2 = 0.1648).

In the white muscles (Fig. 4M, N, O, Table 2), while temperature effects occurred at day 5 (AICc = 10.0%; p = 0.015), it was only strongly evident at day 28 (AICc < 0.01%; p < 0.0001) of Cd exposure. At this latter time, Cd accumulated about 11.9–fold more in the white muscles of 14°C fish in comparison to 4°C fish.

Table 2. Cadmium concentrations in the gills, livers, kidneys, guts and muscle of 4ºC- or 14ºC-acclimated banded killifish after 2, 5, and 28 days of exposure to control (0 µg L^{-1}), low (0.5 µg L^{-1}) and high (5 µg L^{-1}) Cd treatments. Data presented as mean ± SEM with n in parentheses

	Control (0 µg L^{-1})		**Low Cd (0.5 µg L^{-1})**		**High Cd (5 µg L^{-1})**	
	4°C	**14°C**	**4°C**	**14°C**	**4°C**	**14°C**
Day 2	(µg g^{-1} dw.)	(µg g^{-1} dw.)	(µg g^{-1} dw.)	(µg g^{-1} dw.)	(µg g^{-1} dw.)	(µg g^{-1} dw.)
Gills	0.078 ± 0.008 (11)	0.049 ± 0.004 (11)	0.144 ± 0.008 (12)	0.541 ± 0.037 (12)	0.824 ± 0.079 (12)	3.670 ± 0.440 (12)
Liver	0.137 ± 0.018 (12)	0.116 ± 0.012 (12)	0.110 ± 0.007 (11)	0.095 ± 0.006 (10)	0.124 ± 0.013 (12)	0.128 ± 0.015 (12)
Kidneys	0.152 ± 0.015 (12)	0.172 0.013 (9)	0.181 ± 0.030 (12)	0.143 ± 0.022 (11)	0.234 ± 0.065 (11)	0.316 ± 0.066 (12)
Gut	0.439 ± 0.060 (12)	0.468 ± 0.053 (12)	0.302 ± 0.034 (12)	0.399 ± 0.069 (12)	0.233 ± 0.035 (12)	0.398 ± 0.058 (12)
Muscle	0.005 ± 0.001 (12)	0.005 ± 0.000 (12)	0.002 ± 0.000 (11)	0.003 ± 0.000 (8)	0.008 ± 0.001 (9)	0.006 ± 0.001 (12)
Day 5						
Gills	0.074 ± 0.006 (12)	0.041 ± 0.006 (12)	0.211 ± 0.021 (12)	1.001 ± 0.126 (12)	1.404 ± 0.067 (12)	5.532 ± 0.564 (12)
Liver	0.080 ± 0.007 (12)	0.084 ± 0.007 (11)	0.090 ± 0.017 (12)	0.114 ± 0.008 (12)	0.101 ± 0.013 (12)	0.179 ± 0.017 (12)
Kidneys	0.207 ± 0.039 (11)	0.213 ± 0.048 (11)	0.166 ± 0.031 (11)	0.189 ± 0.020 (12)	0.189 ± 0.025 (12)	0.320 ± 0.052 (12)
Gut	0.356 ± 0.090 (12)	0.416 ± 0.075 (12)	0.263 ± 0.045 (12)	0.482 ± 0.054 (12)	0.310 ± 0.052 (12)	0.629 ± 0.143 (12)
Muscle	0.005 ± 0.001 (11)	0.005 ± 0.001 (11)	0.003 ± 0.000 (11)	0.007 ± 0.001 (8)	0.004 ± 0.001 (12)	0.007 ± 0.001 (8)
Day 28						
Gills	0.074 ± 0.005 (12)	0.043 ± 0.007 (11)	0.825 ± 0.075 (12)	3.004 ± 0.352 (12)	9.247 ± 1.425 (12)	14.605 ± 1.948(12)
Liver	0.082 ± 0.013 (12)	0.136 ± 0.025 (12)	0.110 ± 0.019 (12)	0.350 ± 0.042 (12)	0.118 ± 0.012 (12)	2.752 ± 0.482 (12)
Kidneys	0.149 ± 0.017 (11)	0.141 ± 0.014 (12)	0.133 ± 0.017 (12)	0.323 ± 0.032 (12)	0.201 ± 0.021 (12)	3.596 ± 0.796 (12)
Gut	0.211 ± 0.033 (12)	0.164 ± 0.034 (12)	0.345 ± 0.043 (12)	0.738 ± 0.166 (12)	0.387 ± 0.076 (12)	6.226 ± 1.122 (12)
Muscle	0.003 ± 0.000 (11)	0.003 ± 0.000 (11)	0.004 ± 0.000 (12)	0.006 ± 0.000 (12)	0.006 ± 0.001 (12)	0.044 ± 0.008 (12)

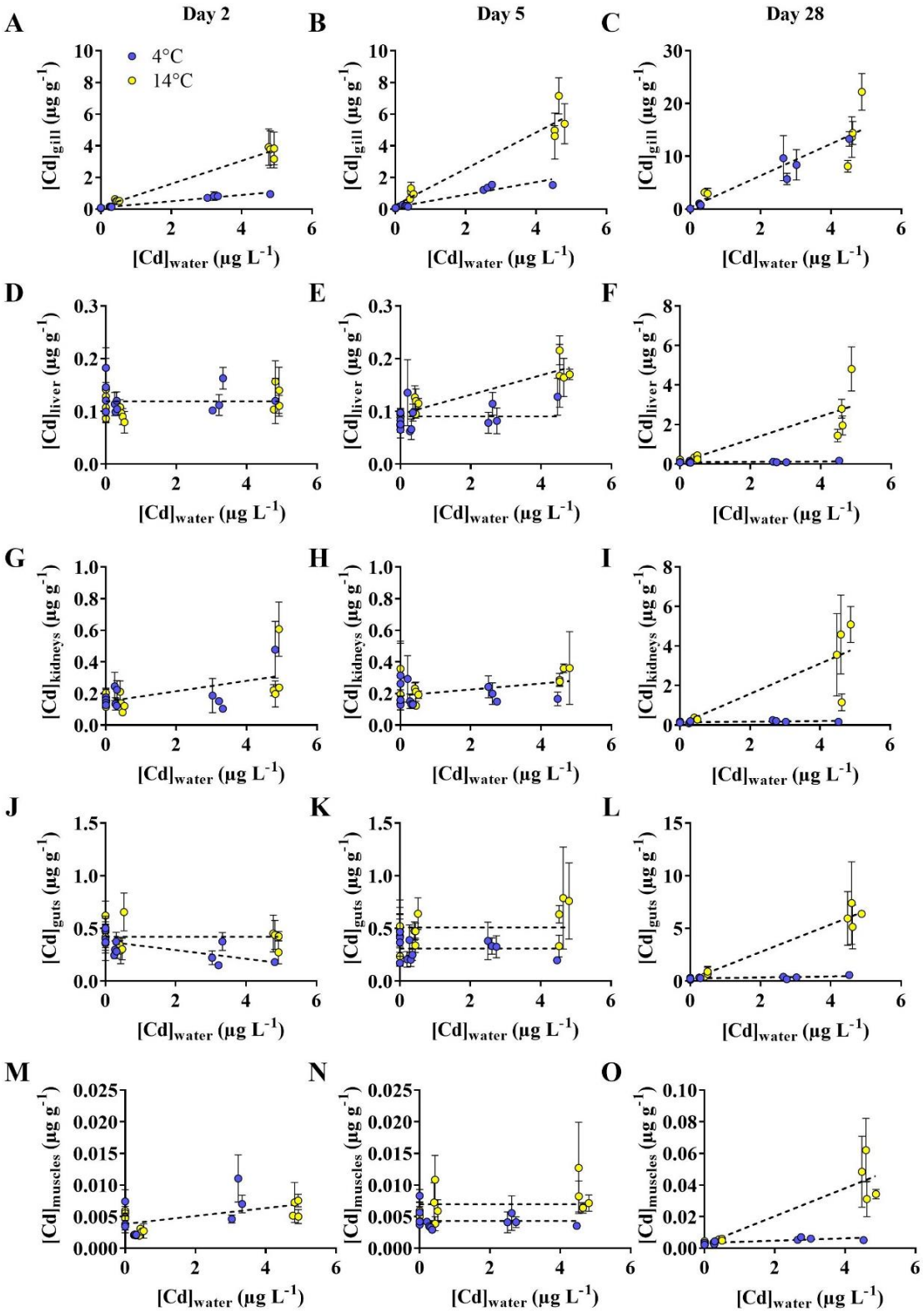
A
Day 2
4°C
14°C
B
Day 5
C
Day 28
D
E
F
G
H
I
J
K
L
M
N
O
[Cd]gill (µg g-1)
[Cd]liver (µg g-1)
[Cd]kidneys (µg g-1)
[Cd]guts (µg g-1)
[Cd]muscles (µg g-1)
[Cd]water (µg L-1)

Figure 4. Cadmium concentration in the gills (A, B, C), livers (D, E, F), kidneys (G, H, I), guts (J, K, L) and muscle (M, N, O) of 4°C- or 14°C-acclimated banded killifish after 2, 5, and 28 days of Cd exposure, as a function of the measured Cd water concentrations. Data are presented as mean ± SEM (n = 1-3 fish in individual aquaria) and dashed lines are linear models (df = 29 to 67).

3.3. Fish conditions

Over the 28-day experiment, the mean FCF and HSI across all conditions (presented as mean ± SEM) were 0.89 ± 0.01 (n = 282) and 4.4 ± 0.07 (n = 277) respectively (Figure 5). Neither parameter was affected by temperature nor Cd exposure, except FCF after 28-days of Cd exposure. For this measurement, there was strong evidence of a small temperature effect (AICc = 1.0%; p = 0.0016), with the 4°C fish having a 1.1x higher FCF (0.899 ± 0.013) than the 14°C fish (0.828 ± 0.012) across the three Cd conditions.

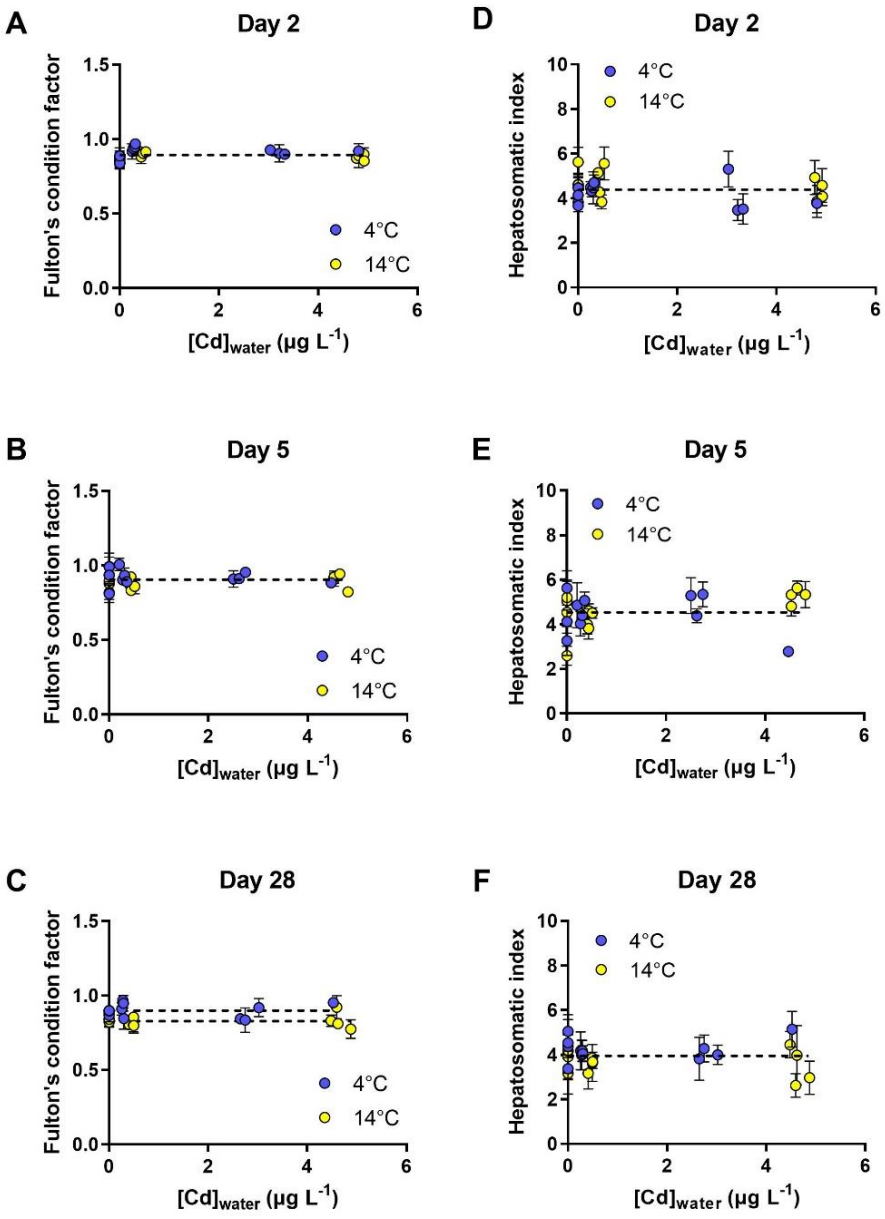

Figure 5. Fulton's condition factor (A, B, C) and hepatosomatic index (D, E, F) of 4ºC- or 14ºC-acclimated banded killifish after 2 , 5, and 28 days of Cd exposure, as a function of the measured Cd water concentration. Each data point (mean ± SEM, n=2-3 fish) represents an individual aquaria and the dashed lines are linear models (df = 31 to 69)

3.4. Ion plasma levels

Ion (Ca^{2+}, Na^{+}, Mg^{2+} and K^{+}) concentrations measured in fish plasma are compiled in Table 3 and presented as a function of dissolved Cd concentrations in Figure 6. The plasma Cd levels are not presented as 72% of the samples fell below the detection limit. Data are presented as mean ± SEM.

Calcium plasma levels (Fig. 6A, B, C, Table 3) appeared to be higher in the 14°C fish than the 4°C fish at day 2 ($p = 0.021$), but the inverse was observed by day 5 ($p = 0.0007$). At both days, there was little evidence that Cd exposure affected Ca^{2+} plasma levels ($p > 0.1$). By day 28, there was weak evidence of a difference between the temperature groups (AICc = 38%; $p = 0.071$), with stable Ca^{2+} plasma levels in the 4°C fish (AICc = 78%; $p = 0.96$) and a 1.5-fold decrease in Ca^{2+} levels in the 14°C fish over the range of Cd water concentration (AICc = 1.8%; $p = 0.0019$; $R^2 = 0.2872$). However, unexposed fish (0µg L^{-1}) showed little to no evidence of a temperature effect in Ca^{2+} levels at day 28 (AICc = 63.47%; $p = 0.2435$).

There was little evidence that temperature affected Na^{+} plasma levels ($p > 0.05$) (Fig. 6D, E, F, Table 3). At days 2 and 5 only, there was strong evidence of a Cd effect ($p < 0.0001$, for both days), with Na^{+} levels increasing by about 1.1 fold over the Cd water concentration range. However, this effect was no longer apparent by day 28 ($p = 0.4491$).

There was very strong evidence that temperature affected Mg^{2+} plasma levels over the entire course of the experiment ($p < 0.0001$ for all days) (Fig. 6G, H, I, Table 3). However, similar to Ca^{2+}, this effect was not straightforward, as Mg^{2+} levels were higher in the 14°C fish than the 4°C fish at day 2, but the inverse was observed by day 5. There

was strong evidence of a Cd effect on Mg^{2+} levels only for the 14°C fish at day 28 ($p = 0.0021$; $R^2 = 0.2671$), with Mg^{2+} levels increasing 1.3-fold over the Cd water concentration range. Unexposed fish (0µg L^{-1}) also showed a strong temperature effect in Mg^{2+} levels at day 28 (AICc = 0.5%; $p = 0.0004$). The mean Mg^{2+} plasma concentrations were 47.9 mg L^{-1} in the 4°C fish and 34.1 mg L^{-1} in the 14°C fish (i.e., a 1.4 fold difference).

Over the entire course of the experiment, K^+ plasma levels (Fig. 6J, K, L, Table 3) appeared little affected by Cd exposure (AICc = 65%; $p > 0.1$ for the zero slope comparison) and temperature (AICc = 88.29% at day 2 and $p > 0.1$, for all days).

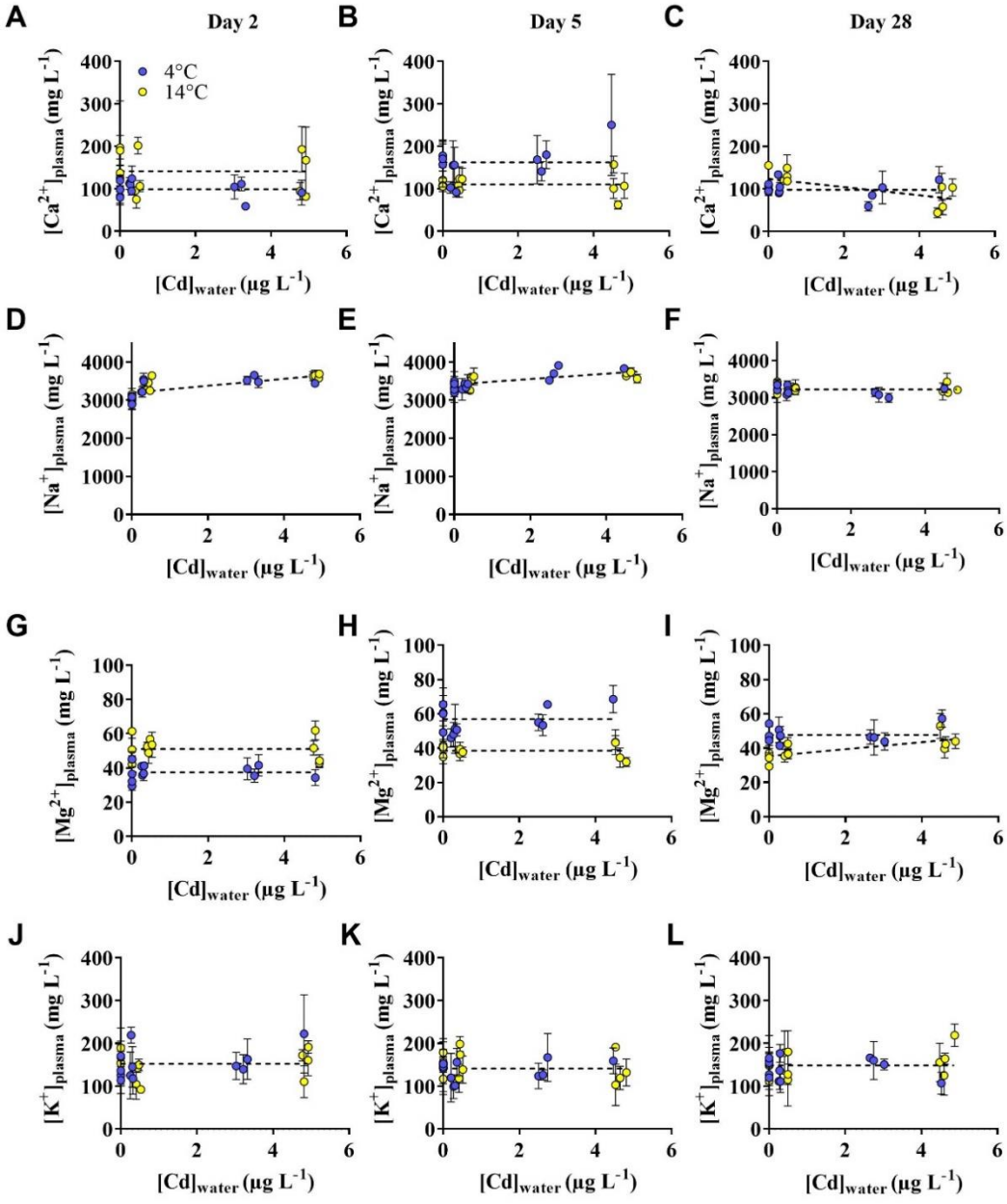
A
Day 2
B
Day 5
C
Day 28
4°C
14°C
[Ca2+]plasma (mg L-1)
[Na+]plasma (mg L-1)
[Mg2+]plasma (mg L-1)
[K+]plasma (mg L-1)
[Cd]water (µg L-1)
D
E
F
G
H
I
J
K
L

Figure 6. Calcium (A, B, C), sodium (D, E, F), magnesium (G, H, I) and potassium (J, K, L) concentrations in plasma (mg L^{-1}) of 4^{o}C- or 14^{o}C-acclimated banded killifish after 2, 5 or 28 days of Cd exposure, as a function of the measured Cd water concentration. Each data point (mean ± SEM, n=1-3 fish) represents fish in an individual aquarium and the dashed lines are linear models (df = 26 to 67).

Table 3. Calcium, sodium, magnesium and potassium concentrations in the plasma of 4ºC- or 14ºC-acclimated banded killifish after 2, 5 and 28 days of exposure to control (0 µg L^{-1}), low (0.5 µg L^{-1}) and high (5 µg L^{-1}) Cd treatments. Data presented as mean ± SEM with n in parentheses.

	Control (0 µg L^{-1})		**Low Cd (0.5 µg L^{-1})**		**High Cd (5 µg L^{-1})**	
	4°C	**14°C**	**4°C**	**14°C**	**4°C**	**14°C**
Day 2						
[Ca^{2+}] (mg L^{-1})	94.76 ± 9.59 (12)	159.60 ± 30.01 (11)	110.31 ± 8.22 (12)	128.86 ± 19.88 (9)	91.37 ± 11.22 (12)	134.17 ± 25.05 (12)
[Na^{+}] (mg L^{-1})	3,009 ± 50 (11)	3,077 ± 51 (11)	3,372 ± 70 (12)	3,410 ± 58 (10)	3,514 ± 53 (11)	3,641 ± 42 (12)
[Mg^{2+}] (mg L^{-1})	35.83 ± 2.15 (12)	49.43 ± 3.17 (10)	38.69 ± 1.29 (12)	52.88 ± 2.10 (11)	37.74 ± 2.47 (12)	50.66 ± 3.03 (11)
[K^{+}] (mg L^{-1})	138.83 ± 22.22 (10)	167.48 ± 10.91 (11)	152.00 ± 21.44 (12)	128.81 ± 12.60 (10)	163.38 ± 21.84 (11)	158.97 ± 14.86 (12)
Day 5						
[Ca^{2+}] (mg L^{-1})	169.50 ± 19.42 (10)	110.72 ± 6.80 (12)	122.86 ± 16.34 (9)	112.98 ± 9.96 (12)	185.06 ± 31.77 (12)	106.75 ± 13.78 (12)
[Na^{+}] (mg L^{-1})	3,366 ± 76 (11)	3,417 ± 61 (11)	3,378 ± 92 (9)	3,502 ± 69 (12)	3,742 ± 55 (12)	3,657 ± 46 (11)
[Mg^{2+}] (mg L^{-1})	59.75 ± 4.50 (11)	39.20 ± 1.81 (12)	49.40 ± 4.08 (10)	37.84 ± 1.49 (12)	60.63 ± 3.09 (12)	38.26 ± 2.72 (12)
[K^{+}] (mg L^{-1})	148.26 ± 13.81 (11)	145.11 ± 12.91 (10)	121.18 ± 14.98 (10)	154.32 ± 16.77 (10)	144.32 ± 16.22 (12)	134.54 ± 16.46 (10)
Day 28						
[Ca^{2+}] (mg L^{-1})	101.30 ± 2.78 (9)	114.29 ± 9.95 (8)	101.80 ± 6.42 (8)	127.22 ± 6.39 (11)	92.78 ± 14.07 (11)	77.38 ± 12.44 (12)
[Na^{+}] (mg L^{-1})	3,312 ± 49 (12)	3,222 ± 77 (10)	3,187 ± 63 (12)	3,254 ± 35 (11)	3,116 ± 67 (12)	3,236 ± 80 (12)
[Mg^{2+}] (mg L^{-1})	47.90 ± 2.58 (12)	34.08 ± 1.99 (10)	46.66 ± 2.27 (10)	37.35 ± 1.58 (11)	48.42 ± 3.22 (12)	44.67 ± 2.78 (12)
[K^{+}] (mg L^{-1})	143.31 ± 14.33 (11)	148.82 ± 22.05 (9)	136.19 ± 14.78 (11)	151.45 ± 20.83 (10)	146.18 ± 13.38 (12)	166.28 ± 19.78 (11)

3.5. Oxidative stress

There was weak to little evidence that Cd exposure and temperature affected oxidative stress in fish gills (data presented in Table A7). Therefore, the focus of this section is on the liver data, which are presented in Table 4, Figure 7 and Figure 8. Below, I first present these data as a function of the Cd concentration in the water (Figure 7), to describe the general effects of temperature on Cd-driven oxidative stress. Then, in order to provide insights into how temperature may affect fish sensitivity to these Cd effects, the data are presented as a function of the Cd concentration in the livers (Figure 8). Data are presented as mean ± SEM.

At days 5 and 28, there was weak to little evidence that protein carbonyl liver levels were affected by waterborne cadmium exposure (AICc = 51% and 58%; p=0.16 and 0.22 at days 5 and 28, respectively) or temperature (AICc = 74 % and 75 %; p = 0.30 and 0.32 at days 2 and 5, respectively) (Fig. 7A, B). This finding did not change when PC levels were expressed as a function of Cd liver concentration (Fig. 8A, B).

There was, however, moderate to very strong evidence of a temperature effect on CAT activity (p = 0.015 at day 5 and AICc < 0.01%; p < 0.0001 at day 28) (Fig. 7C, D). Indeed, the 14°C fish had a higher CAT activity baseline (112 ± 15 and 129 ± 8 μmol^{-1} min^{-1} mg^{-1} protein at day 5 and 28, respectively) in comparison to the 4°C fish (100 ± 7 and 113 ± 6 μmol^{-1} min^{-1} mg^{-1} protein at days 5 and 28, respectively). While Cd exposure had no effect on CAT activity in the 4°C fish (p = 0.7945 at day 5 and AICc = 76.1% ; p = 0.7990 at day 28), the 28-day CAT activity in the 14°C fish increased by a

factor of 1.2 over the Cd water concentration range (AICc = 12.14%; p = 0.0151; R^2 = 0.1616). Alternatively, when expressed as a function of Cd liver concentration, there was strong evidence of a temperature effect on CAT activity at day 28 only (AICc = 3.601%; p = 0.0052) with the 14°C fish having a higher CAT activity by a factor of 1.1, 1.3 and 1.4 fold on average for the control, low and high groups, respectively. At day 28, there was also strong evidence that Cd in the liver increased CAT activity in the 14°C fish only (Fig. 8D) (AICc = 0.3278%; p = 0.0003; R^2 = 0.3188).

Prior to Cd exposures, MDA liver levels were similar in 4°C fish (1.44 ± 0.20 nmol mg^{-1} protein, n = 8) and the 14°C fish (1.77 ± 0.31 nmol mg^{-1} protein, n = 11) (Welch's t-test, p = 0.3881). Following a 28-day Cd exposure, MDA levels in the 14°C fish increased by a factor of 3.1 from 0 to 5 μg L^{-1} of dissolved Cd, although the two parameters were weakly correlated (R^2 = 0.1048) (Fig 7F, Table 3). On the other hand, MDA levels remained stable in the 4°C fish (AICc = 48.89%; p = 0.1211) (Fig 7F, Table 3). When expressed as a function of the Cd concentration in the liver (Fig 8F), there was little evidence of a temperature effect on lipid peroxidation levels in the livers (AICc = 91.73%; p = 0.9487).

Table 4. Catalase (CAT) activity, protein carbonyl (PC) levels and malondialdehyde (MDA) levels in the livers of 4°C- or 14°C-acclimated banded killifish after 5 or 28 days of exposure to the control, low and high Cd exposure groups. Data presented as mean ± SEM with n in parentheses.

	Control (0 µg L^{-1})		**Low Cd (0.5 µg L^{-1})**		**High Cd (5 µg L^{-1})**	
	4°C	**14°C**	**4°C**	**14°C**	**4°C**	**14°C**
CAT activity (µmol/min/mg protein)						
Day 5	100.09 ± 7.10 (4)	112.19 ± 15.10 (5)	115.27 ± 6.45 (12)	145.91 ± 7.40 (11)	112.14 ± 5.73 (12)	126.27 ± 7.39 (12)
Day 28	113.28 ± 5.76 (12)	128.78 ± 7.98 (12)	105.76 ± 7.92 (12)	133.99 ± 6.78 (12)	107.78 ± 5.14 (12)	152.73 ± 7.88 (12)
PC levels (nmol mg^{-1} protein)						
Day 5	7.59 ± 1.04 (4)	7.37 ± 0.59 (5)	7.78 ± 0.61 (11)	6.26 ± 0.48 (12)	7.43 ± 0.58 (12)	8.13 ± 0.70 (12)
Day 28	7.04 ± 0.40 (11)	8.48 ± 1.00 (12)	8.08 ± 0.81 (12)	9.13 ± 0.83 (12)	7.48 ± 0.67 (12)	7.34 ± 0.71 (12)
MDA levels (nmol mg^{-1} protein)						
Day 0	1.44 ± 0.20 (8)	1.77 ± 0.31 (11)				
Day 28	1.59 ± 0.26 (8)	1.10 ± 0.27 (7)	1.92 ± 0.30(9)	2.24 ± 0.15 (6)	1.40 ± 0.22 (9)	3.37 ± 0.59 (7)

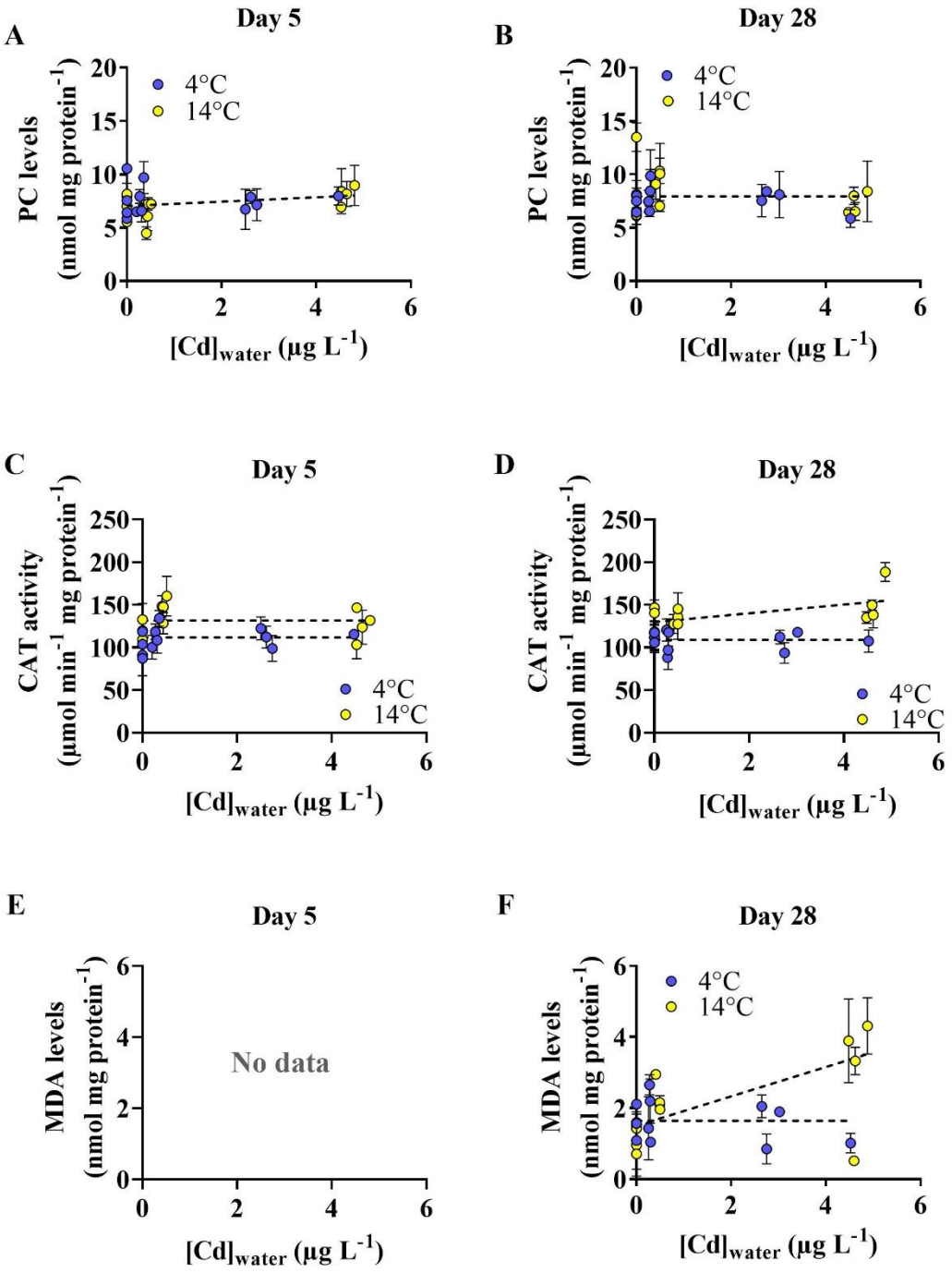
A
Day 5
PC levels (nmol mg protein-1)
4°C
14°C
[Cd]water (µg L-1)
B
Day 28
C
Day 5
CAT activity (µmol min-1 mg protein-1)
D
Day 28
E
Day 5
MDA levels (nmol mg protein-1)
No data
F
Day 28

Figure 7. Protein carbonyl (PC) levels (A, B), catalase (CAT) activity (C, D) and malondialdehyde (MDA) levels (E, F) in the livers of 4°C- or 14°C-acclimated banded killifish after 5 or 28 days of Cd exposure, as a function of the measured Cd water concentrations. Each data point (mean ± SEM, n=1-3 fish) represents an individual aquaria and the dashed lines are linear models (df = 18 to 69). Note that day 5 MDA levels were not measured due to constraint on tissue availability.

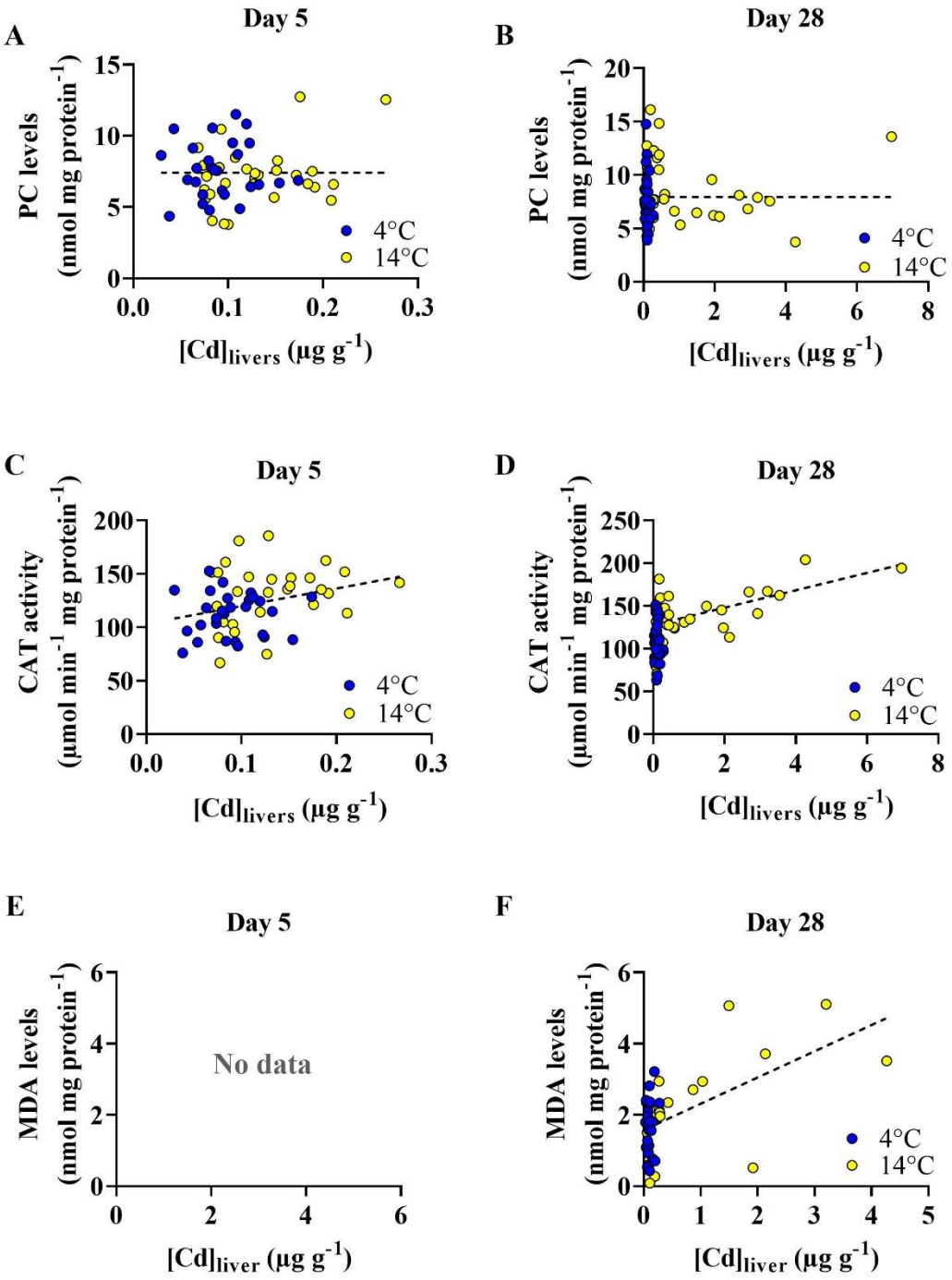
A
Day 5
PC levels (nmol mg protein-1)
[Cd]livers (µg g-1)
4°C
14°C
B
Day 28
PC levels (nmol mg protein-1)
[Cd]livers (µg g-1)
4°C
14°C
C
Day 5
CAT activity (µmol min-1 mg protein-1)
[Cd]livers (µg g-1)
4°C
14°C
D
Day 28
CAT activity (µmol min-1 mg protein-1)
[Cd]livers (µg g-1)
4°C
14°C
E
Day 5
MDA levels (nmol mg protein-1)
No data
[Cd]liver (µg g-1)
F
Day 28
MDA levels (nmol mg protein-1)
[Cd]liver (µg g-1)
4°C
14°C

Figure 8. Protein carbonyl (PC) levels (A, B), catalase (CAT) activity (C, D) and malondialdehyde (MDA) levels (E, F) in the livers of 4°C- or 14°C-acclimated banded killifish after 5 or 28 days of Cd exposure, as a function of the measured Cd water concentrations. Each data point represent a single fish and dashed lines are linear models (df = 18 to 69). Note that day 5 MDA levels were not measured due to constraint on tissue availability.

4. DISCUSSION

My study aimed to investigate the effects of a winter cold temperature on metal bioaccumulation and toxicity in fish. I hypothesized that, due to thermodynamic effects on biochemical rates, Cd bioaccumulation occurs quicker in warm-acclimated fish in comparison to cold-acclimated fish. Yet, I also hypothesized that cold-acclimated fish are more sensitive to Cd due to cold stress. By day 28, cold-acclimated fish accumulated lower levels of Cd in all tissues and showed reduced Cd toxicity. However, with the exception of CAT activity, sensitivity to Cd was similar in cold- and warm-acclimated fish.

The cold-acclimated fish were accidentally exposed to a rapid rise in temperature on day 11.5 after which they were quickly brought back to their acclimation temperature of 4°C. There was no evidence that this temperature surge at day 11.5 affected the fish and their response to Cd at day 28. This agrees with the general understanding of the oxidative stress system as a dynamic system (Lushchak, 2011) that responds quickly and attenuates quickly. Thus, while exposure to a rapid change in temperature can lead to oxidative damage in fish (Ritchie and Friesen, 2022), it is very unlikely that such effect would persist over two weeks. Furthermore, fish that have broad thermal tolerance range similar to the banded killifish, such as *Cyprinodon variegatus* (Baker et al., 2020) and *Oryzias latipes* (Hemmer-Brepson et al., 2014), have been shown to only express marginal changes in oxidative stress response (Ritchie and Friesen, 2022) even following an acute temperature challenge (Baker et al., 2020). The cold-acclimated fish

in the present study showed no detectable increase in levels of lipid peroxidation or enzyme activity that could occur with a rise in temperature.

4.1. Temperature effects on banded killifish

In our study, the condition (FCF and HIS) of the banded killifish remained mostly unaffected by temperature, except for a slightly lower condition factor (FCF) in the 14°C fish on day 28. Thus, cold-acclimated fish were able to maintain their energy stores possibly due to lower metabolic requirements at colder temperatures and prioritizing energy conservation which is essential in cold winter environments (Castaldo et al., 2021; Studd et al., 2021). Similarly, a lower condition at warmer temperatures has been previously reported in yellow perch and juvenile common carp (Castaldo et al., 2021; Grasset et al., 2016).

In our study, levels of plasma ions in control fish remained globally unaffected by temperature, with some inconsistent effects observed on Ca and Mg plasma levels. Indeed, Ca levels in cold-acclimated control fish were only consistent with previous findings on days 2 and 28 with higher levels detected at warmer temperatures (Burton, 1986). Alternatively, Mg levels in cold-acclimated control fish were only consistent with previous findings on days 5 and 28 with lower levels detected at warmer temperatures (Burton, 1986). Previous reports on the effect of temperature on plasma ion concentrations are variable for freshwater fish (Burton, 1986; Gonzalez and McDonald, 2000). Nonetheless, levels of Ca, Na and K in freshwater fish plasma have been shown to typically either be unaffected or rise with increasing temperature, while Mg plasma

levels have been shown to either typically be unaffected or fall with rising temperature (Burton, 1986; Gonzalez and McDonald, 2000; Han et al., 2018).

Temperature also did not appear to affect PC levels or lipid peroxidation in banded killifish. However, hepatic CAT activity decreased with decreased temperature. Similarly, a study found that the CAT activity in the liver of *Oreochromis niloticus* was also lowest at the lowest exposed temperature (20°C vs 32°C) (Abdel-Tawwab and Wafeek, 2017). The authors suggested that this lower CAT activity might be attributed to possibly lower ROS production at lower temperatures, which may also be the case for banded killifish in the present study.

Overall, our data suggest that the extended cold temperature exposure conducted in our study did not particularly affect the energy stores, ion homeostasis and oxidant/antioxidant balance in the banded killifish. This observation may be due to a relatively wide temperature tolerance range of banded killifish. These fish have been shown to have an upper thermal limits >25°C (Wismer et al., 1987), and can tolerate a temperature as low as 0.5°C in freshwater (Ahokas and Sorg, 1977).

4.2. Cadmium bioaccumulation in banded killifish

4.2.1. General observations

In the present study, Cd was detected in all tissues of fish exposed to all Cd treatments, including controls. The accumulation factors (Cd levels in Cd-exposed vs. control fish) were particularly high in the gills. For example, by day 28, Cd concentrations in the cold-acclimated fish were on average 125x (gills), 1.8x (guts), 1.4x

(livers), 1.3x (kidneys) and 2x (muscle) higher in the high-Cd treated fish than in the control fish. These Cd accumulation levels were even higher in the warm-acclimated fish: 340x (gills), 38x (guts), 26x kidneys, 20x (livers) and 15x (muscle). When comparing the Cd levels in the various tissues, the following trends seem to emerge over the course of the experiment: gills > guts > kidneys ~ liver > muscle. Cadmium bioaccumulation was first detected in the gills, as expected considering this tissue is a main uptake route for waterborne Cd (Kamunde, 2009; McGeer et al., 2012). By the end of day 28, gills were still the tissue with the highest Cd concentration. Cadmium bioaccumulation in the livers and kidneys was only detected after days 5 or 28, consistent with these organs being long-term storage sites for Cd (Chowdhury et al., 2003). Similarly, previous fish studies have shown that Cd does accumulate highest amongst the gills, livers and kidneys, with much lower levels found in the muscles (Chowdhury et al., 2005; Harrison and Klaverkamp, 1989; Hollis et al., 2001; Kraal et al., 1995; Vergauwen et al., 2013a). The guts also contained high levels of Cd by the end of the 28 day experiment. Significant accumulation in the guts have also been observed in studies using waterborne Cd exposures (Franklin et al., 2005; Kraal et al., 1995; Vergauwen et al., 2013a). Although it is possible that some Cd uptake could occur through the gut lumen from food-absorption, it would only be expected to play a minor role for a waterborne exposure since freshwater fish have a low drinking rate (Flik et al., 1995; Kraemer et al., 2006) and excess food was netted out as much as possible post-feeding in the present study. Rather, under this waterborne exposure scenario, the Cd-gut levels are typically attributed to the intestines being a route of Cd excretion and

sequestration (Harrison and Klaverkamp, 1989; Kraal et al., 1995; Kraemer et al., 2006; Vergauwen et al., 2013a).

4.2.2. Temperature effects

In our experiment, the relative Cd tissue distribution appeared to be little affected by temperature. However, as expected, a colder temperature consistently resulted in lower levels of Cd bioaccumulation within tissues. More precisely, after 28 days of exposure to the highest Cd concentration, the 14°C fish accumulated on average 1.6x (gills), 16x (gut), 23x (liver), 18x (kidneys), and 7.3x (muscle) more Cd than the 4°C fish. A positive temperature effect (i.e., higher Cd bioaccumulation at higher temperatures) typically occurs at both whole body (Abdel-Tawwab and Wafeek, 2014; Pilehvar et al., 2019) and tissue levels (Abdel-Tawwab and Wafeek, 2014). For example, Pillet et al., (2021) showed that a 1-day exposure to a sublethal concentration of Cd (2.9 µg Cg L^{-1} in a Cu/Zn/Cd mixture) resulted in a lower Cd-gill level in *Cyprinus carpio* exposed at 10°C compared to 20°C. This temperature effect could be attributed to increased Cd uptake rates in fish exposed at higher temperatures (Douben, 1989), likely due to the profound influence of temperature on metabolic rate and biochemical reaction rates. Indeed, a higher temperature will increase energy demands and thus oxygen consumption rate, which may increase the organism's exposure to Cd via increased gill ventilation (Nikinmaa, 2014; Pörtner, 2001; Sokolova and Lannig, 2008). Additionally, because warming increases chemical reaction rates (Arrhenius law), increased metal uptake rates by transporter proteins can be expected at higher temperatures (Nikinmaa,

2014; Sokolova and Lannig, 2008). However, bioaccumulation is the net sum of both uptake and elimination processes, and colder temperatures could also reduce elimination rates (Douben, 1989; Sokolova and Lannig, 2008). Thus, Cd bioaccumulation may not be ultimately lower at colder temperature, but simply slower. In support of this notion, the difference in Cd-gill content between the 14 and 4°C fish decreased from on average 4.5x at day 2 down to 1.6x (and not statistically different) at day 28. While, this trend was not observed in other tissues, the comparison between temperature groups was limited due to the much lower Cd bioaccumulation levels being reached in the 4°C fish over the course of the experiment. To better compare bioaccumulation rates at different temperatures, one would need to prolong the Cd exposure past the 28-day exposure that was tested in the present study. Overall, very few studies have addressed the effect of temperature on the dynamics of Cd uptake and elimination. While the general consensus is that increased temperature appear to increase metal bioaccumulation in aquatic organisms (see review by Sokolova and Lanning, 2008), some reported exceptions suggest a complexity that warrants further research. Notably, Vergauwen et al. (2013a) found that zebrafish exposed to potentially lethal Cd concentration (5µM or 562 µg L^{-1}) accumulated more Cd in their gills at 12°C than at warmer temperatures (18 to 34°C). The authors speculated that a defence mechanism, involving retaining Cd at the gills to prevent its distribution to other vital tissues, could be weakened at higher temperatures (Vergauwen et al., 2013a). However, this speculation isn't consistent with the gills being an important site of Cd toxicity and the cold temperature used in their experiment was extreme for zebrafish, probably bringing them very close to their tolerance limit.

4.3. Cadmium toxicity in banded killifish

4.3.1 General observations

To my knowledge, only one study has reported a toxicity value for Cd in the banded killifish (Rehwoldt et al., 1972). A 96-h LC_{50} (i.e. the lethal concentration to cause 50% mortality at 96h of exposure) of 110µg L^{-1} was reported at a water hardness of 55mg L^{-1} and a temperature of 28 °C (Rehwoldt et al., 1972). Our preliminary tests conducted at a hardness of 25 mg L^{-1} and at 19°C showed no mortality at 100 µg Cd L^{-1} after 96h. Only after 14 days did some mortality occur in this treatment (2 out of 4 fish). Water hardness is a major ameliorative factor against Cd toxicity (Niyogi et al., 2008; Pascoe et al., 1986). Thus, the lower acute Cd toxicity observed in our preliminary study might be the result of its much lower temperature (almost 10°C lower). However, other toxicity modifying parameters (e.g. water pH and fish age) that could contribute to the observed difference in Cd toxicity levels also differed or were simply not reported by Rehwold et al (1972). Nonetheless, in comparisons to other fish species (when adjusted to a hardness of 50mg L^{-1}), banded killifish have a moderate sensitivity to Cd (CCME, 2014).

The overall condition of the fish (FCF and HSI) remained mostly unaffected by Cd exposure. This observation is in agreement with other studies that have shown an absence of Cd effect on fish condition (Castaldo et al., 2021; Grasset et al., 2016).

In our study, Cd affected ion regulation and oxidative stress in the banded killifish. After 28 days of exposure, we observed a 1.5-fold decrease in Ca plasma levels in the presence of up to 5 µg L^{-1} Cd (in the 14°C fish only). Cadmium is well documented to

cause hypocalcaemia in fish, which has been detected in acute toxicity studies (typically up to 96-h exposures) for a wide range of Cd exposure concentrations in various species (Garcia-Santos et al., 2006; Silva and Martinez, 2014). Hypocalcaemia induced by Cd exposure is thought to result from inhibition of Ca uptake (Silva and Martinez, 2014; Verbost et al., 1988; Verbost et al., 1989). In contrast to Ca, Cd exposure led to an increase in the plasma levels of Na (days 2 and 5 by 1.1-fold) and Mg (day 28, in the 14°C fish only, by 1.3-fold) in our study. Decreased Ca levels with concomitant increased Mg levels, possibly as a secondary effect or through kidney dysfunction, have also been previously reported in a freshwater fish exposed to Cd concentrations as low as 6.4 µg L^{-1} (Giles, 1984; Haux and Larsson, 1984; Pratap et al., 1989). The observed Na effects are puzzling, as Na levels are usually reported to either be unperturbed or decreased in response to Cd exposure (Garcia-Santos et al., 2006; McGeer et al., 2000; Pratap et al., 1989; Silva and Martinez, 2014; Vergauwen et al., 2013b).While an increased Na influx has been previously reported by Reader and Morris (1988) in response to Cd, a simultaneous stimulated efflux resulted in an overall net sodium loss (Reader and Morris, 1988).Alternatively, it was also suggested that elevated plasma Na levels may be as a result of loss of Na from tissues to plasma (Larsson et al., 1976). Similar to previous studies, K levels were unaffected by Cd exposure (Pratap et al., 1989; Silva and Martinez, 2014). Overall, although Cd has clearly been shown to cause ionregulatory disturbances, the outcome of plasma ion concentrations following a Cd exposure can vary and is dependent on other factors (such as exposure concentration,

duration of exposure, and water quality) (Garcia-Santos et al., 2006; Giles, 1984; McGeer et al., 2000; Pratap et al., 1989).

In the present study, Cd accumulation in the banded killifish resulted in oxidative stress in the liver of warm-acclimated fish, but not in the gills, despite a higher Cd concentration in the latter tissue. Specifically, a 28-day Cd exposure led to increased hepatic CAT activity and lipid peroxidation (as determined by MDA levels) in the warm-acclimated fish only. On the other hand, PC levels were unaffected by Cd in tissues of fish exposed to both temperature regimes. Exposure to Cd have been shown to induce the production of antioxidant enzymes in fish, such as catalase, and cause oxidative damage (e.g. through lipid peroxidation and protein carbonylation) (Cuypers et al., 2010; McGeer et al., 2012; Pretto et al., 2011). The absence of such effects in the gills suggests that this tissue has a lower sensitivity to oxidative stress compared to the liver. Higher Cd exposure concentrations are typically necessary to observe these effects in the gills. For example, Cd exposures (236 µg L^{-1} and 414 µg L^{-1}) for up to 14 days led to changes in antioxidant enzyme activities (e.g. CAT) in the gills of *Rhamdia quelen* (Pretto et al., 2011).

4.3.2 Temperature effects

In our study, Cd toxicity was lower in the banded killifish acclimated at 4°C than at 14°C. More precisely, while Cd increased CAT activity and MDA levels in the livers of warm-acclimated fish, cold-acclimated fish did not experience any noticeable oxidative stress. The same observation could be made for disturbance of Ca and Mg

regulation at day 28. While the 14°C fish showed changes in Ca and Mg at day 28, the 4°C levels remained unaffected by Cd exposure. Overall, the temperature effects on Cd toxicity observed in our study are consistent with the Cd bioaccumulation data (i.e., increased at higher temperatures), suggesting that Cd toxicity simply follows Cd bioaccumulation patterns. Indeed, there was no clear indication that cold-acclimated fish were more sensitive than the warm-acclimated fish, or vice-versa. The only sensitivity (i.e., Cd toxicity at a given Cd bioaccumulation) difference was observed in liver CAT activity at day 28, with the warm-acclimated fish showing Cd-induced increases in CAT activity, while the cold-acclimated fish were unaffected. However, the comparison was conducted at very different Cd liver levels, as the 4°C fish accumulated much less Cd than the 14°C (from 0.04 to 0.28µg L^{-1} and from 0.13 to 6.97 µg L^{-1}, for Cd exposed fish at day 28, respectively). In general, fish sensitivity could not be adequately compared between both temperature groups, because of the much lower Cd tissue levels in the 4°C fish in comparison to the 14°C fish. A longer exposure duration, leading to greater Cd accumulation levels at both temperature, would be necessary to adequately test the sensitivity hypothesis.

Cold temperatures have the potential to cause deleterious effects in metal-exposed fish (Hallare et al., 2005; Lemly, 1993). Indeed, cold-stressed (5°C below temperature optimum: 21°C) zebrafish embryos exposed to Cd had decreased hatching success and increased mortality in comparison to heat-stressed embryos (7°C above temperature optimum: 33°C) (Hallare et al., 2005). The authors attributed this increased response at a lower temperature to the additional stress inflicted by the cold (Hallare et al., 2005).

Nonetheless, increased metal toxicity at colder temperatures is an uncommon outcome and the results of the present study follows the more common pattern of increased metal bioaccumulation and toxicity at higher temperatures (Sokolova and Lannig, 2008).

5. CONCLUSION

My study is one of few to investigate the combined effects of winter cold temperature and metal bioaccumulation and toxicity in fish. By the end of the exposures at day 28, the cold-acclimated fish had lower Cd levels in all tissues (gills, livers, kidneys, guts and muscles) in comparison to the warm-acclimated fish. In agreement with these lower bioaccumulation levels, Cd toxicity was also lower in the cold-acclimated fish. There was also little evidence that cold and warm-acclimated fish displayed differences in their sensitivity to Cd, but this assessment was limited by the low bioaccumulation levels in the cold-acclimated fish. A longer experiment (winter typically lasts at least 3-months) would be necessary for such comparison. Nonetheless, the present study suggests that fish are not at greater risk of metal exposure during the winter months, contrary to my hypothesis. Therefore, current environmental risk assessments for Cd appear to be protective in the winter.

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Appendix

A1 – 28-day range-finding preliminary test

A1.1 Cd exposures

Fish from each temperature acclimation group (either 4 or 14°C) were separated into groups to be exposed for 28 days to three different waterborne Cd concentrations (0, 2.5 or 5µg L^{-1} Cd) at their respective acclimation temperature. The concentrations were chosen with the prospect that they would be sublethal. Each treatment condition was replicated 2 times using glass aquaria (50cm x 43cmx 28cm) containing 33.4L of flow-through test water with n=3 fish per aquarium. Aquaria were held within two temperature-controlled water baths (244cm x 122cm x 30cm) to maintain a constant temperature of either 4°C or 14°C using chillers (+/- 0.6C, DBM-250, 1/3 HP Arctica, JBJ Lighting).

All test solutions were prepared in 220-L polyethylene drums, by spiking UNB deCl freshwater with a 1 g L^{-1} stock solution of Cd ($CdCl_2$ • $2.5H_2O$, ACS, 79.5-81.0%, Alfa Aesar). The solutions were prepared the day before use, to allow them to reach thermodynamic equilibrium. They were then continuously pumped into the test aquaria, using peristaltic pumps (Masterflex L/S) and tubing (Masterflex PharMed #14). Aquaria of both temperature regimes, within a Cd exposure group, were fed by the same source of Cd. The incoming water was brought to the target temperature by coiling the tubing in the respective water baths before the water reached the temperature-controlled aquaria.

Fish were transferred from their temperature acclimation systems to the cadmium exposure systems 3 days prior to the start of the cadmium exposure, to allow them to become accustomed to their new surroundings. During the 28-day experiment, fish were fed daily *ad libitum* freshwater fish flakes (Omega One) and excess food was removed from the aquaria daily. Water temperature was measured every day. Water flow was measured on days 0, 3, 6, 16 and 23.

Fish were rinsed during 2min in Cd-free water before euthanization in 0.5 g/L MS-222 (Syndel) buffered with sodium bicarbonate (Fisher Chemical™). They were then quickly blot dried and weighed. Fish liver and gills were quickly dissected and flash frozen in liquid nitrogen to practice the dissection procedure. The carcass was then measured for their total and standard length. Gills, and livers were stored at -80°C and used as practice tissues only.

Table A1. Range-finding preliminary test results for mortality (ratio), whole body weight (g) and total length (cm) of the banded killifish (*Fundulus diaphanus*) acclimated to either 4 or 14°C and then exposed to a concentration of waterborne cadmium of either 0µg L^{-1}, 2.5µg L^{-1} or 5µg L^{-1} for 28 days.

Temperature	[Cd]waterborne (µg L^{-1})	Mortality (ratio)	Whole body weight (g)	Total length (cm)
4°C	0	0/6	2.13 ± 0.27	6.22 ± 0.39
	2.5	0/6	2.43 ± 0.29	7.02 ± 0.19
	5	1/6	2.27 ± 0.27	6.66 ± 0.30
14°C	0	0/6	2.75 ± 0.08	7.08 ± 0.12
	2.5	0/6	2.89 ± 0.12	7.18 ± 0.09
	5	0/6	3.23 ± 0.35	7.38 ± 0.15

A2 – Detailed statistical analyses results

Table A2. Statistical analyses using the extra sum-of-squares F tests and the Akaike Information Criterion (AIC) corrected for sample size (AICc) (for comparison when the assumptions of the F tests were no met) for the global slope comparison and the zero-slope comparison of the average cadmium (Cd) concentration (µg L^{-1}) in the water at days 0, 2, 5, 13, 21 and 28 of waterborne Cd exposures and the average temperature over the 28 days for the control (0 µg L-1), low (0.5 µg L^{-1}) and high (5 µg L^{-1}) sublethal Cd exposure groups acclimated to either 4°C or 14°C.

Water quality measurement	Control (0 µg L^{-1})		Low (0.5 µg L^{-1})		High (5 µg L^{-1})	
[Cd] µg L^{-1}	*Global slope comparison – Is there a difference between the temperature groups*					
	N/A		F-test, p <0.0001 AICc < 0.01%		F-test, p <0.0001 AICc < 0.01%	
	Zero slope comparison – Is the slope different from 0					
	4°C	14°C	4°C	14°C	4°C	14°C
	N/A		F-test, p = 0.0038 AICc = 3.396% Slope = -0.004950 R^2 = 0.3219	F-test, p = 0.5293 AICc = 74.9%	F-test, p = 0.0591 AICc = 33.79%	F-test, p = 0.0122 AICc = 10.1% Slope = -0.009152 R^2 = 0.2530

Table A3. Statistical analyses using the extra sum-of-squares F tests and the Akaike Information Criterion (AIC) corrected for sample size (AICc) (for comparison when the assumptions of the F tests were no met) for the global slope comparison and the zero-slope comparison of the average calcium (Ca) concentration (µg L^{-1}) in the water at days 0, 2, 5, 13, 21 and 28 of waterborne Cd exposures and the average temperature over the 28 days for the control (0 µg L-1), low (0.5 µg L^{-1}) and high (5 µg L^{-1}) sublethal Cd exposure groups acclimated to either 4°C or 14°C

Water quality measurement	Control (0 µg L^{-1})		Low (0.5 µg L^{-1})		High (5 µg L^{-1})	
[Ca] µg L^{-1}	*Global slope comparison – Is there a difference between the temperature groups*					
	F-test, p = 0.9202 AICc = 91.3%		F-test, p = 0.9616 AICc = 91.67%		F-test, p = 0.7403 AICc = 89.22%	
	Global slope comparison – Is there a difference between the exposure groups					
	F-test, p = 0.9882 AICc = 98.46%					
	Zero slope comparison – Is the slope different from 0					
	4°C	14°C	4°C	14°C	4°C	14°C
	F-test, p <0.0001 AICc <0.01% Slope = 60.77 R^2 = 0.7639					

Table A4. Statistical analyses using the extra sum-of-squares F tests and the Akaike Information Criterion (AIC) corrected for sample size (AICc) (for comparison when the assumptions of the F tests were no met) for the global slope comparison and the zero-slope comparison of the average sodium (Na) concentration (µg L^{-1}) in the water at days 0, 2, 5, 13, 21 and 28 of waterborne Cd exposures and the average temperature over the 28 days for the control (0 µg L-1), low (0.5 µg L^{-1}) and high (5 µg L^{-1}) sublethal Cd exposure groups acclimated to either 4°C or 14°C

Water quality measurement	Control (0 µg L^{-1})		Low (0.5 µg L^{-1})	High (5 µg L^{-1})
[Na] µg L^{-1}	*Global slope comparison – Is there a difference between the temperature groups*			
	F-test, p = 0.9332 AICc = 91.42%		F-test, p = 0.7367 AICc = 89.17%	F-test, p = 0.0889 AICc = 45.05%
	Global slope comparison – Is there a difference between the exposure groups			
	F-test, p = 0.5932 AICc = 94.68%			
	Zero slope comparison – Is the slope different from 0			
	4°C	14°C	4°C	14°C
	F-test, p <0.0001 AICc <0.01% Slope = 22.80 R^2 = 0.5908			

Table A5. Statistical analyses using the extra sum-of-squares F tests and the Akaike Information Criterion (AIC) corrected for sample size (AICc) (for comparison when the assumptions of the F tests were no met) for the global slope comparison and the zero-slope comparison of the average magnesisum (Mg) concentration (µg L^{-1}) in the water at days 0, 2, 5, 13, 21 and 28 of waterborne Cd exposures and the average temperature over the 28 days for the control (0 µg L-1), low (0.5 µg L^{-1}) and high (5 µg L^{-1}) sublethal Cd exposure groups acclimated to either 4°C or 14°C

Water quality measurement	Control (0 µg L^{-1})		Low (0.5 µg L^{-1})		High (5 µg L^{-1})	
[Mg] µg L^{-1}	*Global slope comparison – Is there a difference between the temperature groups*					
	F-test, p = 0.8358 AICc = 90.43%		F-test, p = 0.4111 AICc = 81.33%		F-test, p = 0.3065 AICc = 75.98%	
	Global slope comparison – Is there a difference between the exposure groups					
	F-test, p = 0.0070 AICc = 4.829%					
	Zero slope comparison – Is the slope different from 0					
	4°C	14°C	4°C	14°C	4°C	14°C
	F-test, p = 0.0088 AICc = 7.74% Slope = 0.4075 R^2 = 0.1399		F-test, p = 0.6570 AICc = 73.8%		F-test, p = 0.1311 AICc = 73.8%	

Table A6. Statistical analyses using the extra sum-of-squares F tests and the Akaike Information Criterion (AIC) corrected for sample size (AICc) (for comparison when the assumptions of the F tests were no met) for the global slope comparison and the zero-slope comparison of the average potassium (K) concentration (µg L^{-1}) in the water at days 0, 2, 5, 13, 21 and 28 of waterborne Cd exposures and the average temperature over the 28 days for the control (0 µg L-1), low (0.5 µg L^{-1}) and high (5 µg L^{-1}) sublethal Cd exposure groups acclimated to either 4°C or 14°C

Water quality measurement	Control (0 µg L^{-1})		Low (0.5 µg L^{-1})		High (5 µg L^{-1})	
[K] µg L^{-1}	*Global slope comparison – Is there a difference between the temperature groups*					
	F-test, p <0.0001		F-test, p = 0.0437 AICc = 27.44%		F-test, p = 0.0724	
	Zero slope comparison – Is the slope different from 0					
	4°C	14°C	4°C	14°C	4°C	14°C
	F-test, p = 0.5145	F-test, p = 0.4756	F-test, p = 0.6035 AICc = 76.2%	F-test, p = 0.2243 AICc = 62.03%	F-test, p = 0.0065 Slope = -0.4141 R^2 = 0.1504	

Table A7. Statistical analyses using the extra sum-of-squares F tests and the Akaike Information Criterion (AIC) corrected for sample size (AICc) (for comparison when the assumptions of the F tests were no met) for the global slope comparison and the zero-slope comparison of the average dissolved organic carbon (DOC) concentration (mg L^{-1}) in the water at days 0, 2, 5, 13, 21 and 28 of waterborne Cd exposures and the average temperature over the 28 days for the control (0 µg L-1), low (0.5 µg L^{-1}) and high (5 µg L^{-1}) sublethal Cd exposure groups acclimated to either 4°C or 14°C

Water quality measurement	Control (0 µg L^{-1})		Low (0.5 µg L^{-1})		High (5 µg L^{-1})	
[DOC] mg L^{-1}	*Global slope comparison – Is there a difference between the temperature groups*					
	F-test, p = 0.1043 AICc = 49.4%		F-test, p = 0.4555 AICc = 82.97%		F-test, p = 0.8911	
	Global slope comparison – Is there a difference between the exposure groups					
	F-test, p = 0.2806 AICc = 84.58%					
	Zero slope comparison – Is the slope different from 0					
	4°C	14°C	4°C	14°C	4°C	14°C
	F-test, p = 0.3137 AICc = 62.87%					

Table A8. Statistical analyses using the extra sum-of-squares F tests and the Akaike Information Criterion (AIC) corrected for sample size (AICc) (for comparison when the assumptions of the F tests were no met) for the global slope comparison and the zero-slope comparison of the average pH in the water at days 0, 2, 5, 13, 21 and 28 of waterborne Cd exposures and the average temperature over the 28 days for the control (0 µg L-1), low (0.5 µg L^{-1}) and high (5 µg L^{-1}) sublethal Cd exposure groups acclimated to either 4°C or 14°

Water quality measurement	**Control (0 µg L^{-1})**		**Low (0.5 µg L^{-1})**		**High (5 µg L^{-1})**	
pH	***Global slope comparison – Is there a difference between the temperature groups***					
	F-test, p = 0.5352 AICc = 85.32%		F-test, p = 0.9947		F-test, p = 0.9300 AICc = 91.39%	
	Global slope comparison – Is there a difference between the exposure groups					
	F-test, p = 0.0446 AICc = 32.5%					
	Zero slope comparison – Is the slope different from 0					
	4°C	**14°C**	**4°C**	**14°C**	**4°C**	**14°C**
	F-test, p = 0.0011 AICc = 1.135% Slope = -0.008368 R^2 = 0.2083		F-test, p <0.0001 Slope = -0.006724 R^2 = 0.2951		F-test, p = 0.0001 AICc = 0.1322% Slope = -0.007933 R^2 = 0.2765	

Table A9. Statistical analyses using the extra sum-of-squares F tests and the Akaike Information Criterion (AIC) corrected for sample size (AICc) (for comparison when the assumptions of the F tests were no met) for the global slope comparison and the zero-slope comparison of fish condition (as Fulton's condition factor (FCF) and hepatosomatic index (HSI)) for days 2, 5 and 28 of the waterborne cadmium (Cd) exposures.

	Day 2		**Day 5**		**Day 28**	
Measurement	***Global slope comparison – Is there a difference between the temperature groups***					
FCF	AICc = 52.26% F-test, p= 0.1264		F-test, p= 0.1840		AICc = 1.04% F-test, p = 0.0016	
HSI	F-test, p= 0.1188		AICc = 42.75% F-test, p = 0.0879		F-test, p= 0.1188	
Measurement	***Zero-slope comparison – Is the slope different from 0***					
	4°C	**14°C**	**4°C**	**14°C**	**4°C**	**14°C**
FCF	F-test, p = 0.8339		F-test, p = 0.7534		AICc = 76.72%; F-test, p = 0.9789	AICc = 76.78%; F-test, p = 0.8550
HSI	F-test, p = 0.1945		AICc = 42.99% F-test, p = 0.1035		F-test, p = 0.6573	

Table A10. Statistical analyses using the extra sum-of-squares F tests and the Akaike Information Criterion (AIC) corrected for sample size (AICc) (for comparison when the assumptions of the F tests were no met) for the global slope comparison and the zero-slope comparison for the tissue (gills, livers, kidneys, guts and muscle) Cd concentration analyses for days 2, 5 and 28 of the waterborne cadmium (Cd) exposures

	Day 2	**Day 5**	**Day 28**
Tissue	***Global slope comparison – Is there a difference between the temperature groups***		
Gills	AICc =<0.01%; F-test, p<0.0001	AICc =<0.01%; F-test, p<0.0001	AICc = 80.43%; F-test, p = 0.4403
Livers	F-test, p = 0.4265	F-test, p = 0.0003	AICc =<0.01%; F-test, p<0.0001
Kidneys	AICc = 90.56%; F-test, p = 0.9631	AICc = 45.18%; F-test, p = 0.0962	AICc < 0.01%; F-test, p < 0.0001
Guts	F-test, p = 0.0245	AICc = 9.331%; F-test, p = 0.0136	AICc < 0.01%; F-test, p < 0.0001
Muscle	F-test, p = 0.0797	AICc = 9.992%; F-test, p = 0.0145	AICc < 0.01%; F-test, p < 0.0001

Table A10. *(Cont'd)*

	Day 2		Day 5		Day 28	
Tissue	***Zero slope comparison – Is the slope different from 0***					
	4°C	**14°C**	**4°C**	**14°C**	**4°C**	**14°C**
Gills	AICc =<0.01% F-test, p<0.0001 R^2 = 0.8268	AICc =<0.01% F-test, p<0.0001 R^2 = 0.7752	AICc =<0.01% F-test, p<0.0001 R^2 = 0.8974	AICc =<0.01% F-test, p<0.0001 R^2 = 0.8229	AICc =<0.01% F-test, p<0.0001 R^2 = 0.7585	
Livers	F-test, p=0.4455		F-test, p = 0.2372	F-test, p <0.0001 R^2 = 0.5089	AICc = 40% F-test, p=0.0845 R^2 = 0.08498	AICc <0.01% F-test, p<0.0001 R^2 = 0.6488
Kidneys	AICc = 0.2159% F-test, p=0.0002 R^2 = 0.1942		AICc = 19.2% F-test, p= 0.0272 R^2 = 0.07073		AICc = 24.86% F-test, p= 0.0385 R^2 = 0.1234	AICc <0.01% F-test, p<0.0001 R^2 = 0.5275
Guts	F-test, p= 0.0140 R^2= 0.1648	F-test, p= 0.5921	AICc = 75.26% F-test, p=0.6986	AICc = 46.82% F-test, p= 0.1169 R^2 = 0.07074	AICc =19.31% F-test, p=0.0271 R^2 = 0.1356	AICc <0.01% F-test, p <0.0001 R^2 = 0.6128
Muscle	F-test, p = 0.0009 R^2 = 0.1641		AICc = 75.43% F-test, p= 0.6919	AICc = 46.8% F-test, p= 0.1140 R^2 = 0.08391	AICc = 0.4326% F-test, p=0.0004 R^2 = 0.3157	AICc <0.01% F-test, p <0.0001 R^2 = 0.5908

Table A 11. Statistical analyses using the extra sum-of-squares F tests and the Akaike Information Criterion (AIC) corrected for sample size (AICc) (for comparison when the assumptions of the F tests were no met) for the global slope comparison and the zero-slope comparison to assess waterborne Cd effects on oxidative stress analyses (protein carbonyl (PC), catalase activity (CAT) and Thiobarbituric acid reactive substances (TBARS) assays) in the livers and gills for days 0, 5 and 28 of the waterborne cadmium (Cd) exposures

		Day 0	**Day 5**	**Day 28**
Assay	**Tissue**	***Global slope comparison – Is there a difference between the temperature groups***		
PC	**Gills**	N/a	F-test, p = 0.7532	AICc = 18.75% F-test, p = 0.0290
	Livers	N/a	AICc = 74.41% F-test, p = 0.2986	AICc = 74.47% F-test, p = 0.3186
CAT	**Gills**	N/a	F-test, p = 0.5406	F-test, p = 0.9629
	Livers	N/a	F-test, p = 0.0148	AICc < 0.01% F-test, p < 0.0001
TBARS	**Livers**	N/a	N/a	AICc = 0.54% F-test, p = 0.0009

Table A11. *(Cont'd)*

		Day 0		Day 5		Day 28	
Assay	**Tissue**	***Is there a temperature difference?***		***Zero slope comparison – Is the slope different from 0***			
		4°C	**14°C**	**4°C**	**14°C**	**4°C**	**14°C**
PC	**Gills**	N/a		F-test, p = 0.8146		AICc = 30.24% F-test, p = 0.0517 R^2 = 0.1132	AICc = 65.69% F-test, p = 0.3145
	Livers	N/a		AICc = 51.45% F-test, p= 0.1548		AICc = 57.92% F-test, p = 0.2225	
CAT	**Gills**	N/a		F-test, p = 0.6702		F-test, p = 0.4710	
	Livers	N/a		F-test, p = 0.7945	F-test, p = 0.5304	AICc = 76.1% F-test, p = 0.7990	AICc = 12.14% F-test, p = 0.0151 R^2 = 0.1616
TBARS	**Livers**	Welch's t-test, p = 0.3881		N/a		AICc = 58.62% F-test, p = 0.1932	AICc = 1.702% F-test, p = 0.0020 R^2 = 0.4203
Assay	**Tissue**	*Is temperature difference for unexposed fish?*					
PC	**Gills**	N/a		N/a		N/a	
	Livers	N/a		N/a		N/a	
CAT	**Gills**	N/a		N/a		N/a	
	Livers	N/a		N/a		AICc = 50.81% F-test, p = 0.1309	
TBARS	**Livers**	N/a		N/a		AICc = 66.2% F-test, p = 0.2148	

Table A 12. Statistical analyses using the extra sum-of-squares F tests and the Akaike Information Criterion (AIC) corrected for sample size (AICc) (for comparison when the assumptions of the F tests were no met) for the global slope comparison and the zero-slope comparison to assess Cd tissue effects on oxidative stress analyses (protein carbonyl (PC), catalase activity (CAT) and Thiobarbituric acid reactive substances (TBARS) assays) in the livers and gills for days 5 and 28 of the waterborne cadmium (Cd) exposures.

		Day 5	Day 28
Assay	Tissue	*Global slope comparison – Is there a difference between the temperature groups*	
PC	Gills	F-test, p = 0.7506	F-test, p = 0.1400
	Livers	F-test, p = 0.2942	AICc = 80.59% F-test, p = 0.4446
CAT	Gills	F-test, p = 0.4810	F-test, p = 0.9458
	Livers	AICc = 46.26% F-test, p = 0.0964	AICc = 3.601% F-test, p = 0.0052
TBARS	Livers	N/a	AICc = 91.73% F-test, p = 0.9487

**There is no day 0 by tissue concentration (including for TBARS) since this measurement is not applicable as the fish were not experimentally exposed to Cd at day 0*

Table A12. *(Cont'd)*

Assay	Tissue	Day 5		Day 28	
		Zero slope comparison – Is the slope different from 0			
		4°C	14°C	4°C	14°C
PC	Gills	F-test, p = 0.8226		F-test, p = 0.6959	
	Livers	F-test, p = 0.2596		AICc = 72.19% F-test, p = 0.6072	
CAT	Gills	F-test, p = 0.8985		F-test, p = 0.6479	
	Livers	AICc = 17.8% F-test, p = 0.0245 R^2 = 0.09023		AICc = 76.63% F-test, p = 0.9189	AICc = 0.3278% F-test, p = 0.0003 R^2 = 0.3188
TBARS	Livers	n/a		AICc = 0.04204% F-test, p <0.0001 R^2 = 0.3215	

**There is no day 0 by tissue concentration (including for TBARS) since this measurement is not applicable as the fish were not experimentally exposed to Cd at day 0.*

Table A13. Statistical analyses using the extra sum-of-squares F tests and the Akaike Information Criterion (AIC) corrected for sample size (AICc) (for comparison when the assumptions of the F tests were no met) for the global slope comparison and the zero-slope comparison to assessed waterborne Cd effects on plasma cation levels (calcium (Ca), sodium (Na), magnesium (Mg) and potassium (K)) for days 2, 5 and 28 of the waterborne cadmium (Cd) exposures.

	Day 2	Day 5	Day 28
Cation	*Global slope comparison – Is there a difference between the temperature groups*		
Ca	F-test, p = 0.0208	F-test, p = 0.0007	AICc = 38.01% F-test, p = 0.0710
Na	F-test, p = 0.8884	F-test, p = 0.0577	F-test, p = 0.3843
Mg	F-test, p <0.0001	F-test, p <0.0001	F-test, p <0.0001
K	AICc = 88.29% F-test, p = 0.7639	F-test, p = 0.5591	F-test, p = 0.5598

Table A13. *(Cont'd)*

	Day 2		Day 5		Day 28	
Cation	*Zero slope comparison – Is the slope different from 0*					
	4°C	14°C	4°C	14°C	4°C	14°C
Ca	F-test, p = 0.3849	F-test, p = 0.6682	F-test, p = 0.1155	F-test, p = 0.6821	AICc = 77.88% F-test, p = 0.9605	AICc = 1.767% F-test, p = 0.0019 R^2 = 0.2872
Na	F-test, p <0.0001		F-test, p <0.0001		F-test, p = 0.4491	
Mg	F-test, p = 0.9732	F-test, p = 0.8990	F-test, p = 0.1629	F-test, p = 0.8271	F-test, p = 0.4200	F-test, p = 0.0021 R^2 = 0.2671
K	AICc = 64.95% F-test, p = 0.3358		F-test, p = 0.8641		F-test, p = 0.4590	
	Is temperature difference for unexposed fish?					
Ca	n/a		n/a		AICc = 63.47% F-test, p = 0.2435	
Na	n/a		n/a		n/a	
Mg	n/a		n/a		AICc = 0.4573% F-test, p = 0.0004	
K	n/a		n/a		n/a	

Table A14. Average cadmium (Cd) concentration ($[Cd]_{water}$; µg L^{-1}) (pre- and post-Cd spike), DOC, pH, calcium concentration ($[Ca]_{water}$; µg L^{-1}), sodium concentration ($[Na]_{water}$; µg L^{-1}), magnesium concentration ($[Mg]_{water}$; µg L^{-1}), and potassium concentration ($[K]_{water}$; µg L^{-1}) in the water at days 0, 2, 5, 13, 21 and 28 of waterborne Cd exposures and the average temperature over the 28 days for the control (0 µg L-1), low (0.5 µg L^{-1}) and high (5 µg L^{-1}) sublethal Cd exposure groups acclimated to either 4°C or 14°C. Data presented as mean ± standard deviation

	Control (0 µg L^{-1})		**Low Cd (0.5 µg L^{-1})**		**High Cd (5 µg L^{-1})**	
	4°C	**14°C**	**4°C**	**14°C**	**4°C**	**14°C**
Overall						
Temperature (°C)	4.1 ± 0.24	14.1 ± 0.17	4.1 ± 0.24	14.1 ± 0.20	3.9 ± 0.12	14.2 ± 0.22
DOC (mg L^{-1})	2.2 ± 0.7	2.0 ± 0.2	2.1 ± 0.5	2.0 ± 0.2	2.0 ± 0.4	2.0 ± 0.3
Day 0						
$[Cd]_{water}$ (µg L^{-1}) *Post-Cd spike*	<0.09	<0.09	0.48 ± 0.02	0.48 ± 0.07	4.94 ± 0.13	4.99 ± 0.08
pH	7.11 ± 0.14	7.04 ± 0.19	7.19 ± 0.09	7.17 ± 0.09	7.26 ± 0.09	7.23 ± 0.11
$[Ca]_{water}$ (µg L^{-1})	6354 ± 25	6380 ± 65	6371 ± 69	6478 ± 103	6447 ± 51	6408 ± 52
$[Na]_{water}$ (µg L^{-1})	11 074 ± 24	11 197 ± 73	11 047 ± 56	11 276 ± 276	11 074 ± 150	11 157 ± 26
$[Mg]_{water}$ (µg L^{-1})	584.7 ± 3.3	589.0 ± 5.3	587.1 ± 3.1	596.5 ± 12.9	588.9 ± 5.9	593.9± 2.7
$[K]_{water}$ (µg L^{-1})	261.7 ± 11.1	290.5 ± 10.3	259.9 ± 13.2	274.9 ± 9.3	275.1 ± 20.1	284.3 ± 14.5
Day 2						
$[Cd]_{water}$ (µg L^{-1})	<0.09	<0.09	0.29 ± 0.03	0.46 ± 0.05	3.60 ± 0.82	4.86 ± 0.08
pH	6.95 ± 0.10	6.91 ± 0.14	7.03 ± 0.07	7.00 ± 0.07	7.05 ± 0.05	7.05 ± 0.04
$[Ca]_{water}$ (µg L^{-1})	6542 ± 56	6529 ± 51	6615 ± 158	6539 ± 142	6543 ± 86	6521 ± 47
$[Na]_{water}$ (µg L^{-1})	11 240 ± 107	11 203 ± 108	11 505 ± 317	11 253 ± 95	11 239 ± 36	11 312 ± 89
$[Mg]_{water}$ (µg L^{-1})	598.5 ± 8.5	599.1 ± 7.8	611.4 ± 10.9	601 ± 7.0	603.2 ± 2.6	604.5 ± 2.1
$[K]_{water}$ (µg L^{-1})	282.1 ± 3.5	292.5 ± 4.8	294.5 ± 19.7	289.4 ± 12.8	286.1 ± 4.8	294.4 ± 4.8

Table A14. *Cont'd*

	Control (0 µg L^{-1})		Low Cd (0.5 µg L^{-1})		High Cd (5 µg L^{-1})	
	4°C	**14°C**	**4°C**	**14°C**	**4°C**	**14°C**
Day 5						
$[Cd]_{water}$ (µg L^{-1})	<0.09	<0.09	0.29 ± 0.06	0.45 ± 0.04	3.08 ± 0.93	4.63 ± 0.13
pH	7.24 ± 0.02	7.26 ± 0.02	7.25 ± 0.04	7.24 ± 0.03	7.26 ± 0.02	7.25 ± 0.03
$[Ca]_{water}$ (µg L^{-1})	6663 ± 115	6623 ± 62	6602 ± 60	6612 ± 78	6554 ± 47	6529 ± 33
$[Na]_{water}$ (µg L^{-1})	11 405 ± 122	11 408 ± 37	11 343 ± 83	11 449 ± 116	11 297 ± 67	11 356 ± 79
$[Mg]_{water}$ (µg L^{-1})	612.2 ± 1.6	614.4 ± 0.7	608.3 ± 3.0	612.1 ± 4.0	606.2 ± 2.0	608.6 ± 5.3
$[K]_{water}$ (µg L^{-1})	287.6 ± 7.6	304.9 ± 11.2	284.2 ± 14.5	289.1 ± 6.3	280.3 ± 2.4	287.4 ± 4.2
Day 13						
$[Cd]_{water}$ (µg L^{-1})	<0.09	<0.09	0.27 ± 0.02	0.44 ± 0.03	3.12 ± 0.93	4.56 ± 0.11
pH	6.98 ± 0.10	6.96 ± 0.13	6.93 ± 0.14	7.04 ± 0.04	6.90 ± 0.20	6.90 ± 0.17
$[Ca]_{water}$ (µg L^{-1})	6864 ± 85	6901 ± 28	6925 ± 27	6861 ± 70	6814 ± 61	6817 ± 56
$[Na]_{water}$ (µg L^{-1})	11 141 ± 108	11 104 ± 108	11 199 ± 115	11 162 ± 59	11 189 ± 72	11 135 ± 47
$[Mg]_{water}$ (µg L^{-1})	610.8 ± 2.2	611.0 ± 0.5	606.9 ± 3.8	609.4 ± 1.9	604.1 ± 3.9	604.3 ± 2.0
$[K]_{water}$ (µg L^{-1})	273.2 ± 3.6	286.2 ± 6.3	271.7 ± 5.2	281.7 ± 7.0	271.9 ± 7.2	273.2 ± 2.5
Day 21						
$[Cd]_{water}$ (µg L^{-1})	<0.09	<0.09	0.21 ± 0.02	0.50 ± 0.01	3.08 ± 1.05	4.67 ± 0.17
pH	6.94 ± 0.09	6.92 ± 0.13	7.05 ± 0.07	7.03 ± 0.09	7.06 ± 0.04	7.00 ± 0.08
$[Ca]_{water}$ (µg L^{-1})	7000 ± 278	6927 ± 114	7152 ± 423	7025 ± 324	7372 ± 734	7190 ± 507
$[Na]_{water}$ (µg L^{-1})	11 663 ± 50	11 668 ± 47	11 703 ± 112	11 690 ± 54	11 703 ± 65	11 569 ± 200
$[Mg]_{water}$ (µg L^{-1})	616.9 ± 1.4	619.0 ± 2.3	610.5 ± 5.9	612.6 ± 1.3	606.9 ± 1.4	603.7 ± 8.6
$[K]_{water}$ (µg L^{-1})	280.1 ± 8.5	298.5 ± 10.8	280.1 ± 7.1	289.9 ± 10.7	272.4 ± 15.9	279.4 ± 5.3
Day 28						
$[Cd]_{water}$ (µg L^{-1})	<0.09	<0.09	0.28 ± 0.02	0.47 ± 0.04	3.24 ± 0.87	4.64 ± 0.17
pH	6.91 ± 0.06	6.75 ± 0.35	6.96 ± 0.04	6.94 ± 0.04	6.99 ± 0.03	6.99 ± 0.02
$[Ca]_{water}$ (µg L^{-1})	8545 ± 109	8450 ± 134	8309 ± 78	8520 ± 228	8456 ± 443	8327 ± 90
$[Na]_{water}$ (µg L^{-1})	11 825 ± 79	11 834 ± 71	11 808 ± 92	12 006 ± 286	12 030 ± 306	11 806 ± 136
$[Mg]_{water}$ (µg L^{-1})	598.9 ± 3.8	600.6 ± 0.7	585.9 ± 1.7	597.7 ± 11.0	592.2 ± 16.7	583.6 ± 4.5
$[K]_{water}$ (µg L^{-1})	276.8 ± 7.8	287.3 ± 7.7	270.3 ± 8.3	288.9 ± 7.4	271.0 ± 6.8	278.3 ± 5.3

A3 – Water parameter figures

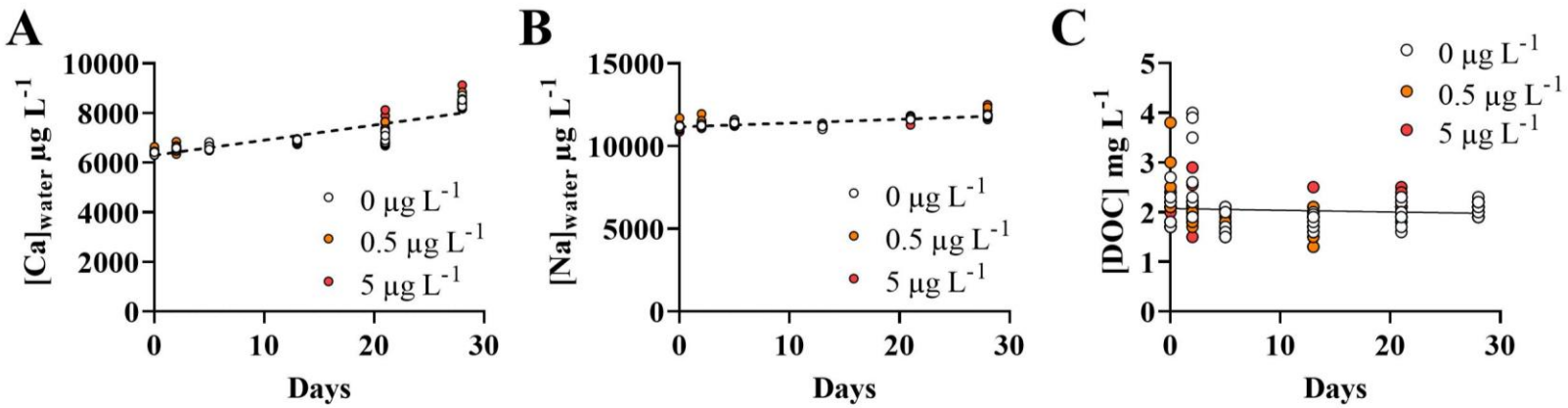

Figure A 1. Calcium (A), sodium (B) and dissolved organ carbon concentration (DOC; C) in the water of the 4°C and 14°C aquaria (n = 4) as a function of Cd exposure days for the control (0 µg L-1), low (0.5 µg L^{-1}) and high (5 µg L^{-1}) exposure aquaria

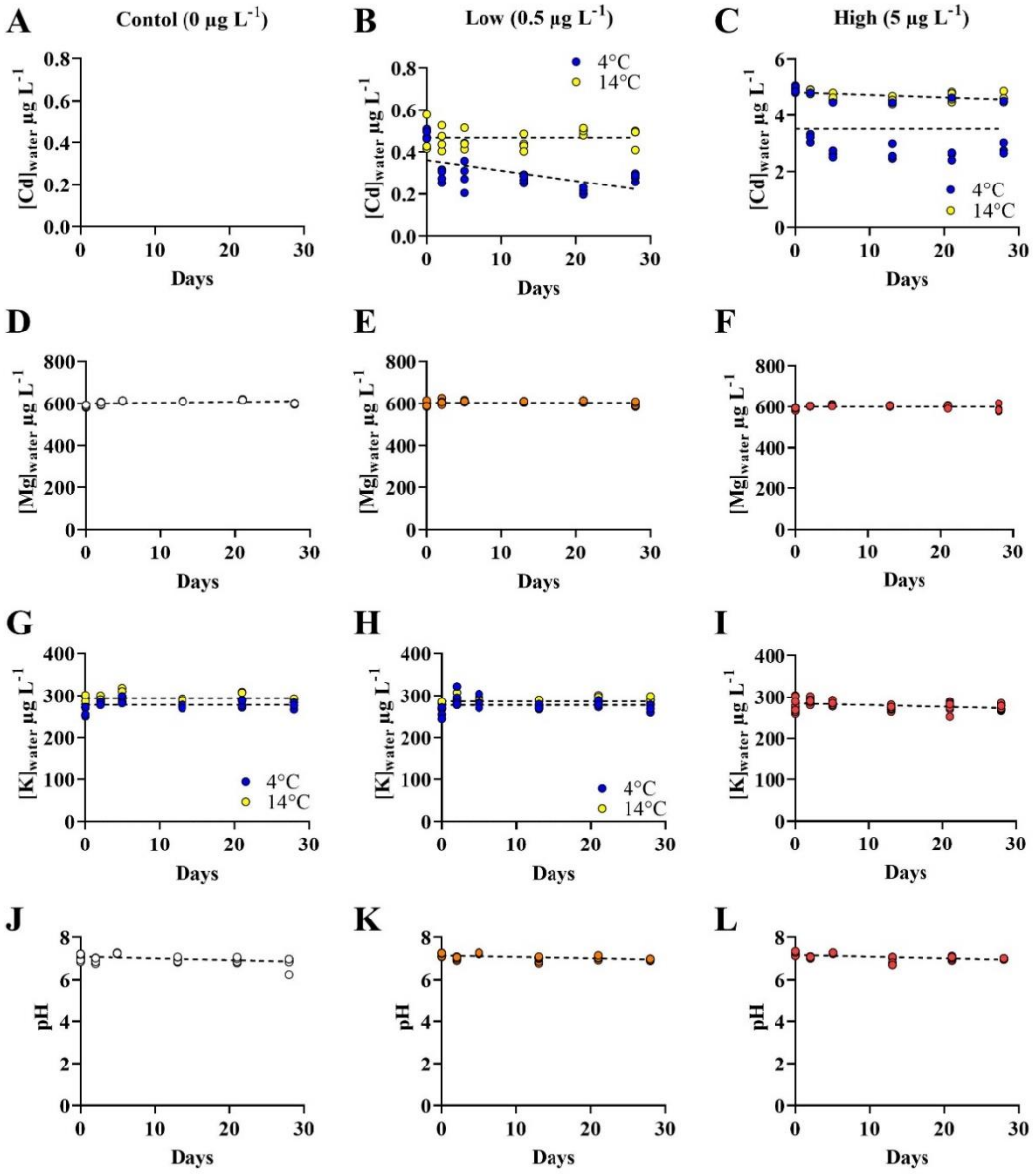

Figure A 2. Cadmium (A, B, C), magnesium (D, E, F), potassium (G, H, I) and pH (J, K, L) levels in the water of 4°C or 14°C aquaria (n = 4) as a function of Cd exposure days for the control (0 µg L^{-1}), low (0.5 µg L^{-1}) and high (5 µg L^{-1}) Cd exposure aquaria.

A4 – Oxidative stress in the gills

Table A 15. Catalase activity (CAT; $\mu mol^{-1}\ min^{-1}\ mg^{-1}$ protein) and protein carbonyl levels (PC; $nmol\ mg^{-1}$ protein) in the gills of 4°C- or 14°C-acclimated banded killifish after 5 r 28 days of Cd exposures for the control (0 $\mu g\ L^{-1}$), low (0.5 $\mu g\ L^{-1}$) and high (5 $\mu g\ L^{-1}$) Cd exposure groups. Data presented as mean ± SEM with n in parentheses

	Control (0 µg L^{-1})		**Low (0.5 µg L^{-1})**		**High (5 µg L^{-1})**	
	4°C	**14°C**	**4°C**	**14°C**	**4°C**	**14°C**
Day 5						
CAT activity ($\mu mol^{-1}\ min^{-1}\ mg^{-1}$ protein)	4.45 ± 0.32 (12)	3.84 ± 0.42 (11)	5.14 ± 0.20 (10)	4.99 ± 0.41 (11)	4.53 ± 0.28 (12)	4.40 ± 0.32 (12)
PC levels ($nmol\ mg^{-1}$ protein)	6.50 ± 0.61 (12)	6.84 ± 0.41 (10)	6.57 ± 0.40 (11)	6.79 ± 0.58 (12)	6.39 ± 0.73 (11)	6.52 ± 0.42 (12)
Day 28						
CAT activity ($\mu mol^{-1}\ min^{-1}\ mg^{-1}$ protein)	4.30 ± 0.35 (12)	4.43 ± 0.42 (12)	4.83 ± 0.44 (12)	4.76 ± 0.31 (12)	4.96 ± 0.33 (12)	4.80 ± 0.41 (12)
PC levels ($nmol\ mg^{-1}$ protein)	6.79 ± 0.43 (11)	7.72 ± 0.44 (12)	8.28 ± 0.48 (11)	7.14 ± 0.37 (12)	6.17 ± 0.36 (12)	8.15 ± 0.60 (12)

Curriculum Vitae

Emily Suominen, MSc candidate

Languages

Speaking Level:	Bilingual (English and French)
Writing Level:	Bilingual (English and French)

Education

09/2019 –To date	**MSc in Biology**, University of New Brunswick, Saint John, Canada
09/2014 – 12/2018	**Honours Bachelor of Science** with Specialization in Biology (COOP), *magna cum laude*, University of Ottawa, Ottawa, Canada
09/2012 – 05/2014	**College diploma in Natural sciences**, Cégep de l'Outaouais, Gatineau, Canada

Work experience

08/2021 – To date	**Animal Care Technician** University of Ottawa (Ottawa, Canada)
02/2020 – 06/2021	**Accessibility invigilator** University of New Brunswick (Saint John, Canada)
09/2019 –12/2020	**Teaching Assistant** University of New Brunswick (Saint John, Canada)
05/2019 – 08/2019	**Laboratory Technician** Michigan State University (Cheboygan, USA)
05/2016 – 09/2018, 01/2019 – 05/2019	**Laboratory Technician** Natural Resources Canada (Ottawa, Canada)
01/2016 – 04/2016	**Monitoring and Reporting of Recovery Activities for Species at Risk Assistant** Environment and Climate Change Canada (Gatineau, Canada)
05/2015 – 12/2015	**Analyst** Ministère du Développement durable, de l'Environnement et de la Lutte contre les changements climatiques (Gatineau, Canada)

Research experience

09/2019 – To date	**Investigating cold temperature effects on cadmium toxicity to freshwater fish**

	MSc project at UNB (Saint John, Canada) Supervisor: Anne Crémazy (anne.cremazy@unb.ca) Co-supervisor: Ben Speers-Roesch (bspeersr@unb.ca)
09/2019 – 04/2020	**Determining metal concentrations in Atlantic Salmon** MSc side project with the Fisheries and Oceans Canada and the Gespe'gewaq Mi'gmaq Resource Council (Saint John, Canada) Direct Supervisor: Anne Crémazy (anne.cremazy@unb.ca) Project Lead: Cindy Breau (Cindy.Breau@dfo-mpo.gc.ca)
05/2019 – 08/2019	**Determining active compounds of alarm cue of sea lamprey** Lab assistant at USGS Hammond Bay Biological Station for Michigan State University (Cheboygan, USA) Direct Supervisor: Mikaela Hanson (hanso106@msu.edu) Principal Investigator: Michael Wagner (mwagner@msu.edu)
09/2018 – 11/2019	**Investigating the effects of acute changes in water temperature and salinity on the swimming performance of *Fundulus heteroclitus*** Directed studies project at the Huntsman Marine Science Center (Saint Andrews, Canada) Supervisor: Ben Speers-Roesch (bspeersr@unb.ca) Co-supervisor: Heather Hunt (Heather.Hunt@unb.ca)
09/2017-04/2018	**Assessing the fate and effects of metals bound to suspended sediments in Sudbury lakes** Honours project at CanmetMining (Ottawa, Canada) Supervisor: Carrie Rickwood (carrie.rickwood@canada.ca) Co-supervisor: Alexandre Poulain (apoulain@uottawa.ca)
05/2016 – 12/2016	**Optimization of algal subcellular fractionation methods** Internship at CanmetMining (Ottawa, Canada) Supervisor: Carrie Rickwood (carrie.rickwood@canada.ca)

Presentations (* presenter)

Suominen, E.*, Speers-Roesch, B., Crémazy, A. 2021. The effects of winter cold on acute and chronic cadmium bioaccumulation and toxicity in the banded killifish (*Fundulus diaphanus*). SETAC North America 42nd Annual Meeting, Virtual

Suominen, E.*, Speers-Roesch, B., Crémazy, A. 2021. The effects of winter cold on acute and chronic cadmium bioaccumulation and toxicity in the banded killifish (*Fundulus diaphanus*). CEW 2021 Annual Conference. Halifax, Canada.

Suominen, E.*, Speers-Roesch, B., Crémazy, A. 2021. The effects of winter cold on acute and chronic cadmium bioaccumulation and toxicity in the banded killifish (*Fundulus diaphanus*). SEB 2021 Annual Conference. Virtual.

Suominen, E.*, Speers-Roesch, B., Crémazy, A. 2021. Les effets de températures hivernales sur la bioaccumulation et la toxicité du cadmium chez le fondule barré (*Fundulus diaphanus*). 2021 Chapitre Saint-Laurent. Virtual.

Suominen, E.*, Speers-Roesch, B., Crémazy, A. 2020. The effects of winter cold on acute and chronic cadmium bioaccumulation and toxicity in the banded killifish (*Fundulus diaphanus*). SETAC North America 41st Annual Meeting. Virtual.

Suominen, E.*, Speers-Roesch, B., Crémazy, A. 2020. Les effets de températures hivernales sur la bioaccumulation et la toxicité aigüe et chronique du cadmium pour le Fondule barré (*Fundulus diaphanus*). EcotoQ student conference, Virtual.

Suominen, E.*, Speers-Roesch, B., Crémazy, A. 2020. The effects of winter on acute and chronic cadmium bioaccumulation and toxicity in the banded killifish (*Fundulus diaphanus*). Departmental MSc proposal presentation. Saint John, Canada

Suominen, E.*, Speers-Roesch, B., Crémazy, A. 2019. Exploring the effects of winter on chronic cadmium bioaccumulation and toxicity in the banded killifish. Canadian Rivers Institute (CRI) Days 3min seminar mini-conference.

Suominen, E.*, Poulain, A., Rickwood, C. 2018. Assessing the fate and effects of metals bound to suspended sediment in Sudbury area lakes. University of Ottawa poster session. Ottawa, Canada

Suominen, E.*, Hunt, H., Speers-Roesch, B. 2018. The effects of acute changes in water temperature and salinity on swimming performance of *Fundulus heteroclitus*. Departmental platform presentations of directed studies projects with collaborators. Saint Andrews, Canada

Rickwood, C.*, King, M., **Suominen, E.**, Prabhakar, G. 2017. Selenate versus Selenite - uptake into algae and trophic-transfer to Daphnia magna. Canadian Ecotoxicity Workshop. Guelph, Canada

Publications

Suominen, E., Speers-Roesch, B., Crémazy, A. 2021. The effects of winter cold on acute and chronic cadmium bioaccumulation and toxicity in the banded killifish (*Fundulus diaphanus*) (MSc Thesis). University of New Brunswick [Under preparation], Saint John, Canada

Gauthier, P. T., Blewett, T. A., Garman, E. R., Schlekat, C. E., Middleton, E. T., Suominen, E. and Crémazy, A. (2021). Environmental risk of nickel in aquatic Arctic ecosystems. *Sci. Total Environ*. 797, 148921.

Environment and Climate Change Canada. 2015. *Species at Risk Act Annual Report for 2015*.45pp.

Technical skills

- Animal Care (aquatic vertebrates and invertebrates)
- Statistics using R software
- Elemental analyses (ICP-OES, ICP-MS)
- Water quality analyses
- DNA extractions
- PCR
- Gel electrophoresis
- Algae cell counting using Coulter counter
- Oxidative stress assays (spectrophotometry)
- Plumbing (for recirculating and flow-through aquatic systems)
- Microscopy (compound and dissection)

Awards and Honours

Maecenas Graduate Scholarship (7894$ CAD)

SETAC North America 41st Annual Meeting Student Attendance Grant (179.10$ USD)

STEM & Social Innovation Award 2019 (7000$ CAD), New Brunswick Innovation Foundation

Merit scholarship 2017 (1000$ CAD), uOttawa

Dean’s Honour List Winter 2017 and Winter 2018, uOttawa