Canadian Nuclear Safety Commission | Commission canadienne de sûreté nucléaire

CMD 25-H3.15

File / dossier : 6.01.07
Date: 2024-12-10
e-Doc PDF: 7409033

Written submission from the Nuclear Transparency Project

Mémoire du Projet de transparence nucléaire

In the Matter of the

À l'égard de

Cameco Corporation, Beaverlodge Project

Cameco Corporation, le projet de Beaverlodge

Application for the Licence Revocation and Transfer of Properties to Saskatchewan Institutional Control Program

Demande de révocation de permis et de transfert de propriétés au programme de contrôle institutionnel de la Saskatchewan

Commission Public Hearing

Audience publique de la Commission

January 30, 2025

30 janvier 2025

Canada

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Submitted via email

December 10, 2024

To President Tremblay and Members of the Canadian Nuclear Safety Commission,

> Re: Cameco's application to be released from CNSC licensing for the Beaverlodge site

We would like to begin by thanking the Commission for this opportunity to provide comments on this matter. We are also grateful for CNSC staff's Commission Member Documents. The new practice of adding a reference package to accompany them was also appreciated and proved very helpful to us in our preparation of this intervention. Finally, we would also like to thank the other intervenors in this matter for their informative submissions on this matter, especially Ya'thi Néné Lands and Resources from whom we have learned a lot about this facility and the region.

NTP's comments have been made possible by CNSC funding through its Participant Funding Program (PFP). These submissions were researched and drafted by NTP founder and coordinator Pippa Feinstein in collaboration with ecotoxicologist and NTP contributor Dr. Shamaila Fraz. They, have been divided into three main parts on the following pages:

About NTP

The Nuclear Transparency Project (NTP) is a Canadian-registered not-for-profit organization dedicated to supporting open, informed, and equitable public discourse on nuclear technologies. NTP advocates for robust public access to data and other types of information and helps to produce accessible analysis of publicly available information, all with a view to supporting greater transparency in the Canadian nuclear sector.

NTP engages with a multi-disciplinary group of experts to address economic, ecological, and social facets of the Canadian nuclear sector, producing public reports, academic articles, and other publicly accessible resources as well as intervening in regulatory decision-making processes. The organization seeks to support youth and early career scholars, especially those from underrepresented communities and groups in the nuclear field. NTP also recognizes a responsibility to model the transparency and accountability practices for which it advocates. It is committed to interdisciplinary, cross-sectoral, and equitable collaborations and dialogue between regulators, industry, civil society, members of host and potential host communities, as well as academics and professionals from Science, Technology, Engineering, and Mathematics (STEM) fields, the social sciences, and humanities.

About this intervention

The Beaverlodge site is one of the oldest Canadian-regulated uranium mining and milling sites. It is located within Nuhenéné and Treaty 10 territory, as well as the homelands of Métis and Cree Peoples. The site is also just outside Uranium City in northwest Saskatchewan. It was opened in 1952 and operated for thirty years until 1982. Beaverlodge was the first uranium mining operation to be formally decommissioned between 1982 and 1985,[1] although the CNSC required additional studies and remediation activities starting in the early 2000s.[2] It is a very large site with over 70 structures or features including a main underground mine and several smaller underground satellite mines, open pit mines, a centralized mill that processed the extracted ore and a centralized tailings area.

To date, 43 structures or features at the Beaverlodge site have been removed from CNSC oversight on the grounds that that they have been successfully decommissioned. This still leaves 27 structures/features that Cameco is now applying to also remove from CNSC oversight. Should their application be approved by the CNSC Tribunal, it will mean there will no longer be any licence issued to Cameco for the Beaverlodge site.

[1] See: https://www.beaverlodgesites.com/.
[2] CNSC staff CMD, online: https://api.cnsc-ccsn.gc.ca/dms/digital-medias/CMD25-H3.pdf/object at p 5.

NTP is primarily interested in assessing the transparency of Beaverlodge decommissioning and monitoring activities to date. As Cameco is applying to remove CNSC oversight, NTP is also interested in verifying whether transparency would be enhanced, or at least protected, should the Beaverlodge site be released from CNSC licensing requirements. In preparing this intervention, we have sought to understand the environmental impacts of the Beaverlodge site and how Cameco proposes to monitor it going forward, should its licence be revoked.

Our review of available materials has also allowed us to provide some analysis of available environmental data relating to ecological conditions at the Beaverlodge site. We offer these with the hope they may also be of interested members of the public, civil society, and Indigenous communities and organizations. Finally, we assess Cameco's approach to public disclosure and communication, making recommendations for how monitoring results and activities should be publicly disclosed in the years to come.

Indigenous jurisdiction and the CNSC's regulatory context

NTP recognizes the sovereignty and jurisdiction of the Indigenous Peoples on whose land the Beaverlodge site is located. We support their interventions in this matter and recognize them as relevant decision-makers when determining allowable activities by nuclear industry in their territories. NTP also recognizes the applicability of Indigenous laws as part of these Nations' governance systems of their homelands.

Neither the Beaverlodge site nor the current hearing extinguishes Indigenous jurisdiction, nor does do these things prove the paramountcy of Canadian law and regulation of the site. A formalized process by which Indigenous Peoples' authority and jurisdiction can be observed is necessary to determine a just outcome of these matters and should be defined by these rights holders.

NTP also notes that questions about Indigeneity are complex and have been made fraught by generations of Canadian colonial lawmaking that sought to break Indigenous legal, governance, and kinship systems. That being said, we urge the CNSC to consult with Nations on protocols for determining Indigenous identity and rights holders in a way that is consistent with Indigenous law and policies developed by the relevant Nations. While this is a difficult task that demands sensitivity, there are examples of it being done ethically and equitably in many jurisdictions and institutions.

PART ONE:
Conditions at the Beaverlodge site

Cameco is applying for the release from their CNSC licence on the basis of their efforts to contain and remediate the Beaverlodge site. However, the application recognizes that there are nuclear substances present above clearance levels, and for this it requests an exemption from CNSC licensing to transfer the site to Saskatchewan's Institutional acProgram.

The *Nuclear Safety and Control Act* and its regulations permit such an exemption to be made provided it would not "pose an unreasonable risk to the environment or the health and safety of persons".[3] However, it is important to note that both Cameco and CNSC staff acknowledge that radionuclide levels at the Beaverlodge site would on their own merit continued CNSC oversight via a license for the facility.

Cameco's site decommissioning framework also provides guidance for assessing site conditions and the need for regulatory oversight of Beaverlodge. According to this framework, facilities' performance objectives must meet a three-point threshold, ensuring they are safe, secure and stable/improving. Each is defined as follows:

> Safe: The site is safe for unrestricted public access. This objective is to ensure that the long-term safety is maintained.
>
> Secure: There must be confidence that long-term risks to public health and safety have been assessed by a qualified person and are acceptable; and
>
> Stable/Improving: Environmental conditions (e.g., water quality) on and downstream of the decommissioned properties are stable and continue to naturally recover as predicted.[4]

The first two largely relate to gamma radiation levels, the permanent closure of boreholes and mine openings, and the removal of mining debris. The last largely relates to surface water quality.[5]

As NTP will outline below, there is not currently sufficient publicly available information or data to determine whether an exemption to CNSC licence is merited, or whether the decommissioning framework threshold has been met.[6] As a result, NTP submits that no determination on Cameco's application should be made by the Commission tribunal until

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[3] *Nuclear Safety and Control Act*, SC 1997, c9 at s 7; and *General Nuclear Safety and Control Regulations*, SOR /2000-202 at s 11.

[4] Cameco CMD, online: https://api.cnsc-ccsn.gc.ca/dms/digital-medias/CMD25-H3-1.pdf/object at p 7.

[5] *Ibid* at p 8.

[6] For a high-level illustration of this specifically, Table 2-1 of Cameco's application outlines which performance indicators it believes have been met – without disclosing any of these specific indicators or how available data proves they have been met. See: Cameco CMD, *ibid* at pp 16-17.

more information is disclosed to allow a meaningful public review. The granting of a short-term licence for another year by the CNSC may be granted to allow for this after which a new hearing can be established to consider Cameco's application more fully on its merits.

Water quality

Water quality acceptance criteria requires trends from past and ongoing water monitoring to be compared against modelled predictions to determine whether: remediation efforts are proving successful on the ground and whether "natural recovery" is occurring downstream from decommissioned properties as predicted in relevant modelling.[7]

Dr. Fraz reviewed Cameco's 2023 annual report to better understand current surface water conditions on and around the Beaverlodge site. She noted the report provides records of water chemistry data that have been averaged for the past four years along with a four-year mean analysis, which showed:

- Six monitoring sites displaying elevated concentrations of copper (Cu). Site ZOR02 measured 2 ug/L of copper, a concentration that has been found to adversely affect fish species. Cu at this concentration has been found to impair salmonoids' olfactory senses which in turn prevent the fish from being able to detect and avoid exposure to other harmful contaminants.[8] Site TL3 measured 1.5 ug/L of Cu, which has been found to reduce the alarm response in juvenile salmonids.[9] Four other sites displayed higher Cu concentrations also with ML1 measuring 1.4 ug/L, CS2 measuring 1.2 ug, and sites TL6 and BL3 measuring 1.1. ug/L;
- One site (TL6) measured 1.6 ug/L of lead (Pb), an elevated concentration for the toxic heavy metal;
- Three sites displayed selenium (Se) concentrations higher than the applicable Saskatchewan Environmental Quality Standard (SEQS) of 2 ug/L: site TL3 measured 2.6 ug/L, TL6 measured 2.5 ug/L, and TL9 measured 2.1 ug/L. Sites TL4 and TL7 measured 1.4 ug/L, sites BL3 and BL4 measured 1.9 ug/L, and site BL5 measured 1.8 ug/L. Studies have found that Se can bioaccumulate at dietary concentrations of 3 ug/L;[10] and
- Concentrations of uranium (U) in surface water were particularly elevated with one site (ZOR-02) measuring 453 ug/L which is 25 times the SEQS of 15 ug/L. Twelve other monitoring sites displayed elevated concentrations of uranium as well: site

[7] Cameco CMD, *ibid* at p 9.
[8] Salmonids sensory mechanism responsible for avoidance responses was impaired by the long-term sub-lethal concentration of 2 µg/L Cu, which could result in further impairment of sensory dependent behaviors essential for survival, or result in mortality if fish are later exposed to higher concentrations. See: Price, 2013 in Appendix A to these submissions.
[9] See: Price, 2013 *ibid* in Appendix A to these submissions
[10] See: Uddin et al, 2024 in Appendix A to these submissions.

> AN5 at 157 ug/L, site DB6 at 86 ug/L , site AC6A at 252 ug/L , site AC14 at 36 ug/L, site TL3 at 191 ug/L, site TL4 at 175 ug/L, site TL6 at 244 ug/L, site TLL7 at 172 ug/L, site TL9 at 126.5 ug/L, site BL3 at 114 ug/L, site BL5 at 107 ug/L, and site BL7 at 105 ug/L. All these values pose concerns relating to their ability for long term sublethal effects and the potential to bioaccumulate in aquatic organisms.[11]

In fact, while many of the values above may seem low, the risks of bioaccumulation and/or biomagnification are concerning. Despite this, neither bioaccumulation nor biomagnification have been addressed in any publicly available materials.

Several of the sites such as TL6 displayed elevated concentrations of multiple contaminants (noted above), raising concerns about complex mixture and cumulative adverse effects which are also not addressed in publicly available materials. Dr. Fraz also identified considerable natural variation in water chemistry (noted in more detail below) between monitoring sites, which can impact the bioavailability of certain contaminants. The complex mixture of contaminants along with natural variation in water chemistry across the Beaverlodge site can thus pose varied effects on fish over a prolonged period, primarily fish organs. More specifically, very low concentrations of metals can elicit chronic sub-lethal toxicity to fish (adults, or early life stages) through mixture effects and cumulative adverse effects. Chronic exposure to heavy metals can also lead to deformities in fish larvae leading to reduced survival and is associated with reduced growth (reduced length and weight) in juveniles. There is also a possibility that these exposures may associate with oxidative damage in the gills, kidneys, liver, gonads of exposed fish. Finally, as these site conditions have been present in the same or higher concentrations for so many decades, effects on aquatic biota have already spanned, and will continue to span, many generations. It remains unclear whether these varied, long-term, cumulative, and potentially multigenerational effects have been taken under consideration by Cameco in their approach to water quality modelling or testing.

Dr. Fraz has also raised concerns of potential underinclusive contaminant parameters as cadmium, aluminum, arsenic, and mercury were monitored in the Eastern Athabasca Regional Monitoring Program as being present in fish chemistry but are not included in Cameco's 2023 annual report. All of these metals could be highly toxic to fish even in low concentrations.

Finally, Dr. Fraz explains there are considerable differences in water chemistry across various monitoring sites. For example, parameters like the alkalinity, hardness, organic carbon and pH at site AC-6A are 85.5, 132, 9.0 mg/L and 7.97 respectively, whereas these parameter values at site AC-8 are 42, 46, 7.2 mg/L and 7.48 respectively. These differences contribute to the free water dissolved fraction versus bound fractions of

[11] See: Kraemer & Evans, 2012, online: https://doi.org/10.1016/j.aquatox.2012.08.012.

organic metals and can also modulate the toxicity, bioaccumulation, and biomagnification of metals. Here, the ERA may have descriptions of models used to account for this variation, predicting potential exposures to fish that take into account these variations as well as the bioavailability of heavy metals present in the environment. There is reference to Cameco's ADEPT model (which may do this) in its ERA summary document, however, without access to the full ERA, the model and its comprehensiveness and limitations remain unknown to the public.

Recommendation 1: before any decision is rendered on Cameco's application, CNSC staff should require the company to disclose its monitoring and modelling methodologies for public review and explain how it accounts for long-term, cumulative, and highly variable water quality.

Proposed long-term monitoring

Dr. Fraz has evaluated Cameco's proposed Long-term Monitoring Plan (LTMP) and associated Field Guide for the Beaverlodge site. Over the course of this review, she identified several issues of potential concern outlined below.

First, the LTMP proposes to drop the TL-6, BL-3, and BL-4 sites from the list of monitoring locations in the plan by arguing that monitoring of downstream sites would be sufficient. However, Dr. Fraz noted these sites in question have quite high contaminant concentrations, several above SEQGs. Any benefits of dropping these sites remain unclear: it would stop Cameco from being able to discern long-term recovery trends for these sites, prevent future conditions from being referenced against historical ones rendering it impossible to measure potential recovery in those areas. Further, without any sufficiently detailed maps accompanying this proposal, the location of downstream monitoring locations remains unknown, preventing the public from being able to determine whether they can reasonably be expected to account for the dropped monitoring sites.

Cameco also asserts in its LTMP that "the current understanding is that, without a substantial additional load to the environment, sediment quality and benthic invertebrate community are expected to continue to recover slowly over time".[12] In fact this is the primary assertion animating the LTMP as well as Cameco's current application for the Beaverlodge site to be released from CNSC oversight. However, publicly available data does not indicate consistent or widespread downward trends in contaminant concentration data across the Beaverlodge site. For example, publicly available data

[12] See Cameco Long-term Monitoring Plan, online: https://www.beaverlodgesites.com/public/Beaverlodge_LTMP_Report.pdf at p 3.

provided for three contaminants of potential concern (COPCs) – selenium, uranium, and radium – do not seem to show a consistent downward decline. This data directly challenges Cameco's assertion that conditions in these pathways and receptors will ameliorate over time.

Additionally, the LTMP states "[e]xtensive watershed modelling" referenced existing monitoring data to assess and predict water quality conditions in the Ace Creek watershed, Fulton Creek Watershed, Beaverlodge Lake, and downstream surface water through the Crackingstone River.[13] While Appendix B to the LTMP provides predictive bounds (minimum and maximum) for U and Se in a couple figures, no comparisons of predictions versus measured concentrations are provided.[14] There is a strong public interest in sharing the model predictions including instances of deviations from predictions, model uncertainties, and limitations. However, for members of the public there is currently no way to determine how models compare with real world conditions. This lack of disclosure prevents us from being able to assess the accuracy and reasonableness of the models being relied upon by Cameco to ensure the Beaverlodge site's containment and recovery.

Cameco also proposes to discontinue any monitoring of sediment or benthic invertebrate species. It remains unclear whether this decision implies that Cameco believes it is not important or necessary to monitor the recovery of sediment quality or the benthic invertebrate community. Or it may indicate feasibility concerns related to sediment and benthic invertebrate monitoring. The former would indicate the lack of an ecosystem approach by Cameco and related regulatory concerns, while the latter would indicate a potential need to revisit funding for the LTMP.

Cameco's LTMP also seems to abandon an ecosystem approach for determining monitoring frequencies. The company proposes that "surface water is the best indicator of overall aquatic environment recovery".[15] As such, the LTMP proposes a water quality sampling frequency of once every three years for the next 15 years with the potential to decrease frequency to every five years after that. It is unclear what data was used in arriving at the decision of water sampling frequency, as available figures all show notable variability in levels of these contaminants at the sampling locations between consecutive years or even within the proposed window of three years.[16]

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[13] *Ibid* at p 4.
[14] *Ibid,* Figure B1.
[15] *Ibid* at p 3.
[16] See in the following figures from the 2023 Annual Report for example: Ra at sites AN-5 (Figure 4.2.1-2), TL-3 (Figure 4.2.2-7), TL-6 (Figure 4.2.2-18), and TL-9 (Figure 4.2.2-29); U at sites AC-6A (Figure 4.2.1-9), AC-14 (Figure 4.2.1-17), TL-3 (Figure 4.2.2-6), CS-2 (Figure 4.2.3-21), and ZOR-2 (Figure 4.3-5); Selenium at sites TL-3 (Figure 4.2.2-9), and TL-6 (Figure 4.2.2-19). Cameco 2023 Annual Report, online: https://www.beaverlodgesites.com/public/Beaverlodge-2023-Annual-Report-06-04-24.pdf.

Finally, the LTMP is silent on any protocols for responding to elevated sampling results where contaminants may be measured in higher concentrations than expected from Cameco predictive models. More specifically, it does not appear as though the discovery of higher-than-expected contaminant concentrations would trigger more frequent sampling. NTP would argue that for contaminants that are measured at levels higher than relevant environmental guidelines, seasonal monitoring should be required. While Cameco says June is the best time for monitoring,[17] we argue that seasonal monitoring is better as it can account for seasonal changes in how contaminants move through the environment influencing ecological contamination.

It is important to underscore NTP's concerns that no water quality sampling methodology is being shared with the public, frustrating our ability to understand the data or factors informing how data is being collected and interpreted. It also deprives the public from understanding how existing data may be informing proposed long-term monitoring plans for the site. It appears from section 3.1.1.1 of the LTMP that community members have expressed the same concerns to Cameco.[18]

Recommendation 2: before any decision is rendered on Cameco's application, CNSC staff should require the company to disclose the scientific and technical basis for determining future monitoring parameters and frequencies for public review.

Fish wellbeing

The Institutional Control Field Guide for the LTMP contains no information relating to fish monitoring methods. Only monitoring frequency is noted and set for once every 15 - 20 years (if not less).[19] Such long temporal gaps in monitoring seem unreasonable as they would effectively prevent the public from being able to discern real conditions at or around the Beaverlodge site for fish. Cameco's excuse for not monitoring fish more relies on its concerns that fish flesh monitoring kills the fish being monitored, believing that fish mortality associated with this type of monitoring is not in the public interest.[20] However, NTP would argue that the public's right to know about the health of local fish for sustainable fisheries would be worth the limited fish kills required for more frequent monitoring.

Monitoring at Cinch Lake, Martin Lake, and Beaverlodge Lake in September 2023 provided a baseline dataset for updating the Healthy Fish Consumption Guideline issued

[17] LTMP, *supra* note 12 at p 4.
[18] LTMP, *supra* note 12 at 3.1.1.1
[19] LTMP, *supra* note 12 at p 9.
[20] *Ibid.*

by the Saskatchewan Health Authority (SHA), for waterbodies near Uranium City. However, this data is not publicly available. NTP argues it is in the public's interest to have access to existing fish health data to understand existing conditions and how they are informing Cameco's proposals relating to monitoring frequency. Access to the ERA and supporting studies might allow a better understanding of monitoring frequencies in the past and how they relate to current proposals for the future, but again the ERA has been denied to the public.

Finally, Cameco seems to display a lack of an ecosystem approach in the LTMP's design. Cameco argues that environmental conditions in waterbodies in and near the Beaverlodge site are "managed" via Saskatchewan Health Authority Healthy Fish Consumption Guidelines for Beaverlodge, Martin, and Cinch Lakes.[21] By extension, the company's proposal to conduct fish chemistry studies every 15 or 20 years in the future is in order to update of healthy fish consumption guidelines. In other words, Cameco's goal for fish monitoring is to manage human consumption of fish, rather than fish wellbeing and ecosystem health in their own rights.

There is no way to see how different trophic levels may be reflected in past or future monitoring plans. And as mentioned above, it appears aquatic invertebrates were monitored in the past but will not be monitored anymore going forward.[22] We have not been able to find any studies to examine the population genetics of the fish from locations in and around the Cameco site, nor have we found any reference to fish reproduction and potential adverse effects on multiple generations of fish. All this frustrates the ability for members of the public to gain a sense of local ecosystem health. The Beaverlodge ERA likely has a more comprehensive list of species to be monitored than the LTMP, but again we have been denied access to the full ERA.

Recommendation 3: before any decision is rendered on Cameco's application, CNSC staff should require the company to disclose a more detailed description of its approach to ensuring fish and ecosystem wellbeing through monitoring for public review.

Gamma radiation

There is only a brief summary of the Beaverlodge radiation risk evaluation on Cameco's website.[23] This summary is not very useful as a public resource due to its lack of detail. It contains a series of assurances without providing any evidence to support them. In order

[21] Cameco CMD, *supra* note 4 at p 4.
[22] LTMP, *supra* note 12.
[23] See: https://www.beaverlodgesites.com/public/BVL_Gamma_Summary.pdf.

to be able to assess Cameco's approach to managing gamma radiation, the public requires descriptions of:

- All radionuclides evaluated;
- Actual measured historical data (including for all sites shown in Cameco's 2023 annual report) of the natural background values compared against anthropogenic activities-related rise in gamma radiation in various environmental components such as surface water, ground water, soil, and sediments and management over the years;
- The dose received by receptors (biota or Valuable Ecological Components and humans) identified in ERA; and
- The calculated hazard quotients to arrive at the conclusion of "acceptable risk".

Recommendation 4: before any decision is rendered on Cameco's application, CNSC staff should require the company to either disclose its entire radiation risk evaluation or else provide for public review a more detailed description of its approach to monitoring for and managing gamma radiation.

PART TWO:
Cameco's approach to public engagement

The current two-year 2023 licence granted to Cameco was meant to provide extra time for the company to prepare materials required for its eventual request to discontinue CNSC oversight. Significantly, this period was also meant to provide more time for Cameco to engage with the public around this final licence revocation process.[24]

In its Facility Licence Manual, uploaded to the Beaverlodge website, Cameco notes that there is a Public Information Program for the Beaverlodge site. The document states,

> The process in which Cameco communicates with the public is described in the BVLPIP [Beaverlodge Public Information Program]. The purpose of this program is to inform identified interested groups about the general nature of the decommissioned Beaverlodge properties and the potential effects of the activities to the safety and health of the public and the environment. It is designed to keep the public informed regarding certain aspects of the properties and foster good relations with northern Saskatchewan communities, regulatory bodies, and the general public.[25]

[24] Cameco CMD, *supra* note 4 at p 1.
[25] See: https://www.beaverlodgesites.com/public/Beaverlodge_Facility_Licence_Manual.pdf a p 14.

Cameco has also uploaded its public disclosure protocol for the Beaverlodge site to its website.[26] The document is interesting in its qualification of public disclosures. In one instance, it "acknowledges our stakeholders' need for timely and accurate information presented in a meaningful way… [because] Cameco believes adhering to this policy will help Cameco foster stakeholder confidence and *loyalty*, ultimately *enhancing support* for activities at the decommissioned Beaverlodge properties." [emphasis added].[27] In another instance, Cameco undertakes to:

> [Build] capacity among residents of northern Saskatchewan to understand the environmental, health and safety aspects of uranium mining, milling, monitoring and reclamation activities, and encourag[e] youth in communities to understand the opportunities for a safe, healthy and rewarding career.[28]

In both these instances, information disclosure is contemplated with the understanding that it will result in increased support for Cameco and its Beaverlodge operations. This problematic approach to public disclosure may explain why Cameco is hesitant to release any information that may not result in 'loyalty' or 'support' for its activities. As the Ya'thi Néné Lands and Resources submissions explained in the 2022 licence renewal proceedings for the Beaverlodge site, some Cameco redactions in a requested report were merely hiding questionable approaches to the protection of the environmental and human health.[29]

As we were preparing for this intervention on behalf of NTP, we made a request to Cameco for a more detailed map of the Beaverlodge site that clearly labelled and described which facilities had been released from CNSC licensing and which remained. We also requested a copy of their ERA and Environmental Protection Review (EPR). However, all three requests were ultimately denied. Cameco maintained their ERA was confidential, argued that the maps included in their Beaverlodge closure report were sufficient, and that since the EPR was not directly referenced in current hearing proceedings, it should not have to be disclosed. We will proceed to outline our continued interest in receiving each of these sources below.

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[26] See: https://www.beaverlodgesites.com/public/PUBLIC.DISCLOSURE.PROTOCOL.FOR.THE.DECOMMISSIONED.BEAVERLODGE.PROPERTIES.pdf.
[27] *Ibid* at p 1.
[28] *Ibid* at pp 1-2.
[29] Here, Ya'thi Néné Lands and Resources received a requested document with redacted portions that had a formatting error in which they could be removed by the reader. These redacted portions showed that Cameco's fish ingestion rates were 50% lower than recommended by Health Canada. See: https://api.cnsc-ccsn.gc.ca/dms/digital-medias/cmd22-h5-15.pdf/object at p 14.

Beaverlodge site map

While several maps of the Beaverlodge site are provided in Cameco materials, none are detailed enough to facilitate a good understanding of the site. From publicly available materials, it is impossible to get a comprehensive conception of the Beaverlodge site, including: site boundaries, exact monitoring locations and types, a comprehensive sense of all facilities (namely, their types, locations, and decommissioning status).

Several sites and monitoring locations are described in Cameco's annual reports, but no maps or coordinates are provided for them, making it impossible to confidently place them within the site's geography and ecology. Further, there is no comprehensive description of each of the properties on site, their decommissioning status, and nearest monitoring locations and results. Some regional maps are provide indicating general areas with monitoring locations but these are insufficient to understand individual specific facilities or monitoring locations. All this prevents the public from being able to gain a functioning understanding of all facilities at the site and how they are managed and monitored.

Recommendation 5: that CNSC require Cameco to publicly disclose a detailed site map for Beaverlodge that includes all 70 properties and their decommissioning status as well as all monitoring locations and natural features such as waterbodies and wetlands.

Recommendation 6: that CNSC require Cameco to provide exact location points for all environmental monitoring sites on the Beaverlodge site

Environmental Risk Assessment

Although the Beaverlodge site was claimed to have been decommissioned between 1982 and 1985, the CNSC required more work to understand and further contain the site in the 2000s.[30] A Quantitative Site Model (QSM) was developed for this purpose, characterizinf the entire Beaverlodge site based on studies conducted between 2009 - 2012. This QSM predicted long-term water quality trends for radium-226, uranium and selenium, and formed the basis for performance indicators at the Beaverlodge site's monitoring locations.[31] This model, however, was not disclosed or even summarized for broader public access.

Subsequent ERAs for the Beaverlodge site have updated the QSM, taking into account additional ecological parameters as well as regulatory developments since the 2000s as well. Cameco explains its ERA

[30] CNSC staff CMD, *supra* note 2 at p 5.
[31] Cameco CMD, *supra* note 4 at p 14.

> was completed in accordance with the CSA N288.6 standard (CSA 2022) and consisted of watershed dispersion modelling, and a pathways assessment to evaluate potential risks to ecological and human receptors on and downstream of the decommissioned properties. The model assumptions were revisited based on the current understanding of the environmental conditions and informed by almost 40 years of monitoring results. The environmental performance indicators related to the assessment of water quality at various monitoring stations were also updated.[32]

Cameco further explained its most recent 2020 ERA used a

> probabilistic modelling approach that included updated environmental monitoring data and allowed for inclusion of a wider range of environmental variability, such as that created by climate change (CanNorth 2020). The model was used to update the performance indicator at each of the water quality monitoring stations. The model and the updated performance indicators have been accepted by the regulatory agencies.[33]

In this way, the ERA provides the most comprehensive characterization of on-site conditions as well as the most comprehensive explanation of Cameco's environmental modelling practices. As discussed in part one of these submissions above, Cameco's understanding and management of the site are grounded in their ERA.

In fact, most assertions relating to the current and future environmental performance of the Beaverlodge site relies on the ERA. It is referenced to support Cameco's claims that the Ace Creek Watershed, Fulton Creek Watershed, and downstream surface water are all "generally expected to gradually improve in the future with the exception of radium-226 in the TMA [tailings management area]".[34] For radium-266, Cameco concedes, "concentrations in the TMA and downstream Greer Lake are expected to continue to increase for the next 15 to 60 years (depending on the waterbody) due to the release of historically precipitated radium from sediments; after the peak is reached, levels are expected to gradually improve over the long-term."[35] Here, Cameco again relies on the ERA to support its claims that these increases will be "localized, with no effects expected in the downstream environment".[36]

Where tailings are not uniformly drained or covered, Cameco notes there may be variability in releases to the environment based on precipitation. Here, their climate

[32] Cameco CMD, *supra* note 4 at p 69.
[33] Cameco CMD, *supra* note 4 at p14.
[34] Cameco CMD, *supra* note 4 at pp 14-15.
[35] Cameco CMD, *supra* note 4 at pp 14-15.
[36] Cameco CMD, *supra* note 4 at pp 15.

change study was conducted to predict future precipitation patters. This is included in the ERA but has not been disclosed to the public. Cameco's application explains,

> The ERA utilized an updated probabilistic modelling approach to account for the range of natural variability seen in model input parameters and more accurately represent expected water quality results. As part of the performance indicator update, a sensitivity analysis was completed by including a wider range of environmental variability, such as that expected from climate change, to assess the potential impact on the performance indicators. Overall, it was found that the climate change scenario did not have a significant effect on the expected recovery of the site in the long term and the updated performance indicators are applicable[37]

As these examples illustrate, the ERA is the most important technical document underlying the environmental assertions in its current application. Denying public access to this document effectively prevents the public from being able to comment on environmental aspects of Cameco's application.

Environmental Protection Review

EPRs are CNSC staff-generated reviews of regulated nuclear sites, canvassing their environmental performance and assessing their compliance with applicable licence terms and regulations. These reports are more detailed than (and generally independent from) more generalized Commission Member Documents. EPRs are usually posted online to the CNSC website, but the report for Beaverlodge has not been posted.[38]

There are additional sources of data, though none provide a detailed picture of the Beaverlodge site specifically:

- For the Country Foods Assessment and Community Based Environmental Monitoring Program, detailed maps and monitoring results not publicly available. Cameco's two-page summary does not provide any meaningful details only unsupported assurances;
- The Eastern Athabasca Regional Monitoring Program and CNSC's Independent Environmental Monitoring Program both share their sampling locations and results online. However, monitoring locations are relatively far from the Beaverlodge site. There are also several other mine sites in close vicinity to Beaverlodge, including the Lorado and Gunnar mine sites. This means that results from these monitoring programs are impossible to attribute solely to Beaverlodge.

[37] Cameco CMD, *supra* note 4 at p 69.
[38] See: https://www.cnsc-ccsn.gc.ca/eng/resources/environmental-protection/reviews/.

PART THREE:
Cameco's confidentiality application

The current proceeding included the CNSC's new process for more transparently rendering decisions concerning proponent applications for confidential treatment of submitted materials. In the past, such determinations were made separately between CNSC staff and project proponents, and not released to the public.

NTP commends the CNSC for making this process more transparent, and for inviting public comments on Cameco's application for confidentiality during the current proceedings. However, no notice was provided at the start of this proceeding that there would, at a later date, be a process by which the public could comment on this confidentiality filing. Further, by the time intervenors were notified, there was just under two weeks available to comment.[39]

Section 12(1) of the CNSC's own *Rules of Procedure* allows for applications for confidentiality to be made on the following grounds:

> **(a)** the information involves national or nuclear security;
> **(b)** the information is confidential information of a financial, commercial, scientific, technical, personal or other nature that is treated consistently as confidential and the person affected has not consented to the disclosure; or
> **(c)** disclosure of the information is likely to endanger the life, liberty or security of a person.[40]

Further, the heading for the application form completed by Cameco indicates a high threshold for Commission findings of confidentiality. It notes that public disclosure will only be prevented in "exceptional" circumstances and that any confidential portions will be "proportional, minimal and not imposed lightly". The application header confirms it is up to the applicant to make their case via an "adequately detailed explanation as to how and why subrule 12(1) applies".[41] Finally, the form specifies that applicants for confidentiality

> must confirm: the importance of protecting the information outweighs the public interest in public hearings and disclosure of evidence; and that the confidentiality measures would affect the public nature of the proceeding only to the extent necessary to adequately protect the given information.

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[39] Canadian Nuclear Safety Commission, Notice of Request for Confidentiality, October 15, 2024, online: https://api.cnsc-ccsn.gc.ca/dms/digital-medias/Cameco-Notice-Request-for-Confidentiality-25-H3-e.pdf/object.
[40] Canadian Nuclear Safety Commission, *Rules of Procedure*, SOR/2000-211, at s 12(1).
[41] See: https://api.cnsc-ccsn.gc.ca/dms/digital-medias/Request-for-Confidentiality-Beaverlodge-CMD25-H3.pdf/object . Note: this application is shared as a non-searchable, non-highlightable PDF document, further frustrating its use by the public.

Cameco has argued for the entirety of its 2020 ERA should be withheld from the public, asserting that section 12(1)(b) of the *Rules of Procedure* apply without explaining why. In fact, Cameco merely copies the language in this subsection with little else. but fail to explain why. Merely relying on text in the CNSC's *Rules of Procedure* to justify a blanket protection against disclosure, stating the report contains "confidential information of a financial, commercial, scientific, technical, personal or other nature that is treated consistently as confidential and was provided by Cameco to the CNSC" does not meet the Commission requirement of an "adequately detailed explanation" for the need for confidentiality.

Cameco claims,

> the disclosure of this information provides details of the development, calibration and validation of models used to support environmental risk assessments at Cameco's operations. The information used to support these assessments originate from monitoring activities and other research endeavours financed and managed by Cameco that would be beneficial to Cameco's competitors.

Given the breadth of environmental information we know the ERA must contain, it remains unclear how content such as lists of valued ecosystem components, monitoring methodologies, or site-wide ecological conditions could be considered proprietary and necessary to maintain for commercial competitiveness. To hold otherwise would deal a significant blow to nuclear transparency.

It is also important to note that ERAs are required to be disclosed by licensees pursuant to REGDOC 3.2.1.[42] While this REGDOC may not be strictly binding on its own, its inclusion in facility licence terms and licence conditions handbooks makes it mandatory as part of the facility's licensing basis. Beaverlodge's current licence and Licence Conditions Handbook contain a requirement to adhere to REGDOC 3.2.1.[43] As such, the disclosure of the 2020 ERA is required by the current Beaverlodge licence. Even Cameco's confidentiality application acknowledges that "if submission of the material is required pursuant to reporting requirements under the NSCA or the regulations under NSCA or the regulations under the NSCA, or pursuant to a licence issued under the NSCA, or if the material is specifically requested by the Commission, it may **not** be withdrawn" [emphasis in original]. As such, by Cameco's own admission, it seems required to disclose its ERA.

[42] REGDOC 3.2.1, online: https://www.cnsc-ccsn.gc.ca/eng/acts-and-regulations/regulatory-documents/published/html/regdoc3-2-1/ at s 2.2.4.
[43] Licence Control Handbook for the Beaverlodge site at p 7, contained in the CNSC staff CMD reference package for these proceedings.

Appendix A

Uddin et al., 2024

Chemosphere 364 (2024) 143214

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Selenium toxicity in fishes: A current perspective

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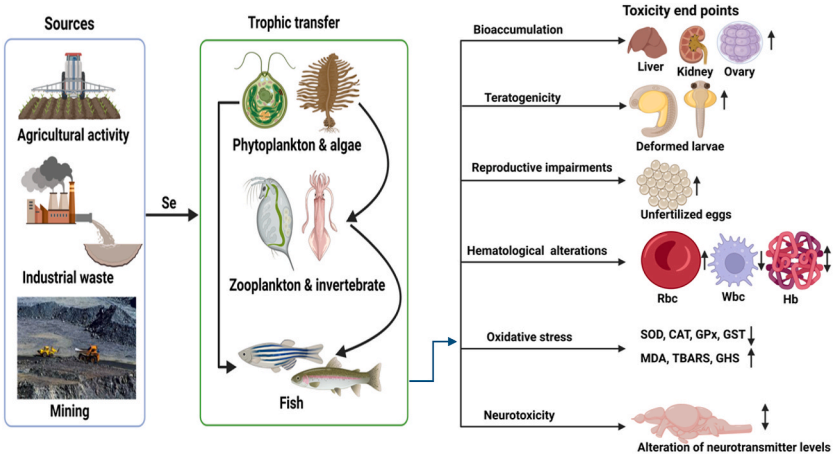
GRAPHICAL ABSTRACT



ARTICLE INFO

Handling editor: Kashif Hayat

ABSTRACT

Anthropogenic activities have led to increased levels of contaminants that pose significant threats to aquatic organisms, particularly fishes. One such contaminant is Selenium (Se), a metalloid which is released by various industrial activities including mining and fossil fuel combustion. Selenium is crucial for various physiological functions, however it can bioaccumulate and become toxic at elevated concentrations. Given that fishes are key predators in aquatic ecosystems and a major protein source for humans, Se accumulation raises considerable ecological and food safety concerns. Selenium induces toxicity at the cellular level by disrupting the balance between reactive oxygen species (ROS) production and antioxidant capacity leading to oxidative damage. Chronic exposure to elevated Se impairs a wide range of critical physiological functions including metabolism, growth and reproduction. Selenium is also a potent teratogen and induces various types of adverse developmental effects in fishes, mainly due to its maternal transfer to the eggs. Moreover, that can persist across generations. Furthermore, Se-induced oxidative stress in the brain is a major driver of its neurotoxicity, which leads

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https://doi.org/10.1016/j.chemosphere.2024.143214
Received 13 July 2024; Received in revised form 14 August 2024; Accepted 27 August 2024
Available online 29 August 2024

to impairment of several ecologically important behaviours in fishes including cognition and memory functions, social preference and interactions, and anxiety response. Our review provides an up-to-date and in-depth analysis of the various adverse physiological effects of Se in fishes, while identifying knowledge gaps that need to be addressed in future research for greater insights into the impact of Se in aquatic ecosystems.

1. Introduction

Trace elements such as chromium (Cr), copper (Cu), iodine (I), iron (Fe), manganese (Mn), selenium (Se), and zinc (Zn) are essential to fishes, playing a crucial role in their growth, metabolism, reproduction, and overall health when present within physiologically optimum range (Lall and Kaushik, 2021; NRC, 2011). Specifically, Se is a vital micronutrient that helps maintain physiological homeostasis in all vertebrates including fishes (Aramli et al., 2023). However, these elements can become toxic when present in supraphysiological concentrations or in certain chemical forms. For example, Se is essential to fishes within a narrow concentration range, however it becomes extremely toxic when its concentration exceeds the physiological threshold, with toxic effects in fishes observed at dietary concentrations above 3 mg/g dry weight (dw) compared to its essential dietary range of 0.1–0.3 mg/g dw (Thomas and Janz, 2011). Moreover, organic forms of Se (e.g., selenomethionine), which are primarily found in the diet, are considered to be more toxic to fishes than its inorganic counterparts (e.g., selenite and selenate), which occur predominantly in the water (Janz et al., 2010). At optimal physiological concentrations, Se is incorporated into various selenoproteins, such as glutathione peroxidases (GPX1, GPX2, GPX3, GPX4, GPX6), thioredoxin reductases (TXNRD1, TXNRD2, TXNRD3), and iodothyronine deiodinases (DIO1, DIO2, DIO3), which have antioxidative functions that protect cells from oxidative damage (Plateau et al., 2017; Mullur et al., 2014).

Geochemically, Se primarily exists in the crustal rock and phosphate-rich soil and is introduced into aquatic ecosystems by both natural sources and anthropogenic activities (Lemly, 2004). Anthropogenic activities such as mining, coal combustion, oil refining wastewaters, and agricultural drainage waters are the major sources of Se contamination in aquatic systems resulting in elevated concentrations which are toxic to aquatic organisms (Mo et al., 2019; Chapman et al., 2010; Janz et al., 2010). Recent studies have reported varying dissolved Se concentrations in surface water of different geographical locations. For instance, investigations revealed the highest Se concentration recorded in Tonle Sap Lake in Cambodia during the dry season to be 17.6 μg/L, while in Najran city, Saudi Arabia, it was found to be 11.44 μg/L (Haque et al., 2016). Moreover, research conducted in the Ibadan metropolis of Nigeria indicated an average Se content in water as high as 46.3 ± 22.4 μg/L, with the highest recorded Se concentration reaching 258 μg/L in irrigation water in Texas (Etim, 2017; Hudak, 2009). Furthermore, studies have observed Se levels ranging from 45 to 341 μg/L in the underground water of Punjab, North-West India (Bajaj et al., 2011).

Selenium can exist in both inorganic and organic forms. In its inorganic forms, Se is commonly found as selenate and selenite. Its organic forms include selenomethionine (Se-Met) and selenocystine (Se-Cyst) (Janz et al., 2010). In aquatic ecosystems, microorganisms, and primary producers absorb inorganic Se, and bio-transform it into organic forms, primarily Se-Met that can bioaccumulate and bio-magnify in the upper trophic levels and cause toxicity to animals, particularly to oviparous species such as birds (Mo et al., 2020). The most well characterized detrimental effects of Se in fishes include teratogenicity, neurological disorders, cognitive impairment, reproductive failure, cardiovascular complications, and behavioural alterations in fishes (Attaran et al., 2019; Naderi et al., 2018a; Pettem et al., 2017; Thomas and Janz, 2015). Furthermore, previous studies also suggest that excessive Se exposure in freshwater fishes can result in growth inhibition, impaired swimming performance, altered energy homeostasis, visual system impairment, morphological deformities, reproductive impairments, alteration of hemato-biochemical parameters, and histopathological changes in vital organs (Al-Din et al., 2022; Dhara et al., 2022; Mushtaq et al., 2022a,b). Because of such wide spectrum of toxic effects, Se can pose serious threat to the long-term sustenance of natural fish populations (Rathore et al., 2021b; McPhee and Janz, 2014). Various toxic effects of Se in fishes are presented in Fig. 1.

Selenium can move through aquatic food chains via bioconcentration and biomagnification. Importantly, lower trophic organisms, such as algae, zooplankton, and benthic invertebrates, exhibit high tolerance to elevated Se exposures. Conversely, fishes are among the most sensitive organisms to Se toxicity. Consequently, these prey items can act as vectors, delivering high dietary Se concentrations to fishes (Janz, 2012; Janz et al., 2010).

Given that fishes are top predators in most aquatic ecosystems and provide approximately 60% of the total animal protein consumed by humans (Kim et al., 2021), Se accumulation poses a significant food safety concern. Several reviews have addressed Se toxicity in aquatic organisms with special focus on bioaccumulation (Ohlendorf et al., 2011), growth and survival (Hamilton, 2004), essentiality and toxicity (Janz, 2012; Janz et al., 2010; Hodson and Hilton, 1983), histopathology (Hung, 2018), and neuropathology (Naderi et al., 2021) in aquatic organisms including fishes. However, recent studies have revealed new insights into the various aspects of Se toxicity in fishes that require a renewed focus and an in-depth discussion. To this end, we reviewed the existing literature on Se toxicity in fishes that were published over the last 10 years. This review mainly focuses on the most up-to-date information on critical aspects of Se toxicity in fishes including bioaccumulation, growth and metabolic functions, antioxidative capacity, reproduction and development, and neurobehavioural performance. Moreover, this review also identifies key knowledge gaps that should be addressed in future research for greater insights into the impact of Se in aquatic ecosystems.

2. Bioaccumulation of selenium in fishes

Fishes are essential bioindicators in aquatic ecosystems, as they accumulate trace metals that reflect environmental contamination (Burger and Gochfeld, 2005). They are often the tertiary predators in aquatic food chain, which makes them susceptible to accumulating toxic substances from both natural and anthropogenic sources. Thus, they play a key role in understanding trophic transfer and biomagnification of contaminants up through the food chain (Nwani et al., 2010). Moreover, from human health perspective, fishes are globally recognized for their high-quality protein, low saturated fats, and high omega-3 fatty acids, which contribute to reduced cholesterol and lower risks of cancers and cardiovascular diseases (Bosch et al., 2016; Storelli, 2008). Collectively, these two aspects highlight the importance of studying trace metal bioaccumulation in fishes, which have important implications for protecting aquatic and public health (Arulkumar et al., 2017).

Assessment of bioaccumulation serves as a crucial indicator for monitoring the geochemical cycling of heavy metals within aquatic ecosystems (Emon et al., 2023). Among the wide array of metals and metalloids, Se is notably recognized as one of the most accumulative toxic metalloids, which is attributed to its capability to substitute sulfur (S) atoms within proteins, thereby forming stable complexes (Lemly, 2004). The bioaccumulation of Se in fishes is influenced by various factors, including exposure pathways (waterborne or dietary) and habitat characteristics (seawater or freshwater). In both laboratory and field studies, irrespective of waterborne and dietary exposure, Se

bioaccumulation profiles consistently reveal the following order: kidney > liver > gonads > spleen > intestine > gill > brain > muscle (Mushtaq et al., 2022a,b; Pan et al., 2022; Acosta-Lizárraga et al., 2020). Furthermore, bioaccumulation of Se in fishes is significantly influenced by water quality parameters such as pH, temperature, hardness, and the presence of other ions or dissolved organic matter. For instance, lower pH levels can increase the bioavailability of selenite, leading to higher Se accumulation in fish tissues, as observed in studies involving freshwater species (Besser et al., 1993). Elevated temperatures often enhance the metabolic rates of fishes, thereby increasing Se uptake in aquatic organisms (Hilton et al., 1980). Increased water hardness, characterized by higher concentrations of calcium and magnesium in the water, can reduce Se bioaccumulation (Holm et al., 2005). Additionally, the presence of other trace elements like arsenic can antagonistically reduce Se bioaccumulation in rainbow trout (*Oncorhynchus mykiss*) (Jamwal et al., 2019), while higher levels of dissolved organic matter can also reduce Se uptake by complexation and thereby rendering it less bioavailable to fishes (Luoma and Presser, 2009). However, Se accumulation in different organs varies from species to species, depending on their detoxification mechanism. Notably, numerous investigations have consistently highlighted the kidney and liver as sites of highest Se bioaccumulation, attributed to their pivotal roles in detoxification and the elimination of toxic substances from the body (Rathore et al., 2021b; Li et al., 2020; Bergés-Tiznado et al., 2019). Waterborne exposure to Se in fishes results in the highest Se accumulation in the gills (Garnero et al., 2018), whereas dietary exposure has been reported to lead to maximum accumulation in the intestine (Chen et al., 2020).

It is important to note that Se accumulation in specific tissues can be used to predict its toxicity in fishes, regardless of the exposure routes. The United States Environmental Protection Agency (USEPA) and the Canadian Council of Ministers of the Environment (CCME) have established tissue-based toxicity thresholds for assessing Se toxicity in natural fish populations. USEPA recommends Se toxicity thresholds of 15.2 mg/kg dw for egg-ovary, 8.5 mg/kg dw for whole body, and 11.3 mg/kg dw for muscle tissue in fishes (USEPA, 2016). In comparison, CCME suggests Se toxicity thresholds of 14.7 μg/g dw for egg-ovary and 6.7 μg/g dw for whole body (CCME, 2016). Moreover, the United States Fish and Wildlife Service proposed more conservative Se toxicity thresholds of 10 mg/kg dw for ovaries and 4 mg/kg dw for whole body of fishes (USFWS, 1990). Although these thresholds have been suggested to be overly conservative (Brix et al., 2000), several studies have documented toxic effects in fishes that were associated with tissue-specific Se accumulation that are comparable or lower than the tissue-based Se toxicity threshold established by different regulatory bodies. For example, whole body Se concentrations of 6 mg/kg and 5 mg/kg dw were found to be associated with impaired growth and increased mortality in fathead minnow (*Pimephales promelas*) and bluegill (*Lepomis macrochirus*), respectively (Ogle and Knight, 1989; USFWS, 1990). Additionally, an ovary Se concentration of 10 mg/kg was reported to be associated with reproductive failure in bluegill (Hermanutz et al., 1992). Thomas and Janz (2015) reported that Se concentrations of 12.7 μg/g dw in eggs led to significant larval deformities and mortality in zebrafish (*Danio rerio*). Similarly, Shi et al. (2018) observed significant larval deformities in Japanese medaka (*Oryzias latipes*) when Se concentrations reached 8 μg/g dw in eggs.

Moreover, the accumulation of Se in the muscle tissues of fishes holds significant importance as an indicator of food safety, considering that muscle is the most ingested tissue by humans (Lee et al., 2019). It is to be noted though that Se accumulation in muscle has consistently been reported to be very low compared to other tissues in fishes (Pan et al., 2022; Chen et al., 2020; Khadra et al., 2019). The Se accumulation profiles outlined in Table 1 emphasize specific target organs crucial for assessing the toxic effects of Se in fishes.

3. Mechanism of selenium toxicity in fishes

Oxidative stress acts as the primary mechanism underlying Se toxicity in fishes (Misra et al., 2012). Both organic and inorganic forms

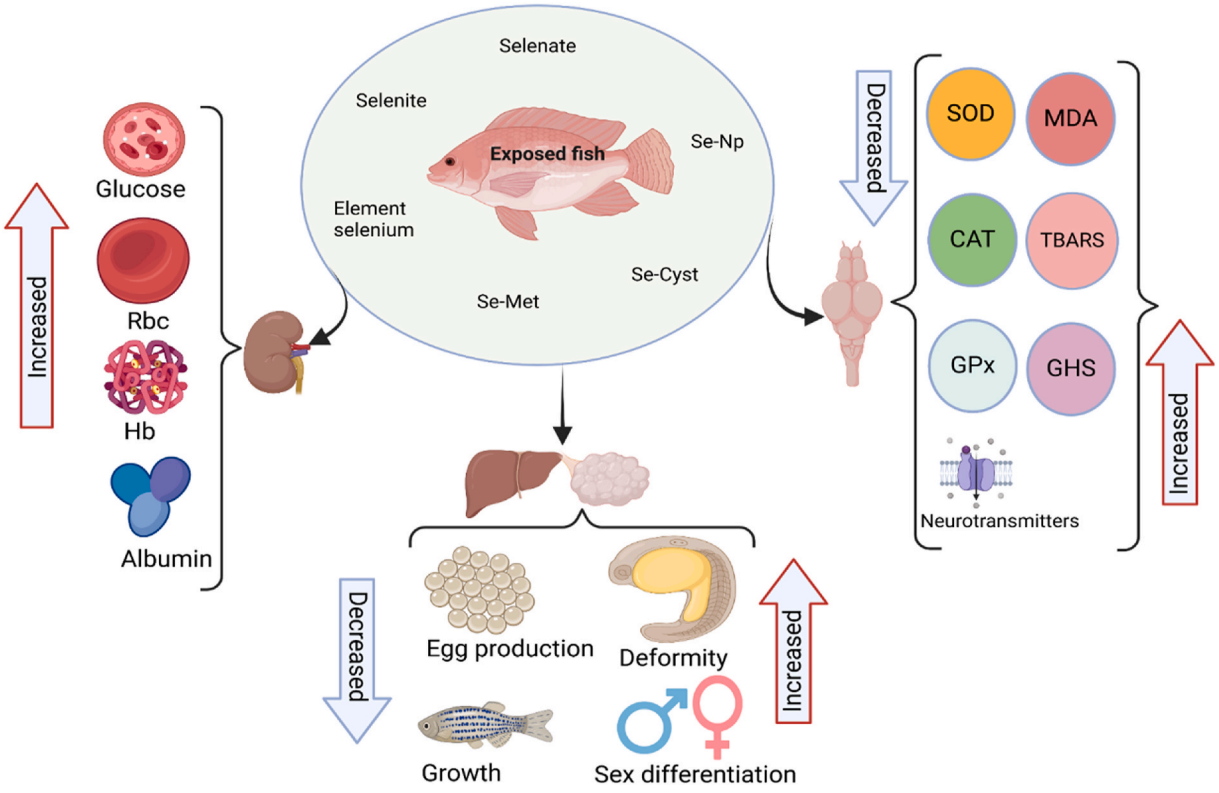

Fig. 1. Toxic effects of selenium in fishes.

Table 1
Bioaccumulation patterns in fishes exposed to selenium.

Laboratory study					
Exposure route	Fish species	Exposure concentration	Exposure periods (days)	Accumulation profiles	Reference
Dietary	*Hypophthalmichthys molitrix*	0, 0.5, 1 mg/kg	96	kidney > liver > pancreas > muscle	Mushtaq et al. (2022a)
Dietary	*Pangasianodon hypophthalmus*	0, 0.5, 1, 2 mg/kg	60	liver > whole-body > muscle > gill	El-Sharawy et al. (2021)
Dietary	*Oreochromis niloticus*	0, 3, 6, 12 μg/g	45	intestine > brain > liver > muscle	Chen et al. (2020)
Dietary	*Oreochromis niloticus*	0, 3, 6, 12 μg/g	90	intestine > liver > brain > muscle	Chen et al. (2020)
Waterborne	*Channa argus*	0, 100, 200 mg/L	56	kidney > liver > spleen > intestine > gill > muscle	Li et al. (2020)
Dietary	*Acanthopagrus schlegelii*	0, 0.34, 0.52, 0.68, 0.91, 1.08, 3.06 mg/kg	56	liver > muscle	Wang et al. (2019)
Dietary	*Carassius auratus*	0, 5, 10, 20 mg/kg	30	kidney > liver > muscle	Bai et al., 2019a
Waterborne	*Channa argus*	0, 50, 100, 200, 400 μg/L	28	kidney > liver > spleen > intestine > gill > muscle	Li et al. (2019)
Waterborne	*Channa argus*	0, 50, 100, 200 μg/L	56	kidney > liver > spleen > intestine > gill > muscle	Li et al. (2019)
Dietary	*Pagrus major*	0, 0.5, 1, 2 mg/kg	45	liver > muscle > whole body	Dawood et al. (2019)
Waterborne	*Oreochromis mossambicus*	0, 5, 10, 25, 50, 100 μg/L	4	liver > gill > brain	Gobi et al. (2018)
Waterborne	*Pseudorasbora parva*	0, 10, 200, 1000 μg/L	28	liver	Ma et al. (2018)
Dietary	*Cyprinus carpio*	0, 0.7 mg/kg	56	liver > muscle	Saffari et al. (2017)
Dietary	*Oreochromis niloticus*	0, 0.25, 0.5, 0.75, 1, 2, 4, 8, 16 mg/kg	70	liver > muscle > gill	Lee et al. (2016)
Waterborne	*Danio rerio*	0, 1 mg/L	4	liver > kidney > brain	Davis et al., (2016)
Dietary	*Oncorhynchus mykiss*	0.75, 1.4, 4.46, 8.94 g/kg	70	kidney > liver > muscle > blood	Pacitti et al. (2015)
Dietary	*Cyprinus carpio*	0, 0.5, 1, 2 mg/kg	56	liver > muscle	Ashouri et al. (2015)

Field study				
Study area	Fish species	Study periods	Accumulation profiles	Reference
Yellow river, China	Various fish species	July–October 2018	liver > gonads > gill > muscle	Pan et al. (2022)
Northern gulf of California	*Merluccius productus*	January–March 2017 & 2018	kidney > liver > gonads > gills > muscle	Lizárraga et al., (2020)
Southwestern Atlantic estuaries	*Genidens barbus*	November 2016	liver > gills > muscle	Carvalho et al., (2019)
La Plata basin, South America	*Prochilodus lineatus*	April & June 2017	liver > gills > muscle	Avigliano et al. (2019)
Southeast gulf of California	*Coryphaena hippurus*	2011–2013	kidney > liver > gonads > muscle	Bergés-Tiznado et al. (2015)
Macedonian rivers	*Squalius vardarensis*	Spring & Autumn	liver > gill	Dragun et al. (2019)
Lake Saint-Pierre, Quebec, Canada	*Perca flavescens*	April 2016	gut > liver > gonads > brain > muscle	Khadra et al. (2019)
Vaal dam, South Africa	*Labeobarbus aeneus, Labeobarbus kimberleyensis, Labeo umbratus, Labeo capensis*	January 2016	liver > muscle	Plessl et al. (2019)
Lakes, Saskatchewan	*Catostomus commersonii*	October 2014	liver > ovary > testis	Urien et al. (2018)
Río Tercero Reservoir, Argentina	*Hoplias malabaricus*	Wet & dry season	gills > intestine > brain ≥ liver ≥ muscle	Garnero et al. (2018)
	Oligosarcus jenynsii		gills > intestine > liver ≥ brain ≥ muscle	
	Rhamdia quelen		intestine > gills > liver ≥ brain ≥ muscle	
	Bryconamericus iheringii		intestine > gills > liver ≥ brain ≥ muscle	
	Astyanax fasciatus		intestine ≥ gills > liver ≥ brain > muscle	
	Odontesthes bonariensis		intestine ≥ gills > liver ≥ muscle > brain	
Coast of port Klang, Malaysia	*Lates calcarifer*	January 2016	liver > muscle	Nasyitah et al. (2018)
	Lutjanus campechanus		liver > muscle	
	Lutjanus griseus		muscle > liver	
East coast of Ireland	*Mytilus edulis*	August 2012	muscle > skin	McEneff et al. (2017)
Six lakes, North Carolina	Bluegill sunfish, Largemouth bass, Redear sunfish	March–May 2015	liver > ovary > testis > muscle	Brandt et al. (2017)
Eastern Pacific	*Istiophorus platypterus*	2011–2013	kidney > liver > gonads > muscle	Bergés-Tiznado et al. (2015)

of Se can oxidize cellular thiols (e.g., glutathione; GSH), a phenomenon well-documented over the past two decades. Organic Se, primarily in the form of Se-Met, generates highly reactive metabolites like methylselenol and selenide anion through the action of methioninase enzymes. The redox cycling of methylselenol in the presence of GSH leads to the production of reactive oxygen species (ROS) such as superoxide anion radicals (Palace et al., 2004). Notably, methioninase can also catalyze Se-Met in the absence of thiol groups, producing initial Se radicals that subsequently form superoxide radicals (Spallholz et al., 2004). In contrast, inorganic Se, primarily in the form of selenite, undergoes reduction by GSH, resulting in the production of hydrogen selenide. This compound is readily oxidized by oxygen, leading to the formation of ROS such as hydrogen peroxide, superoxide, and hydroxyl radicals. Furthermore, selenite's primary metabolite, selenodiglutathione, is highly unstable and is reduced by GSH to form glutathioselenol, which spontaneously dismutase into hydrogen selenide and elemental Se.

Mechanism of Se toxicity in fishes is presented in Fig. 2.

A secondary mechanism of Se toxicity in fishes is the ability of Se to substitute for S in S- containing amino acids, specifically methionine (Met) and cystine (Cyst), forming Se-Met and Se-Cyst during protein synthesis. This occurs due to the chemical similarities between Se and S (Janz et al., 2010). This inappropriate substitution disrupts the formation of disulfide (S–S) bonds, which are crucial for the stabilization of the tertiary structure of proteins (Lemly, 2004; Maier and Knight, 1994), leading to the functional impairment of key enzymes including GPXs, TXNRDs, protein disulfide isomerase, sulfite oxidase, and methionine synthase (Sunde, 1984).

4. Effects of selenium exposure on oxidative stress response

In fishes, the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway plays a crucial role in combating oxidative stress by upregulating key antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), and glutathione peroxidase (GPX). These enzymes work synergistically to convert superoxide radicals into hydrogen peroxide, which is then broken down into water and oxygen, serving as the primary defense against highly toxic ROS (Kim and Kang, 2016a). For example, grass carp (*Ctenopharyngodon idella*) fed with Se-supplemented diet at doses of 0, 0.3, 0.6, 0.9 and 1.2 mg/kg for 10 weeks showed significantly elevated hepato-pancreatic expression of Nrf2 gene, which also resulted in the simultaneous upregulation of GPX1 and CAT genes (Yu et al., 2020). Interestingly, dietary interventions, such as the inclusion of *Taraxacum mongolicum* polysaccharide, have been found to enhance the Nrf2-mediated antioxidant response, thereby improving the overall antioxidative and immune status of fishes (Li et al., 2024; Yu et al., 2022). GPX, a Se-dependent enzyme, is particularly important for reducing lipid peroxides, thereby protecting against lipid peroxidation and ferroptosis. However, when Se levels exceed physiological thresholds, this delicate balance is disrupted, leading to oxidative stress as the production of ROS overwhelms the protective functions of antioxidant enzymes (Kim and Kang, 2017b). This ROS-driven oxidative stress impairs essential biological molecules, including lipids, proteins, and DNA (Kim et al., 2017b, 2021; Lee et al., 2019; Kim and Kang, 2016b). Monitoring the responses of these antioxidant enzymes provides a sensitive and reliable indicator for assessing oxidative stress in fishes exposed to Se. As shown in Table 2, exposure to elevated Se leads to alterations in the activity of SOD, CAT, GST, and GPX in fishes.

SOD is a crucial antioxidant enzyme that catalyzes the conversion of two superoxide anions into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), thereby protecting organisms by eliminating excess ROS and maintaining the redox balance of the immune system. Most of the literature reported that chronic exposure to Se at different concentrations ranging from 0.5 to 3.0 mg/kg resulted in increased SOD activities in different organs of fishes including liver (Ghaniem et al., 2022; Wangkahart et al., 2022; Lin et al., 2021), gills (Gobi et al., 2018; Kumar et al., 2018a) and blood (Ghaniem et al., 2022; Abd El-Kader et al., 2021). It has been reported that the increased SOD activities were induced by an antioxidant reaction to prevent oxidative damage caused by ROS production. However, several studies have also reported a decrease in SOD activity in various organs of different fish species exposed to Se, indicating that Se at elevated level can suppress the antioxidant defense system and thereby increase oxidative stress (Li et al., 2020; Kumar and Singh, 2019; Neamat-Allah et al., 2019; Ma et al., 2018).

CAT is another important antioxidant enzyme involved in the detoxification of hydrogen peroxide which minimizes oxidative stress and protects cells from damage. Wangkahart et al. (2022) reported a significant increase in CAT activity in *O. niloticus* exposed to dietary Se. Similarly, CAT activities were noticed to be increased in the liver of *Pangasianodon hypophthalmus* due to increase ROS production (El-Sharawy et al., 2021). In contrast, waterborne exposure of Se to *Channa argus* results in lower CAT activities indicating increased oxidative stress (Li et al., 2020).

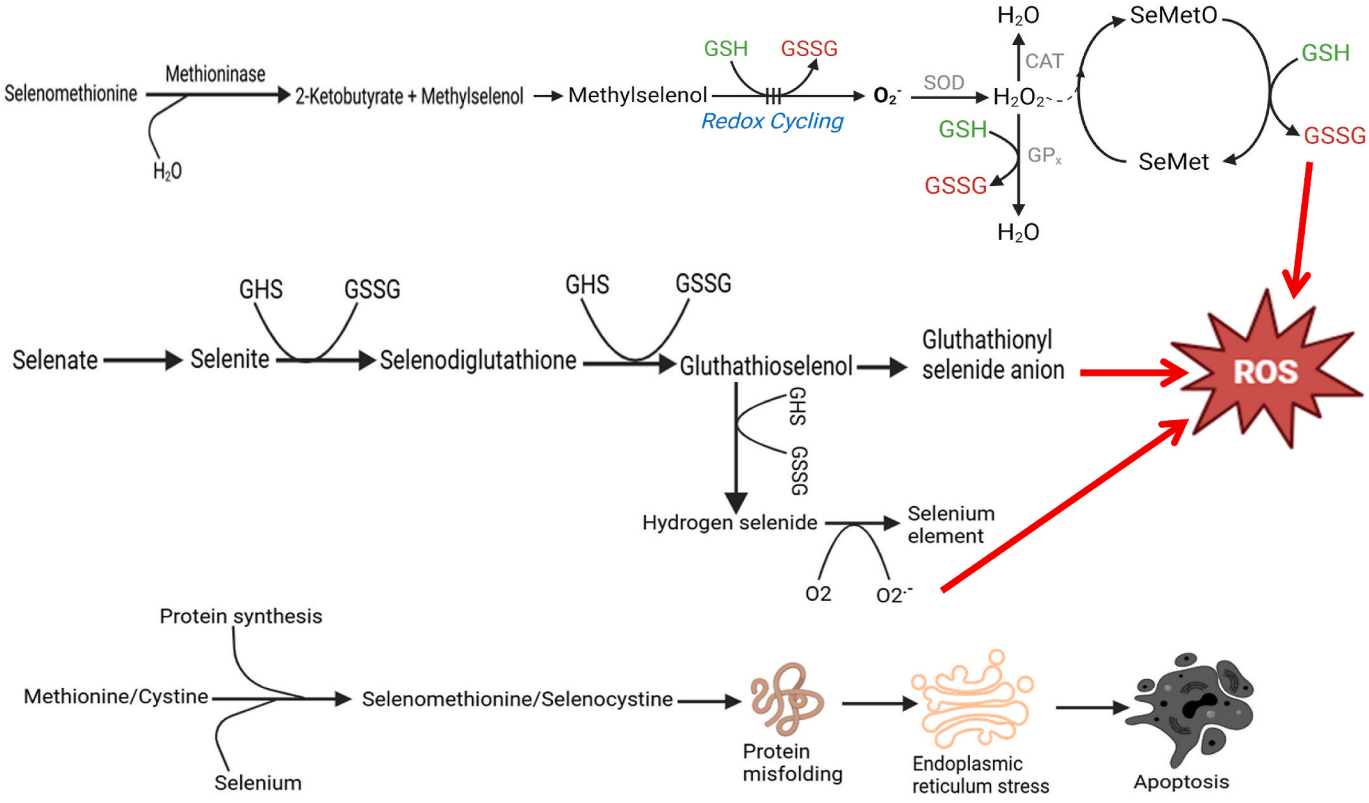

Fig. 2. Mechanism of selenium toxicity in fishes.

Table 2
Antioxidant enzymes response in fishes exposed to selenium.

Exposure route	Fish species	Target organs	Exposure concentration	Exposure periods (days)	Response concentration	Response	Reference
Superoxide dismutase (SOD)							
Dietary	*Oreochromis niloticus*	Liver	0, 1, 3, 5 mg/kg	56	1 mg/kg	↑	Wangkahart et al. (2022)
Dietary	*Hypophthalmichthys molitrix*	Liver	0, 0.5, 1 mg/kg	96	–	x	Mushtaq et al. (2022a)
		Whole body	0, 0.5, 1 mg/kg	96	–	x	
Dietary	*Oreochromis niloticus*	Blood	0, 1 mg/kg	65	1 mg/kg	↑	Ghaniem et al. (2022)
Waterborne	*Oreochromis mossambicus*	Gill	0, 10, 100 μg/L	4	100 μg/L	↓	Gopi et al. (2021)
		Liver	0, 10, 100 μg/L	4	100 μg/L	↓	
Dietary	*Nibea coibor*	Blood	0.21, 0.53, 0.79, 1.11, 1.45, 1.72 mg/kg	56	0.79,1.11 mg/kg	↑	Lin et al. (2021)
		Liver	0.21, 0.53, 0.79, 1.11, 1.45, 1.72 mg/kg	56	0.79, 1.11, 1.45 mg/kg	↑	
Dietary	*Pangasianodon hypophthalmus*	Liver	0, 0.5, 1, 2 mg/kg	60	1, 2 mg/kg	↑	El-Sharawy et al. (2021)
Dietary	*Dicentrarchus labrax*	Blood	0, 0.25, 0.5, 1 mg/kg	90	0.25, 0.5, 1 mg/kg	↑	Abd El-Kader et al. (2021)
Dietary	*Megalobrama amblycephala*	Liver	0.10, 0.42, 0.67, 1.06, 1.46 mg/kg	56	0.67,1.06 mg/kg	↑↓	Jingyuan et al. (2020)
Waterborne	*Channa argus*	Liver	0, 100, 200 mg/L	56	100, 200 mg/L	↓	Li et al. (2020)
		Spleen	0, 100, 200 mg/L	56	100, 200 mg/L	↓	
Dietary	*Danio rerio*	Muscle	1.63, 3, 10 mg/kg	28	3, 10 mg/kg	↑↓	Bai et al. (2019a)
Dietary	*Acanthopagrus schlegelii*	Blood	0, 0.34, 0.52, 0.68, 0.91, 1.08, 3.06 mg/kg	56	0.68, 0.91, 1.08, 3.06 mg/kg	↑	Wang et al. (2019)
		Liver	0, 0.34, 0.52, 0.68, 0.91, 1.08, 3.06 mg/kg	56	0.68, 0.91, 1.08, 3.06 mg/kg	↑	
Dietary	*Oreochromis niloticus*	Liver	0, 0.7 mg/kg	63	0.7 mg/kg	↓	Neamat-Allah et al. (2019)
Dietary	*Pangasianodon hypophthalmus*	Liver	0, 1, 2 mg/kg	60	1, 2 mg/kg	↓	Kumar and Singh (2019)
		Gill	0, 1, 2 mg/kg	60	1, 2 mg/kg	↓	
		Kidney	0, 1, 2 mg/kg	60	1, 2 mg/kg	↓	
Waterborne	*Oreochromis mossambicus*	Liver	0, 5, 10, 25, 50, 100 μg/L	4	25, 50, 100 μg/L	↑	Gobi et al. (2018)
		Gill	0, 5, 10, 25, 50, 100 μg/L	4	25, 50, 100 μg/L	↑	
Waterborne	*Pangasius hypophthalmus*	Liver	0, 4.5, 5, 5.5, 6.0 mg/L	4	4.5, 5, 5.5, 6.0 mg/L	↑	Kumar et al. (2018a)
		Gill	0, 4.5, 5, 5.5, 6.0 mg/L	4	4.5, 5, 5.5, 6.0 mg/L	↑	
		Brain	0, 4.5, 5, 5.5, 6.0 mg/L	4	4.5, 5, 5.5, 6.0 mg/L	↑	
Waterborne	*Pangasius hypophthalmus*	Liver	0, 2.5, 3.0, 3.5, 4.0 mg/L	4	4.0 mg/L	↑	Kumar et al. (2018a)
		Gill	0, 2.5, 3.0, 3.5, 4.0 mg/L	4	–	x	
		Brain	0, 2.5, 3.0, 3.5, 4.0 mg/L	4	3, 4 mg/L	↑	
Waterborne	*Pseudorasbora parva*	Liver	0, 10, 200, 1000 μg/L	28	10, 200, 1000 μg/L	↓	Ma et al. (2018)
Dietary	*Cyprinus carpio*	Liver	0, 0.7 mg/kg	56	0.7 mg/kg	↑	Saffari et al. (2017)
Dietary	*Argyrosomus regius*	Liver	0, 1, 2, 3 mg/kg	63	1, 2, 3 mg/kg	↑	Mansour et al. (2017)
Dietary	*Oreochromis niloticus*	Blood	0, 0.25, 0.5, 0.75, 1, 2, 4, 8, 16 mg/kg	70	8 mg/L	↑	Lee et al. (2016)
Dietary	*Cyprinus carpio*	Liver	0, 0.5, 1, 2 mg/kg	56	1, 2 mg/kg	↑	Ashouri et al. (2015)
(Catalase) CAT							
Dietary	*Oreochromis niloticus*	Liver	0, 1, 3, 5 mg/kg	56	1, 3 mg/kg	↑	Wangkahart et al. (2022)
Dietary	*Hypophthalmichthys molitrix*	Liver	0, 0.5, 1 mg/kg	96	–	x	Mushtaq et al. (2022a)
		Whole body	0, 0.5, 1 mg/kg	96	–	x	
		Muscle	0, 0.5, 1 mg/kg	96	–	x	
Dietary	*Oreochromis niloticus*	Blood	0, 1 mg/kg	65	1 mg/kg	↑	Ghaniem et al. (2022)
Waterborne	*Oreochromis mossambicus*	Gill	0, 10, 100 μg/L	4	100 μg/L	↓	Gopi et al. (2021)
		Liver	0, 10, 100 μg/L	4	100 μg/L	↓	
		Brain	0, 10, 100 μg/L	4	100 μg/L	↓	
Dietary	*Oreochromis niloticus*	Blood	0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/kg	56	0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/kg	↑	Naiel et al. (2021)
Dietary	*Nibea coibor*	Blood	0.21, 0.53, 0.79, 1.11, 1.45, 1.72 mg/kg	56	0.53, 0.79 mg/kg	↑	Lin et al. (2021)
		Liver	0.21, 0.53, 0.79, 1.11, 1.45, 1.72 mg/kg	56		x	
Dietary	*Pangasianodon hypophthalmus*	Liver	0, 0.5, 1, 2 mg/kg	60	0.5, 1, 2 mg/kg	↑	El-Sharawy et al. (2021)
Dietary	*Dicentrarchus labrax*	Blood	0, 0.25, 0.5, 1 mg/kg	90	0.25, 0.5, 1 mg/kg	↑	Abd El-Kader et al. (2021)
Dietary	*Oreochromis niloticus*	Liver	0, 3, 6,12 μg/g	90	3, 6,12 μg/g	↑	Chen et al. (2020)
		Liver	0, 3, 6,12 μg/g	45	6,12 μg/g	↑	
Waterborne	*Channa argus*	Liver	0, 100, 200 mg/L	56	100, 200 mg/L	↓	Li et al. (2020)

(*continued on next page*)

Table 2 (*continued*)

Exposure route	Fish species	Target organs	Exposure concentration	Exposure periods (days)	Response concentration	Response	Reference
		Spleen	0, 100, 200 mg/L	56	100, 200 mg/L	↓	
Dietary	*Megalobrama amblycephala*	Liver	0.10, 0.42, 0.67, 1.06, 1.46 mg/kg	56	0.67,1.06 mg/kg	↑	Jingyuan et al. (2020)
Dietary	*Carassius auratus*	Liver	0, 5, 10, 20 mg/kg	30	5, 10, 20 mg/kg	↑	Bai et al. (2019b)
Waterborne	*Channa argus*	Liver	0, 50, 100, 200, 400 μg/L	28	200, 400 μg/L	↑	Li et al. (2019)
		Spleen	0, 50, 100, 200, 400 μg/L	28	200, 400 μg/L	↑	
waterborne	*Channa argus*	Liver	0, 50, 100, 200 μg/L	56	–	x	Li et al. (2019)
		Spleen	0, 50, 100, 200 μg/L	56	–	x	
Dietary	*Acanthopagrus schlegelii*	Blood	0, 0.34, 0.52, 0.68, 0.91, 1.08, 3.06 mg/kg	56	0.68, 0.91, 1.08, 3.06 mg/kg	↑	Wang et al. (2019)
		Liver	0, 0.34, 0.52, 0.68, 0.91, 1.08, 3.06 mg/kg	56	0.68, 0.91, 1.08, 3.06 mg/kg	↑	
Dietary	*Oreochromis niloticus*	Liver	0, 0.7 mg/kg	63	0.7 mg/kg	↓	Neamat-Allah et al. (2019)
Dietary	*Pangasianodon hypophthalmus*	Liver	0, 1, 2 mg/kg	60	1, 2 mg/kg	↓	Kumar and Singh (2019)
		Gill	0, 1, 2 mg/kg	60	1, 2 mg/kg	↓	
		Kidney	0, 1, 2 mg/kg	60	1, 2 mg/kg	↓	
Waterborne	*Oreochromis mossambicus*	Liver	0, 5, 10, 25, 50, 100 μg/L	4	5, 10, 25, 50, 100 μg/L	↑↓	Gobi et al. (2018)
		Gill	0, 5, 10, 25, 50, 100 μg/L	4	5, 10, 25, 50, 100 μg/L	↑↓	
Waterborne	*Pangasius hypophthalmus*	Liver	0, 4.5, 5, 5.5, 6.0 mg/L	4	4.5, 5, 5.5, 6.0 mg/L	↑	Kumar et al. (2018a)
		Gill	0, 4.5, 5, 5.5, 6.0 mg/L	4	4.5, 5, 5.5, 6.0 mg/L	↑	
		Brain	0, 4.5, 5, 5.5, 6.0 mg/L	4	4.5, 5, 5.5, 6.0 mg/L	↑	
Waterborne	*Pangasius hypophthalmus*	Liver	0, 2.5, 3.0, 3.5, 4.0 mg/L	4	2.5, 3.0, 3.5, 4.0 mg/L	↑	Kumar et al. (2018a)
		Gill	0, 2.5, 3.0, 3.5, 4.0 mg/L	4	2.5, 3.0, 3.5, 4.0 mg/L	↑	
		Brain	0, 2.5, 3.0, 3.5, 4.0 mg/L	4	2.5, 3.0, 3.5, 4.0 mg/L	↑	
Dietary	*Cyprinus carpio*	Liver	0, 0.7 mg/kg	56	0.7 mg/kg	↑	Saffari et al. (2017)
Dietary	*Piaractus mesopotamicus*	Liver	0.72,0.94,1.15, 1.57,2.51 mg/kg	65	–	x	Takahashi et al. (2017)
Dietary	*Argyrosomus regius*	Liver	0, 1, 2, 3 mg/kg	63	2, 3 mg/kg	↑	Mansour et al. (2017)
Dietary	*Cyprinus carpio*	Liver	0, 0.5, 1, 2 mg/kg	56	2 mg/kg	↑	Ashouri et al. (2015)
Glutathione peroxidase (GPX)							
Dietary	*Oreochromis niloticus*	Liver	0, 1, 3, 5 mg/kg	56	1, 3, 5 mg/kg	↑	Wangkahart et al. (2022)
Dietary	*Hypophthalmichthys molitrix*	Liver	0, 0.5, 1 mg/kg	96	–	x	Mushtaq et al. (2022a)
		Whole body	0, 0.5, 1 mg/kg	96	–	x	
		Muscle	0, 0.5, 1 mg/kg	96	–	x	
Dietary	*Oreochromis niloticus*	Blood	0, 1 mg/kg	65	1 mg/kg	↑	Ghaniem et al. (2022)
waterborne	*Oreochromis mossambicus*	Gill	0, 10, 100 μg/L	4	10, 100 μg/L	↑	Gopi et al. (2021)
		Liver	0, 10, 100 μg/L	4	10, 100 μg/L	↑	
Dietary	*Carassius auratus*	Blood	0, 0.1, 0.5,1 mg/kg	60	0.1, 0.5,1 mg/kg	↑	Seyedi et al. (2021)
Dietary	*Dicentrarchus labrax*	Blood	0, 0.25, 0.5, 1 mg/kg	90	0.25, 0.5, 1 mg/kg	↑	Abd El-Kader et al. (2021)
Dietary	*Pangasianodon hypophthalmus*	Liver	0, 0.5, 1, 2 mg/kg	60	0.5, 1, 2 mg/kg	↑	El-Sharawy et al. (2021)
Dietary	*Nibea coibor*	Blood	0.21, 0.53, 0.79, 1.11, 1.45, 1.72 mg/kg	56	0.53, 0.79, 1.11, 1.45,1.72 mg/kg	↑	Lin et al. (2021)
		Liver	0.21, 0.53, 0.79, 1.11, 1.45, 1.72 mg/kg	56	0.53, 0.79, 1.11, 1.45,1.72 mg/kg	↑	
Waterborne	*Channa argus*	Liver	0, 100, 200 mg/L	56	200 mg/L	↓	Li et al. (2020)
		Spleen	0, 100, 200 mg/L	56	–	x	
Dietary	*Megalobrama amblycephala*	Liver	0.10, 0.42, 0.67, 1.06, 1.46 mg/kg	56	0.42, 0.67, 1.06, 1.46 mg/kg	↑	Jingyuan et al. (2020)
Dietary	*Danio rerio*	Gill	0, 2.5, 3.0, 3.5, 4.0 mg/kg	4	–	x	Bai et al., 2019a
Dietary	*Carassius auratus*	Brain	0, 2.5, 3.0, 3.5, 4.0 mg/kg	4	3, 4 mg/kg	↑	Bai et al., 2019a
Waterborne	*Channa argus*	Liver	0, 50, 100, 200, 400 μg/L	28	100, 200, 400 μg/L	↑	Li et al. (2019)
		Spleen	0, 50, 100, 200, 400 μg/L	28	100, 200, 400 μg/L	↑	
Waterborne	*Channa argus*	Liver	0, 50, 100, 200 μg/L	56	50, 100, 200 μg/L	↑	Li et al. (2019)
		Spleen	0, 50, 100, 200 μg/L	56	–	X	
Dietary	*Acanthopagrus schlegelii*	Blood	0, 0.34, 0.52, 0.68, 0.91, 1.08, 3.06 mg/kg	56	0.68, 0.91, 1.08, 3.06 mg/kg	↑	Wang et al. (2019)
		Liver	0, 0.34, 0.52, 0.68, 0.91, 1.08, 3.06 mg/kg	56	0.68, 0.91, 1.08, 3.06 mg/kg	↑	
Dietary	*Oreochromis niloticus*	Liver	0, 0.7 mg/kg	63	0.7 mg/kg	↓	Neamat-Allah et al. (2019)
Dietary	*Oncorhynchus mykiss*	Liver	0, 1 mg/kg	56	1 mg/kg	↑	Kohshahi et al. (2019)
		Blood	0, 1 mg/kg	56	1 mg/kg	↑	
Waterborne	*Oreochromis mossambicus*	Liver	0, 5, 10, 25, 50, 100 μg/L	4	5, 10, 25, 50, 100 μg/L	↑	Gobi et al. (2018)
		Gill	0, 5, 10, 25, 50, 100 μg/L	4	5, 10, 25, 50, 100 μg/L	↑	

(*continued on next page*)

Table 2 (*continued*)

Exposure route	Fish species	Target organs	Exposure concentration	Exposure periods (days)	Response concentration	Response	Reference
Dietary	*Piaractus mesopotamicus*	Liver	0.72,0.94,1.15, 1.57,2.51 mg/kg	65	1.15,1.57 mg/kg	↑	Takahashi et al. (2017)
Dietary	*Cyprinus carpio*	Liver	0, 0.7 mg/kg	56	0.7 mg/kg	↑	Saffari et al. (2017)
Dietary	*Tor putitora*	Liver	0, 0.68 mg/kg	70	0.68 mg/kg	↑	Khan et al. (2016)
		Muscle	0, 0.68 mg/kg	70	0.68 mg/kg	↑	
		Gill	0, 0.68 mg/kg	70	0.68 mg/kg	↑	
		Brain	0, 0.68 mg/kg	70	0.68 mg/kg	↑	
Dietary	*Lates calcarifer*	Muscle	2, 3, 4, 5, 7 mg/kg	60	3, 4, 5, 7 mg/kg	↑	Ilham et al. (2016)
Dietary	*Pelteobagrus fulvidraco*	Liver	0, 0.23, 6.5 mg/kg	56	0.23, 6.5 mg/kg	↑	Hu et al., (2016)
Dietary	*Cyprinus carpio*	Liver	0, 0.5, 1, 2 mg/kg	56	1, 2 mg/kg	↑	Ashouri et al. (2015)
Glutathione transferases (GST)							
Waterborne	*Oreochromis mossambicus*	Gill	0, 10, 100 μg/L	4	10, 100 μg/L	↑	Gopi et al. (2021)
		Liver	0, 10, 100 μg/L	4	10, 100 μg/L	↑	
Waterborne	*Channa argus*	Liver	0, 100, 200 mg/L	56	100, 200 mg/L	↓	Li et al. (2020)
		Spleen	0, 100, 200 mg/L	56	100, 200 mg/L	↓	
Dietary	*Oreochromis niloticus*	Liver	0, 3, 6,12 μg/g	90	3, 6,12 μg/g	↑	Chen et al. (2020)
Dietary	*Oreochromis niloticus*	Liver	0, 3, 6,12 μg/g	45	3, 6,12 μg/g	↑	Chen et al. (2020)
Dietary	*Carassius auratus*	Liver	0, 5, 10, 20 mg/kg	30	5, 10, 20 mg/kg	↓	Bai et al., 2019a
Waterborne	*Oreochromis mossambicus*	Liver	0, 5, 10, 25, 50, 100 μg/L	4	10, 25, 50, 100 μg/L	↑↓	Gobi et al. (2018)
		Gill	0, 5, 10, 25, 50, 100 μg/L	4	10, 25, 50, 100 μg/L	↑	
Waterborne	*Pangasius hypophthalmus*	Liver	0, 4.5, 5, 5.5, 6.0 mg/L	4	4.5, 5, 5.5, 6.0 mg/L	↑	Kumar et al. (2018a)
		Gill	0, 4.5, 5, 5.5, 6.0 mg/L	4	4.5, 5, 5.5, 6.0 mg/L	↑	
		Brain	0, 4.5, 5, 5.5, 6.0 mg/L	4	4.5, 5, 5.5, 6.0 mg/L	↑	
Waterborne	*Pangasius hypophthalmus*	Liver	0, 2.5, 3.0, 3.5, 4.0 mg/L	4	2.5, 3.0, 3.5, 4.0 mg/L	↑	Kumar et al. (2018a)
		Gill	0, 2.5, 3.0, 3.5, 4.0 mg/L	4	4.0 mg/L	↑	
		Brain	0, 2.5, 3.0, 3.5, 4.0 mg/L	4	2.5, 3.0, 3.5, 4.0 mg/L	x	
Waterborne	*Pseudorasbora parva*	Liver	0, 10, 200, 1000 μg/L	28	10, 200, 1000 μg/L	↓	Ma et al. (2018)
Dietary	*Pangasianodon hypophthalmus*	Liver	0, 1, 2 mg/kg	60	1, 2 mg/kg	↓	Kumar et al. (2018b)
		Gill	0, 1, 2 mg/kg	60	1, 2 mg/kg	↓	
		Kidney	0, 1, 2 mg/kg	60	1, 2 mg/kg	↓	
Dietary	*Piaractus mesopotamicus*	Liver	0.72,0.94,1.15, 1.57,2.51 mg/kg	65	0.94,1.15, 1.57 mg/kg	↑	Takahashi et al. (2017)

↑ = increased; ↓ = decreased; x = no changed.

GST is usually triggered in fishes by exposure to environmental toxins (Kim and Kang, 2016c). GST functions in the second stage of detoxification metabolism by conjugating to xenobiotics and clearing them from the cells. Thus, GST plays a key role in homeostasis and foreign body dissociation, protecting tissues from oxidative stress (Mushtaq et al., 2022a,b). GPX is an enzyme that assists peroxide conversion to less toxic hydroxyl compounds that protect cells from damage caused by oxygen. Many authors have reported that exposure to Se alters GST and GPX activities by inducing oxidative stress (El-Sharawy et al., 2021; Gopi et al., 2021; Gobi et al., 2018; Ma et al., 2018; Takahashi et al., 2017).

Malondialdehyde (MDA), thiobarbituric acid reactive substances (TBARS), and reduced glutathione levels (GHS) are recognized as crucial indicators of oxidative damage in fishes exposed to environmental toxins (Shah and Mraz, 2020). Table 3 summarizes the oxidative damage indicators (MDA, TBARS, and GHS) in fishes exposed to Se. Oxidative stress triggers lipid peroxidation of cell membranes and DNA damage, quantified by MDA levels. Elevated ROS signify toxic reactions, inducing oxidative stress by disrupting the balance between ROS generation and antioxidant capacity. Excessive ROS can escalate lipid peroxidation, leading to MDA production, serving as direct evidence of free radical-induced damage in fishes (Lin et al., 2021). Changes in MDA content indirectly gauge the disruption level of the biofilm system. Chronic dietary Se exposure below 1 mg/kg enhances antioxidant enzyme activities and diminishes MDA content in the serum, liver, gill, and muscle, indicating improved antioxidant capacity in fishes (Ghaniem et al., 2022; Wang et al., 2019). However, MDA content shows an upward trajectory with increasing dietary Se levels beyond 1.11 mg/kg, likely due to oxidative stress (Bai et al., 2019a). Additionally, waterborne Se exposure ranging from 50 μg/L to 200 mg/L leads to increased MDA levels in the liver, spleen, and serum of various fishes, attributed to increased ROS generation and lipid peroxidation (Li et al., 2019; Ma et al., 2018).

TBARS level serves as a widely used indicator of lipid peroxidation in fishes experiencing oxidative stress induced by environmental toxins, including Se above the physiological threshold (Kumar and Singh, 2019; Ponton et al., 2016). Fishes, with their high content of polyunsaturated fatty acids (PUFA), are particularly prone to oxidative damage, making TBARS a valuable biomarker of oxidative stress (Lauriano et al., 2016). Both waterborne and dietary exposure to Se in *O. mossambicus* and *O. niloticus*, respectively, have been linked to increased TBARS levels, indicating lipid peroxidation and oxidative damage in the liver and gills (Gopi et al., 2021; Chen et al., 2020). The level of GHS is also recognized as an indicator of oxidative damage in fishes exposed to Se. Most studies report that various fish species exposed to Se up to 1 mg/kg or 1 mg/L exhibit increased GHS levels in different internal organs, indicating higher antioxidative capacity and lower oxidative stress (Naiel et al., 2021; Gobi et al., 2018; Ma et al., 2018).

5. Effects of selenium on metabolic functions

Metabolic markers are critical for assessing the health and physiological stress in fishes, influenced by internal and external factors (Uddin et al., 2023). Blood biochemical alterations are key indicators of pollutant exposure (Kucukbay et al., 2009). As an accessible biological fluid, blood reflects changes in physiological states. Thus, hematological investigations are vital for monitoring fish health, with trace elements in the blood significantly affecting physiological functions (Shahjahan et al., 2018). Metal stress has been shown to alter metabolic markers, including enzyme levels, lipid peroxidation products, and hematological parameters (Ates et al., 2008). These changes are influenced by factors such as the type of metal or fish species, water quality, and exposure duration (Orun et al., 2008). Primary stress responses due to metals exposure are characterized by the release of catecholamines and corticosteroids, whereas the secondary stress responses to hematological changes and its associated biochemistry as reliable biomarkers in fishes

Table 3
Oxidative damage indicators in fishes exposed selenium.

Exposure route	Fish species	Target organs	Exposure concentration	Exposure periods (days)	Response concentration	Response	Reference
Malondialdehyde (MDA)							
Dietary	*Oreochromis niloticus*	Liver	0, 1, 3, 5 mg/kg	56		x	Wangkahart et al. (2022)
Dietary	*Oreochromis niloticus*	Blood	0, 1 mg/kg	65	1 mg/kg	↓	Ghaniem et al. (2022)
Dietary	*Dicentrarchus labrax*	Blood	0, 0.25, 0.5, 1 mg/kg	90	0.25, 0.5, 1 mg/kg	↓	Abd El-Kader et al. (2021)
Dietary	*Pangasianodon hypophthalmus*	Liver	0, 0.5, 1, 2 mg/kg	60	0.5, 1, 2 mg/kg	↓	El-Sharawy et al. (2021)
Dietary	*Nibea coibor*	Blood	0.21, 0.53, 0.79, 1.11, 1.45, 1.72 mg/kg	56	0.79 mg/kg	↓	Lin et al. (2021)
		Liver	0.21, 0.53, 0.79, 1.11, 1.45, 1.72 mg/kg	56	0.79 mg/kg	↓	
Waterborne	*Channa argus*	Liver	0, 100, 200 mg/L	56	100, 200 mg/L	↑	Li et al. (2020)
Waterborne	*Channa argus*	Spleen	0, 100, 200 mg/L	56	100, 200 mg/L	↑	
Dietary	*Megalobrama amblycephala*	Liver	0.10, 0.42, 0.67, 1.06, 1.46 mg/kg	56	0.42, 0.67, 1.06, mg/kg	↓↑	Jingyuan et al. (2020)
Dietary	*Carassius auratus*	Liver	0, 5, 10, 20 mg/kg	30	5, 10, 20 mg/kg	↑	Bai et al., 2019b
Waterborne	*Channa argus*	Liver	0, 50, 100, 200 μg/L	56	50, 100, 200 μg/L	↑	Li et al. (2019)
Waterborne	*Channa argus*	Liver	0, 50, 100, 200, 400 μg/L	28	200, 400 μg/L	↑	Li et al. (2019)
Waterborne	*Channa argus*	Spleen	0, 50, 100, 200, 400 μg/L	28	200, 400 μg/L	↑	
Dietary	*Oreochromis niloticus*	Liver	0, 0.7 mg/kg	63		x	Neamat-Allah et al. (2019)
Dietary	*Acanthopagrus schlegelii*	Blood	0, 0.34, 0.52, 0.68, 0.91, 1.08, 3.06 mg/kg	56	0.68, 0.91, 1.08, 3.06 mg/kg	↓	Wang et al. (2019)
		Liver	0, 0.34, 0.52, 0.68, 0.91, 1.08, 3.06 mg/kg	56	0.34, 0.68, 0.91, 1.08, 3.06 mg/kg	↓	
Dietary	*Oreochromis niloticus*	Liver	0.53,0.86,1.04,1.22 mg/kg	42	–	x	Durigon et al. (2019)
		Gill	0.53,0.86,1.04,1.22 mg/kg	42	1.04,1.22 mg/kg	↓	
		Muscle	1.04,1.22 mg/kg	42	0.86, 1.04,1.22 mg/kg	↓	
Waterborne	*Oreochromis mossambicus*	Liver	0, 5, 10, 25, 50, 100 μg/L	4	5, 10, 25, 50, 100 μg/L	↑	Gobi et al. (2018)
		Gill	0, 5, 10, 25, 50, 100 μg/L	4	5, 10, 25, 50, 100 μg/L	↑	
Waterborne	*Pseudorasbora parva*	Liver	0, 10, 200, 1000 μg/L	28	10, 200, 1000 μg/L	↑	Ma et al. (2018)
Dietary	*Cyprinus carpio*	Liver	0, 0.7 mg/kg	56	0.7 mg/kg	↓	Saffari et al. (2017)
Dietary	*Pelteobagrus fulvidraco*		0, 0.23, 6.5 mg/kg	56	0.23, 6.5 mg/kg	x	Hu et al., (2016)
Waterborne	*Carassius auratus*	Blood	0, 2, 3, 4 mg/L	5	4 mg/L	↑	Choi et al. (2015)
Waterborne	*Channa argus*	Spleen	0, 50, 100, 200 μg/L	56	50, 100, 200 μg/L	↑	
Dietary	*Cyprinus carpio*	Liver	0, 0.5, 1, 2 mg/kg	56	2 mg/kg	↓	Ashouri et al. (2015)
Thiobarbituric acid reactive substances (TBARES level)							
Dietary	*Hypophthalmichthys molitrix*	Liver	0, 0.5, 1 mg/kg	96	–	x	Mushtaq et al. (2022a)
		Blood	0, 0.5, 1 mg/kg	96	–	x	
		Muscle	0, 0.5, 1 mg/kg	96	0.5, 1 mg/kg	↑	
Waterborne	*Oreochromis mossambicus*	Gill	0, 10, 100 μg/L	4	10, 100 μg/L	↑	Gopi et al. (2021)
		Liver	0, 10, 100 μg/L	4	10, 100 μg/L	↑	
Dietary	*Oreochromis niloticus*	Liver	0, 3, 6,12 μg/g	90	6,12 μg/g	↑	Chen et al. (2020)
Dietary	*Oreochromis niloticus*	Liver	0, 3, 6,12 μg/g	45	6,12 μg/g	↑	Chen et al. (2020)
Dietary	*Argyrosomus regius*	Liver	0, 1, 2, 3 mg/kg	63	1, 2, 3 mg/kg	↓	Mansour et al. (2017)
Glutathione (GSH levels)							
Waterborne	*Oreochromis mossambicus*	Gill	0, 10, 100 μg/L	4	10, 100 μg/L	↑	Gopi et al. (2021)
		Liver	0, 10, 100 μg/L	4	10, 100 μg/L	↑	
Dietary	*Oreochromis niloticus*	Blood	0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/kg	56	0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/kg	↑	Naiel et al. (2021)
Dietary	*Megalobrama amblycephala*	Liver	0.10, 0.42, 0.67, 1.06, 1.46 mg/kg	56	0.67,1.06 mg/kg	↑↓	Jingyuan et al. (2020)
Waterborne	*Pseudorasbora parva*	Liver	0, 10, 200, 1000 μg/L	28	10, 200, 1000 μg/L	↑	Ma et al. (2018)
Waterborne	*Oreochromis mossambicus*	Liver	0, 5, 10, 25, 50, 100 μg/L	4	10, 25, 50, 100 μg/L	↑	Gobi et al. (2018)
		Gill	0, 5, 10, 25, 50, 100 μg/L	4	10, 25, 50, 100 μg/L	↑	
Dietary	*Piaractus mesopotamicus*	Liver	0.72,0.94,1.15, 1.57,2.51 mg/kg	65	–	x	Takahashi et al. (2017)
Waterborne	*Danio rerio*	Liver	0, 1 mg/L	4	1 mg/L	↑	Davis et al., (2016)
		Kidney	0, 1 mg/L	4	1 mg/L	↑	
		Brain	0, 1 mg/L	4	1 mg/L	↑	

↑ = increased; ↓ = decreased; x = no changed.

has been well documented (Rebl et al., 2021).

Hematological properties [red blood cell (RBC), white blood cell (WBC), hematocrit (Hct), hemoglobin (Hb), glucose, cholesterol, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP)] are important metabolic markers for evaluating the health status of fishes following exposure to various environmental stresses, chemical toxicity, and bacterial infections (Kim et al., 2020; Kim and Kang, 2017a). Se above the physiological optimum results in alteration of metabolic markers in fishes including RBC, WBC, Hb, Hct, AST, ALT, ALP, albumin, globulin, and

total protein. Alterations of metabolic markers of fishes in relation to Se exposure route (waterborne and dietary) are presented in Table 4. Several studies have consistently reported that chronic dietary exposure to Se within the range of 0.5–1 mg/kg for a duration of 30–90 days leads to increase levels of RBCs, Hb, and Hct in different fish species (Al-Din et al., 2022; Ghaniem et al., 2022; Abd El-Kader et al., 2021). These elevated parameters signify an enhancement in the health status of the fishes, attributed to improved oxygen availability in cellular tissues, thereby preventing anemia.

The antioxidant properties of Se contribute to the protection of RBC membranes, thereby prolonging their lifespan and defending against oxidative damage caused by ROS. This mechanism aids in reducing anemia, membrane disruption, cell hemolysis, and degeneration (Khan et al., 2016). Similar enhancements in hematological parameters have been observed in fishes fed with nano-Se, including Common carp (Saffari et al., 2017), European seabass (Dawood et al., 2019), and Nile tilapia (Rathore et al., 2021a). The elevated levels of Hb and Hct resulting from nano-Se exposure are attributed to the increased oxygen-carrying capacity to meet the higher respiratory and metabolic demands (de Azevedo et al., 2015).

Several studies have reported an increased in WBCs counts in various fish species exposed to Se (Al-Din et al., 2022; Mushtaq et al., 2022a,b; Yeganeh et al., 2016). This elevation in WBCs indicates the activation of cell-mediated immunity, particularly under stressful conditions (Fiúza et al., 2015). However, it is noteworthy that exposure to waterborne Se has shown contrasting effects, including a significant decrease in RBC count, Hct, and Hb levels (Dhara et al., 2022). Such decreases may be an indication of an increasing rate of erythrocyte destruction in the hematopoietic organ, potentially leading to anemia (Yeganeh et al., 2016).

Blood glucose is used as a stress indicator in fishes; high glucose levels often indicate high stress because high cortisol levels stimulate the dissolution of liver glycogen to provide energy during the stress process. The significant utilization of blood glucose in Se treated fishes may be due to enhanced energy demand by releasing more glucose via glycogenolysis (Dhara et al., 2022). The activity of transaminases (ALT and AST) in serum serves as an indicator of stress in fishes, with increased concentrations reflecting responses to stressful conditions (Bitiren et al., 2004). Moreover, elevated liver enzyme activities can lead to liver and kidney damage, resulting in necrosis and higher ALP levels in blood, which can cause skeletal disorders such as osteoporosis and hepatic cell ruptures (Bitiren et al., 2004). AST and ALT also play essential roles in nitrogen metabolism within cells, facilitating the transfer of amino acids to liver cells and monitoring toxic effects (Abdel-Tawwab, 2016). Additionally, ALP is involved in the transport of phosphorylated intermediates through cells and carbohydrate metabolism (Yousef et al., 2003).

Several studies have indicated that the observed increase in AST, ALT, and ALP serum activities in Se-exposed fishes may be attributed to cellular cytotoxicity and damage to liver and kidney tissues. However, contradictory findings have been reported, with no significant differences found in AST, ALT, and ALP activities in various fish species fed different levels of Se (Abd El-Kader et al., 2021; Ziaei-Nejad et al., 2021; Jingyuan et al., 2020). Conversely, Se supplementation led to decrease activities of AST, ALT, and ALP in certain fish species, indicating a potential positive influence on liver health (Mushtaq et al., 2022a,b; Naiel et al., 2021; Saffari et al., 2017). These findings underscore that feeding fishes with Se supplemented diets could positively influence the health of liver.

Detecting total protein levels in fish blood is vital for assessing overall health and immunity status, as proteins play critical roles in cell function, metabolism, hormone secretion, and regulating physiological processes within the fish body. *O. niloticus* exposed to Se for 65 days exhibited an increase in total protein levels (Ghaniem et al., 2022; Naiel et al., 2021). This elevation may be attributed to the high protein content induced by Se's role in increasing selenoprotein levels intracellularly. Conversely, other species of fishes exposed to different concentrations of Se showed lower levels of total protein (Dhara et al., 2022; Yeganeh et al., 2016). This reduction in total protein levels may be associated with hypoalbuminemia, which could be related to cellular degradation, imperfect protein synthesis, and protein loss due to pathological changes in the kidney (Hamed, 2015).

Among serum proteins, albumin and globulin are crucial indicators of the immune status of experimental animals (Naderi et al., 2017a). Elevated levels of albumin can help protect blood vessels from leaking during times of stress, while globulins contain various immunological components (Uribe et al., 2011). An increase in albumin and globulin levels is associated with higher organic Se levels in fish diets, which can enhance their production in the liver (Abdel-Tawwab et al., 2007).

Studies have shown that increasing the amount of Se up to 2 mg/kg in the diet significantly increases total protein and globulin levels, consistent with findings on African catfish and Common carp by Abdel-Tawwab et al. (2007) and Ashouri et al. (2015), respectively. Furthermore, triglycerides serve as a source of energy for various metabolic processes, with excess amounts being stored as fat in adipose tissue. Therefore, the observed elevation in serum triglyceride levels in Se-treated fishes could be attributed to the degradation of stored fats to produce the required energy to counteract the toxic effects of Se (Dhara et al., 2022; Naderi et al., 2017b).

6. Effects of selenium on growth and reproduction

Appropriate nutrition is essential for maintaining the overall growth, reproductive performance, and health status of fishes (Rohani et al., 2022; Islam et al., 2021; Jahan et al., 2021). Food deprivation can lead to physiological impairments in fishes, potentially resulting in reproductive issues such as reduced fertility and hatching rates due to nutrient deficiencies (Wu, 2022; Volkoff and London, 2018). Among various nutrients, trace metals play a significant role in fulfilling the nutritional demands of fishes for various physiological processes, including growth and reproduction (Taslima et al., 2022). Se is a key trace element that plays essential roles in regulating reproductive hormones, growth, and metabolism within physiological thresholds in animals, including fishes (Abdollahi-Mousavi et al., 2024; Mushtaq et al., 2022a,b; Saffari et al., 2022).

The dietary requirement of Se in fishes are highly species-specific (Jahanbakhshi et al., 2021). For example, the dietary requirement of Se for *Oncorhynchus mykiss*, *O. niloticus*, *Ictalurus punctatus*, and *Carassius auratus* is reported to be 0.15–0.38, 1.06–2.06, 0.25, and 0.73–1.19 μg/g diet dw, respectively (Jahanbakhshi et al., 2021; Khalil et al., 2019; Nazari et al., 2017; Zhu et al., 2017). Prabhu et al. (2016) reported that diets containing varying levels of Se, ranging from 0.2 to 12 mg/kg, exhibited beneficial effects on the physiological and immunological responses of fishes. Environmentally relevant waterborne exposure to inorganic Se (selenite) can also affect the fish growth. For example, freshly hatched zebrafish embryos exposed to waterborne selenite concentrations of 10–100 μg/L for 30 days exhibited reduced length and body weight compared to their counterparts which were raised in normal water (Uddin et al., 2023; unpublished data).

In contrast, Se exposure above dietary requirements level can lead to Se accumulation, causing adverse effects on reproductive performance, growth inhibition, tissue damage, and mortality (Zhu et al., 2017). Cheng et al. (2022) reported that long-term exposure to 57.01 or 117.67 μg/L of sodium selenite for 4 months markedly inhibited the growth of adult zebrafish. Similarly, dietary Se-Met at 58.63 μg/g (dw) disturbed the sexual differentiation and development of zebrafish larvae after 90 days of exposure (Mo et al., 2020). Dietary exposure to high Se levels (30.02 and 59.76 mg/kg dw) caused significant Se accumulation in the ovaries of female *Procambarus clarkii*, reducing their spawning rate by inhibiting the secretion of 17β-estradiol (Mo et al., 2019). Additionally, Mo et al. (2020) reported that exposure to high dietary Se-Met levels (10.80 mg/kg dw) interfered with the growth hormone/insulin-like growth factors (GH/IGFs) and hypothalamus-pituitary-gonad-liver

Table 4
Alterations of metabolic markers in fishes exposed to selenium.

Exposure route	Fish species	Exposure concentration	Exposure periods (days)	Response concentration	Response	Reference
Red blood cell (RBC)						
Dietary	*Oreochromis niloticus*	0, 1, 3, 5 mg/kg	56	1 mg/kg	↓	Wangkahart et al. (2022)
Dietary	*Hypophthalmichthys molitrix*	0, 0.5, 1 mg/kg	96	–	x	Mushtaq et al. (2022a)
Dietary	*Oreochromis niloticus*	0, 0.5,1 mg/kg	70	0.5, 1 mg/kg	↑	Al-Din et al. (2022)
Waterborne	*Channa punctata*	0, 1.5, 3 mg/L	30	1.5, 3 mg/L	↓	Dhara et al. (2022)
	Ctenopharyngodon idella	0, 0.798, 1.596 mg/L	30	0.798, 1.596 mg/L	↓	
Dietary	*Oreochromis niloticus*	0, 1 mg/kg	65	1 mg/kg	↑	Ghaniem et al. (2022)
Dietary	*Labeo rohita*	0, 0.5 mg/kg	60	0.5 mg/kg	↑	Pavithra et al., (2021)
Dietary	*Dicentrarchus labrax*	0, 0.25, 0.5, 1 mg/kg	90	0.25, 0.5, 1 mg/kg	↑	Abd El-Kader et al. (2021)
Dietary	*Rutilus caspicus*	0, 1 mg/kg	28	–	x	Zahmatkesh et al. (2020)
Dietary	*Oreochromis niloticus*	0, 0.7 mg/kg	63	–	x	Neamat-Allah et al. (2019)
Dietary	*Oreochromis niloticus*	0, 2, 4, 8 mg/kg	90	2, 8 mg/kg	↑,↓	Iqbal et al. (2017)
Dietary	*Piaractus mesopotamicus*	0.72, 0.94, 1.15, 1.57, 2.51 mg/kg	65	1.15, 1.57 mg/kg	↑	Takahashi et al. (2017)
Dietary	*Argyrosomus regius*	0, 1, 2, 3 mg/kg	63	–	x	Mansour et al. (2017)
Waterborne	*Cyprinus carpio*	0, 0.054 mg/L	28	0.054 mg/L	↓	Yeganeh et al. (2016)
Dietary	*Tor putitora*	0, 0.68 mg/kg	70	0.68 mg/kg	↑	Khan et al. (2016)
White blood cell (WBC)						
Dietary	*Oreochromis niloticus*	0, 1, 3, 5 mg/kg	56	–	x	Wangkahart et al. (2022)
Dietary	*Hypophthalmichthys molitrix*	0, 0.5, 1 mg/kg	96	0.5, 1 mg/kg	↑	Mushtaq et al. (2022a)
Dietary	*Oreochromis niloticus*	0, 1 mg/kg	65	–	x	Ghaniem et al. (2022)
Dietary	*Oreochromis niloticus*	0, 0.5,1 mg/kg	70	1 mg/kg	↑	Al-Din et al. (2022)
Dietary	*Labeo rohita*	0, 0.5 mg/kg	60	0.5 mg/kg	↓	Pavithra et al., (2021)
Dietary	*Dicentrarchus labrax*	0, 0.25, 0.5, 1 mg/kg	90	0.25, 0.5, 1 mg/kg	↑	Abd El-Kader et al. (2021)
Dietary	*Rutilus caspicus*	0, 1 mg/kg	28	–	x	Zahmatkesh et al. (2020)
Dietary	*Argyrosomus regius*	0, 1, 2, 3 mg/kg	63	1, 2, 3 mg/kg	↑	Mansour et al. (2017)
Dietary	*Oreochromis niloticus*	0, 2, 4, 8 mg/kg	90	–	x	Iqbal et al. (2017)
Waterborne	*Cyprinus carpio*	0, 0.054 mg/L	28	0.054 mg/L	↑	Yeganeh et al. (2016)
Hemoglobin (Hb)						
Dietary	*Oreochromis niloticus*	0, 1, 3, 5 mg/kg	56	1 mg/kg	↓	Wangkahart et al. (2022)
Dietary	*Hypophthalmichthys molitrix*	0, 0.5, 1 mg/kg	96	1 mg/kg	↑	Mushtaq et al. (2022a)
Waterborne	*Channa punctata*	0, 1.5, 3 mg/L	30	1.5, 3 mg/L	↓	Dhara et al. (2022)
	Ctenopharyngodon idella	0, 0.798, 1.596 mg/L	30	0.798, 1.596 mg/L	↓	
Dietary	*Oreochromis niloticus*	0, 1 mg/kg	65	1 mg/kg	↑	Ghaniem et al. (2022)
Dietary	*Oreochromis niloticus*	0, 0.5,1 mg/kg	70	1 mg/kg	↑	Al-Din et al. (2022)
Dietary	*Dicentrarchus labrax*	0, 0.25, 0.5, 1 mg/kg	90	0.25, 0.5, 1 mg/kg	↑	Abd El-Kader et al. (2021)
Dietary	*Labeo rohita*	0, 0.5 mg/kg	60	0.5 mg/kg	↑	Pavithra et al., (2021)
Dietary	*Rutilus caspicus*	0, 1 mg/kg	28	–	x	Zahmatkesh et al. (2020)
Dietary	*Oreochromis niloticus*	0.53,0.86,1.04,1.22 mg/kg	42	–	x	Durigon et al. (2019)
Dietary	*Oreochromis niloticus*	0, 2, 4, 8 mg/kg	90	4, 8 mg/kg	↓	Iqbal et al. (2017)
Dietary	*Argyrosomus regius*	0, 1, 2, 3 mg/kg	63	–	x	Mansour et al. (2017)
Waterborne	*Cyprinus carpio*	0, 0.054 mg/L	28	0.054 mg/L	↓	Yeganeh et al. (2016)
Dietary	*Tor putitora*	0, 0.68 mg/kg	70	0.68 mg/kg	↑	Khan et al. (2016)
Dietary	*Lates calcarifer*	2, 3, 4, 5, 7 mg/kg	60	–	x	Ilham et al. (2016)
Glucose						
Dietary	*Hypophthalmichthys molitrix*	0, 0.5, 1 mg/kg	96	–	x	Mushtaq et al. (2022a)
Waterborne	*Channa punctata*	0, 1.5, 3 mg/L	30	1.5, 3 mg/L	↑	Dhara et al. (2022)
	Ctenopharyngodon idella	0, 0.798, 1.596 mg/L	30	0.798, 1.596 mg/L	↑	
Dietary	*Oreochromis niloticus*	0, 0.5,1 mg/kg	70	0.5 mg/kg	↑	Al-Din et al. (2022)
Dietary	*Oreochromis niloticus*	0, 1 mg/kg	65	1 mg/kg	↓	Ghaniem et al. (2022)
Dietary	*Carassius auratus*	0, 0.3, 0.6, 0.9 mg/kg	63	–	x	Jahanbakhshi et al. (2021)
Dietary	*Megalobrama amblycephala*	0.10, 0.42, 0.67, 1.06, 1.46 mg/kg	56	–	x	Jingyuan et al. (2020)
Dietary	*Rutilus caspicus*	0, 1 mg/kg	28	–	x	Zahmatkesh et al. (2020)
Dietary	*Pangasianodon hypophthalmus*	0, 1, 2 mg/kg	60	1, 2 mg/kg	↓	Kumar and Singh (2019)
Dietary	*Oreochromis niloticus*	0, 0.7 mg/kg	63	–	x	Neamat-Allah et al. (2019)
Dietary	*Oncorhynchus mykiss*	0, 1 mg/kg	60	–	x	Nazari et al., 2017
Dietary	*Pelteobagrus fulvidraco*	0, 0.23, 6.5 mg/kg	56	0.23, 6.5 mg/kg	↑↓	Hu et al., (2016)
Waterborne	*Cyprinus carpio*	0, 0.054 mg/L	28	0.054 mg/L	↑	Yeganeh et al. (2016)
Alkaline phosphatase (ALP)						
Dietary	*Hypophthalmichthys molitrix*	0, 0.5, 1 mg/kg	96	0.5, 1 mg/kg	↓	Mushtaq et al. (2022a)
Dietary	*Oreochromis niloticus*	0, 1, 3, 5 mg/kg	56	–	x	Wangkahart et al. (2022)
Dietary	*Carassiusa uratus*	0, 0.3, 0.6, 0.9 mg/kg	63	0.3, 0.6, 0.9 mg/kg	↑	Jahanbakhshi et al. (2021)
Dietary	*Acanthopagrus latus*	0, 0.5, 1, 2 mg/kg	56	0.5 mg/kg	↓	Ziaei-Nejad et al. (2021)

(*continued on next page*)

Table 4 (*continued*)

Exposure route	Fish species	Exposure concentration	Exposure periods (days)	Response concentration	Response	Reference
Dietary	*Megalobrama amblycephala*	0.10, 0.42, 0.67, 1.06, 1.46 mg/kg	56	–	x	Jingyuan et al. (2020)
Dietary	*Rutilus caspicus*	0, 1 mg/kg	28	–	x	Zahmatkesh et al. (2020)
Dietary	*Danio rerio*	1.63, 3, 10 mg Se/kg	28	3, 10 mg Se/kg	↑	Bai et al., 2019b
Dietary	*Carassius auratus*	0, 5, 10, 20 mg/kg	30	5, 10, 20 mg/kg	↑	Bai et al., 2019b
Dietary	*Oreochromis niloticus*	0, 0.7 mg/kg	63	0.7 mg/kg	↑	Neamat-Allah et al. (2019)
Dietary	*Cyprinus carpio*	0, 0.7 mg/kg	56	0.7 mg/kg	↓	Saffari et al. (2017)
Waterborne	*Cyprinus carpio*	0, 0.054 mg/L	28	0.054 mg/L	↑	Yeganeh et al. (2016)
Dietary	*Cyprinus carpio*	0, 0.5, 1, 2 mg/kg	56	–	x	Ashouri et al. (2015)
Waterborne	*Carassius auratus*	0, 2, 3, 4 mg/L	5	3, 4 mg/L	↑	Choi et al. (2015)
Aspartate transaminase (AST)						
Dietary	*Oreochromis niloticus*	0, 0.5,1 mg/kg	70	0.5,1 mg/kg	↑	Al-Din et al. (2022)
Dietary	*Hypophthalmichthys molitrix*	0, 0.5, 1 mg/kg	96	0.5, 1 mg/kg	↓	Mushtaq et al. (2022a)
Dietary	*Oreochromis niloticus*	0, 1 mg/kg	65	1 mg/kg	↑	Ghaniem et al. (2022)
Dietary	*Oreochromis niloticus*	0, 1, 3, 5 mg/kg	56	1 mg/kg	↓	Wangkahart et al. (2022)
Dietary	*Dicentrarchus labrax*	0, 0.25, 0.5, 1 mg/kg	90	–	x	Abd El-Kader et al. (2021)
Dietary	*Oreochromis niloticus*	0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/kg	56	0.2, 0.3, 0.4, 0.5, 0.6 mg/kg	↓	Naiel et al. (2021)
Dietary	*Carassius auratus*	0, 0.3, 0.6, 0.9 mg/kg	63	–	x	Jahanbakhshi et al. (2021)
Dietary	*Acanthopagrus latus*	0, 0.5, 1, 2 mg/kg	56	–	x	Ziaei-Nejad et al. (2021)
Dietary	*Megalobrama amblycephala*	0.10, 0.42, 0.67, 1.06, 1.46 mg/kg	56	–	x	Jingyuan et al. (2020)
Dietary	*Rutilus caspicus*	0, 1 mg/kg	28	–	x	Zahmatkesh et al. (2020)
Dietary	*Carassius auratus*	0, 5, 10, 20 mg/kg	30	10, 20 mg/kg	↑	Bai et al., 2019b
Dietary	*Oreochromis niloticus*	0, 0.7 mg/kg	63	0.7 mg/kg	↑	Neamat-Allah et al. (2019)
Dietary	*Cyprinus carpio*	0, 0.7 mg/kg	56	0.7 mg/kg	↓	Saffari et al. (2017)
Dietary	*Argyrosomus regius*	0, 1, 2, 3 mg/kg	63	1, 2, 3 mg/kg	↑	Mansour et al. (2017)
Waterborne	*Cyprinus carpio*	0, 0.054 mg/L	28	–	x	Yeganeh et al. (2016)
Dietary	*Pelteobagrus fulvidraco*	0, 0.23, 6.5 mg/kg	56	–	x	Hu et al., (2016)
Dietary	*Cyprinus carpio*	0, 0.5, 1, 2 mg/kg	56	1, 2 mg/kg	↑	Ashouri et al. (2015)
Waterborne	*Carassius auratus*	0, 2, 3, 4 mg/L	5	3, 4 mg/L	↑	Choi et al. (2015)
Alanine transaminase (ALT)						
Dietary	*Oreochromis niloticus*	0, 0.5,1 mg/kg	70	0.5,1 mg/kg	↑	Al-Din et al. (2022)
Dietary	*Oreochromis niloticus*	0, 1 mg/kg	65	–	x	Ghaniem et al. (2022)
Dietary	*Hypophthalmichthys molitrix*	0, 0.5, 1 mg/kg	96	0.5, 1 mg/kg	↓	Mushtaq et al. (2022a)
Dietary	*Oreochromis niloticus*	0, 1, 3, 5 mg/kg	56	3 mg/kg	↓	Wangkahart et al. (2022)
Dietary	*Dicentrarchus labrax*	0, 0.25, 0.5, 1 mg/kg	90	–	x	Abd El-Kader et al. (2021)
Dietary	*Carassius auratus*	0, 0.3, 0.6, 0.9 mg/kg	63	–	x	Jahanbakhshi et al. (2021)
Dietary	*Oreochromis niloticus*	0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/kg	56	0.2, 0.3, 0.4, 0.5, 0.6 mg/kg	↓	Naiel et al. (2021)
Dietary	*Acanthopagrus latus*	0, 0.5, 1, 2 mg/kg	56	0.5, 2 mg/kg	↓ ↑	Ziaei-Nejad et al. (2021)
Dietary	*Megalobrama amblycephala*	0.10, 0.42, 0.67, 1.06, 1.46 mg/kg	56	–	x	Jingyuan et al. (2020)
Dietary	*Rutilus caspicus*	0, 1 mg/kg	28	–	x	Zahmatkesh et al. (2020)
Dietary	*Carassius auratus*	0, 5, 10, 20 mg/kg	30	10, 20 mg/kg	↑	Bai et al., 2019b
Dietary	*Oreochromis niloticus*	0, 0.7 mg/kg	63	0.7 mg/kg	↑	Neamat-Allah et al. (2019)
Dietary	*Argyrosomus regius*	0, 1, 2, 3 mg/kg	63	1, 2, 3 mg/kg	↑	Mansour et al. (2017)
Dietary	*Cyprinus carpio*	0, 0.7 mg/kg	56	0.7 mg/kg	↓	Saffari et al. (2017)
Dietary	*Pelteobagrus fulvidraco*	0, 0.23, 6.5 mg/kg	56	–	x	Hu et al., (2016)
Waterborne	*Cyprinus carpio*	0, 0.054 mg/L	28	0.054 mg/L	↑	Yeganeh et al. (2016)
Dietary	*Cyprinus carpio*	0, 0.5, 1, 2 mg/kg	56	2 mg/kg	↑	Ashouri et al. (2015)
Hematocrit (Hct)						
Waterborne	*Channa punctata*	0, 1.5, 3 mg/L	30	1.5, 3 mg/L	↓	Dhara et al. (2022)
	Ctenopharyngodon idella	0, 0.798, 1.596 mg/L	30	0.798, 1.596 mg/L	↓	
Dietary	*Oreochromis niloticus*	0, 1 mg/kg	65	1 mg/kg	↑	Ghaniem et al. (2022)
Dietary	*Oreochromis niloticus*	0, 0.5,1 mg/kg	70	–	x	Al-Din et al. (2022)
Dietary	*Oreochromis niloticus*	0, 1, 3, 5 mg/kg	56	–	x	Wangkahart et al. (2022)
Dietary	*Hypophthalmichthys molitrix*	0, 0.5, 1 mg/kg	96	0.5, 1 mg/kg	↑	Mushtaq et al. (2022a)
Dietary	*Labeo rohita*	0, 0.5 mg/kg	60	0.5 mg/kg	↑	Pavithra et al., (2021)
Dietary	*Rutilus caspicus*	0, 1 mg/kg	28	1 mg/kg	↑	Zahmatkesh et al. (2020)
Dietary	*Pagrus major*	0, 0.5, 1, 2 mg/kg	45	1, 2 mg/kg	↑	Dawood et al. (2019)
Dietary	*Oreochromis niloticus*	0, 0.7 mg/kg	63	–	x	Neamat-Allah et al. (2019)
Dietary	*Piaractus mesopotamicus*	0.72,0.94,1.15, 1.57, 2.51 mg/kg	65	1.15, 1.57 mg/kg	↑	Takahashi et al. (2017)
Dietary	*Tor putitora*	0, 0.68 mg/kg	70	0.68 mg/kg	↑	Khan et al. (2016)
Dietary	*Lates calcarifer*	2, 3, 4, 5, 7 mg/kg	60	5, 7 mg/kg	↓	Ilham et al. (2016)
Waterborne	*Cyprinus carpio*	0, 0.054 mg/L	28	0.054 mg/L	↓	Yeganeh et al. (2016)
Cholesterol						

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Table 4 (*continued*)

Exposure route	Fish species	Exposure concentration	Exposure periods (days)	Response concentration	Response	Reference
Waterborne	*Channa punctata*	0, 1.5, 3 mg/L	30	1.5, 3 mg/L	↑	Dhara et al. (2022)
	Ctenopharyngodon idella	0, 0.798, 1.596 mg/L	30	0.798, 1.596 mg/L	↑	
Dietary	*Oreochromis niloticus*	0, 1 mg/kg	65	1 mg/kg	↓	Ghaniem et al. (2022)
Dietary	*Oreochromis niloticus*	0, 1, 3, 5 mg/kg	56	3 mg/kg	↓	Wangkahart et al. (2022)
Dietary	*Acanthopagrus latus*	0, 0.5, 1, 2 mg/kg	56	0.5 mg/kg	↓	Ziaei-Nejad et al. (2021)
Dietary	*Megalobrama amblycephala*	0.10, 0.42, 0.67, 1.06, 1.46 mg/kg	56	1.06, 1.46 mg/kg	↓	Jingyuan et al. (2020)
Dietary	*Rutilus caspicus*	0, 1 mg/kg	28	–	x	Zahmatkesh et al. (2020)
Dietary	*Oreochromis niloticus*	0.53,0.86,1.04,1.22 mg/kg	42	–	x	Durigon et al. (2019)
Dietary	*Argyrosomus regius*	0, 1, 2, 3 mg/kg	63	2, 3 mg/kg	↓	Khalil et al. (2019)
Dietary	*Oncorhynchus mykiss*	0, 1 mg/kg	60	–	x	Nazari et al., 2017
Dietary	*Argyrosomus regius*	0, 1, 2, 3 mg/kg	63	3 mg/kg	↑	Mansour et al. (2017)
Dietary	*Pelteobagrus fulvidraco*	0, 0.23, 6.5 mg/kg	56	–	x	Hu et al., (2016)
Dietary	*Cyprinus carpio*	0, 0.5, 1, 2 mg/kg	56	2 mg/kg	↓	Ashouri et al. (2015)
Albumin						
Waterborne	*Channa punctata*	0, 1.5, 3 mg/L	30	1.5, 3 mg/L	↑	Dhara et al. (2022)
	Ctenopharyngodon idella	0, 0.798, 1.596 mg/L	30	0.798, 1.596 mg/L	↑	
Dietary	*Oreochromis niloticus*	0, 0.5,1 mg/kg	70	–	x	Al-Din et al. (2022)
Dietary	*Oreochromis niloticus*	0, 1, 3, 5 mg/kg	56	–	x	Wangkahart et al. (2022)
Dietary	*Dicentrarchus labrax*	0, 0.25, 0.5, 1 mg/kg	90	–	x	Abd El-Kader et al. (2021)
Dietary	*Oreochromis niloticus*	0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/kg	56	0.2, 0.3, 0.4, 0.5, 0.6 mg/kg	↑	Naiel et al. (2021)
Dietary	*Acanthopagrus latus*	0, 0.5, 1, 2 mg/kg	56	1, 2 mg/kg	↓↑	Ziaei-Nejad et al. (2021)
Dietary	*Megalobrama amblycephala*	0.10, 0.42, 0.67, 1.06, 1.46 mg/kg	56	1.06 mg/kg	↑	Jingyuan et al. (2020)
Dietary	*Rutilus caspicus*	0, 1 mg/kg	28	–	x	Zahmatkesh et al. (2020)
Dietary	*Pangasianodon hypophthalmus*	0, 1, 2 mg/kg	60	1, 2 mg/kg	↑	Kumar and Singh (2019)
Dietary	*Oreochromis niloticus*	0, 0.7 mg/kg	63	–	x	Neamat-Allah et al. (2019)
Dietary	*Oncorhynchus mykiss*	0, 1 mg/kg	60	–	x	Nazari et al., 2017
Dietary	*Argyrosomus regius*	0, 1, 2, 3 mg/kg	63	2, 3 mg/kg	↑	Mansour et al. (2017)
Dietary	*Pelteobagrus fulvidraco*	0, 0.23, 6.5 mg/kg	56	–	x	Hu et al., (2016)
Dietary	*Cyprinus carpio*	0, 0.5, 1, 2 mg/kg	56	2 mg/kg	↓	Ashouri et al. (2015)
Globulin						
Dietary	*Oreochromis niloticus*	0, 0.5,1 mg/kg	70	–	x	Al-Din et al. (2022)
Waterborne	*Channa punctata*	0, 1.5, 3 mg/L	30	1.5, 3 mg/L	↓	Dhara et al. (2022)
	Ctenopharyngodon idella	0, 0.798, 1.596 mg/L	30	0.798, 1.596 mg/L	↓	
Dietary	*Oreochromis niloticus*	0, 1, 3, 5 mg/kg	56	–	x	Wangkahart et al. (2022)
Dietary	*Dicentrarchus labrax*	0, 0.25, 0.5, 1 mg/kg	90	0.25, 0.5, 1 mg/kg	↑	Abd El-Kader et al. (2021)
Dietary	*Oreochromis niloticus*	0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/kg	56	0.2, 0.3, 0.4, 0.5, 0.6 mg/kg	↑	Naiel et al. (2021)
Dietary	*Acanthopagrus latus*	0, 0.5, 1, 2 mg/kg	56	–	x	Ziaei-Nejad et al. (2021)
Dietary	*Rutilus caspicus*	0, 1 mg/kg	28	–	x	Zahmatkesh et al. (2020)
Dietary	*Pangasianodon hypophthalmus*	0, 1, 2 mg/kg	60	1, 2 mg/kg	↑	Kumar and Singh (2019)
Dietary	*Oreochromis niloticus*	0, 0.7 mg/kg	63	0.7 mg/kg	↑	Neamat-Allah et al. (2019)
Dietary	*Oncorhynchus mykiss*	0, 1 mg/kg	60	1 mg/kg	↓	Nazari et al., 2017
Dietary	*Argyrosomus regius*	0, 1, 2, 3 mg/kg	63	2, 3 mg/kg	↑	Mansour et al. (2017)
Dietary	*Pelteobagrus fulvidraco*	0, 0.23, 6.5 mg/kg	56	–	x	Hu et al., (2016)
Dietary	*Cyprinus carpio*	0, 0.5, 1, 2 mg/kg	56	2 mg/kg	↑	Ashouri et al. (2015)
Total protein						
Dietary	*Oreochromis niloticus*	0, 0.5,1 mg/kg	70	–	x	Al-Din et al. (2022)
Waterborne	*Channa punctata*	0, 1.5, 3 mg/L	30	1.5, 3 mg/L	↓	Dhara et al. (2022)
	Ctenopharyngodon idella	0, 0.798, 1.596 mg/L	30	0.798, 1.596 mg/L	↓	
Dietary	*Oreochromis niloticus*	0, 1 mg/kg	65	1 mg/kg	↑	Ghaniem et al. (2022)
Dietary	*Oreochromis niloticus*	0, 1, 3, 5 mg/kg	56	–	x	Wangkahart et al. (2022)
Dietary	*Carassius auratus*	0, 0.3, 0.6, 0.9 mg/kg	63	–	x	Jahanbakhshi et al. (2021)
Dietary	*Oreochromis niloticus*	0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/kg	56	0.2, 0.3, 0.4, 0.5, 0.6 mg/kg	↑	Naiel et al. (2021)
Dietary	*Acanthopagrus latus*	0, 0.5, 1, 2 mg/kg	56	1, 2 mg/kg	↑	Ziaei-Nejad et al. (2021)
Dietary	*Megalobrama amblycephala*	0.10, 0.42, 0.67, 1.06, 1.46 mg/kg	56	0.67, 1.06 mg/kg	↑	Jingyuan et al. (2020)
Dietary	*Rutilus caspicus*	0, 1 mg/kg	28	–	x	Zahmatkesh et al. (2020)
Dietary	*Pangasianodon hypophthalmus*	0, 1, 2 mg/kg	60	1, 2 mg/kg	↑	Kumar and Singh (2019)
Dietary	*Oreochromis niloticus*	0, 0.7 mg/kg	63	0.7 mg/kg	↑	Neamat-Allah et al. (2019)
Dietary	*Oncorhynchus mykiss*	0, 1 mg/kg	60	–	x	Nazari et al., 2017
Dietary	*Argyrosomus regius*	0, 1, 2, 3 mg/kg	63	2, 3 mg/kg	↑	Mansour et al. (2017)
Dietary	*Tor putitora*	0, 0.68 mg/kg	70	0.68 mg/kg	↑	Khan et al. (2016)

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Table 4 (*continued*)

Exposure route	Fish species	Exposure concentration	Exposure periods (days)	Response concentration	Response	Reference
Waterborne	*Cyprinus carpio*	0, 0.054 mg/L	28	0.054 mg/L	↓	Yeganeh et al. (2016)
Dietary	*Cyprinus carpio*	0, 0.5, 1, 2 mg/kg	56	2 mg/kg	↑	Ashouri et al. (2015)
Triglyceride (TG)						
Waterborne	*Channa punctata*	0, 1.5, 3 mg/L	30	1.5, 3 mg/L	↑	Dhara et al. (2022)
	Ctenopharyngodon Idella	0, 0.798, 1.596 mg/L	30	0.798, 1.596 mg/L	↑	
Dietary	*Oreochromis niloticus*	0, 1 mg/kg	65	–	x	Ghaniem et al. (2022)
Dietary	*Carassius auratus*	0, 0.3, 0.6, 0.9 mg/kg	63	–	x	Jahanbakhshi et al. (2021)
Dietary	*Acanthopagrus latus*	0, 0.5, 1, 2 mg/kg	56	–	x	Ziaei-Nejad et al. (2021)
Dietary	*Megalobrama amblycephala*	0.10, 0.42, 0.67, 1.06, 1.46 mg/kg	56	0.67 mg/kg	↓	Jingyuan et al. (2020)
Dietary	*Rutilus caspicus*	0, 1 mg/kg	28	–	x	Zahmatkesh et al. (2020)
Dietary	*Pagrus major*	0, 0.5, 1, 2 mg/kg	45	1, 2 mg/kg	↓	Dawood et al. (2019)
Dietary	*Oreochromis niloticus*	0.53,0.86,1.04,1.22 mg/kg.	42	–	x	Durigon et al. (2019)
Dietary	*Argyrosomus regius*	0, 1, 2, 3 mg/kg	63	2, 3 mg/kg	↓	Khalil et al. (2019)
Dietary	*Oncorhynchus mykiss*	0, 1 mg/kg	60	1 mg/kg	↑	Nazari et al., 2017
Dietary	*Cyprinus carpio*	0, 0.5, 1, 2 mg/kg	56	–	x	Ashouri et al. (2015)

↑ = increased; ↓ = decreased; x = no changed.

(HPGL) systems in zebrafish, resulting reduction of the percent early vitellogenic oocyte and spermatid in female and male individuals respectively. Berntssen et al. (2018) noted that Se consumption exceeding 5 μg/g diet (dw) in fishes increases the potential for physiological toxicities and reduces overall growth and reproductive fitness. Naiel et al. (2023) reported that treatment with diet containing 1 mg/kg selenium-nanoparticle (SeNp) for 6 months resulted in increased eggs production and frequency of spawning in red tilapia (*Oreochromis* sp.). In contrast, male catfish *Sorubim cuspicaudus* fed with 2.57 mg/kg Se-Met diet for a period of 14 weeks showed no significant difference in length and weight gain (Hoya-Flórez et al., 2024). Se is a crucial dietary component that significantly impacts the growth and reproduction in fishes, thus understanding the dietary requirements of Se in terms of essentiality and toxicity is very important for sustainable aquaculture practices and maintaining the sustainability of aquatic ecosystems (Sumana et al., 2023).

7. Developmental effects of selenium

The early developmental stages of fishes, particularly embryos and larvae, are notably susceptible to pollutants such as heavy metals and metalloids. Consequently, they are widely recognized as significant bio-indicators for evaluating the toxicity of these substances on aquatic organisms (Rahman et al., 2020; Jezierska et al., 2009). Se is an essential trace element and a well-documented teratogen at supra-nutritional levels in oviparous vertebrates, including fishes (Massé et al., 2015; Thomas & Janz, 2014, 2015). Among various endpoints, teratogenicity is considered a crucial indicator of Se toxicity in fishes (Witeska et al., 2014).

Selenium induced developmental toxicity primarily occurs in fishes due to the maternal deposition of Se from the liver into eggs during vitellogenesis, followed by the metabolism of Se from egg albumen or yolk by the embryo and fish larvae. Maternal exposure to Se-Met, the predominant dietary form of Se, results in developmental toxicity in fishes, manifesting as spinal curvatures (lordosis, kyphosis, scoliosis), craniofacial malformations, incidences of edema (yolk sac, pericardial), in developing larvae (Cheng et al., 2022; Mo et al., 2020, 2021; Penglase et al., 2014; Thomas and Janz, 2014). The deposition of protein bound Se-Met in embryos through maternal transfer is subsequently utilized in energy production and/or protein synthesis in the larvae. The gradual release of Se-Met during protein catabolism could potentially delay the onset and progression of Se toxicity in early life stages, resulting in developmental deformities rather than immediate mortality (Thomas and Janz, 2016). Additionally, Se-Met catabolism has been associated with oxidative stress in developing embryos, potentially impacting embryo hatchability.

Waterborne exposure to Se-Met induces malformations including the absence of fins, pericardial edema, spinal deformities, and defects in cardiovascular and ocular development (Zhao et al., 2022; Janz, 2012). However, several field, and laboratory-based studies have reported that maternal Se transfer results in increased mortality and/or deformities in F1 generation fishes (Thomas and Janz, 2015; Janz et al., 2010). Uddin et al. (2023; unpublished data) found embryonic and larval deformities in zebrafish following waterborne exposure to Se-Met. Additionally, high mortality and/or deformities were observed after microinjection of excess Se-Met in pallid sturgeon (*Scaphirhynchus albus*), shovelnose sturgeon (*Scaphirhynchus platorynchus*), and white sturgeon (*Acipenser transmontanus*) (Papoulias et al., 2014; Lin et al., 2021). Mo et al. (2021) found that exposing female zebrafish to selenite resulted in adverse developmental effects on their offspring. On the contrary, zebrafish embryos exposed to waterborne selenite at concentrations of 10–100 μg/L did not show any larval deformities (Uddin et al., 2023; unpublished study). Moreover, waterborne exposure to SeNP in zebrafish embryos at concentrations of 15–25 μg/mL, led to the development of pericardial edema, tail malformation, and decreased heart rate (Kalishwaralal et al., 2016a). It is important to note that larvae are typically more sensitive to Se than embryos since embryos possess protective hard chorion layers and perivitelline fluid that can impede Se entry (Kong et al., 2013; Mhadhbi et al., 2010). Table 5 illustrates Se-induced developmental toxicity in fishes. These developmental deformities in fishes hold ecotoxicological relevance due to their potential to directly impair swimming, feeding, and reproductive capacities, ultimately contributing to the reduction in population size and diversity over time (Lemly, 2002).

8. Neurobehavioural effects of selenium

Fish behaviours serve as a critical indicator in eco-toxicology for monitoring water pollution (Brodin et al., 2013). Previous research has demonstrated that various environmental toxicants can significantly alter behaviours of fishes, affecting their learning and memory functions, exploration tendencies, predator-prey interactions, swimming abilities, and overall activity levels (Hong and Zha, 2019; Sandoval-Herrera et al., 2019). These behavioural changes are closely linked to growth, reproduction, and population dynamics in fishes (Jacquin et al., 2020). However, it is essential to understand that changes in behaviours at individual levels can have long-term adverse effects on population and community structures, potentially increasing their susceptibility to extinction (Ward et al., 2017, 2020).

Although Se has neuroprotective properties, high concentrations can

Table 5
Effect of selenium on embryonic and larval development of fishes.

Fish species	Exposure concentration	Exposure periods (days)	Response concentration	Phenotypic alterations	Reference
Danio rerio	0, 12.5, 25, 50, 100 μg/L	120	100 μg/L	ROS levels & apoptotic cells increased	Cheng et al. (2023)
Danio rerio	0, 12.5, 25, 50, 100 μg/L	120	100 μg/L	increased mortality, elevated malformation rate, reduced body length	Cheng et al. (2022)
Danio rerio	0, 0.25, 0.5, 1, 2 μM	4	0.5, 1, 2 μM	lower hatching rate, high mortality & deformities	Zhao et al. (2022)
Danio rerio	0.15, 0.2, 0.3, 0.4 mg/L	5	0.2, 0.3, 0.4 mg/L	delayed hatchability with pericardial edema and tail malformation	Vaishnavi et al. (2019)
Pimephales promelas	30, 90, 270, 810, 2430, 7290, 21,870, 65,610 μg/L	6	810, 2430, 7290, 21870, 65610 μg/L	reduced hatchability and survival, increased severity of deformities	Gerhart et al. (2019)
Oryzias latipes	0, 10, 20 μg/g	7	20 μg/g	pericardial edema and craniofacial changes	Shi et al. (2018)
Danio rerio	0, 8, 16, 32 μg/g	6	16, 32 μg/g	increased deformities, mortality, decreased hatchability	Thomas and Janz (2016)
Danio rerio	0, 10 μg/g	57	10 μg/g	reduced fitness, survivability, lower hatching rate	Raine et al. (2016)
Oryzias latipes	0, 0.5, 5, 50 μM	1	5, 50 Mm	significantly reduced larval survival and hatching rate	Kupsco and Schlenk (2016)
Oryzias latipes	0, 12.5, 25, 50 μg/g	6	12.5, 25, 50 μg/g	lower hatching success with high mortality	Chernick et al. (2016)
Danio rerio	0, 5, 25 μg/mL	1	5, 25 μg/mL	increased mortality, pericardial edema, cardiac arrhythmia	Kalishwaralal et al. (2016b)
Oncorhynchus mykiss	0, 0·5, 1·2 mg/kg	72	–	no significant difference in hatching and mortality	Dicharry et al., (2015)
Danio rerio	0, 3.4, 9.8, 27.5 μg/g	90	9.8, 27.5 μg/g	increased mortality & deformities	Thomas and Janz (2015)

cause neuronal damage and neurotoxicity (Naderi et al., 2017a). The primary mechanism of Se-induced neurotoxicity is its ability to increase ROS production, leading to oxidative stress, which can adversely affect the brain and central nervous system (CNS) (Ellwanger et al., 2016). Given the brain's high oxygen consumption and lipid-rich composition, it is particularly vulnerable to oxidative stress, which can disrupt various neural signaling pathways and thereby alter fish behaviours (Salim, 2017).

Neurotoxicity of Se can manifest through alterations in monoamine neurotransmitter systems, such as dopamine and serotonin, which are crucial for regulating social behaviour, anxiety, stress responses, and learning and memory functions in vertebrates (Naderi et al., 2017a; Vinceti et al., 2014). For example, adult zebrafish (*Danio rerio*) exposed to environmentally relevant concentrations of Se-Met (3.6–34.1 μg/g dw) for 90 days exhibited dysregulations in serotonergic systems in the brain, which was associated with impairments in social learning, reduced group preference, and increased anxiety-like behaviours, mainly in the highest treatment group (Attaran et al., 2020). Interesting, offspring of female zebrafish treated with dietary Se (34.1 μg/g dw) also demonstrated similar behavioural impairments and disruption of serotonergic signaling in the brain (Attaran et al., 2021). In addition, Attaran et al. (2019) reported that chronic dietary exposure to Se-Met at a concentration of 31.5 μg/g dw for 60 days resulted in disruption of serotonergic neurotransmission, and impaired antipredator and social behaviours in zebrafish. Furthermore, adult zebrafish chronically exposed to different concentrations of dietary Se (0, 3.5, 11.1, 27.4, 63.4 μg/g) showed impaired latent learning performance and associative learning behaviour, which were likely mediated by the alterations in the dopaminergic neurotransmission in the brain (Naderi et al., 2017, 2018b). Interestingly, chronic maternal (60 days) exposure to dietary Se was also reported to alter the latent learning performance in adult zebrafish offspring likely via the dysregulation of the dopaminergic system (Naderi et al., 2018a). These studies indicate that environmentally relevant exposure to dietary Se can adversely affect the cognitive and social behaviours in fishes and these effects can be transmitted intergenerationally affecting the next generation even without direct exposure to elevated Se. It is important to note though that behavioural effects of Se can be dependent of species tested and exposure dose. For example, fathead minnows (*Pimephales promelas*) fed with diets containing Se (2.9–6.8 μg/g wet weight) for 70 days did not show any significant alterations in their escape responses - a routine behaviour critical to predator-prey interactions (Anderson et al., 2019).

Direct waterborne exposure to elevated Se can also cause adverse neurobeahavioural effects in embryonic and larval fish. For example, Uddin et al. (2023; unpublished data) found that zebrafish embryos at 1-h post-fertilization (hpf) exposed to Se-Met at concentrations of 5 μg/L and 10 μg/L exhibited impaired light-dark preference, following dysregulation of key genes in the dopaminergic and serotonergic pathways. Additionally, 1 hpf zebrafish embryos exposed to selenite at environmentally relevant concentrations (0, 10, 50, 100 μg/L) for 30 days showed reflexive movement impairment at 5 days post-fertilization (dpf), thigmotactic disruption at 15 dpf, social preference interruption at 21 dpf, and reduced novel object recognition ratio at 30 dpf (Uddin et al., 2023; unpublished data). Social preference behaviour is crucial for obtaining updated information about the habitat, which directly relates to fish fitness, as it plays a vital role in foraging, mating, territorial defense, and predator avoidance (Hoppitt and Laland, 2013). Scototaxis and thigmotaxis are well established index of anxiety like behaviours in fishes (Maximino et al., 2010). Various behavioural effects in fishes exposed to elevated Se are summarized in Table 6.

Brain acetylcholinesterase (AChE) activity serves as a notable biomarker of neurotoxicity, essential for the deactivation of acetylcholine at nerve endings and the proper functioning of sensory and neuromuscular systems (Song et al., 2006), thus playing a pivotal role in locomotion (Drever et al., 2011). Locomotor activity, a fundamental behaviour, is integral to various fitness-related functions such as feeding, social interactions, reproduction, and responses to predation threats. Consequently, alterations in locomotor activity induced by toxicants can negatively impact fitness (Salahinejad et al., 2023). Moreover, fishes with impaired locomotor activity may struggle to effectively respond to challenges in aquaculture environments (José et al., 2007). Hariharan et al. (2024) observed that zebrafish larvae exposed to SeNp at concentrations ranging from 0 to 0.6 μg/mL and selenite at concentrations from 0 to 10 μg/mL for 6 days exhibited reduced locomotor activity and increased anxiety levels, although these concentrations are not environmentally relevant. Thomas and Janz (2011) reported a significant reduction in critical swimming speed in adult zebrafish following a 60-day dietary exposure to environmentally relevant concentrations of Se (3.7, 9.6, and 26.6 μg/g dw). Li et al. (2021) found that two-month-old zebrafish exposed to environmentally relevant concentrations of Se exhibited disruptions in dopamine, serotonin, and acetylcholine signaling pathways, accompanied by altered

Table 6
Summary of studies on effects of selenium on various behaviours of fishes.

Selenium species	Fish species	Developmental stages	Exposure concentration	Exposure period (days)	Environmentally relevant study	Behaviour affected	Effect direction	Reference
Selenite	*Danio rerio*	larvae	0, 0.2, 0.4, 0.6 μg/mL	6	no	Light preference locomotion Anxiety	↓ ↓ ↑	Hariharan et al. (2024)
SeNp	*Danio rerio*	larvae	0, 1, 5, 10 μg/mL	6	no	Light preference locomotion Anxiety	↓ ↓ ↑	Hariharan et al. (2024)
SeMet	*Danio rerio*	adult	0, 3.6, 12.8, 34.1 μg/g	90	yes	Anxiety Group preference Social learning preference	↑ ↓ ↓	Attaran et al. (2021)
SeMet	*Danio rerio*	adult	0, 3.6, 12.8, 34.1 μg/g	90	yes	Social learning preference	↓	Attaran et al. (2020)
Ph_2Se_2	*Ctenopharyngodon*	juvenile	0, 3 mg/kg	30	no	locomotion	x	Baldissera et al. (2020)
Ph_2Se_2	*Danio rerio*	adult	0, 0.1, 0.25, 0.5, 1, 2 Mm	30	no	locomotion thigmotaxis	x ↓	Ferreira et al. (2019)
SeMet	*Danio rerio*	adult	2.1, 11.6, 31.5 μg/g	60	yes	Fear response Group preference	↓ ↓	Attaran et al. (2019)
SeMet	*Danio rerio*	adult	0, 3.5, 11.1, 27.4 μg/g	60	yes	Latent learning performance	↓	Naderi et al. (2018a)
SeMet	*Danio rerio*	adult	0, 2.3, 9.7, 32.5, 57.7 μg/g	30	no	Latent learning performance	↓	Nazari et al., 2017
SeMet	*Danio rerio*	adult	0, 3.5, 11.1, 27.4, 63.4 μg/g	60	no	Associated learning performance	↓	Naderi et al. (2018b)

SeMet: Selenomethionine; SeNp: Selenium-nano particle; Ph_2Se_2: Diphenyl diselenide; ↑ = increased; ↓ = decreased; x = no changed.

locomotor activity and novel area preference. Similarly, 20-day post-hatch fathead minnows exposed to various concentrations of Se in the form of Se-Met in their diet for 60 days exhibited reduced swimming speeds (McPhee and Janz, 2014). Additionally, zebrafish (*Danio rerio*) embryos exposed to a 0.5 μM concentration of Se from 1 hpf to 96 hpf exhibited decreased swimming speed and distance, as well as a diminished touch response (Zhao et al., 2022). Adult zebrafish showed alterations in their repeated swimming performance upon exposure to different concentrations of Se (1.3, 3.4, 9.8 or 27.5 μg/g dw) through diet for 90 days (Thomas et al., 2013). Notably, a significant reduction in AChE activity is commonly observed in fishes exposed to Se (Modesto and Marinez, 2010), and the accumulation of acetylcholine resulting from AChE inhibition may influence fleeing and reproductive behaviour in fishes (Bretaud et al., 2000).

9. Conclusions and future directions

Selenium is an essential micronutrient to aquatic organisms but poses significant risks to aquatic ecosystems when present above threshold levels. This review examined the diverse detrimental effects of Se in fishes, including metabolic effects, reproductive failures and teratogenicity, and neurobehavioural impairments. Bioaccumulation of Se emerges as a pivotal indicator for monitoring its geochemical cycling within aquatic environments. Regardless of exposure routes, be it through diet or waterborne sources, fishes exhibit the highest Se accumulation in their kidney, liver, and gonads. Maternal transfer of Se from the liver to eggs during vitellogenesis induces developmental toxicity, leading to spinal curvatures, craniofacial malformations, and incidences of edema in the offspring. Moreover, Se disrupts different metabolic markers of fishes, impacting oxygen carrying capacity and overall immunity. Oxidative stress ensues from Se accumulation, attributed to the generation of ROS. To counteract oxidative damage, fishes activate antioxidant responses involving SOD, CAT, GST, GPX. Additionally, Se alters behaviours by perturbing neurotransmitter systems, including dopamine and serotonin, and inhibiting AChE, leading to behavioural and cognitive disorders. In summary, Se exposure in fishes results in a spectrum of toxicities encompassing bioaccumulation, reproductive toxicity, teratogenicity, alterations of metabolic and oxidative stress markers, and neuro-behavioural toxicity, underscoring the importance of understanding its impacts on aquatic ecosystems.

Despite significant advancements in understanding Se toxicity in fishes, critical gaps remain in the existing literature. Many studies use Se exposure concentrations that are not environmentally relevant, failing to reflect real-world scenarios in aquatic environments. Additionally, research often relies on model organisms to establish toxicity thresholds, which may not apply to other species due to the species-specific nature of the essentiality and toxicity of Se. The uptake and depuration of Se in fishes are highly species-specific and depend on their diet and feeding habits. Therefore, a single toxicity threshold cannot sufficiently protect all fish species. Moreover, the essentiality and toxicity thresholds vary among freshwater, brackish water, and marine fishes. Furthermore, most studies focus on early developmental stages and reproductive toxicity, neglecting the fact that nutritional requirements and sensitivity to Se can vary significantly across different developmental stages of fishes. Additionally, the speciation of Se needs to be accounted for in toxicity thresholds for aquatic life, as organic Se is significantly more toxic than inorganic Se. In most studies, the no observed effect concentration (NOEC) and low observed effect concentration (LOEC) of Se have been determined based solely on developmental endpoints in fishes, often neglecting behavioural endpoints. Recent research, however, indicates that even lower concentrations of Se can significantly alter behavioural endpoints without affecting developmental endpoints. Moreover, dietary exposure studies typically involve spiking commercial diets with Se, which does not accurately represent natural environmental conditions. The USEPA has established chronic Se water quality criteria values for the protection of aquatic organisms, which are 1.5 μg/L for lentic and 3.1 μg/L for lotic water environment In contrast, the CCME has set a more stringent guideline for the protection of aquatic life, with a threshold of 1 μg Se/L for freshwater, while no specific guideline exists for saltwater. However, due to anthropogenic activities, Se concentrations in the environment frequently exceed these established guidelines, posing a significant threat to aquatic ecosystems.

To address these limitations, we propose the following future research directions-future studies should reflect realistic environmental conditions to better understand Se-induced toxicity in fishes; research should determine essential and toxicity thresholds for fishes on a species-specific basis, independent of their taxonomic relationships; conduct studies across different life stages of fishes, including larvae, fry,

juveniles, and adults, to identify potential Se toxicity thresholds; design experiments that better mimic natural conditions by exposing different prey organisms, such as black worms, to Se and subsequently feeding these to fishes; develop tissue-based toxicity thresholds of Se for fishes to safeguard the health and well-being of aquatic organisms. Behavioural end points should be taken into consideration during revising national water quality guidelines. All environmental biologist and toxicologist should move together to enhance the ecological relevance and accuracy of future research on Se toxicity in fishes, ultimately contributing to the conservation and management of aquatic ecosystems.

CRediT authorship contribution statement

Md Helal Uddin: Writing – original draft, Resources, Investigation, Data curation, Conceptualization. **Jinnath Rehana Ritu:** Writing – review & editing, Conceptualization. **Sravan Kumar Putnala:** Writing – review & editing. **Mahesh Rachamalla:** Writing – review & editing. **Douglas P. Chivers:** Writing – review & editing, Supervision. **Som Niyogi:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: MD HELAL UDDIN reports was provided by University of Saskatchewan. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

MD HELAL UDDIN was supported by the Dean's Graduate Scholarship of the University of Saskatchewan. This work was supported by the discovery grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) to Douglas P Chivers and Som Niyogi.

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Taslima, 2022

Toxicology Reports 9 (2022) 858–868

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Impacts of heavy metals on early development, growth and reproduction of fish – A review

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ARTICLE INFO

Handling Editor: Lawrence Lash

Keywords:
Aquaculture
Embryos
Larvae
Pollution
Trace elements

ABSTRACT

Heavy metals pollution causes a threat to the aquatic environment and to its inhabitants when their concentrations exceed safe limits. Heavy metals cause toxicity in fish due to their non-biodegradable properties and their long persistence in the environment. This review investigated the effects of heavy metals on early development, growth and reproduction of fish. Fish embryos/larvae and each developmental stage of embryo respond differently to the intoxication and vary from species to species, types of metals and their mode of actions, concentration of heavy metals and their exposure time. Many of the heavy metals are considered as essential nutrient elements that positively improve the growth and feed utilization of fishes but upon crossing the maximum tolerable limit these metals cause not only a hazard to fish health but also to human consumers and the disruption of ecological systems. Reduced gonadosomatic index (GSI), fecundity, hatching rate, fertilization success, abnormal shape of reproductive organs, and finally failure of reproduction in fish have been attributed to heavy metal toxicity. In summary, this review sheds light on the manipulation of fish physiology by heavy metals and seeks to raise sensitivity to the prevention and control of aquatic environmental contamination, particularly from heavy metals.

1. Introduction

Heavy metals pollution is a great concern to aquatic environments because they impart a wide range of toxicities with serious impacts to the aquatic faunal communities [1,2]. Most of the heavy metals accumulated in aquatic water bodies are originate from anthropogenic activities such as agricultural cultivation, erosions of landfills, docking and embarking activities, sewage from industrial and domestic wastewater and some natural processes [1,3]. The uncontrolled population growth, intensive agricultural activities and heavy industrialization result in a wide range of pollutants which eventually inflict serious consequences on aquatic ecosystems as well as associated faunal and floral communities [4–6]. Commonly, trace amount of heavy metals (non-degradable) cause serious difficulties in aquatic systems as a result of their assimilation, deposition and even incorporation at a specific concentration in abiotic substances and ultimately, accumulated into the body of associated aquatic organisms [7]. Heavy metals accumulate into the tissues of aquatic organisms throughout different aquatic food chains where they can be concentrated; bioaccumulated metals can result in substantial human health hazards upon consumption of these contaminated aquatic foods [8]. The rapid growth of industrialization across the cities results in the release of effluents contaminated with toxic metals including chromium (Cr), nickel (Ni), copper (Cu), lead (Pb), iron (Fe), and zinc (Zn). In broad, metals can be classified as biologically essential and nonessential. Metals like aluminum (Al), cadmium (Cd), mercury (Hg), tin (Sn) and lead (Pb) have no records of specific biological functions and therefore their toxicities rise with high concentration. On the other hand, essential metals (Cr, Zn, Ni, Cu, Co, Fe) have established biological functions and toxicities occur in response to either their deficiencies or excessive concentrations. Essential metals positively improved the growth and feed utilization of several species [9–15] but when maximum allowable/tolerable limit these metals are exceeded, they hamper the normal physiological and ecological systems in the aquatic environment [16,17], causing toxicity within the organisms and

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https://doi.org/10.1016/j.toxrep.2022.04.013
Received 9 January 2022; Received in revised form 11 April 2022; Accepted 14 April 2022
Available online 20 April 2022

ultimately causing a substantial threat to human health [1,8]. Most of these heavy metals are highly carcinogenic in nature and in addition they can cause serious health complexities like liver disorders, cardiovascular difficulties, kidney dysfunctions and in extreme cases death. Heavy metal pollution severely disrupts the physiology of several aquatic organisms, especially fish [4,18,19]. Heavy metal contamination greatly changed the hemato-biochemical scenario of fish and also resulted several deformities (cellular and nuclear) in different blood cells [19–21]. Genetic damages as a result of heavy metal toxicities have also been recorded by several studies [18]. Heavy metals contamination significantly hampers the reproductive performances of fish [22–24]. Investigations have reported several reproductive compromises including reduced GSI, fecundity, hatching rate, fertilization success, abnormal shape of reproductive organs, and finally overall reproductive success in response to a variety of heavy metals [25–30]. Moreover, heavy metals severely affected the embryonic and larval development of fish through resulting number of complexities such as increased heart rate, reduced cardiac activity, increased mortality rate, deformed shape, vertebral column deformities etc. in different developmental stages of embryo [11,31–35]. Despite the destructive impacts of several heavy metals on fish physiology and reproductive performance in fishes, few if any generalized or comprehensive patterns of these responses are available. The current review focuses on the aggregation of up-to-date information about the impacts of heavy metals on embryonic and larval development, growth, reproductive performance with an emphasis of the most commercially important aquaculture species.

2. Heavy metals effects on embryonic and larval development of fish

Early developmental stages of fish, specifically embryos and larvae, are more susceptible to pollutants such as heavy metals than juvenile and adult fish are, and are widely used as bio-indicators to determine the toxicity of such chemicals to the aquatic organisms [36,37]. Various endpoints such as developmental malformations (teratogenicity), physiological and biochemical alterations, behavioural and functional deformities are used to assess and predict the toxicity of heavy metals to fish population [35]. Fish embryos/larvae at each developmental stage of embryo (blastula, gastrula, segmentation, hatching etc.) respond differently to the intoxication and vary from species to species, types of metals and their mode of actions, concentration of heavy metals and their exposure time etc. [38,39]. For instance, hatching and embryo survival of African catfish (*Clarias gariepinus*) were unaffected by Cd exposure at a concentration ranging from 0.05-5 mg/L. Another study reported that embryo and larvae survival, hatching of Ide (*Leuciscus idus)* were significantly affected by Cd exposure (100 μg/L; [35,40]. The types of deformities in different fish species due to expose to different heavy metals are summarized in Table 1.

Most of the literature reported reduced embryonic and larval survival, reduced and delayed hatching, stunted growth rate and morphological abnormalities such as skeletal deformities, vascular system abnormalities, reduction in pigmentation, eye anomalies etc. among different fish species exposed to lethal and sub-lethal doses of essential (Cu, Zn) and non-essential (Cd, Cr, Hg and Pb) heavy metals [32,38,40–42]. Cardiovascular endpoints such as hyper or hypo dystrophia, positioning abnormality, incomplete or abnormal heart looping, tubular heart, oedemata, megalocardia etc. are important parameters to assess the toxicity of heavy metals in embryos and larvae, revealing species-dependent differences in the responses to various heavy metals. For example, Cu exposure significantly increased heart rate in zebrafish embryo [31], whereas cardiac activity is reduced in red sea bream [32] and zebrafish [43] embryos exposed to Cd. Larvae are less tolerant to heavy metals than the embryo since embryos have protective hard chorion layers and perivitelline fluid that can impede the entry of heavy metals [44,45]. Catalase (CAT, the enzyme which converts relatively toxic hydrogen peroxide to oxygen activity is significantly reduced in

Table 1
Effect of heavy metals on embryonic and larval development of fish.

Species	Dose	Exposure period	Alterations/ type of deformities	References
Cd				
Odontesthes bonariensis	0.25, 2.5 μg/l	10 days	Reduced embryo and larval survivability	[41]
Oncorhynchus mykiss	2 μg/l	4 days	Larval erythroblasts with MN, NB and BN	[52]
Danio rerio	60 ppb	7 dpf	Decreased diameter of the saccule otolith, otoliths with numerous fiber between knobs	[53]
Cyprinus carpio	0.3, 0.06 mg/l	60 days	Lowest survival and growth rate, malformation in the yolk sac, curvature in vertebral column, body shortening, and cardiac edema	[49]
Leuciscus idus	0.1 mg/l	21 dah	Lowest survival, body length, body perimeter area, swim bladder	[35]
Oryzias latipes	0.18–19.8 μg/l	10 days	Spinal deformities (kyphosis, lordosis and C-shaped larvae)	[47]
Silurus soldatovi	0.0001–30 mg/l	144 h	Spinal curvature	[34]
Gambusia affinis	0.4 mg/l	30 days	Spinal (kyphosis, lordosis and scoliosis)	[46]
Pagrus major	0–3.2 mg/l	-	Cardiac edema, blastodermal lesions and skeletal deformities (spinal curvature, degenerated and hooked tails, fins lesions)	[32]
Rhamdia quelen	0.0005–0.018 mg/l	21 dah	Deformed spinal column	[50]
Oncorhynchus mykiss	0.05, 0.25, 0.50 & 2.50 μg/l	56 days	Premature hatching, delayed hatching, lower larval growth	[54]
Danio rerio	3.3, 6.7 & 13.3 μM	80 hpf	Edema (pericardial, yolk sac), decreased pericardial area and length of tail, lordosis	[55]
Cyprinus carpio	0.2 mg/l	30 days	Growth retardation	[56]
Clarias gariepinus	0.05–5.00 mg/l	5 days	Reduction of pigmentation, 100% mortality in 1.5 and 5.0 mg/l	[40]
Cyprinus carpio	5–50 mg/l	-	Swelling of eggs with increasing concentration	[57]

(*continued on next page*)

Table 1 (*continued*)

Species	Dose	Exposure period	Alterations/ type of deformities	References
Melanotaenia fluviatilis	0.033–3.3 mg/l	2 h	Spinal abnormalities	[58]
Cr				
Odontesthes bonariensis	4, 40 μg/l	10 days	Reduced embryo and larval survivability, morphological alteration (C-shaped body)	[41]
Danio rerio	50, 500 mg/l	4 days	Increased embryo mortality and heart rate of the hatched eggs	[51]
Clarias gariepinus	11–114 mg/l	5 days	Abnormal body axis, reduced larval survivability and growth	[40]
Cu				
Oryzias melastigma	0.32 mg/l	7 days	Skeletal and vascular system abnormalities (anemia, hemorrhage), reduction of pigmentation, absence of eye	[11]
Odontesthes bonariensis	22, 220 μg/l	10 days	Reduced embryo and larval survivability	[41]
Danio rerio	50, 500 mg/l	4 days	Increased embryo mortality and heart rate of the hatched eggs	[51]
Leuciscus idus	0.1 mg/l	21 days	Vertebral curvatures, yolk sac deformities, shorten body length, body perimeter area, swim bladder perimeter area	[35]
Carassius auratu	0.1–1 mg/l	24 hah	Scoliosis and tail curvatures	[44]
Oryzias latipes	6.95–23.1 μg/l	10 days	Spinal deformities (kyphosis and lordosis), yolk-sac mal-absorption, abnormal cardiovascular system	[47]
Fundulus heteroclitus	0.0005–0.004 mg/l	50 days	Vertebral deformities and inflammatory masses	[59]
Oncorhynchus mykiss	0.22 mg/l	4 days	Increased mortality of embryos	[48]
Danio rerio	0.068-0.244 mg/l	120 haf	Lateral line deformities (fewer functional neuromasts)	[31]
Danio rerio	50-1000 μg/l	3 dpf	Low hatching rate, higher heart rate, larger yolk sac	[31]
Cyprinus carpio	0.2 mg/l	-	First developmental retardation, Retardation of hatching	[60]
Cyprinus carpio	0.2 mg/l	20 day	Curvature of the spine, C-shaped larva, deformed yolk sac, shortened body	[61]
Cyprinus carpio	0.2 mg/l	30 days	Growth retardation	[56]
Clarias gariepinus	0.15–2.5 mg/L	5 days	Reduction of pigmentation	[40]
Cyprinus carpio	2 mg/l	-	Larvae with axial and lateral curvatures of spine, C shaped larvae, eye anomalies, deformed yolk sac, cardiac edema	[62]
Hg				
Danio rerio	20 and 30 mg/l	-	Abnormal fin, flexure of the posterior tail region	[38]
Pb				
Clarias gariepinus	0.1–0.5 mg/L	48–168 h	Irregular head, notochord defects, yolk-sac edema, spinal curvatures etc.	[42]
Zn				
Odontesthes bonariensis	211, 2110 μg/L	10 days	Cumulative embryo survival was significantly reduced to 40% at day 6 and 10% at day 2 respectively	[41]
Danio rerio	50, 500 mg/l	4 days	Majority of eggs were dead within 48 hr because of its severe toxicity, the heart rate of the hatched eggs increased with increasing concentration	[51]
Pagrus major	0.1, 0.3, 0.5, 0.7, 1.0, 1.5, 2.0, 2.5 mg/l	10 days	Low hatching rate, high mortality, abnormal pigmentation, hooked tail, spinal deformity, pericardial edema, and visceral hemorrhage	[33]
Oncorhynchus mykiss	0.3 mg/l	4 days	Increased mortality of embryos	[48]
Melanotaenia fluviatilis	0.33–33.3 mg/l	2 h	Spinal deformities	[58]

MN; micronucleus, NB; nuclear bud, BN; bi-nucleated

the larvae compared to embryos, which might contribute to the resistance of embryos to heavy metals.

Toxicity levels of heavy metals in embryos and larvae of freshwater fish are different from marine fish because of salinity differences. At higher salinity levels, the bioavailability of the toxic forms of heavy

metals in water decreases. Information is limited about the toxic effects of heavy metals on marine fish embryos and larvae. Low hatchability, high mortality, morphological abnormalities etc. are reported in embryos and larvae of marine fish exposed to different heavy metals [11, 32]. Environmental cues especially high temperature is known to cause developmental deformities in fishes and it has been reported that combined application of high temperature (24-32ºC) and heavy metal such as Cd causes intense increase in skeletal deformities in juvenile mosquito fish (*Gambusia affinis*) than Cd or temperature alone [46]. High temperature increases the metabolic activity of fish, increasing the potentiality of metal ion action (Cd in this case) on cellular enzyme and cell membrane.

The mode of action (especially changes in enzyme and DNA) of each heavy metal exposure in embryo and larvae are at early stage of investigation and gaining importance among the researchers investigating molecular mechanisms of their effects in fish. Superoxide dismutase (SOD) and catalase (CAT) enzymes are known to convert reactive oxygen species to non-toxic oxygen in the liver. It has been found that in embryos and larvae of goldfish (*Carassius auratus*), these enzymatic activities were significantly inhibited due after exposure of high Cu concentration (1.0 mg/L), causing oxidative stress responsible for lipid peroxidation [44]. Moreover, Cd and Cu exposure to 2 dph larvae of Japanese medaka (*Oryzias latipes*) induced significant DNA damage [47] determined by Comet assay (a reliable method to assess genotoxicity in all stages of fish).

There are numerous reports on the effect of single heavy metal on the ontogenic development embryos and larvae. Because most of the open water environment is contaminated with mixtures of heavy metals (from anthropogenic and geogenic sources), it is important to evaluate the combined effects of those heavy metals on embryonic and larval development. The combined effect of Cu-Zn and Cd-Zn has been investigated in Rainbow trout *Oncorhynchus mykiss* [48] and common carp *Cyprinus carpio* [49] embryos respectively, revealing increased embryonic mortality and physical deformities (e.g. vertebral column deformities). Hg and Pb toxicity resulted defects of important organs of fish such as abnormal and irregular fins, head, tails and several spinal difficulties [38,42]. Moreover, Zn contamination negatively affected the hatching success and survival of several fish species as well as hampered the normal formation and pigmentation of several organs [33,35,41,48].

Supplementation of vitamin C with the dry feed to the embryo and larvae of common carp (*Cyprinus carpio*) exposed to mixture of Zn and Cd increased the ontogenic development and quality and quantity of the larvae through the improvement of immune system [49]. It has been reported that Cd exposure under conditions of high alkalinity can significantly increase the hatching, survival rate and growth of larvae of Silver catfish *Rhamdia quelen* [50].

3. Impact of heavy metals on growth performance of fish

Nutritional adequacy is prerequisite sustainable aquaculture. The overall growth, health status and reproductive performances of various aquaculture species especially fish are dependant on appropriate nutrition [63–65]. Among the various candidates that contribute nutritional demand of various aquaculture species, heavy metals play important roles in this regard. Various types of trace metals significantly contribute to different physiological processes including growth of fish (Table 2). Several trace metals such as Mn, Fe, Co, Cu, Cr and Zn are known to be important minerals with positively influences on the physiology and metabolism of fish [9,10]. Cr has been regarded as very important trace element that improved the health status of several animals through upgrading the physiology as well as their metabolism [66,67]. Cr directly involved in nutrient (protein, lipid and carbohydrates) metabolism significantly influences the growth and feed utilization of several fish species [68,69]. Moreover, Cr also altered the fatty acid profile in blood through participating in fatty acid metabolism in various animals [70,71]. It has been found that Cr supplementation lowered the

Table 2
Impacts of heavy metals on growth performance of fish.

Species	Doses (mg/kg)	Exposure time (days)	Effects	References
As				
Oncorhynchus mykiss	26–77 µg/kg	30	Growth reduced accompanied by slower feeding rate, reduced FCE	[105]
Cd				
Mystus seenghala	1/3 of LC_{50}	112	Lowered average wet weight, body length and condition factor while higher FCR	[106]
Ictalurus punctatus	0.5, 2, 6 µg/L	180	Negatively impacted on growth (length and weight)	[107]
Pelteobagrus fulvidraco	0, 50 and 200 µg/L	56	Growth retardation; decreased WG and SGR in both 50 and 200 µg/L	[108]
Oreochromis niloticus	0, 25, 50	84	Lowest BW and WG at 50 mg/kg	[109]
Danio rerio	30 µg/L	35	Reduced growth and survival rate	[110]
Danio rerio	30 µg/l	35	Inhibited body weight, SGR and survival rate	[111]
Oreochromis niloticus	0.5	56	Reduced growth and feed intake	[112]
Oncorhynchus mykiss	1 and 3 µg/l	30	Condition Factor (K), SGR, BWG decreased, while FCR increased	[113]
Ctenopharyngodon idella	0, 5, 500 µg/l	56	Reduction in growth	[114]
Pelteobagrus fulvidraco	0.25, 4.92, 48.57, 474.7	28	WG, SGR, FI, PER declined with increasing dietary Cd	[115]
Cr				
Pangasianodon hypophthalmus	2, 4, & 8	60	The growth and feed utilization increased significantly in the fish fed with 2 and 4 mg/kg supplemented diets	[10]
Labeo rohita	0.4, 0.8 & 1.2	60	Improved %WG, SGR, FER and PER and %ANPU at 0.8 mg kg^{-1}	[116]
Oreochromis niloticus	4.57 mg/L	60	WG, SGR reduced	[117]
Platichthys stellatus	0, 50, 100, 200, 400 ppb	28	DLG, DWG, CF, and HSI decreased	[118]
Megalobrama amblycephala	0.2, 0.4, 0.8, 1.6, 3.2 & 12.0	77	Highest FW and SGR; lowest FCR in fish fed with 0.4 mg/kg	[119]
Sebastes schlegelii	0, 30, 60, 120 & 240	28	Decreased growth performance	[120]
Larmichthys crocea	5, 10, 20, 40 & 80	70	Higher survival and SGR in fish fed the diet with 5 mg/kg	[101]
Cyprinus carpio	0.5, 1.0, 2.0	56	produced superior %WG, SGR, FCR and	[121]

(*continued on next page*)

Table 2 (*continued*)

Species	Doses (mg/kg)	Exposure time (days)	Effects	References
			PER at a level 0.5 mg/kg	
Oreochromis niloticus	200, 400, 600, 800, 1000 & 1200 ppb	72	increased FI at 400 ppb and 600 ppb	[122]
Cyprinus carpio	0.5, 1.0, 2.0	63	higher FBW, % WG, SGR and lower FCR at 0.5 mg/kg	[121]
Ctenopharyngodon idellus	0.2, 0.4, 0.8, 1.6 & 3.2	70	improved WG, FER, PER and PR at 0.8 mg kg^{-1}	[123]
Channa punctatus	2 & 4	60	BWG was comparatively less in fish exposed to 4 mg/L than the 2 mg/L and control	[124]
Cu				
Cyprinus carpio	0.05 & 0.1	90	Significantly reduced SGR, WG, PER and increased FCR	[125]
Megalobrama amblycephala	1.43 & 9.13	70	Improved growth performance	[126]
Oreochromis niloticus	25, 50 & 75 μg/L	90	Decrease in FW, WG, and HSI	[127]
Cyprinus carpio	0, 1.5 & 3.0	60	Decrease in WG, length, CF and increase in FCR	[128]
Poecilia vivipara	5 & 9 μg/L	365	Exposure to 9 μg/L Cu reduced fish body weight and length	[129]
Pagrus major	2	60	Increased FBW, WG, SGR, FI, FER, PER, PG and PR	[97]
Pagrus major	2, 4, 6, 8	60	Highest final body weight, WG, SGR, FI, protein gain at levels of 2 and 4 mg/kg	[97]
Channa punctatus	3.7, 4.7, 5.7, 6.7, 7.7 & 8.7	84	Fish fed diet with 6.7 mg kg^{-1} copper had highest AWG, PER, PG and best FCR	[130]
Cyprinus carpio	20, 30, 40 & 70 μg/l	28	Decrease in TL, WG and CF, and increase in HSI	[131]
Carassius carassius	0.30 & 0.60	20	High-concentration (0.60 mg/L) hindered the growth	[132]
Poecilia reticulata	0, 0.004, 0.013, 0.019, 0.029	56	Decrease in FW, SGR, and increase in FCR	[133]
Lateolabrax japonicus	0 & 4	56	Higher FI, SGR, PER	[100]
Huso huso	1.1, 3.5, 7.1, 9.7, 13.1, 25.1, 49.9 & 195	84	Weight gain of fish fed 10 and 13 mg/kg diets was higher than others.	[96]
Ctenopharyngodon idella	2.26, 3.75, 5.25, 6.70 & 8.33	56	increased %WG and FI at up to 3.75 mg/kg	[134]
Ctenopharyngodon idella	2.26, 3.75, 5.25, 6.70, & 8.33	56	PWG and FI increased with dietary Cu levels up to 3.75 mg/kg	[134]
Megalobrama amblycephala	0, 3, 6, 9, 25, 50, 100 & 150	56	Higher WG, SGR in fish fed diets supplemented with 3–6 mg/kg	[135]
Synechogobius hasta	0, 0.15 & 0.3	15	WG and SGR declined	[67]
Sebastes schlegeli	0, 50, 125, 250 & 500	60	reduced the growth rate	[136]
Oncorhynchus mykiss	35.7 & 54.1 μg/l	56	fish exposed to higher Cu concentrations growing slower	[137]
Fe				
Clarias gariepinus	0.2, 0.4, 0.8, 1.2 & 1.6	49	Improved WG, % WG, SGR, FCR in fish fed the Fe supplemented diet	[138]
Ctenopharyngodon idella	12.15, 35.38, 63.47, 86.43, 111.09, 136.37	60	FBW, PWG, SGR and FI increased significantly up to 207 63.47 mg/kg diet and then decreased significantly	[139]
Cyprinus carpio	53.9, 90.0, 115.6, 146.1, 176.0, 215.8 & 266.0	60	Improved %WG, FE, PER in fish fed the diet up to 90.0 mg/kg	[140]
Epinephelus coioides	0, 50, 100, 150, 200 &250	56	highest WG and FE in fish fed the diet supplemented with 100 mg/kg	[141]
Ictalurus puctatus	40, 336 & 671	70	Best growth at 40 and 336 mg/kg diet	[142]
Ictalurus punctatus	0, 30 & 300	112	Increased WG and survival; better FCR in fish fed the diet up to 300 mg/kg	[143]
Zn				
Oreochromis niloticus	80	42	Improved growth parameters (WG, %WG, and SGR) and feed utilization (FCR and PER)	[9]
Cyprinus carpio	15.3, 26.9, 40.8, 58.2, 68.9 & 92.5	42	Enhanced %WG, FE, PER and LPV with dietary levels up to 40.8 mg/kg	[93]
Salmo salar	50, 180	180	Increased SGR at higher	[144]

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Table 2 (*continued*)

Species	Doses (mg/kg)	Exposure time (days)	Effects	References
			concentration, better FCR	
Pb				
Chanos chanos	0, 42.64, 63.97 & 85.2	40	WG, LG, SGR, FE, and FCR declined significantly at the highest concentration	[145]
Catla catla,				
Labeo rohita				
Cirrhina mrigala	1/3rd of LC50	60	Lesser WG, FI and FCE	[146]

ANPU; apparent net protein utilization, FCR; feed conversion ratio, LPV; lipid productive value, FE; feed efficiency, FER; feed efficiency ratio, PER; protein efficiency ratio, FBW; final bodyweight, WG; weight gain, SGR; specific growth rate, FI; feed intake, FER; feed efficiency ratio, PER; protein efficiency ratio, PG; protein gain, PR; protein retention

cholesterol, triglycerides level in blood and increased the high density lipoprotein (HDL) cholesterol level [72,73]. Dietary Cr significantly influenced the expression of several genes related to glucose metabolism, lipogenesis, apparently playing a key role in growth enhancement [74]. Cr supplementation in diet significantly improved the growth and feed utility of striped catfish (*Pangasianodon hypophthalmus*) upto 4 mg/kg but greater concentrations resulted in lower growth with higher micronucleus frequencies (Fig. 1) [10]. On the contrary, presence of Cr in excess level led to several toxicities and therefore, reduced the growth

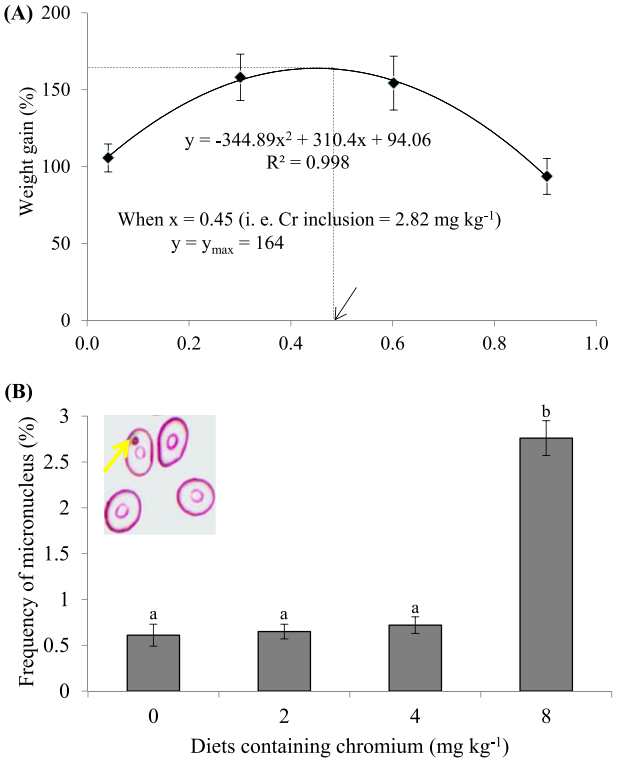

Fig. 1. Effects of dietary Cr on (A) weight gain (WG) and (B) frequency of formation of micronucleus (MN) in the erythrocytes of striped catfish. The analyzed dietary Cr concentration was log transformed for better visualization. Requirement derived with the polynomial regression method for WG was 2.82. Values with different alphabetical superscripts differ significantly ($p < 0.01$) among different diets.

and feed palatability of several species [75–77]. Zn is an essential trace element that plays a significant role in the life processes of animals including fish [78–80]. Zn acts as a co-factor of several metallo-enzymes (carbonic anhydrase, alkaline phosphatase, alcohol dehydrogenase etc.) ensuring the availability and activities of those important enzymes to stimulate digestion and metabolism of nutrients [81–83]. Zn also regulates the nucleic acid metabolism, protein synthesis and anti-oxidative enzymes functionalities of fish [84]. The anti-oxidative roles of Zn were well demonstrated in several studies [85,86]. Dietary Zn supplementation considerably improved the growth of fish through upgrading muscle morphology [9]. Dietary Zn provisions also influence the whole body composition of fish muscle. Zn significantly enhanced the lipid content and lowered the moisture and ash level of fish carcass [87]. However, Zn deficiency hampers the nucleic acid and protein biosynthetic pathways [66,88], impairment of bone development [87] and various other pathological effects [89]. On the other hand, excess amount of Zn resulted various negative impacts such as growth and reproductive performance reduction [90], oxidative stress [91] and poor feed utilization [92–94]. Moreover, Zn toxicity resulted in delayed hatching, malformations in bone calcification and growth defects [95]. Cu is an essential element that plays a pivotal role in various physiological as well as biological systems such as hemoglobin and bone formation, control the activities of myelin in the nervous system and finally acts as an activists of many important enzymatic action including cytochrome oxidase, lysyl oxidase, dopamine hydroxylase ferroxidase, tyrosinase and Cu-Zn superoxidase dismutase [93,96]. Various studies revealed that dietary Cu supplementation significantly improve the growth, oxidative status and immune system of several aquatic species [96–99]. In the very recent years, aquaculture nutritionists find out the outstanding role of Cu particles has caught the attention aquaculture personnel as potentially interesting feed supplement [100,101]. On the contrary, dietary Cu toxicity exhibited several adverse effects including reduced growth, greater FCR, lower feed efficiency [102,103]. Fe, an essential element that helps to maintain the normal activities of different organs and tissues of animals including fish because of its active role in physiological processes like oxygen gas transportation, cellular respiratory activities, and lipid peroxidation processes. Fe modulated the immune system of animals and thus protects against various infectious agents and also actively participates in the synthesis of steroid and DNA, drug metabolism and electron transportation [104].

4. Heavy metals effect on reproduction of fish

Reproduction is essential to all animals and successful reproductive performance among the most important determinants of survival at the species level [147–149]. Heavy metals pollution negatively affects the reproductive performance of fish resulting low quality gametes that may influence not only success rate of fertilization but also hatching as well as survival rate of the offspring (Table 3) [150]. Various types of heavy metals accumulated into the fish body from the environment and their continuous accumulation disrupt the formation and activities of various tissues and organs including reproductive organs [62]. Heavy metals caused anomalies in reproductive cell/organ development. Arsenic (As) pollution seriously affected the reproductive performances of fish through inhibition of spermatogenesis and oogenesis including reduced egg and sperm quality and quantity, hatching and fertilization rate [22–24]. Cd is a potent hazardous metal that resulted several dysfunctions of reproductive process of fish. Various studies demonstrated several difficulties in reproductive performance of fish such as abnormal oocytes structure, empty follicle and loosing follicular line, retraction as well as condensation of cytoplasm, total GSI reduced and so on [27]. Moreover, Cd toxicities cause shrinkage of spermatic lobules and fibrosis in testis, lower sperm motility and viability as well as reduced fertilization rate [26,150–153]. Cr has been regarded as one of the most biologically potent heavy metals due to its summative destructive effects on living organisms [154]. Long term exposure to Cr drastically reduced

Table 3
Effects of heavy metals on reproductive performances of fish.

Fish species	Doses	Exposure period (days)	Effects	References
As				
Anguilla japonica	0.1, 100 μM	15	Inhibited spermatogenesis via steroidogenesis suppression	[24]
Danio rerio	-	68	Reduced reproductive output, egg production, number of spawns, average number of eggs per spawn and hatching rate	[23]
Anguilla japonica	10^{-5} M	6	Inhibited the spermatogenesis, necrosis of testicular fragments	[22]
Cd				
Oryzias melastigma	10 μg/L	30	irregular oocytes, partly adhesion, empty follicle, and increased follicular atresia, cytoplasmic retraction, cytoplasm condensed form, karyoplasm clumping, loose follicular lining	[27]
Gasterosteus aculeatus	1 μg/L	90	GSI decreased in prolonged exposure	[161]
Odontesthes bonariensis	0.25 μg/L	14	Testis showed fibrosis and shrinkage of the spermatic lobules, pyknotic cells, reduce of the length of the spermatic lobules	[26]
Cyprinus carpio	50, 100, 150 & 200 ppm	3	Sperm quality (motility and viability) and fertilization rate decreased at 100 ppm or more	[153]
Acipenser baerii	0–100 mg/L	4 h	Percentage of motile sperm was reduced from 10 mg/l to higher conc.	[151]
Oncorhynchus mykiss	10, 100 and 500 mg/l	4 h	Altered sperm motility characteristics and hatching rates	[152]
Acipenser ruthenus	0.1, 5.0 mg/L	2 h	Sperm motility parameters (motility and velocity) inhibited in higher conc.	[150]
Cr				
Oryzias melastigma	½ of 96LC50	60	After long-term exposure amount of spawning decreased	[155]
Odontesthes bonariensis	4 μg/L	14	Testis showed fibrosis and shrinkage of the spermatic lobules, pyknotic cells in the testis	[26]
Oryzias latipes	4 mg/L	90	Decreases in gonad weight, GSI and fecundity, reduced number of mature oocyte and mature spermatozoa in testes	[156]
Acipenser ruthenus	0.1, 5.0 mg/L	2 h	Sperm motility parameters (motility and velocity) inhibited in higher conc.	[150]
Channa punctatus	4 mg/L	30	Decreased the percentage of vitellogenic oocytes	[124]
Cu				
Poecilia reticulata	0, 5, 10 mg/L	56	Lowest reproductive success, prolonged parturition time and highest mortality rate at 10 mg/l	[28]
Oreochromis niloticus	1, 2, 4 mg/kg	4	Decrease in sperm motility rate, VCL, VAP, and VSL,	[29]
Odontesthes bonariensis	22 μg/L	14	Fibrosis and shrinkage of the spermatic lobules, pyknotic cells in the testis, reduce of the length of the spermatic lobules	[26]
Xiphophorus helleri	0.04, 0.08, 0.12 & 0.16 ppm	100	Decreased GSI, gonad not developed in high concentrations (0.12 and 0.16 ppm)	[160]
Carassius auratus	0.25, 0.05, 0.075 & 0.1 ppm	100	Decreased GSI, reduced the fecundity	[160]
Danio rerio	100, 500 & 1000 μg/g	260	1000 μg produce decrease in GSI but not significant.	[159]
Hg				
Acipenser baerii	0-100 mg/L	4 h	Percentage of motile sperm reduced from 1 mg/l to higher conc and complete obstruction in 100 mg/l.	[151]
Oncorhynchus mykiss	1, 10, 100 mg/l	4 h	Inhibition of sperm motility	[152]
Dicentrarchus labrax	0.01, 0.1, 1, 10 & 100 ppm	-	Exposure to100 ppm completely inhibited sperm motility	[158]
Oryzias latipes	40 μg/L	8	Testicular atrophy and arrested spermiation	[157]
Pimephales promelas	0.87 to 3.93 μg/g diet	250	Lowered GSI, Reduced the reproductive success	[162]
goldfish	1, 10 & 100 μg/L	-	Reduced curvilinear velocity, percentage of motile sperm, and flagella length	[163]
Pimephales promelas	0.88, 4.11 & 8.46 μg/g	-	Delayed spawning, and days to spawning Reduced the instantaneous rate of reproduction, GSI and reproductive efforts	[43]
Oreochromis niloticus	0.08 to 0.54 μg/g	210	The normal morphology of the testes was altered, Decreased spermatogenesis	[164]
Pb				
Oryzias melastigma	50 μg/L	30	Irregular oocytes, partly adhesion,	[27]

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Table 3 (*continued*)

Fish species	Doses	Exposure period (days)	Effects	References
			empty follicle, increased follicular atresia, loose follicular lining	
Carassius gibelio	8, 13, 24 & 49 mg/kg	365	Decreased GSI, affected ovarian steroidogenesis, gametogenesis, ovulation	[30]
Zn				
Clarias magur	50, 200, 300 mg/kg	60	The highest GSI and fecundity at 50 mg/l	[25]
Oryzias melastigma	100 μg/L	30	Irregular oocytes, partly adhesion, empty follicle, and increased follicular atresia, loose follicular lining	[27]
Odontesthes bonariensis	211 μg/L	14	Fibrosis and shrinkage of the spermatic lobules, pyknotic cells in the testis, reduced the length of the spermatic lobules,	[26]
Cyprinus carpio	10, 50, 100, 200, 500, 1000 and 2000 ppm	-	Decreased the motility of sperm, inhibitory influence on VSL, low fertilization rate	[165]

GSI; gonad-somatic index,

the spawning success [155], fibrotic and pyknotic testis [26], significantly reduced the GSI, fecundity, lowered number of oocytes and matured spermatozoa [156], hampered the motility of sperm [150] and finally gradual decrease of vitellogenic oocytes [124]. Various studies revealed that reduced GSI, fecundity, hatching rate, fertilization success, abnormal shape of reproductive organs, and finally overall reproductive success resulted from the toxicities created by Cu and Hg [28,29,151,157–160]. Pd and Zn resulted similar deformities as well as negative impacts in *Carassius gibelio* [30], *Odontesthes bonariensis* [26]; *Oryzias melastigma* [27] and *Clarias magur* [25].

5. Conclusion and future perspectives

Heavy metals contamination is a serious threat to entire aquatic ecosystems including associated flora and fauna. The devastating impacts of heavy metals on aquatic organisms specifically fish result an irreparable loss in aquaculture industry. In this review, destructive effects of heavy metals on fish focusing the embryonic and larval development, growth and reproduction of commercially important species are discussed very concisely with a view to using it as a tool for further genotoxicity related experiments by the researchers of the associated areas. Heavy metals resulted in severe deformities in several aquatic organisms that will ultimately pose a substantial threat to associated consumers. To enlarge the sustainability of the aquaculture sector and to produce safe fish for human consumption, regular monitoring of the fish and associated environment should be done by the appropriate authorities at the local government, state, and national levels. A well-established framework should be developed as soon as possible to mitigate this great problem.

CRediT authorship contribution statement

Khanam Taslima: preparation of the first draft of the manuscript. Md Al-Emran, Mohammad Shadiqur Rahman, Jabed Hasan, Zannatul Ferdous and Md Fazle Rohani: data collection and preparation of the Tables. Md Shahjahan: conceptualization, edited the manuscript and final approval. All authors have read the final version and approved the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Sharing of data is not permissible for this article. The data that support the outcomes of this study are available on request from the corresponding author [M Shahjahan].

Acknowledgement

This study was supported by the grants from Aqua-drugs project of Bangladesh Fisheries Research Institute and University Grants Commission of Bangladesh (2018/666/UGC) to the corresponding author (Md Shahjahan) which is gratefully acknowledged.

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Price 2013

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December 17, 2013

Prepared by Michael H.H. Price

For SkeenaWild Conservation Trust

Citation: Price, M.H.H. 2013. Sub-lethal metal toxicity effects on salmonids: a review. Report prepared for SkeenaWild Conservation Trust. Smithers, BC. 64 pages.

SkeenaWild Conservation Trust is a regionally based organization. We are dedicated to bringing together governments, First Nations and members of the public in the Skeena Watershed to sustain the long-term health and resilience of the wild salmon ecosystem, while optimizing economic returns to First Nations and local communities.

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Executive summary

Heavy metals are common pollutants of aquatic systems, often associated with human activities. Hard-rock mining, agriculture, urbanization, and industrialization have enabled the release of significant quantities of heavy metals to aquatic ecosystems, sometimes with profound ecological harm.

While metals naturally leach from rock when they are exposed to weathering processes, human activities often speed-up metal leaching through the excavation and exposure of vast quantities of rock from mineral deposits (such as during metal- and coal-mining). Metal leaching can be further accelerated by acidic drainage, which occurs when acid-generating rock is exposed to air and/or water. Acid mine drainage is typically produced in tailings ponds and waste rock dumps at metal- and coal-mining sites, and is characterized by acidic water and high concentration of dissolved metals. These acidic waters can then dissolve and mobilize more heavy metals as they flow across the landscape and contact other minerals and exposed rock. Acid mine drainage is a major source of water contamination in many mining districts on Earth.

Fish are particularly vulnerable to metals because of sensitive organs that are continuously in contact with the environment, and because metals are highly soluble in water. Most metals can disrupt the essential functions of the fish gill (responsible for gas and ion exchange) and the olfactory system (a fish's sense of smell). Even relatively low concentrations of heavy metals can cause harm to fish. The olfactory system specifically plays an essential role in the survival of fish. While there is a wealth of scientific information describing the concentrations of metals that cause death in freshwater fish, much less reported are the potential sub-lethal effects (i.e., negative impacts that do not cause immediate or direct death) on salmon and trout from low metal concentrations.

Herein is a synthesis of the relevant scientific literature describing the 7 most cited heavy metals, and the corresponding lowest concentrations reported to cause sub-lethal effects on salmonids. These metals are: aluminum (Al), cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), silver (Ag), and zinc (Zn). The lowest concentrations of these metals reported in the literature are compared with the water quality guidelines of British Columbia, so as to determine whether salmonids residing in freshwater systems adjacent to resource extraction industries are protected from metal toxicants.

All of the metals that were examined can negatively impact salmonids to some degree at concentrations below the levels known to cause lethality. Sub-lethal concentrations can alter behaviours related to predator avoidance, foraging, migration, and social interactions, and can cause the physical impairment associated with growth and development, swimming efficiency, and immune system responses. However, most of the metal concentrations reported to invoke sub-lethal effects are above the regulatory limits in British Columbia. Water quality guidelines assigned for Al, Cd, Pb, and Ag would protect against all of the effect concentrations reported in the literature.

There are several instances where sub-lethal effects on salmonids from metals such as Cu, Ni, and Zn have been reported at concentrations below the water quality guidelines of

British Columbia. All of these sub-lethal effects involve either the avoidance of metal-contaminated water by fish or an impaired sense of smell. Several studies report the loss of smell in juvenile rainbow trout, coho salmon, and chum salmon exposed to Cu at concentrations below provincial guidelines. These copper-exposed fish failed to detect near-by predators, and did not exhibit the typical anti-predator response; thus, these fish were more vulnerable to predators, and had lower survival compared to unexposed fish. The disruption of such an anti-predator response in salmonids at ecologically-relevant concentrations below government guidelines is a very real scenario that may have conservation implications – especially considering that British Columbia is the largest producer of Cu in Canada.

Water quality criteria for the protection of aquatic life in British Columbia are not legislated, but rather serve as environmental benchmarks. Specific industrial projects apply for permits to pollute, and the resulting metal concentrations in receiving waters of discharge may be higher than the provincial criteria. Two such examples are the open-pit copper mines (Noranda Bell and Granisle) located on Babine Lake in the Skeena River watershed, where the maximum authorized discharge for dissolved Cu from mine waste-water into the lake is five-fold higher than the regulatory guidelines for each mine.

The impact of heavy metals on fish is complex and depends on the chemical characteristics of water. Acidity (pH), hardness ($CaCO_3$), and organic matter are all complicating factors in the determination of metal toxicity. Thus, differences in acidity, hardness, and/or organic matter of test water may at least partly explain why the effect concentrations for a given metal and fish species can be dissimilar between studies.

There are at least four limitations when applying the reported effect concentrations on salmonids to real-life scenarios: i) the effect concentrations reported in this review are more often the lowest *detected effect*, not the actual *lowest effect concentration*, ii) scientific studies rarely reflect natural exposure conditions, iii) laboratory studies tend to examine metals in isolation, which may not be environmentally realistic or relevant for assessing actual impacts on fish, and iv) dietary metal concentrations are not incorporated into Canada's water quality guidelines despite the likely simultaneous occurrence of both waterborne and dietary routes of metal toxicity.

Research is needed not only to determine threshold concentrations for salmonids, but also to compare the effect concentrations derived from laboratory studies with natural environments, and examine the effects of metal mixtures and dietary toxicity on salmonids. Ultimately, a shift in research emphasis from the routine single metal - single organism - perspective, to population, community, and ecosystem scale is required to achieve a full understanding of the sub-lethal metal toxicity effects on salmonids.

Introduction

Heavy metals are widely occurring pollutants commonly associated with human activities. Hard-rock mining, agriculture, urbanization, and industrialization have mobilized significant quantities of heavy metals to aquatic ecosystems (Boyd 2010; Tierney et al. 2010), sometimes with profound ecological harm (Downs and Stocks 1977; Balistrieri et al. 2002). Impacts to freshwater fish from heavy metals have been particularly well documented (Woody et al. 2010; Dennis and Clair 2012).

Heavy metals enter aquatic systems through natural weathering and leaching processes, which can be greatly accelerated by humans. Metals naturally leach from rock when they are exposed to air and/or water, and the resulting chemical reactions mobilize them into biologically available forms (Wilkin 2007). However, human activities often speed-up this process through the excavation of vast quantities of rock from mineral deposits (such as during metal- and coal-mining), and the subsequent exposure of that material to weathering processes (Kelly 1988; Moore and Luoma 1990; Hogsden and Harding 2012). The leaching process can be further accelerated by acidic drainage, which occurs when sulphide minerals previously "locked" in rock are exposed to air and water, and naturally oxidize without the presence of sufficient quantities of neutralizing minerals (Wilkin 2007). Acid mine drainage is typically produced in tailings ponds and waste rock dumps at mining sites, and is characterized by acidic water (low pH) and high concentration of dissolved metals. Bacteria contribute to metal leaching by catalyzing the reactions and speeding-up the rate in which water becomes acidified. These acidic waters can then dissolve and mobilize more heavy metals as they flow across the landscape and contact other minerals and exposed rock. Acid mine drainage is a major source of water contamination in many mining districts on Earth (Hogsden and Harding 2012).

Fish are extremely vulnerable to metal toxicants because fish have sensitive organs that are continuously in contact with the environment, and because metals are highly soluble in water. For example, the fish gill is a sophisticated, yet delicate, organ with multiple physiological functions that range from gas exchange to excretion of nitrogenous waste (Hogstrand and Wood 1998). High concentrations of most metals can disrupt these functions, damage the gill structurally, and cause suffocation and death (Mallat 1985). Even relatively low concentrations of heavy metals can fatally impair physiological functions (such as the regulation of ions; ionoregulation) of the gill (Wood 1992). The olfactory organ, and its associated nerve cells, is also directly exposed to the environment and thus highly susceptible to damage by metal toxicants in water. Heavy metals can interfere with a fish's sense of smell (olfaction) by blocking the effects stimulated by natural odorants or by directly damaging olfactory receptor sites (Hara et al. 1983; Klaprat et al. 1988). Olfaction plays an essential role in the survival of fish, initiating behaviours such as food gathering, predator avoidance, schooling, defense, navigation between ocean and freshwater habitats, and reproduction, and low concentrations of heavy metals can alter such behaviours and reduce survival (Sandahl et al. 2007; Tierney et al. 2010; McIntyre et al. 2012).

Freshwater and anadromous salmonids (fish in the Salmonidae family, which includes salmon and trout) are key elements of ecosystems (Gende et al. 2002; Hocking and

Reynolds 2011); they play an important role in the cultural foundation of human societies (Campbell and Butler 2010), and coastal economies (Schindler et al. 2010). While the ecological threats posed by metal-mining and other resource-extraction industries are not limited to salmonids, lost and degraded salmon and trout populations threaten a range of human values that define our well-being and sustain our quality of life. Concerns regarding the possible effects of heavy metals on salmonid populations have been raised. However, published findings have generally focused on heavily polluted systems or metal concentrations that cause direct lethality. For example, the Coeur d'Alene mining district in northern Idaho, U.S.A. (grossly disturbed by mining for over 100 years), has been routinely studied for its widespread contamination of water and soils, and impairment of salmon and trout populations (Moore and Luoma 1990; Woody et al. 2010; Mebane 2012). Furthermore, we have a fairly robust understanding of the concentrations for most metals that cause death in fish over short time-periods (e.g., Chapman 1978a; Chapman and Stevens 1978; Buhl and Hamilton 1990; Hansen et al. 2002a). Yet much less reported are the potential sub-lethal effects (i.e., negative impacts that do not cause immediate or direct death of fish) on salmon and trout from low metal concentrations.

Canada is a resource-dependent country with a long tradition of mining activity, and poor record for environmental protection (Lemly 1994). In the western-most province of British Columbia (home to the largest wild salmon abundance in the country, and a national leader in mineral production; copper (Cu) specifically), there are 9 metal-mines in operation, at least 18 proposed, and more than 60 additional locations considered significant exploration projects with the potential to become mines (BCMoM 2013a; Figure 1). The British Columbia government has explicitly proposed to create 8 new mines, and expand 9 existing ones, by 2015. Indeed, "the Province will move quickly and decisively to leverage today's high commodity prices and gain a competitive edge over other global mining jurisdictions" (BCMoM 2013b). Given the speed of the proposed metal-mine development in British Columbia, and the abundance and importance of salmonids to humans and ecosystems in the region, there is an urgent need to assess whether such developments pose a risk to salmonids in aquatic environments.

The following is a synthesis of the known literature on metal toxicity effects on salmonids, and an examination as to whether such metals pose a significant risk. The review is not intended to comprehensively cover the literature regarding all heavy metals and their effects. Instead, this review is focused on sub-lethal effects of the most common metal pollutants, and the resulting lowest concentrations reported to cause an effect (i.e., effect concentrations). To assess whether metals in aquatic environments pose a risk to salmonids in British Columbia, effect concentrations were compared to government regulatory guidelines for the protection of aquatic life. The report is structured such that the described metals are in alphabetical order, each with its suite of reported effects on salmonids. All concentrations described are for waterborne toxicants that are assumed dissolved (as opposed to total concentrations), unless otherwise noted. Dissolved concentrations are ecologically important because they represent the mobile and biologically available amount of a given metal in water, whereas total metal concentrations are the total amount of a given metal in water whether in an available form or not; a dissolved metal concentration is a sub-set of the total metal concentration.

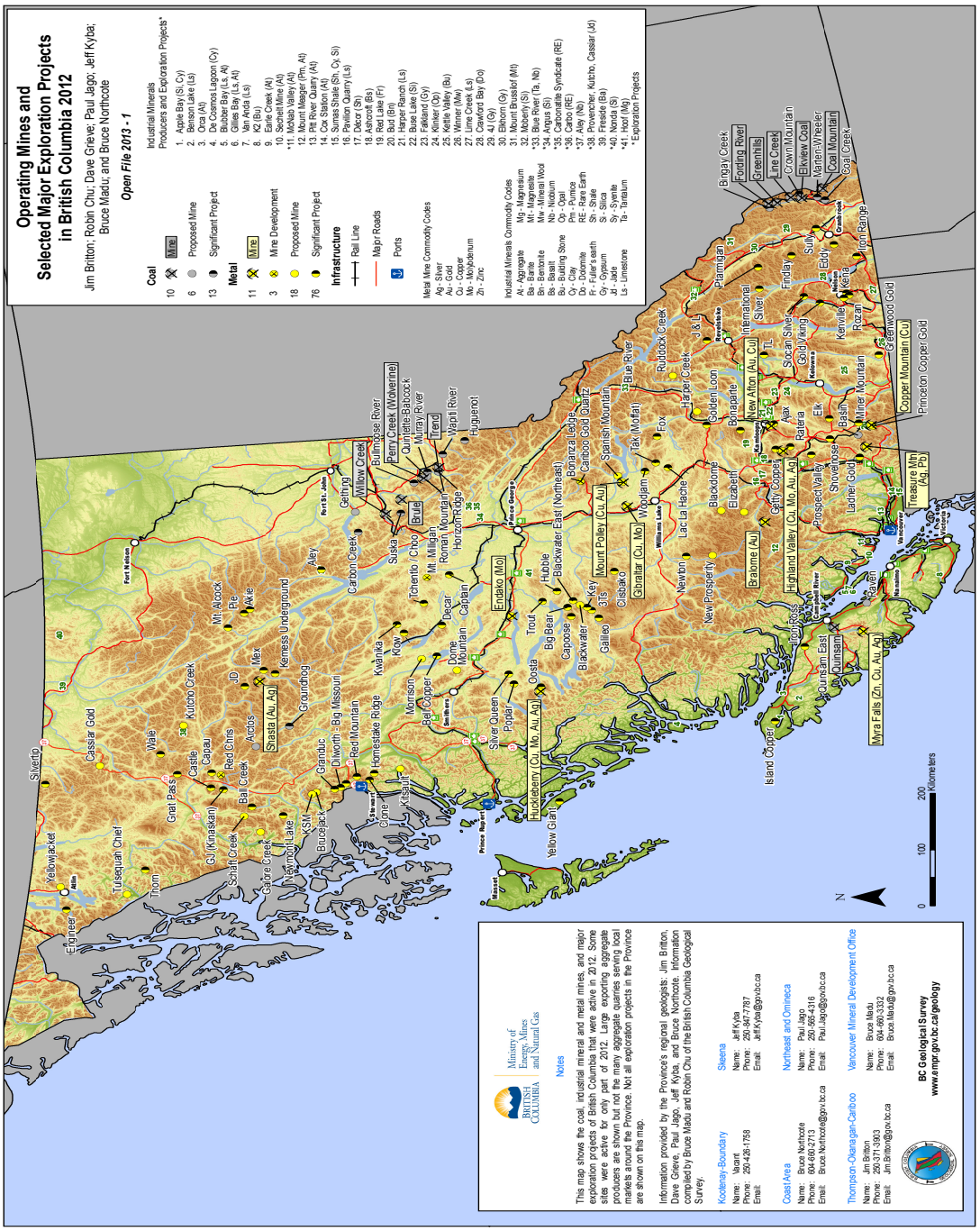

Figure 1. Map of British Columbia and all of the operating, proposed, and potential metal- and coal-mine projects as of 2012 (BCMoM 2013a).

Effect concentrations of metals

Aluminum (Al)

Aluminum is the third most abundant element in the Earth's crust, yet this metal is not known to be biologically essential to aquatic life (Gensemer and Playle 1999). Human communities in North America have been reminded about the importance of Al as a toxicant by recent oil shale mining development, where Al is liberated from the mineral Dawsonite (which is present in some oil shale ore) as a bi-product (Freeman and Everhart 1971). The behaviour of Al in water is highly varied, forming a variety of sensitive complexes that exhibit different effects on fish. In freshwater, labile (cationic/inorganic) forms of Al may be most toxic. Although Al is relatively insoluble at pH 6 to 8, the solubility increases under more acidic and alkaline conditions, at lower temperatures, and in the presence of complexing ligands (Driscoll and Postek 1996). It is the interaction between Al, pH, and calcium (Ca), which generally determines the level of toxicity to fish. For example, in hard (high Ca^{2+} concentration) fresh water, Ca protects fish against the toxic effects of Al so that negative effects occur only at high Al concentrations (Gensemer and Playle 1999).

The fish gill is a multifunctional organ involved in ion regulation and respiration; as such, it is the primary site of toxicity for metals such as Al (Exley et al. 1991; Gensemer and Playle 1999; Monette et al. 2008). Aluminum accumulates both on the surface and within the fish gill epithelium during exposure, which can result in increased branchial permeability and active ion uptake inhibition (Youson and Neville 1987; Booth et al. 1988; Wilkinson and Campbell 1993). Increased permeability of the gill may specifically be caused by the displacement of Ca ions by Al at binding sites. Calcium ions help bind intercellular junctions, and the displacement of Ca by Al results in the weakening of these otherwise tight junctions (Booth et al. 1988; Freda et al. 1991; Monette et al. 2008). Salmonids may be particularly vulnerable to ion regulatory disturbance due to their complex life history of separate freshwater and saltwater phases, and the physiological adaptations required for each (Hoar 1988; McCormick et al. 1998). Mortality rates for juvenile Atlantic salmon (*Salmo salar*) are reportedly high when exposed for days-to-weeks to cationic Al concentrations >45 µg/L (Kroglund et al. 2008), though much higher concentrations (i.e., 5,200 µg/L) are noted to cause death within 96 hours to 50% of juvenile rainbow trout (Freeman and Everhart 1971; Table 1).

Physical impairment

Growth and swim speed

Sub-lethal levels of Al can affect the feeding behavior, growth, and swim speed of salmonids. For example, reduced growth rates have been observed in juvenile brown trout (*Salmo trutta*) exposed to total Al concentrations greater than 27 µg/L (unknown dissolved concentration) in waters with pH below 5.5 (Sandler and Lynam 1987; Table 1). Juvenile rainbow trout exposed to 30.0 µg/L total Al (unknown dissolved concentration) in waters of 5.2 pH showed a 30% reduction in the maximum sustainable swimming speed within 7 days, and these effects were roughly two times greater than for fish exposed only to low pH (i.e., 5.2; Wilson and Wood 1992). In a separate study, juvenile rainbow trout (*Oncorhynchus mykiss*) pre-exposed to 38 µg/L total Al at 5.2-5.4

pH for 36 days suffered impaired swim speed, and the maximum swim speed remained depressed even when fish were subsequently placed in waters with pH of 6.5 and 0 µg/L total Al (Wilson et al. 1994).

Mucous production

Aluminum can accumulate rapidly on the gill lamellae surface of juvenile rainbow trout, and may gradually penetrate within the gill cells themselves over time (Wilson and Wood 1992). Juvenile rainbow trout exposed to 38 µg/L Al at pH 5.2 for 5 days showed a five-fold increase in the number of mucous cells present in the filamental epithelium compared to fish exposed to 0 µg/L Al in waters with pH 5.2 and 6.5 (Wilson et al. 1994). After 34 days exposure to 38 µg/L Al at pH 5.2, juvenile rainbow trout showed a four-fold increase in mucous cells compared to unexposed fish (Wilson et al. 1994), suggesting that fish do not acclimate to Al toxicity. Gill hyperplasia, which is an abnormal increase in cell numbers that can lead to respiratory impairment, may result from Al toxicity. Specifically, positively charged Al binds to the negatively charged fish gill epithelium, causing irritation that results in excessive mucous production, which can then clog gill membranes and lead to severe respiratory impairment (Rosseland and Staurnes 1994; Sparling and Lowe 1996; Klöppel et al. 1997). At minimum, low Al concentrations, especially in waters with pH between 5.0 and 5.6, will cause fitness degradation, and reduce the ability of salmonids to adequately deal with other stressors, such as smoltification (Dennis and Clair 2012).

Migration

Exposure to low levels to Al during long-term and episodic (single or re-occurring episodes lasting several days) events may disrupt the downstream migration of juvenile salmonids and reduce survival in seawater. Several studies have reported that non-lethal Al concentrations can compromise the ability of juvenile Atlantic salmon to balance body fluids (osmoregulation) during smoltification (Staurnes et al. 1995; Magee et al. 2001, 2003; Kroglund et al. 2007). Juvenile Atlantic salmon exposed for three months to 6 (+/-2) µg/L Al showed a 20-30% reduction in survival compared to control fish (Kroglund and Finstad 2003). Juvenile Atlantic salmon exposed to 28-64 µg/L inorganic Al for 2 to 5 days in acidic water (pH 5.4-6.3) also showed reduced seawater tolerance compared to control fish (Monette et al. 2008). Concentrations of inorganic Al of 5-10 µg/L is predicted to cause a 25%–50% reduction in the survival of Atlantic salmon when smolts are exposed for as few as 3 days during seaward migration (Kroglund et al. 2008).

Olfaction

Aluminum may cause physical alteration in the olfactory epithelium of salmonids and influence the electrical properties of olfactory sensory neurons. For example, juvenile rainbow trout exposed to 9.5 µg/L Al in acidic water (pH 4.7) for 2 weeks resulted in loss of receptor cell cilia, anatomically altered olfactory knobs, and clumped microvilli compared to control fish, and showed reduced olfactory nerve responses compared to fish only exposed to acidic water (Klaprat et al. 1988).

Regulatory limits

The government of British Columbia's water quality criteria pertaining to Al for the protection of aquatic life is dependent on pH and exposure duration (BCMoE 2013). Based on the above examples of low effect concentrations, the guidelines appear low enough to protect salmonids from chronic lethal and sub-lethal effects (Figure 2).

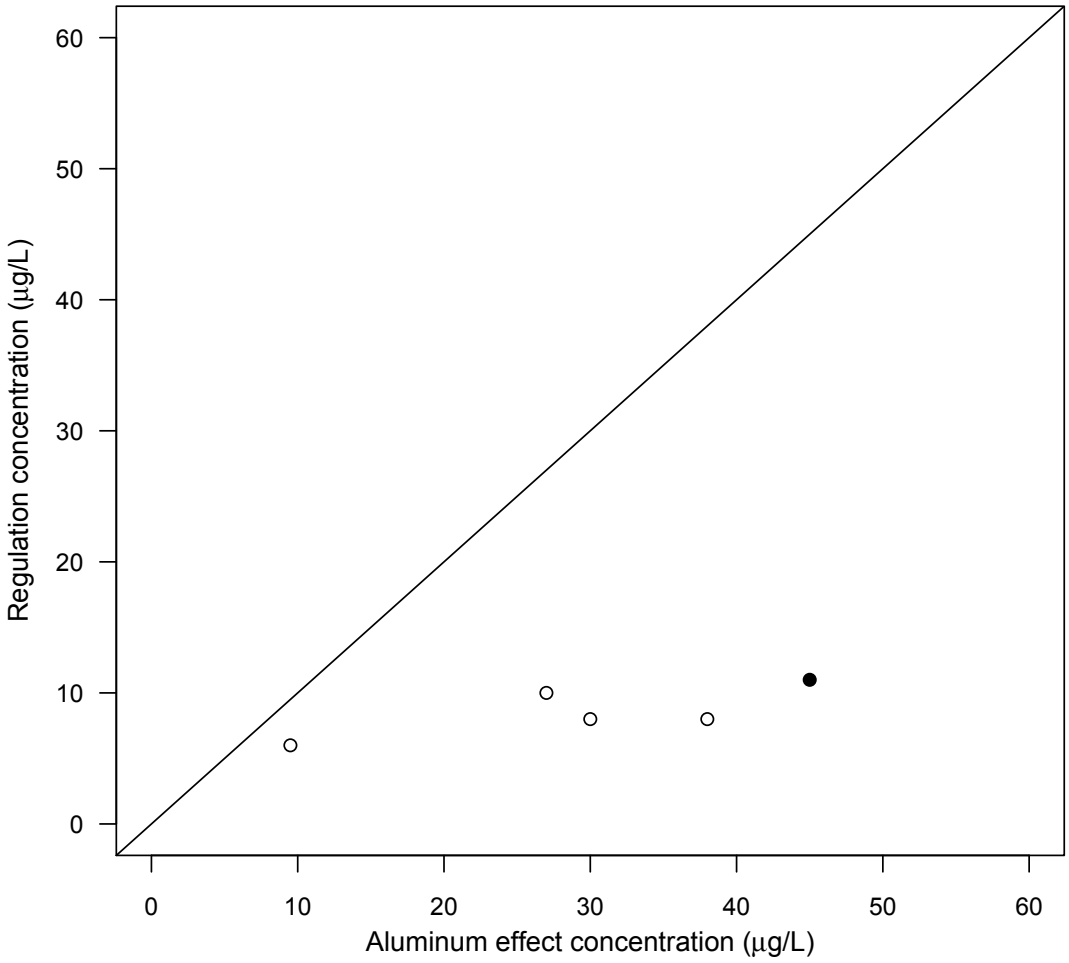

Figure 2. Lowest total and dissolved concentrations of waterborne aluminum (Al) observed to cause chronic sub-lethal (open circle) and lethal (closed circle) effects on salmonids plotted against the British Columbia government's water quality criteria for the protection of aquatic life (Regulation concentration; BCMoE 2013). Circles located above the 1:1 line show Al concentrations that cause an effect on fish at levels below regulation.

Cadmium (Cd)

Cadmium is a biologically non-essential heavy metal that occurs naturally in ores together with Cu, lead (Pb), and zinc (Zn), which is extremely toxic to salmonids at low concentrations (U.S.EPA 2001; Jarup 2003). Major human uses of Cd are in the manufacture of batteries and plastic stabilizers, and the primary sources of Cd pollution include smelter fumes and dusts, fertilizers, and municipal wastewater and sludge discharges (Eisler 1985). Cadmium concentrations are often highest in the localized regions of smelters, mines, or in urban industrialized areas, and wild salmonids are most likely to be affected by Cd in adjacent freshwater systems through waterborne and/or dietary exposure (Franklin et al. 2005). Background levels of Cd in uncontaminated aquatic systems range several orders of magnitude, from 0.05 μg/L to 0.2 μg/L (Korte 1983; Eisler 1985), whereas Cd concentrations in polluted waters are known to reach 200 μg/L (Jezierska et al. 2009).

The toxic nature of Cd is due to its actions as a Ca-antagonist; waterborne Cd mimics Ca, which can cause an imbalance and deficiency of Ca, and eventual death (Wood 2001). The two most important sites of Cd absorption in fish are the gills and the gastrointestinal tract (Szebedinszky et al. 2001). Waterborne Cd enters the gill epithelium through the same pathway as Ca, and effectively blocks active Ca uptake (Verbost et al. 1987, 1989; Playle et al. 1993). Importantly, the pathological effects of Cd are less severe in waters with high Ca. For example, waterborne Ca (in $CaCO_3$; measured as water hardness) can have a strong protective effect against waterborne-Cd toxicity by protecting Ca uptake, and by competitively inhibiting Cd binding to the gills (Playle et al. 1993; Hollis et al. 2001). An additional uptake route of waterborne Cd of fish is through the olfactory epithelium, which contains ciliated olfactory sensory neurons, and is in direct contact with surface waters (McIntyre et al. 2008). Olfactory sensory neurons are responsible for sensory inputs that convey important information about a fish's surrounding environment (McIntyre et al. 2008, 2012). Sub-lethal Cd accumulation in the olfactory system can cause significant behavioural effects relevant to a fish's ability to smell (Scott et al. 2003). Food may also be a significant route for Cd toxicity (Farag et al. 1999; Szebedinszky et al. 2001; Meyer et al. 2005), yet there remains insufficient knowledge on the risk of diet-borne Cd to salmonids. Cadmium concentrations lethal to salmonids range from 0.4 μg/L for juvenile rainbow trout (Hansen et al. 2002a) to 30 μg/L for juvenile sockeye salmon (*Oncorhynchus nerka*; Servizi and Martens 1978).

Sensory impairment

Predation

Low concentrations of Cd for relatively short exposure periods can affect the chemosensory function in prey fish. For example, juvenile rainbow trout exposed to 2 μg/L Cd for 7 days showed a significant reduction in normal predator avoidance behaviour when presented with an alarm substance (i.e., predator skin extract; Scott et al. 2003). The same exposure concentration and duration also inhibited the normal physiological response to stress (the release of plasma cortisol) of juveniles compared to unexposed fish. Importantly, these effects were present after two days in Cd-free water, which suggests that disruptive effects may persist well after exposure has ceased. Fish

exposed to 0.5 μg/L Cd for 7 days also showed a small, but statistically insignificant, disruption of the behavioural response (Scott et al. 2003).

Foraging

Foraging behaviour can be a sensitive indicator of metal toxicant stress on fish (Atchison et al. 1987; Little et al. 1990; Scherer et al. 1992). Several authors have reported a significant relationship between chronic sub-lethal Cd toxicity and reduced predation success in salmonids. For example, adult lake trout (*Salvelinus namaycush*) exposed to 0.5 μg/L Cd for 106-112 days showed a significant reduction in the number of prey captured and consumed compared to unexposed fish (Kislalioglu et al. 1996). A similar study reported that adult lake trout exposed to 0.5 μg/L Cd for 9 months showed reduced predation success compared to control fish when presented with unexposed rainbow trout prey, and foraging success decreased with increasing Cd concentration (Scherer et al. 1997). This same study revealed that unexposed lake trout showed the highest predation success when presented with juvenile rainbow trout previously exposed to 0.5 μg/L Cd for 9 months, though the results were not significantly different from controls. Finally, research by Riddell et al. (2005a, b) showed that exposure to 0.5 μg/L Cd for 30 days can alter the net energy available to juvenile brook trout (*Salvenius fontinalis*) by increasing the activity of individuals, and reducing their prey capture efficiency. Specifically, the capture efficiency of Cd-induced fish declined by 20%, and the activity of individuals increased by 25%, compared to unexposed fish (Riddell et al. 2005b).

Although the particular mechanisms that may have caused the reduction in predation success were not investigated, disruption of the olfactory system during waterborne exposure to Cd may play a role. Cadmium can accumulate in the olfactory system and inhibit olfactory functions in fishes, such as foraging (Hara 1986), and prey detection of predators such brook trout or lake charr may in some way be dependent on olfaction.

Social interactions

The social behaviour of individual fish, and dominance hierarchies within populations, can be altered by sub-lethal levels of Cd. Dominance hierarchies form between a pair and among groups of salmonids living in the confined or natural environment, owing to competition over limited resources such as food or mates (McGeer et al. 2011). Juvenile rainbow trout exposed to 2 μg/L Cd for 24 hours displayed significantly less aggressive attacks during agonistic encounters with non-exposed fish, and had a reduced ability to socially compete and become dominant even after 3 days depuration in clean water (Sloman et al. 2003a). Fish exposed to 0.8 μg/L Cd for 24 hours also showed a decreased tendency to become dominant compared to non-exposed fish, but the results were not statistically significant. When groups of rainbow trout were exposed to Cd during hierarchy formation, hierarchies developed faster than among non-exposed controls (Sloman et al. 2003a). In a separate study, exposure of juvenile rainbow trout to 3.3 μg/L Cd for 24 hours resulted in less aggressive competition than between control fish, and dominance amongst individuals was less easily determined (Sloman et al. 2003b). Furthermore, all Cd pre-exposed fish became subordinate when paired with non-exposed fish.

Both the decreased aggression of individual exposed fish, and faster formation of hierarchies among groups, may in part be attributed to a disruption in the olfactory system. Olfaction is thought to play an important role in the social interactions of salmonids (Brown and Brown 1993; Griffiths and Armstrong 2000), and an inability to detect odours in the water may reduce aggression amongst exposed fish, which in turn may increase the rate of hierarchy formation. However, interference by Cd toxicity with other physiological mechanisms linked to social behaviour, such as neurotransmitters (Winberg and Nilsson 1993) and hormone concentrations (Sloman et al. 2001), is thought to also play a role (Sloman et al. 2003a).

Avoidance

Salmonids can detect and respond to sub-lethal Cd levels. Lake whitefish (*Coregonus clupeaformis*) exposed to 0.2 µg/L Cd demonstrated a dichotomous response pattern where most fish showed avoidance to the source metal while a significant number appeared to be attracted (McNicol and Scherer 1991). The authors postulate that these opposing reactions may be an indication that such concentrations can disorient fish.

Physical impairment

Development

Sub-lethal Cd concentrations can reduce the growth and development of salmonids. Atlantic salmon alevins exposed to 0.47 µg/L Cd showed a significant reduction in growth compared to unexposed fish, and the results further indicated that these fish had a lower growth response threshold around 0.13 µg/L Cd (Rombough and Garside 1982). Rainbow trout alevins exposed to 0.25 µg/L Cd for 56 days weighed significantly less than fish exposed to the same Cd concentration for 35 days (Lizardo-Daudt and Kennedy 2008), and juveniles exposed to 1.0 µg/L Cd for 30 days showed a reduction in growth compared to control fish (Ricard et al. 1998). Finally, juvenile bull trout (*Salvelinus confluentus*) exposed to 0.79 µg/L Cd for 56 days showed a 28% reduction in weight change compared to unexposed fish (Hansen et al. 2002b).

In terms of biological performance (measured as reduced biomass in the population), exposure of juvenile brown trout to 0.87 µg/L Cd, and Atlantic salmon alevins to 1.0 µg/L Cd, caused a 20% and 38% reduction in biomass, respectively, compared with control groups (Rombough and Garside 1982; Brinkman and Hansen 2007). Additionally, juvenile brook trout exposed to 0.5 µg/L Cd for 30 days showed significantly poorer biological health, as measured by condition factor, compared to unexposed fish (Riddell et al. 2005b). The condition of exposed fish declined by 12-18% over a 30-day period, whereas the condition of control fish increased by 34%. The authors hypothesized that Cd-exposed fish shift their preference from nutritionally-rich pelagic prey to nutritionally-poor benthic prey (Riddell et al. 2005b). Importantly, an exposed fish's proximity to contaminated sediment may further exacerbate the sub-lethal effects of Cd on these individuals by intensifying or prolonging exposure through a combination of trophic transfer and altered foraging behavior (Cummins and Wuycheck 1971; Riddell et al. 2005b).

Reproduction

Sub-lethal Cd levels can negatively affect the reproductive functioning in salmonids. Rainbow trout eggs exposed to 0.05 µg/L Cd have been shown to hatch prematurely compared to unexposed eggs; yet exposure of eggs to 2.5 µg/L Cd resulted in delayed hatching, with >90% of eggs having hatched on the last day of the hatching period (Lizardo-Daudt and Kennedy 2008). Exposure of female juvenile rainbow trout to 5 µg/L Cd for 72 hours decreased egg yolk formation (vitellogenesis), and caused endocrine disruption in estrogenic pathways, which are signals that contribute to the function of the reproductive system (Vetillard and Bailhache 2005). Finally, a significant number of adult male brook trout exposed to 3.4 µg/L Cd for 24 weeks showed distressed activity and eventual death in the presence of female spawning behaviour compared to control fish (Benoit et al. 1976).

Immune response

Cadmium can affect stress in salmonids. Juvenile rainbow trout exposed to 1 µg/L Cd for 2 days showed elevated plasma cortisol levels compared to control fish, and a similar response was observed after 30 days (Brodeur et al. 1998; Ricard et al. 1998). Cortisol production is a general adaptation response of fish to stress (Brodeur et al. 1998).

Regulatory limits

The government of British Columbia does not have water quality criteria pertaining to Cd for the protection of aquatic life. Instead, the government relies on the guidelines approved by the government of Canada and the Canadian Council of Ministers of the Environment (CCME 2013). Similar to all metals except Al, guidelines are predicated on the concentration of $CaCO_3$ (hardness) in water. Based on the above examples of acute and chronic low effect concentrations, the federal guidelines appear low enough to protect salmonids from lethal and sub-lethal toxicity (Figure 3).

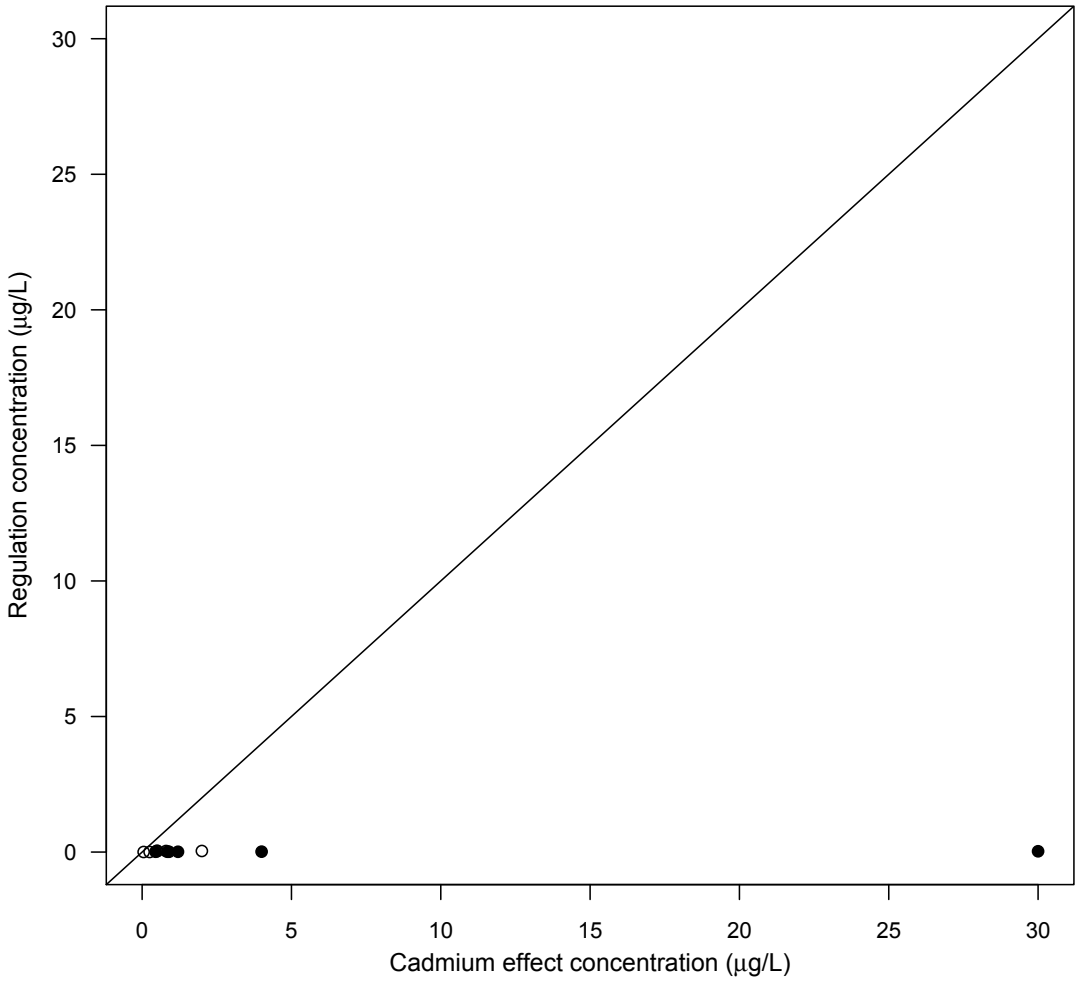

Figure 3. Lowest dissolved concentrations of waterborne cadmium (Cd) observed to cause acute and chronic sub-lethal (open circle) and lethal (closed circle) effects on salmonids plotted against the Canadian Council of Ministers of the Environment criteria for the protection of aquatic life (Regulation concentration; CCME 2013). Circles located above the 1:1 line show Cd concentrations that cause an effect on fish at levels below regulation.

Copper (Cu)

Copper is a biologically essential heavy metal that occurs naturally. Because of its abundance and availability, Cu was one of the first metals to be worked by humans 7,000 to 8,000 years ago (Schroeder et al. 1966), and continues to be widely used in building materials, automobile parts, and pesticides (Davis et al. 2001). Input of Cu into aquatic systems is primarily the result of industrial discharges from metal mines, smelters, municipal sewage, and agricultural pesticides and fertilizers (Eisler 1998a). Consequently, Cu is one of the most pervasive contaminants in urban and agricultural watersheds where salmonids reside (Baldwin et al. 2011).

Copper is one of the most toxic heavy metals in aquatic systems (Eisler 1998a). It is a neurobehavioral toxicant that interferes with the ability of fish to detect and respond to chemical signals in aquatic environments (Sandahl et al. 2007), and elevated concentrations can decrease growth, reproduction, and survival of salmonids (U.S.EPA 2007). These effects can manifest over a period of minutes to hours, and can be temporary or permanent. Importantly, a large body of scientific literature has shown that behaviors of salmonids can be compromised at concentrations of Cu that are at or slightly above ambient (i.e., background) levels (Hecht et al. 2007). Acute lethality in salmonids can occur at Cu concentrations that range 9-17 µg/L for juvenile rainbow trout (Chapman 1978a; Marr et al. 1999) to 103-240 µg/L for juvenile sockeye salmon (Davis and Shand 1978).

Sensory impairment

Olfaction

Olfactory impairment can manifest within minutes, with recovery rates being time and dose dependent. The inhibitory effects of 5 µg/L Cu on juvenile coho salmon (*Oncorhynchus kisutch*) have been observed within 10 minutes, and a 30-minute exposure was sufficient to produce the maximal reduction in odor detection (Baldwin et al. 2003). A 7-day continuous exposure of 4.4 µg/L Cu to juvenile coho produced similar results (Sandahl et al. 2004), but also suggests that the olfactory system of salmonids may not be able to acclimate to continuous Cu exposure (Hecht et al. 2007). While olfactory system recovery may be relatively quick (i.e., ≤1 day) when exposure time and concentrations are low (Baldwin et al. 2003; Sandahl et al. 2006), recovery can take weeks or months where sensory cell death occurs (Evans and Hara 1985; Moran et al. 1992; Sandahl et al. 2007). Hansen et al. (1999a) showed that a 4-hour exposure to 25 µg/L Cu caused a significant loss of olfactory receptor neurons in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout. Impairment recovery was quickest for fish exposed to lower Cu concentrations, yet no recovery was evident in Chinook and rainbow trout exposed to >50 µg/L and >100 µg/L, respectively (Hansen et al. 1999a). Similarly, Hara (1981) reported that no recovery was evident in fish exposed to 320 µg/L, and Hara et al. (1976) reported that recovery rates for juvenile rainbow trout exposed to 50 µg/L Cu were slower with increasing exposure times.

Several studies have estimated effect thresholds for reduced sensory responses in juvenile coho salmon exposed to Cu. Sandahl et al. (2004) produced threshold estimates for juvenile coho salmon of 4.4 µg/L and 11.1 µg/L Cu exposure, which corresponded to

reductions in odor recognition of 20% and 50%, respectively. Baldwin et al. (2003) estimated a 25% reduction in olfactory response of juvenile coho exposed to a concentration of Cu that ranged from 2.3 µg/L to 3.0 µg/L. Finally, Hecht et al. (2007) estimated a 29.2% reduction in olfactory response, and 31.8% reduction in alarm response, for juvenile coho exposed to between 0.44 µg/L and 1.42 µg/L Cu. Although these benchmark concentrations are derived using data from coho salmon, thresholds are considered applicable to other salmonids given the similar range of olfactory toxicity responses to comparable Cu exposures (Hecht et al. 2007; Baldwin et al. 2011).

Social interactions

The social behaviour of individual fish may influence the accumulation of Cu in the olfactory system. Within a dominance hierarchy, the social status of juvenile rainbow trout was found to affect the uptake of Cu. For example, sub-ordinate fish had a greater tendency to accumulate Cu from the water, and these fish consequently displayed higher tissue burdens when exposed to 30 µg/L Cu for 48 hours (Sloman et al. 2002).

Avoidance

Where distinct Cu gradients are present (e.g., near a point-source discharge), salmonids may use their sense of smell to detect and avoid contaminated waters. Several studies have reported that juvenile salmonids rearing in freshwater avoid Cu concentrations ranging from 0.7 µg/L to 7.3 µg/L (Sprague et al. 1965; Giattina et al. 1982; Hansen et al. 1999b; Svecevicius 2007), with Chinook salmon, rainbow trout, and Atlantic salmon, all displaying avoidance behavior in waters with Cu concentrations $\leq$ 2.4 µg/L. A recent study estimated that Cu concentrations as low as 0.84 µg/L for rainbow trout and 0.91 µg/L for Chinook salmon produced an avoidance response in 20% of the test population (Meyer and Adams 2010). An avoidance response to Cu-contaminated water may ensure that fish select favorable habitat conditions for survival, but also indicates that fish habitat is lost when contaminated (Saucier et al. 1991; Baldwin et al. 2003).

Long-term sub-lethal Cu exposure may impair a fish's avoidance response to higher Cu concentrations. For example, juvenile Chinook salmon exposed to 2 µg/L Cu for 25 to 30 days showed no preference for clean water versus contaminated water, and failed to avoid waters with Cu concentrations higher than 2 µg/L, including a failure to avoid Cu-contaminated water of 21 µg/L (Hansen et al. 1999b). Prior to acclimation to 2 µg/L Cu, Chinook salmon consistently avoided waters up to 21 µg/L Cu (Hansen et al. 1999b). The failure to avoid higher Cu concentrations suggests that the sensory mechanism responsible for avoidance responses was impaired by the long-term sub-lethal concentration of 2 µg/L Cu, which could result in further impairment of sensory-dependent behaviors essential for survival, or result in mortality if fish are later exposed to higher concentrations.

Migration

Sub-lethal Cu exposure may delay the upstream migration of salmonids to spawning habitat, and induce downstream movement by adults away from spawning grounds. The upstream spawning migration of Atlantic salmon has been reported to be interrupted by Cu concentrations of 20 µg/L (Sprague et al. 1965; Sutterlin and Gray 1973), with reverse

downstream migrations occurring whenever Cu concentrations exceeded 16.8 μg/L to 20.6 μg/L (Sprague et al. 1965; Saunders and Sprague 1967; Hecht et al. 2007). Copper levels higher than 38.4 μg/L are thought to completely prevent upstream migration by spawning Atlantic salmon (Saunders and Sprague 1967). There is also observational evidence that the spawning migration of Chinook salmon may be interrupted at Cu concentrations between 10 μg/L and 25 μg/L (Hecht et al. 2007). Furthermore, the effectiveness of home-stream water as an attractant to Atlantic salmon can be altered by Cu concentrations as low as 44 μg/L (Sutterlin and Gray 1973).

Low levels of Cu exposure can disrupt the downstream migration of juvenile salmonids and reduce survival in seawater. For example, yearling coho salmon exposed to ≥5 μg/L Cu exhibited delayed downstream migration to the ocean and reduced seawater survival, compared to unexposed control fish (Lorz and McPherson 1976). Migration success for juveniles decreased more with higher Cu concentrations and increasing exposure time. A 40% reduction in downstream migration success over a distance of 6.4 km was observed for juvenile coho exposed to 30 μg/L Cu for 72 hours, and a 76% decline in survival occurred for juveniles exposed to 20 μg/L Cu for 144 hours followed by transfer to seawater, compared to control fish (Lorz and McPherson 1976). Juvenile coho exposed to 15 μg/L Cu for 7 days in freshwater followed by transfer to seawater resulted in 40% mortality compared to 100% survival of unexposed fish (Schreck and Lorz 1978). Finally, juvenile sockeye salmon exposed to 30 μg/L Cu in freshwater for 144 hours and transferred to seawater for 24 to 48 hours also demonstrated incomplete smoltification and increased mortality compared to control fish (Davis and Shand 1978).

Predation

Low levels of Cu can cause a loss in sensory capacity for salmonids, and interfere with a fish's ability to detect and respond to chemical signals. Juvenile salmon in natural environments typically alter their behaviour when alerted by the smell of predators to avoid being captured; studies show that low levels of Cu can disrupt this anti-predator response. For example, exposure of 5 μg/L Cu impaired the neurophysiological response of juvenile coho to odorants within minutes (Baldwin et al. 2003). Similar impairment of olfactory function has been reported for juvenile steelhead trout exposed to 5 μg/L Cu for 3 hours (Baldwin et al. 2011), juvenile chum salmon (*Oncorhynchus keta*) exposed to 3 μg/L Cu for 4 hours (Sandahl et al. 2006), and juvenile coho exposed to 3.6 μg/L for 7 days (Sandahl et al. 2004). When the chemical odor is conspecific skin extract (i.e., a chemical cue of predator threat), unexposed fish reduce their swimming speed on average by 75% as an anti-predator response. However, juvenile coho exposed to 2.0 μg/L Cu for 3 hours and then presented with conspecific skin extract showed significant impairment of predator avoidance behaviours; fewer fish became motionless compared to pre-exposure (Sandahl et al. 2007). In a separate study, upstream predator cues presented to juvenile coho previously exposed to 5.0 μg/L Cu for 3 hours did not elicit an alarm response in contrast to control fish (McIntyre et al. 2012). Importantly, Cu-exposed juvenile coho were more vulnerable to predation by cutthroat trout, as measured by attack latency, survival time, and capture success rate; and, pre-exposing predators to similar Cu concentrations did not improve the evasion success of coho prey (McIntyre et al. 2012).

Physical impairment

Growth and swim speed

Sub-lethal Cu exposure can alter swimming and feeding behaviour. Rainbow trout fry exposed to 9.0 µg/L Cu showed a 10% reduction in critical swim speed, and the same effect was observed with juvenile rainbow trout exposed to 5.0 µg/L Cu in low pH water (Waiwood and Beamish 1978a, b). Juvenile brook trout exposed to 6 µg/L Cu showed a three-fold increase in swimming activity within minutes compared to pre-exposure activity levels (Drummond et al. 1973). However, the increase in activity did not equate to an increase in feeding behaviour, as these same fish showed a 40% reduction in foraging after 2 hours of exposure to 6 µg/L Cu (Drummond et al. 1973). The concomitant decrease in feeding behaviour with increased activity may best be explained by the need of fish to increase water flow across the gills for oxygen diffusion due to suffocation from gill damage and/or clogging of the lamellae with mucus, which is a direct effect of Cu toxicity on fish (Scarfe et al. 1982).

Several authors have reported reduced growth rates in Cu-exposed fish. For example, juvenile brook trout exposed to 3.4 µg/L Cu for 1-23 weeks showed a reduction in growth by 15-25% compared to control fish (McKim and Benoit 1971). Rainbow trout fry exposed to 4.6 µg/L Cu for 20 days experienced significantly reduced growth during the same period, and a 40-day exposure to 9.0 µg/L Cu resulted in a 45% reduction in mean body mass relative to control fish (Marr et al. 1996). A reduction in growth by 20% relative to control fish occurred in juvenile rainbow trout exposed to 4.0 µg/L Cu, and the same effect was observed in fish exposed to 2.0 µg/L Cu in low pH water (Waiwood and Beamish 1978a). Cu-induced growth suppression may be associated with depressed appetite and decreased food consumption (Lett et al. 1976; Waiwood and Beamish 1978b), but may more likely involve a metabolic cost related to metal detoxification (Dixon and Sprague 1981; Marr et al. 1995, 1996).

Immune response

Low level Cu exposure can stress fish, suppress resistance to pathogens, and increase susceptibility to secondary stressors. Brook trout fry exposed to 6 µg/L Cu for 5-20 hours showed increased cough frequencies, which is indicative of stress (Drummond et al. 1973). Juvenile coho salmon exposed to 18.2 µg/L for 30 days showed significantly reduced immune response to *Vibrio anguillarum*, the etiological agent of the fish disease known as vibriosis (Stevens 1977). Finally, coho fry exposed to 13.8 µg/L Cu for 7 days showed reduced survival after handling and confinement (Schreck and Lorz 1978), an indication that Cu exposure may increase the vulnerability of salmonids to secondary stressors such as disease and predator pursuits.

Reproduction

Low-level Cu concentrations are able to disrupt the reproductive performance and spawning behaviour of exposed fish. For example, adult brown trout exposed to 10 µg/L Cu for 4 days and then presented with female pheromones produced significantly less milt than control fish, and control fish demonstrated more pre-spawning behaviours than exposed fish (Jaensson and Olsen 2010).

Regulatory limits

Although British Columbia's water quality criteria for Cu do adequately protect salmonids from lethal effects, they often do not protect salmonids from acute and chronic sub-lethal effects based on the above examples of low effect concentrations (Figure 4). Several effects have been documented in fish exposed to Cu concentrations that are lower than the set criteria. However, it is important to note that the government criteria are based on *total* Cu, whereas the above examples of low effect concentrations exclusively pertain to *dissolved* Cu; the actual concentration of Cu that is dissolved, and thus available as a toxicant to fish in freshwater, tends to be lower than the total Cu concentration.

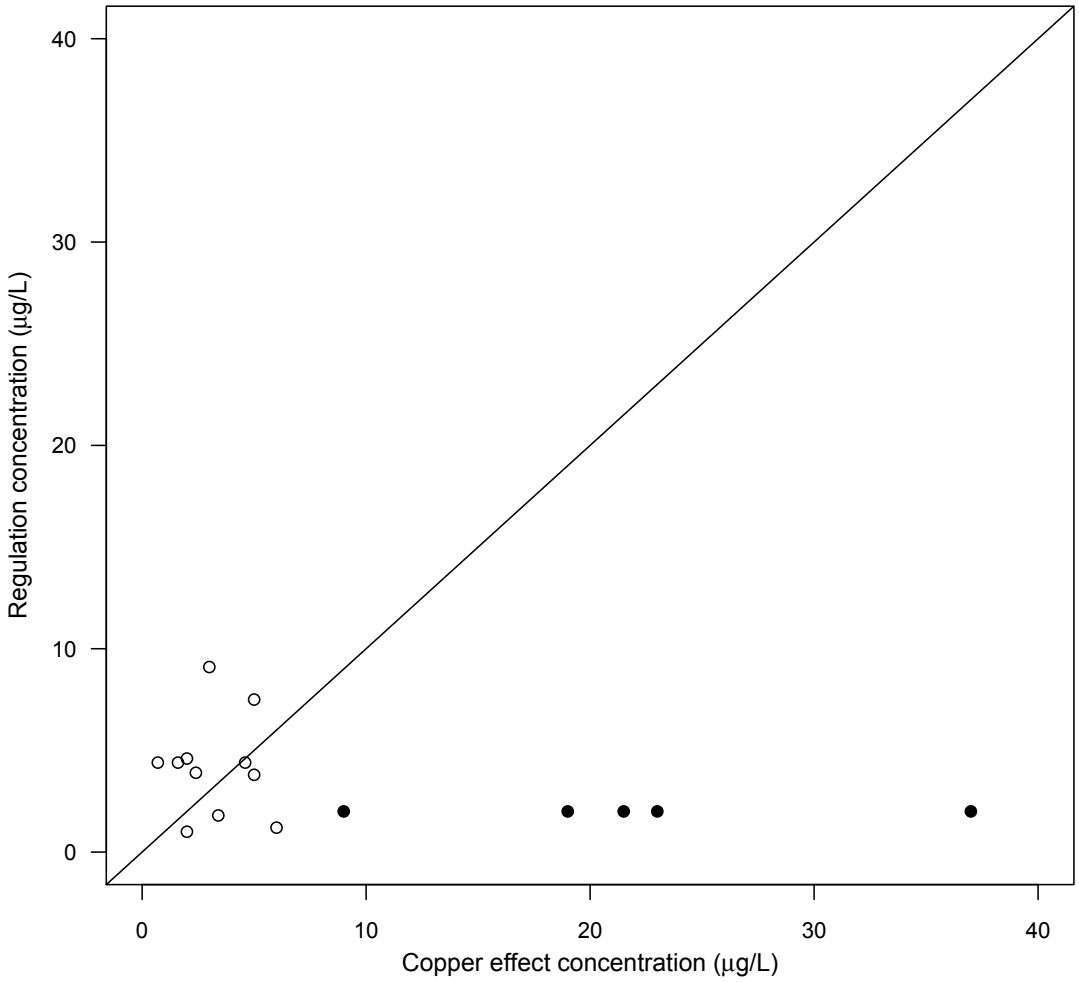

Figure 4. Lowest dissolved concentrations of waterborne copper (Cu) observed to cause acute and chronic sub-lethal (open circle) and lethal (closed circle) effects on salmonids plotted against the British Columbia government's water quality criteria for the protection of aquatic life (Regulation concentration; BCMoE 2013). Circles located above the 1:1 line show Cu concentrations that cause an effect on fish at levels below regulation.

Lead (Pb)

Lead is a biologically non-essential element that is present in nearly all surface waters (Eisler 1988). Most lead measurements from pristine aquatic systems in British Columbia are less than 1 µg/L (Nagpal 1987). Naturally occurring Pb has three oxidative states: metal, Pb (ii), and Pb (iv). While Pb (ii) is the primary state found in water, Pb (iv) is found in extreme conditions. Lead in the form of Pb (iv) compounds can also be produced artificially and released into the environment. For example, tetraethyl Pb (which was a widely used agent in gasoline) has been one of the principal sources of anthropogenic Pb due the subsequent release of emissions from gasoline and waste oil combustion (Nagpal 1987). The manufacture of Pb chemicals and batteries, incineration of refuse, and the effluent generated from mining, smelting, milling, sewage treatment facilities, leachate from landfills, and agricultural run-off are also primary sources of Pb in aquatic environments (Eisler 1988).

The major route of uptake for Pb in fish occurs across the gill (Hodson et al. 1978). Lead is similar to Cd in that the metal is a Ca-antagonist and neurotoxin (Sorensen 1991) that may affect the behaviour of salmonids (Sloman et al. 2003b). Lead accumulates in the bones and tissues of fish, and in high enough concentrations can impair the function of the liver, kidney, and spleen (Haider 1964), and can cause spinal deformities and death (Davies and Everhart 1973). Concentrations between 1,000 µg/L for juvenile rainbow trout (Rogers et al. 2003) and 3,362 µg/L for juvenile brook trout can cause death to 50% of fish in 96 hours (Holocombe et al. 1976).

Physical impairment

Lead exposure can inhibit essential physiological functions in salmonids. The exposure of juvenile rainbow trout to 13 µg/L Pb for 2 weeks caused a reduction in red blood cell enzyme (delta-aminolevulinic acid dehydratase; ALA-D) activity, and the activity was significantly reduced after 4 weeks compared to control fish; at 4 months, enzyme activity of exposed fish was reduced to 60% of control fish levels (Hodson 1976). The effect of Pb on ALA-D increases both with concentration and exposure time (Hodson 1976). Delta-aminolevulinic acid dehydratase is responsible for the production of hemoglobin, an essential oxygen-transport protein in red blood cells. In a follow-up study, Hodson et al. (1977) reported a 20% reduction in ALA-D activity in juvenile rainbow trout exposed to 10 µg/L Pb after only 2 weeks compared to control fish. Red blood cell enzyme activity of juvenile brook trout was inhibited by 20-45% only during exposure to 90-100 µg/L Pb for 2 weeks; 50-60 µg/L Pb over the same time period had little effect (Hodson et al. 1977). The maximum acceptable toxicant concentration for juvenile rainbow trout exposed to Pb has been estimated at between 3.0 µg/L and 13 µg/L in waters of alkalinity between 26 mg/L and 90 mg/L (Davies and Everhart 1973; Hodson 1976).

Development

Salmonids exposed to Pb can develop physical abnormalities. Juvenile rainbow trout exposed for 6 weeks during the eyed-egg stage to 7.6 µg/L Pb in soft water developed blacktail abnormalities (Davies et al. 1976). Roughly 40% of fish exposed as eggs to 13.2 µg/L Pb over the same time period developed blacktails, and 5.5% and 3.6% also

developed eroded caudal fins and curved spines, respectively (Davies et al. 1976). Physical deformities in juvenile brook trout increased with each generation exposed to Pb. For example, the percentage of second-generation alevins at hatch with curved spines was 5% when exposed to 119 µg/L total Pb (unknown dissolved concentration) compared to control fish, whereas the percentage of third-generation alevins at hatch with curved spines increased to 21% (Holocombe et al. 1976).

Reproduction

Sub-lethal Pb exposure can cause endocrine dysfunction in fish. For example, Ruby et al. (1993) reported decreased transformation of spermatogonia to spermatocytes in sexually maturing male rainbow trout exposed to 10 µg/L Pb for 12 days. Additionally, two-year old female rainbow trout exposed to 10 µg/L Pb for 12 days showed significantly reduced oocyte (cells from which eggs develop) growth compared to control fish (Ruby et al. 2000).

Regulatory limits

The government of British Columbia's criteria pertaining to Pb for the protection of aquatic life are low enough to protect salmonids from sub-lethal effects, based on the above examples of chronic low effect concentrations (Figure 5), and are very conservative to protect fish from lethal effects (Table 2; not shown on Figure 5).

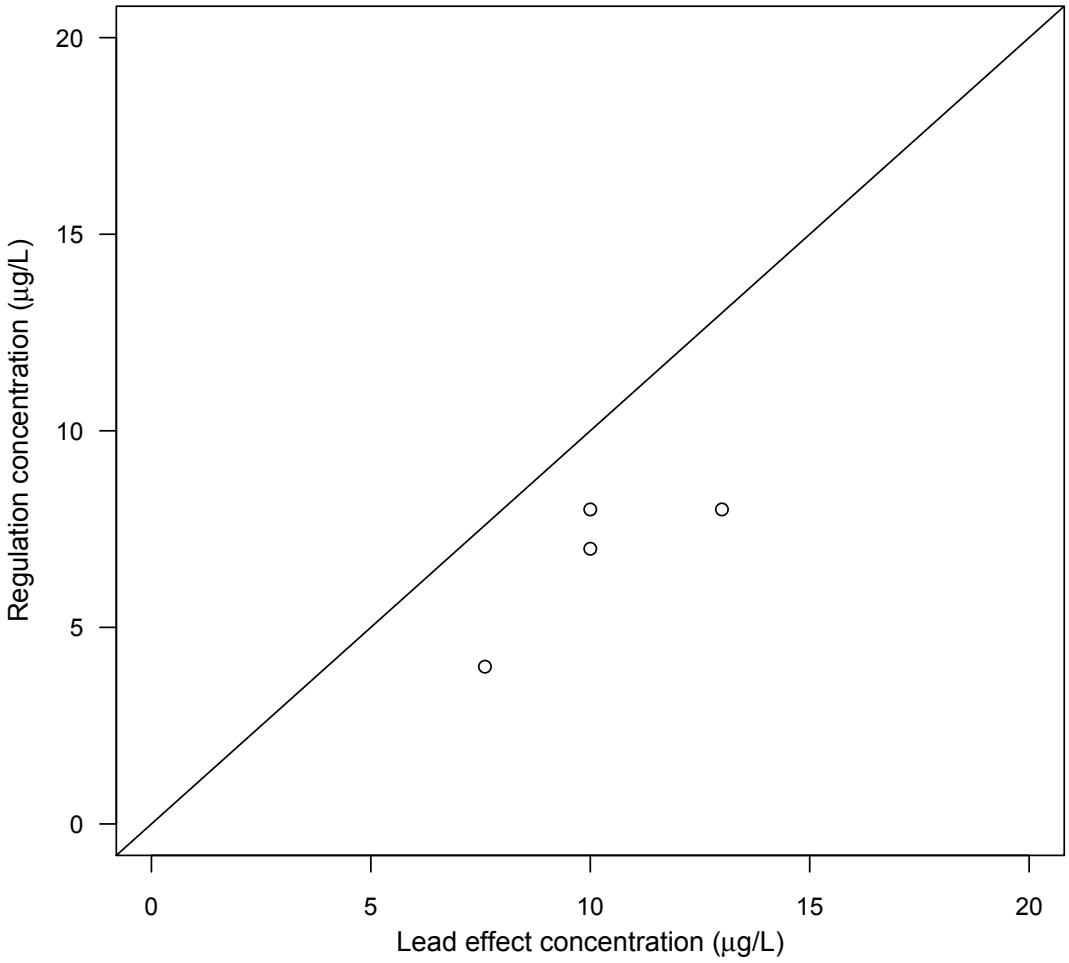

Figure 5. Lowest dissolved concentrations of waterborne lead (Pb) observed to cause chronic sub-lethal effects on salmonids (lethal concentrations exceed the figure scale) plotted against the British Columbia government's water quality criteria for the protection of aquatic life (Regulation concentration; BCMoE 2013). Circles located above the 1:1 line show Pb concentrations that cause an effect on fish at levels below regulation.

Nickel (Ni)

Nickel is a biologically essential element for the normal growth of fish (Eisler 1998b). The metal is a common component of natural freshwaters due to erosion and weathering, and levels of Ni generally range 1-10 µg/L in unpolluted areas (U.S.EPA 1980a), though human activities have contributed greatly to the more recent loadings in terrestrial and aquatic ecosystems. Mining, smelting, refining, fossil fuel combustion, and waste incineration are some of the most common contributors of Ni to the environment (Eisler 1998b). Nickel concentrations are comparatively elevated in fishes near Ni smelters, Ni-Cd battery plants, sewage outfalls, metal mines, and generally heavily polluted areas.

Nickel is a respiratory toxicant, and the gill is a key site of toxicity in fish; this is in contrast to most other metals that are ionoregulatory toxicants (Pane et al. 2003). Significant structural alterations to the brachial epithelium (i.e., swelling of the gill surface) have been observed in salmonids exposed to Ni (Hughes et al. 1979), which can lead to diffusive limitations of high performance gas exchange during intense swimming episodes (Pane et al. 2005). The large swelling of the respiratory surface is thought to be the result of profound Ni-induced disturbances in blood gases and acid–base balance, such as observed in juvenile rainbow trout by Pane et al. (2003). Concentrations of Ni as high as 8,100 µg/L can cause death to 50% of juvenile rainbow trout within 96 hours (Nebeker et al. 1985).

Sensory impairment

Avoidance

Salmonids respond to Ni in different ways at sub-lethal concentrations. At 6 µg/L total Ni (unknown dissolved concentration), juvenile rainbow trout showed a 40% increase in time spent in the area of the experimental tank with toxicant water compared to control fish; yet these same fish detected and avoided the toxicant water when total Ni concentrations reached 10-19 µg/L (Giattina et al. 1982). The concentration that caused a 50% reduction in the amount of time fish spent in an area relative to control times was estimated at 23.9 µg/L total Ni (unknown dissolved concentration; Giattina et al. 1982).

Physical impairment

Locomotion

Chronic exposure of fish to sub-lethal Ni concentrations can result in respiratory toxicity in the form of altered gill morphology, and impaired swim performance and oxygen consumption patterns. For example, juvenile rainbow trout exposed to 384 µg/L Ni for 12 and 24 days resulted in small (~7%; not statistically significant) decreases in critical swim speed compared to un-exposed fish (Pane et al. 2005). After 34 days of exposure to 394 µg/L Ni, juvenile rainbow trout showed 33% reduction in maximal oxygen consumption rate, and 38% decrease in aerobic activity, compared to control fish (Pane et al. 2005). Importantly, Pane et al. (2005) report that the aerobic capacity of exposed fish remained depressed despite a subsequent clean-water exposure period of 38 days, and suggest that such an impairment may reduce the overall fitness of juvenile rainbow trout by impairing predator avoidance, prey capture, and migration success.

Regulatory limits

The government of British Columbia does not have water quality criteria pertaining to Ni; instead, the government relies on the guidelines approved by the Canadian Council of Ministers of the Environment (CCME 2013). Based on the above examples of low effect concentrations, the federal guidelines at times are not low enough to protect salmonids from sub-lethal effects (Figure 6). There are two examples where juvenile rainbow trout exhibited non-normal behaviour when exposed to acute concentrations of Ni that were lower than the regulatory concentration. The first involved an attraction response to 6 µg/L Ni, and the second involved an avoidance response by 50% of fish to 23.9 µg/L Ni; the regulatory concentration based on the same water hardness as the study is set at 25 µg/L. Importantly, the concentrations that were shown to evoke a response in fish were measured as *total* Ni, and the actual *dissolved* concentrations were likely lower.

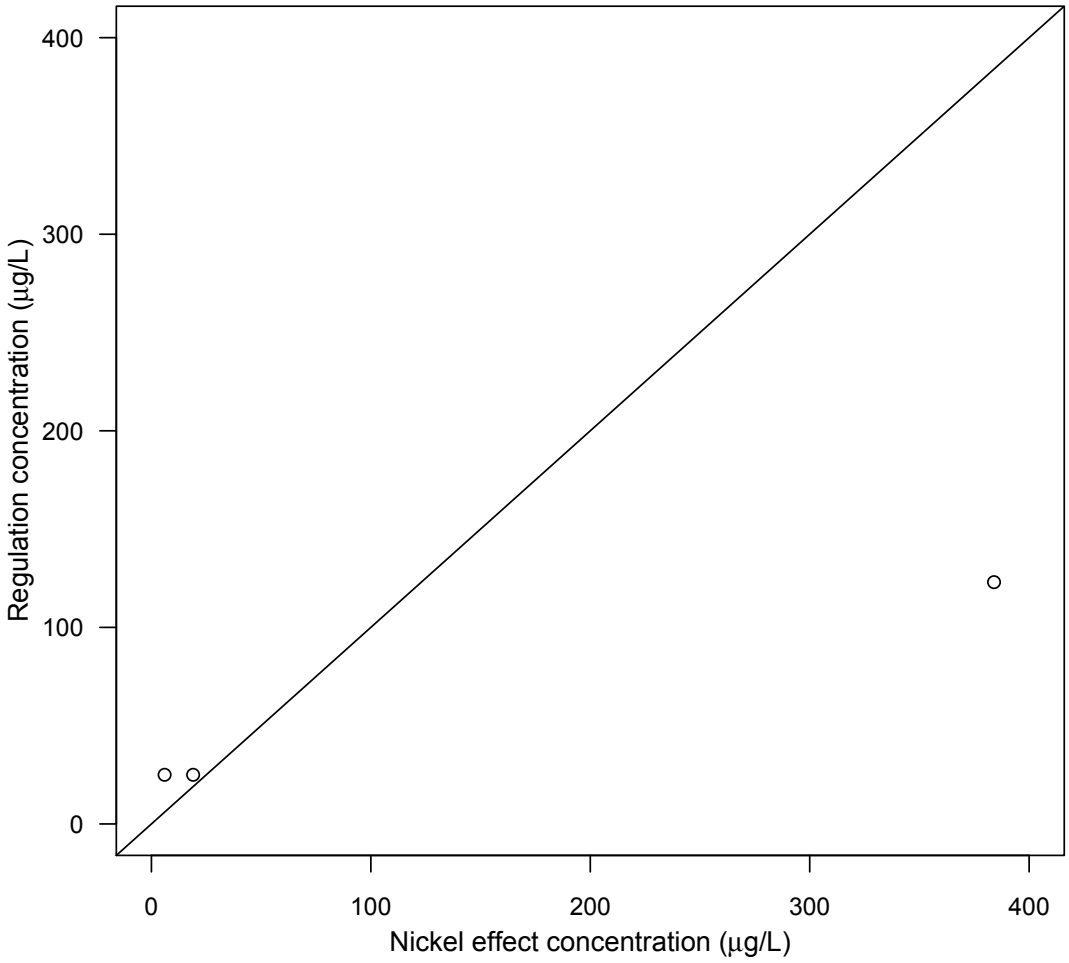

Figure 6. Lowest total concentrations of waterborne nickel (Ni) observed to cause acute and chronic sub-lethal effects on salmonids (lethal concentrations exceed the figure scale) plotted against the Canadian Council of Ministers of the Environment criteria for the protection of aquatic life (Regulation concentration; CCME 2013). Circles located above the 1:1 line show Ni concentrations that cause an effect on fish at levels below regulation.

Silver (Ag)
Silver is a rare and biologically non-essential element that is one of the most toxic metals known to aquatic organisms when in its ionic form (Ag^+; Davies et al. 1978; Hogstrand et al. 1996; Galvez and Wood 2002). Silver is commonly recovered as a byproduct from the smelting of Ni, and in the ores of Cu, Pb, gold (Au), platinum (Pt), and Zn, but is also naturally elevated in crude oil (Eisler 1996). In recent times, the principal industrial use of Ag was in the manufacture of photographic imaging materials, electrical and electronic products, coins, jewelry, and medicinal products such as antiseptics and germicides (Eisler 1996). Silver is commonly found in low concentrations (range = 0.09-0.55 µg/L) in natural waters, yet concentrations in biota tend to be highest near sites of sewage effluent and metal mines (U.S.EPA 1980b).

The mechanism of Ag toxicity in salmonids involves the blockage of sodium (Na^+) and chloride (Cl^-) transport at the gills (Wood et al. 1999). This inhibition can result in reductions in plasma Na^+ and Cl^- levels, and the decrease in plasma ions will eventually lead to circulatory failure and death of the fish (Hogstrand and Wood 1998; Morgan et al. 2005). Concentrations of Ag as low as 6.5 µg/L can cause death to 50% of juvenile rainbow trout within 96 hours (Davies et al. 1978).

Physical impairment
Development and mobility
Sub-lethal Ag exposure can alter feeding behaviour, growth, and swim speed. Food consumption by juvenile rainbow trout exposed to 5 µg/L Ag decreased by 23% compared to unexposed fish (Galvez and Wood 2002). These same fish were significantly smaller than unexposed fish after 10 days of exposure, and weighed 22% less than control fish after 23 days. Additionally, specific growth rates of exposed fish were reduced by 70% compared to unexposed fish, which further resulted in food-conversion efficiencies of exposed fish that were 58% lower than those measured in unexposed fish (Galvez and Wood 2002). In two separate studies, juvenile rainbow trout exposed to 0.1 µg/L and 0.17 µg/L Ag were significantly smaller (in mean length and weight) than unexposed fish after 60 days (Davies et al. 1978; Nebeker et al. 1983). The maximum acceptable toxicant concentration based on the lowest significant effect level for these fish was estimated to be < 0.1 µg/L Ag (Nebeker et al. 1983). Premature hatching of eggs and retarded sac-fry development as a result of exposure to 0.17 µg/L Ag was reported in rainbow trout by Davies et al. (1978). Finally, with regards to mobility, 5 days exposure to 5 µg/L Ag reduced the critical swim speed of juvenile rainbow trout by 14% compared to control fish (Galvez and Wood 2002).

Physiological response
Juvenile rainbow trout exposed to 5 µg/L Ag for 5 and 10 days showed reduced plasma Na^+ concentrations of 23% and 18%, respectively, compared to unexposed fish; however, plasma Na^+ concentrations returned to control concentrations after 15 days exposure (Galvez and Wood 2002). Similarly, mean plasma Cl^- concentrations were significantly reduced in fish exposed to 0.1 µg/L Ag on day 15, and 5 µg/L Ag on days 5 and 10; juveniles exposed to 5 µg/L Ag showed reductions in plasma Cl^- concentrations of 21% and 17% by days 5 and 10, respectively (Galvez and Wood 2002).

Regulatory limits

The government of British Columbia's criteria pertaining to Ag for the protection of aquatic life is low enough to protect salmonids from chronic lethal and sub-lethal effects, based on the above examples of low effect concentrations (Figure 7).

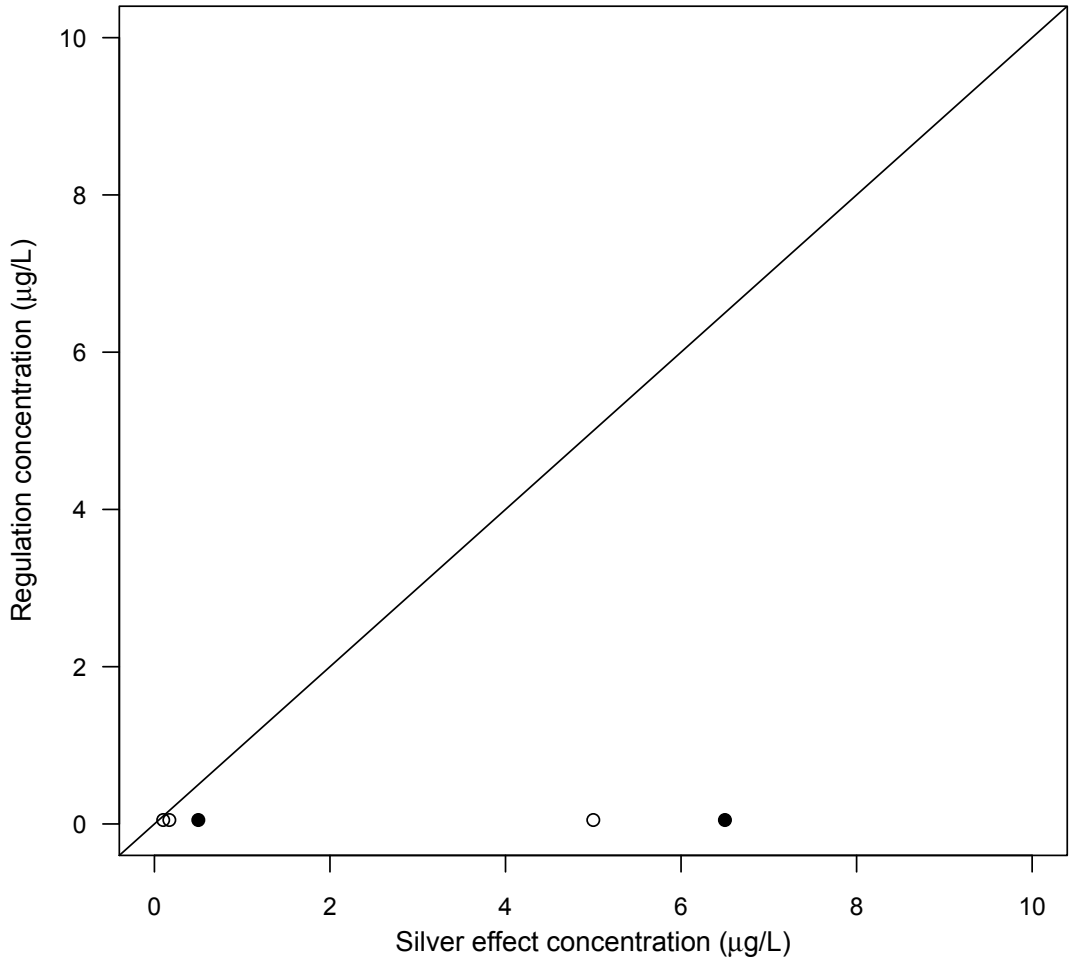

Figure 7. Lowest dissolved concentrations of waterborne silver (Ag) observed to cause chronic sub-lethal (open circle) and lethal (closed circle) effects on salmonids plotted against the British Columbia government's water quality criteria for the protection of aquatic life (Regulation concentration; BCMoE 2013). Circles located above the 1:1 line show Ag concentrations that cause an effect on fish at levels below regulation.

Zinc (Zn)

Zinc is a ubiquitous element that is biologically essential for the normal growth, physiology, and development of fish in minute quantities, but becomes toxic when in excess of cellular requirements (Wood 2001). Zinc is also one of the most common contaminants in aquatic systems, and tends to occur in elevated concentrations adjacent to areas of urban run-off, industrial discharges, and soil erosion (Bowen et al. 2006). The primary anthropogenic sources of Zn in the environment are from mining activities and metal smelters, though the production and use of Zn in die castings metal, alloys, rubber, and paints may also lead to its release to receiving systems through various waste streams.

Zinc is a Ca-antagonist, and sub-lethal concentrations of waterborne Zn can competitively inhibit the uptake of Ca^{2+} by fish at the gill (Hogstrand et al. 1995). Zinc and Ca^{2+} compete for the same sites on the gills of fish, hence the protective effect of increased water hardness ($CaCO_2$) on fish exposed to Ca-antagonist metals such as Zn. However, as the concentration of Zn relative to Ca increases in freshwater systems, the more Zn will effectively bind to sites on the fish's gill and outcompete Ca. The result is an accumulation of Zn on the gills, a decrease in branchial ionoregulation (i.e., the maintenance of the concentrations of the various ions in the body fluids relative to one another), and eventual death (Skidmore 1970). Concentrations between 93 µg/L for juvenile rainbow trout (Chapman 1978a) and 749 µg/L for juvenile sockeye salmon (Chapman 1978b) can cause death to 50% of fish in 96 hours.

Sensory impairment

Avoidance

Sub-lethal Zn exposure may induce avoidance of rearing habitat for salmonids. Estimates of threshold concentrations for avoidance (i.e., the lowest concentration that causes at least 50% of fish to show significant avoidance) of juvenile rainbow trout to Zn are reported to be 8.6 µg/L (95% confidence limits range 7.3-10.3 µg/L; Sprague 1968). Importantly, a decrease in water temperature raised the avoidance threshold for fish. For example, the threshold avoidance for juvenile rainbow trout exposed to 17°C water was 7.3 µg/L, whereas exposure to 9.5°C resulted in an estimated threshold avoidance of 8.4 µg/L; though the differences were not statistically significant (Sprague 1968). Juvenile Atlantic salmon exposed under similar laboratory conditions also showed avoidance to Zn, with an estimated threshold concentration of 53 µg/L (range = 27-104 µg/L; Sprague 1964). The difference in avoidance thresholds between the two species is thought to be the result of differences in behaviour characteristics (i.e., while Atlantic salmon tend to be less mobile, rainbow trout are active swimmers that may become more aware of toxicant gradients), rather than a difference in sensory perception (Sprague 1968).

Physical impairment

Immune response

Sub-lethal exposure of salmonids to waterborne Zn can induce physiological stress and reduce immune responses. For example, juvenile rainbow trout exposed to 81 µg/L Zn for 1 day showed significantly higher plasma glucose levels compared to control fish, and the rise in glucose was attributed to, and a sign of, stress (Wagner and McKeown 1982).

In a separate study of juvenile rainbow trout, fish exposed to 10 μg/L Zn for 30 days showed significantly inhibited immune response compared to control fish (Sanchez-Dardon et al. 1999).

Regulatory limits

There is one example of where juvenile rainbow trout exhibited avoidance behaviour when exposed to a chronic concentration of Zn that was lower than British Columbia's regulatory concentration (Figure 8). At 8.6 μg/L, 50% of fish avoided the toxicant compared to control fish; the regulatory concentration based on the same water hardness as the study is set at 33 μg/L. Regulatory concentrations do adequately protect salmonids from acute mortality.

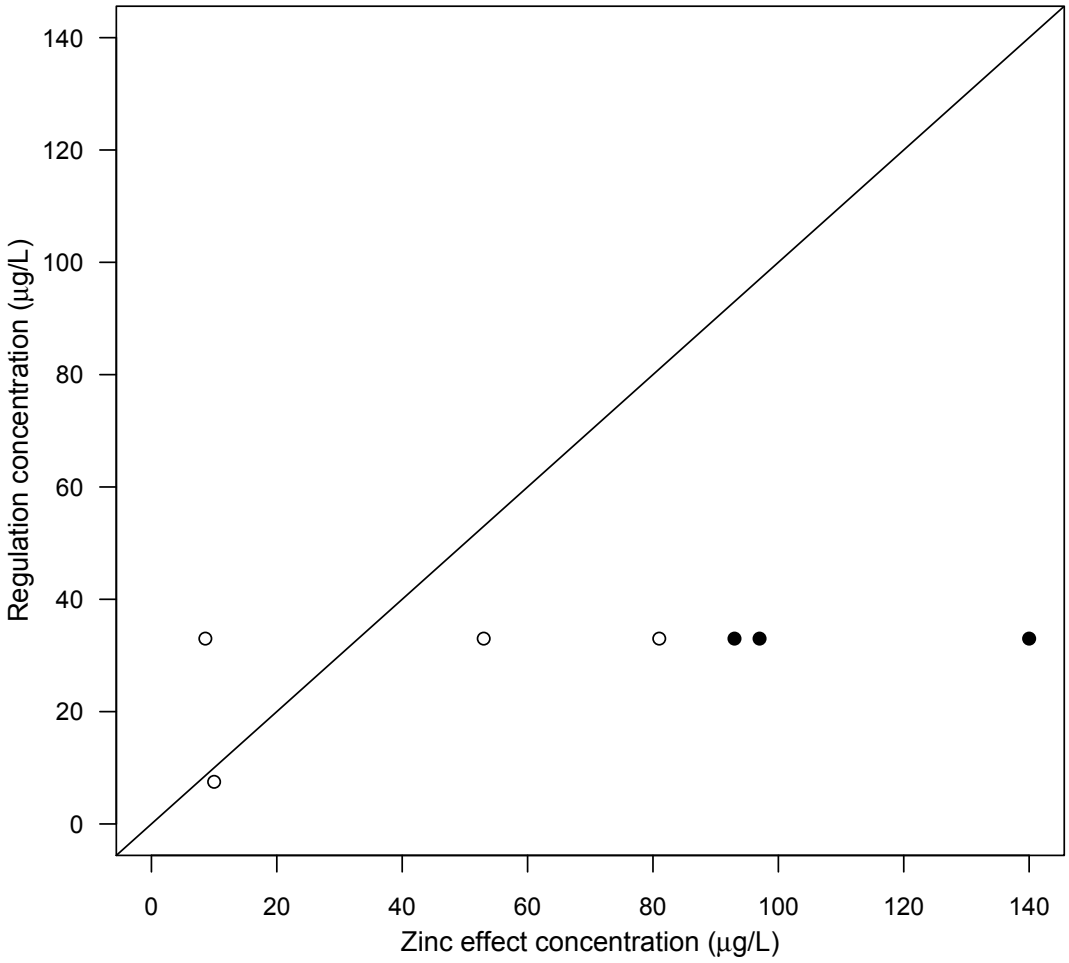

Figure 8. Lowest dissolved concentrations of waterborne zinc (Zn) observed to cause acute and chronic sub-lethal (open circle) and lethal (closed circle) effects on salmonids plotted against the British Columbia government's water quality criteria for the protection of aquatic life (Regulation concentration; BCMoE 2013). Circles located above the 1:1 line show Zn concentrations that cause an effect on fish at levels below regulation.

Metal Mixtures

One complicating factor in an assessment of the toxicity potential for any particular heavy metal is that, unlike laboratory studies that often examine metals in isolation, multiple metals typically occur and interact in aquatic systems (Boyd 2010). Toxicological studies that focus on the effects of single metals may not be environmentally realistic or relevant for assessing actual impacts on fish. Combinations of heavy metals may behave in three ways: additively (one metal acts independently from another, and the toxic effect of each metal in combination is the same as the effect of the individual metals), synergistically (different metals interact, and the toxic effect of the combined metals is greater than the additive effects of the individual metals), or antagonistically (different metals interact, but the toxic effect of the combined metals is less than the additive effects of the individual metals; Boyd 2010).

Additive effects

Mixtures of metals can illicit responses similar to the individual metals. Adult Chinook salmon preferred to spawn in waters relatively free of metals contamination compared to an adjacent tributary polluted with Cd (7 µg/L), Cu (2 µg/L), Pb (23 µg/L), and Zn (2,200 µg/L) in the Coeur d'Alene River, Idaho (Goldstein et al. 1999). Similarly, a study of adult Atlantic salmon during their spawning migration upstream in the Miramichi River, New Brunswick, reported that 22% of spawning fish avoided upstream waters with sub-lethal Cu (20 µg/L), and Zn (260 µg/L) by returning prematurely downstream (Saunders and Sprague 1967). The concentrations of these metal mixtures are similar to those avoided for individual metals. However, juvenile brown trout exposed to low levels of Pb (<1.7 µg/L), in a mixture of Cd (0.6 µg/L), Cu (6.5 µg/L), and Zn (32 µg/L) for 30 minutes showed a significant avoidance response compared to unexposed fish (Woodward et al. 1995); the Pb concentration used in this study was considerably lower than those cited above and the 26 µg/L Pb reported to induce avoidance behaviour in juvenile rainbow trout by Giattina and Garton (1983). A similar study with juvenile cutthroat trout reported avoidance behaviour when exposed to low levels of Pb (0.6 µg/L) in a mixture of Cd (0.30 µg/L), Cu (6.0 µg/L), and Zn (28 µg/L). Importantly, these same fish did not avoid water containing only 0.6 µg/L Pb (Woodward et al. 1997). Only when Cu or Zn were added did fish show an avoidance response, which suggests that Cu and/or Zn are the metals that fish are negatively responding to.

Synergistic effects

Metal mixtures can illicit responses in salmonids at lower concentrations than the individual metals. For example, the LC50 (lowest concentration that causes death in 50% of fish) for bull trout ranged from 0.83 to 0.88 µg/L Cd when exposed only to Cd, whereas the LC50 in a mixture with Zn was 0.51 µg/L Cd (Hansen et al. 2002a). A study that examined a mixture of Cu and Zn reported that the combination of the two metals reduced the avoidance threshold of fish by an order of magnitude below that for each metal tested individually. In combination, 0.4 µg/L Cu and 6.1 µg/L Zn produced an avoidance reaction in juvenile Atlantic salmon, whereas the individual thresholds were 2.3 µg/L Cu, and 53 µg/L Zn (Sprague 1964). Sprague and Ramsay (1965) also reported that lethal concentrations of mixtures of Cu and Zn on juvenile Atlantic salmon act two or three times faster than the metals singly. The threshold of avoidance for juvenile

rainbow trout exposed to metal mixtures has been estimated at 1.2 µg/L Cu, 0.11 µg/L Cd, 0.32 µg/L Pb, and 5 µg/L Zn (Hansen et al. 1999c), which is less than the single-metal avoidance concentrations for Cu and Zn, and may indicate metal interactions and synergy. With regards to physiological effects, while sub-lethal concentrations of Cd alone (and not Pb alone) induced disturbances to the normal Ca^{2+} influx at the gill of juvenile rainbow trout, the addition of Pb plus Cd exacerbated these effects in a synergistic fashion (Birceanu et al. 2008). Finally, mixtures of Cu and Al, and Cu and iron (Fe) were more than additive in their toxicity to ova of brown trout (Sayer et al. 1991).

Antagonistic effects

Metals such as Zn and Pb can reduce the negative effects of metals in isolation when combined in a mixture. Juvenile rainbow trout exposed to individual doses of sub-lethal concentrations of Cd, Zn, and mercury (Hg) experienced reduced immune system responses compared to control fish (Sanchez-Dardon et al. 1999). However, when Zn was combined with either Cd or Hg, the immune responses of exposed fish were similar to unexposed fish (i.e., no changes occurred). Finally, accumulation of Pb and Cd on the gills of juvenile rainbow trout were less than additive (i.e., antagonistic effect) when combined in a metal mixture likely because of the competition between these metals for binding sites (Birceanu et al. 2008).

Discussion

Sub-lethal toxicity of salmonids is a common consequence of low concentrations of heavy metals in aquatic systems. Indeed, all of the metals that were examined can negatively influence the physiology of salmonids to some degree at concentrations far below lethal levels. Sub-lethal concentrations can alter behaviours related to predator avoidance, foraging, migration, and social interactions, and can cause the physical impairment associated with growth and development, swimming efficiency, and immune system responses. Despite several complicating factors for studies of toxicant impacts on fish, metals such as Cu can cause effects at concentrations below regulatory limits in British Columbia, which is a concern for wild salmonid populations there.

Both the provincial and federal governments of Canada assign water quality guidelines for the protection of aquatic life, which includes fishes. While the two sets of guidelines are generally comparable, federal criteria tend to be more sensitive to metal toxicity for aquatic life (CCME 2013; BCMoE 2013). In British Columbia, federal guidelines are referred to in circumstances when guidelines for a particular metal have not been set; Cd and Ni are two such examples.

Most of the metal concentrations reported to invoke sub-lethal effects on salmonids are above the regulatory limits in British Columbia. For example, water quality guidelines assigned for Al, Cd, Pb, and Ag would protect against all of the effect concentrations that were found reported in the literature. However, there are several instances where sub-lethal effects on salmonids from metals such as Cu, Ni, and Zn have been reported at concentrations below the water quality guidelines of British Columbia and the federal government of Canada (Figure 9). All of these sub-lethal effects involve either avoidance behaviour or impaired olfaction. Avoidance of polluted water is one of the most sensitive responses of fish to toxicants, which enables them to survive in the perturbed environment (Sprague and Drury 1969), yet ultimately results in lost habitat. Alternatively, the inhibition of olfactory responses to predators by juvenile rainbow trout, and juvenile chum and coho salmon (Sandahl et al. 2006, 2007; Baldwin et al. 2011; McIntyre et al. 2012) is arguably the most ecologically severe sub-lethal consequence of metal toxicity. Copper-exposed salmonids are more vulnerable to predators (McIntyre et al. 2012), and have lower survival compared to unexposed fish. The disruption of such an anti-predator response in salmonids at effect concentrations below that assigned by the government of British Columbia is a very real scenario that may have implications for wild salmonid populations – especially considering that British Columbia is the largest producer of Cu in Canada.

Water quality criteria for the protection of aquatic life in British Columbia are not legislated, but rather serve as environmental benchmarks (BCMoE 2013). Specific industrial projects apply for permits to pollute, and the resulting metal concentrations in receiving waters of discharge may be higher than the provincial criteria. Two such examples are the open-pit copper mines (Noranda Bell and Granisle) located on Babine Lake in the Skeena River watershed, where the maximum authorized discharge for dissolved Cu from mine waste-water into the lake is five-fold higher than the regulatory guidelines for each mine (Remington 1996).

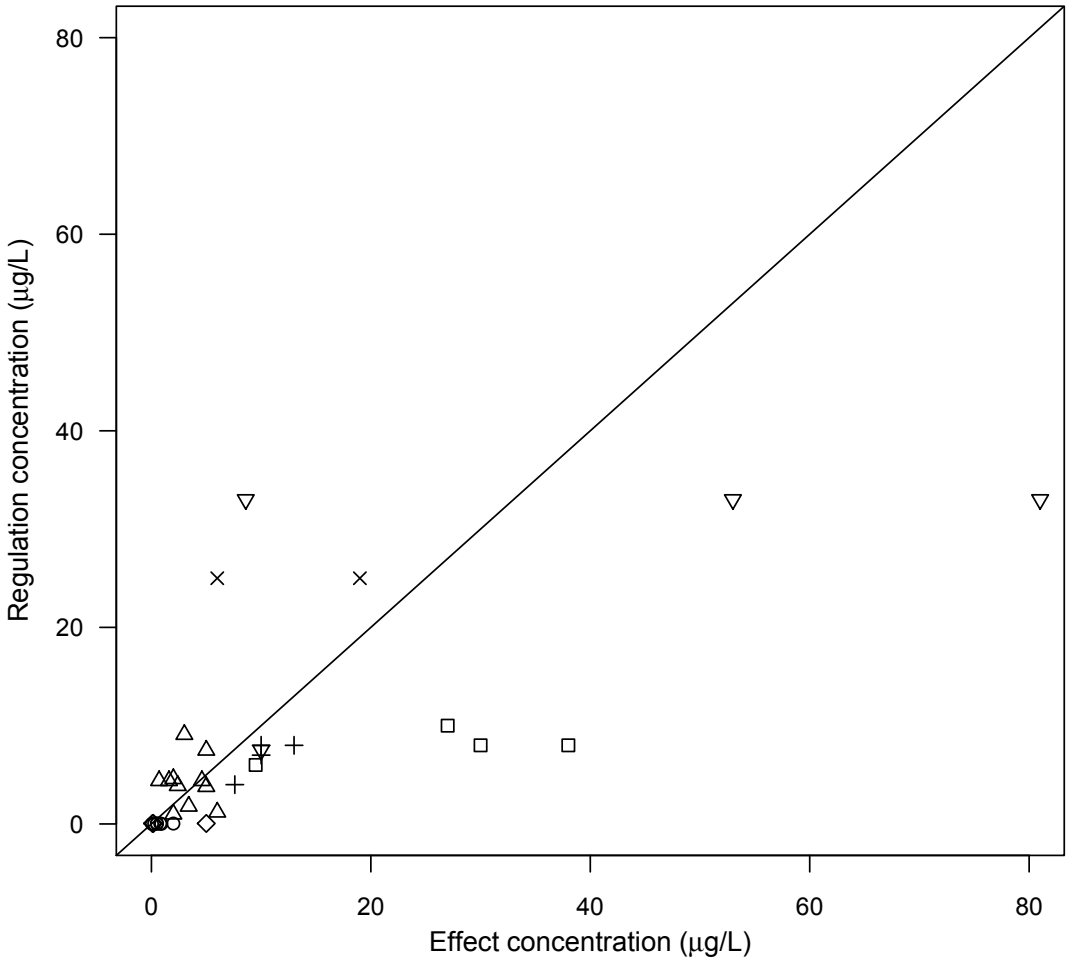

Figure 9. Lowest dissolved concentrations of waterborne metals observed to cause acute and chronic sub-lethal effects on salmonids plotted against the British Columbia government (BCMoE 2013) and Canadian Council of Ministers of the Environment (CCME 2013) water quality criteria for the protection of aquatic life (Regulation concentration). Symbols are represented as following: Al (□), Cd (◊), Cu (△), Ni (×), Pb (+), and Zn (∇); those located above the 1:1 line show concentrations that cause an effect on fish at levels below regulation.

The impact of heavy metals on fish is complex and depends on the chemical characteristics of water. Acidity (pH), hardness ($CaCO_3$), and organic matter are complicating factors in the determination of metal toxicity. For example, acidification of surface waters can increase the toxicity of metals to fish (Cusimano et al. 1986: Spry and Wiener 1991). The solubility of metals such as Al, are particularly susceptible to speciation as a direct function of pH, with the more toxic forms developing in acidic water (Freeman and Everhart 1971). Likewise, water hardness can influence the toxicity of metals to fish. Most heavy metals become more toxic in softer water, and this is likely due (in part) to the decrease in Ca^{2+} ions associated with softer water (Wood et al. 1999; Morgan et al. 2005; Monette et al. 2008). Some heavy metals enter the fish gill epithelium through the same pathway as Ca, and can effectively block active Ca uptake and result in ion imbalance of fish (Verbost et al. 1987, 1989; Playle et al. 1993). An increase in Ca via increasing water hardness can help out-compete metals at cellular binding sites that might otherwise result in the weakening of the tight junctions responsible for ion regulation (Booth et al. 1988; Freda et al. 1991; Monette et al. 2008). Thus, a difference in acidity and/or hardness of test water may at least partly explain why the effect concentrations for a given metal and fish species are often dissimilar between studies.

While acidity and hardness can influence the toxicity of metals to fish, the amount of organic matter in water (particularly in the form of dissolved organic carbon; DOC) may have a greater effect. In a study on the toxic effects of Cu on juvenile coho for example, the olfactory capacity of fish was partially restored by increasing DOC, whereas it was not affected by a change in acidity, and only slightly improved with increasing water hardness (McIntyre et al. 2008). A separate study showed that LC50s (the lowest concentration shown to cause an effect in 50% of the test population) can vary widely depending on the amount of DOC in the water (Ryan et al. 2004; Sciera et al. 2004), which has not been shown for acidity or hardness. Additionally, the effect of Cu on the olfactory system of adult brown trout and juvenile Chinook salmon was considerably reduced as a result of the amount of DOC in the test water (Jaensson and Olsen 2010; Kennedy et al. 2012). As a result, it has been recommended that DOC concentrations be considered when evaluating the potential impact of Cu on fish olfaction. However, one complication with this recommendation is that other heavy metals can potentially reduce the amelioratory effects of DOC through competition for binding sites with DOC (Kennedy et al. 2012).

There are at least four limitations when applying the reported effect concentrations on salmonids to real-life scenarios. First, the effect concentrations reported in this review are more often the lowest *detected effect*, not the actual *lowest effect concentration*. While the lowest *detected effect* describes the lowest concentration of a metal that was tested and found to cause an effect on fish, the *lowest effect concentration* is the actual lowest concentration of a metal that can cause an effect on fish. For example, Sandahl et al. (2007) showed that juvenile coho exposed to $\geq$2 μg/L Cu for 3 hours exhibited a suppression in predator avoidance behaviour (a lowest *detected effect*); yet, concentrations below 2 μg/L were not tested. Thus, uncertainty remains as to the precise threshold for olfactory impairment. This is also true for the olfactory impairment of

juvenile rainbow trout (Baldwin et al. 2011), and juvenile chum salmon (Sandahl et al. 2006). Further research is needed to determine threshold concentrations for most metals on salmonids.

Second, scientific studies rarely reflect natural exposure conditions. Most of the studies reported in this review were performed in a laboratory, where conditions for fish are near optimal. Parameters such as water flow, temperature, and food all tend to be favorable for fish and constant throughout the experimental period (Pyle and Merza 2007). However, fish in natural settings are typically forced to cope with sub-optimal conditions, and are frequently exposed to multiple stressors (Hecht et al. 2007); these added stressors may or may not alter the toxic effects of heavy metals. Not only can the chemical properties of water influence the availability of toxicants (Newman and Unger 2003), but the nutritional status of fish may influence the uptake of toxicants from the environment (Holmstrup et al. 2010). Thus, the measured toxicity of a particular metal at a given concentration in the laboratory may be less than for fish in contaminated waters.

Third, laboratory studies tend to examine metals in isolation, which may not be environmentally realistic or relevant for assessing actual impacts on fish. This is because fish are more often exposed to an assortment of metals, as well as organic chemical pollutants, in contaminated aquatic systems (Boyd 2010). Of the three ways that metals can behave (antagonistically, additively, or synergistically) when combined in a mixture, the greatest concern for fish is one of synergy. There are examples of mixtures of Cd/Zn, Cd/Pb, Cd/Cu/Pb/Zn, Cu/Al, Cu/Fe, and Cu/Zn with resulting effects on bull trout, rainbow trout, brown trout, and Atlantic salmon that were more than additive (Sprague 1964; Sprague and Ramsay 1965; Sayer et al. 1991; Hansen et al. 1999c; Birceanu et al. 2008). Although a review of the relevant literature on mixed metals suggests that studies more often report synergistic effects than the other two behaviour types (an indication that laboratory studies may underestimate sub-lethal effects on salmonids), future research is needed.

Finally, dietary metal concentrations are not incorporated into Canada's water quality guidelines despite the likely simultaneous occurrence of both waterborne and dietary routes of metal toxicity. The results reported in this literature synthesis only describe waterborne effects of metals on fish; yet, the consumption of metal-contaminated prey is also a common route of toxicity for predatory animals such as salmonids. Dietary Cu may at times be more important than waterborne Cu at reducing survival of salmonids during early life stages (Woodward et al. 1994). Importantly, waterborne and dietary metal exposures occur simultaneously in aquatic environments, and sub-lethal toxic effects of waterborne metals in salmonids may be exacerbated by dietary uptake. For example, the switch in feeding preference from motile to non-motile (benthic) prey by juvenile brook trout as a result of Cd-exposure is hypothesized to exacerbate the effects of Cd by intensifying or prolonging exposure through a combination of trophic transfer and altered foraging behavior (Riddell et al. 2005b). Yet, the water quality guidelines for heavy metals assigned by the governments of British Columbia or Canada do not factor the toxic effects of chronic dietary loading in the regulatory context. This may be because

there is insufficient knowledge on the risk of diet-borne metals to salmonids, and is an important area of future research.

To conclude, heavy metals are common persistent pollutants of aquatic ecosystems that can routinely cause sub-lethal effects in salmonids. Sub-lethal concentrations can alter behaviours related to predator avoidance, foraging, migration, and social interactions, and impair growth and development, swimming efficiency, and immune system responses. Within the regulatory context of the government of British Columbia and Canada's water quality guidelines, Cu is the metal of highest concern for wild populations. Research is needed not only to determine threshold concentrations for salmonids, but also to compare the effect concentrations derived from laboratory studies with natural environments, and examine the effects of metal mixtures and dietary toxicity on salmonids. Ultimately, a shift in research emphasis from the routine single metal - single organism - perspective, to population, community, and ecosystem scale is required to achieve a full understanding of the sub-lethal metal toxicity effects on salmonids.

Acknowledgements
I thank M. Cleveland, G. Knox, and J. Moore for helpful comments that greatly improved this report, SkeenaWild Conservation Trust for the patience, support, and resources required to compile all relevant information, and Mountain Equipment Co-op for funding.

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Appendix Table. Lowest waterborne metal concentrations observed to cause sub-lethal and lethal effects in various species of salmonids during specified life-cycle periods. Species abbeviations are: rainbow trout (RbT), Atlantic salmon (AtS), brown trout (BnT), lake charr (LkC), brook trout (BkT), bull trout (BlT), cutthroat trout (CtT), arctic grayling (ArG); Chinook salmon (CkS), coho salmon (CoS), sockeye salmon (SkS), and chum salmon (CmS). Phase refers to the life-cycle periods: egg (E), juvenile (J), and adult (A). All concentrations are measured as *dissolved* unless denoted [t], which refers to *total* metal concentration, and * signifies the concentration that causes death in 50% of fish exposed. Source numbers refer to corresponding literature cited.

Metal	Species (phase)	Effect	Effect concentration (µg/L)	Water hardness (mg/L)	Exposure duration	Source
Al		**Sub-lethal effects**				
	RbT					
	(J)	Impaired olfactory nerves	9.5	75	14 d	85
	(J)	Reduced swim speed	30.0[t]	-	7 d	182
	AtS					
	(J)	Reduced survival	6.0	-	-	88
	BnT					
	(J)	Reduced growth	>27.0	-	42 d	138
		Direct lethality				
	RbT					
	(J)	Death	5,200*	41	40 d	47
Cd		**Sub-lethal effects**				
	RbT					
	(E)	Premature hatching	0.05	7	294 d	94
	(J)	Reduced growth	0.25	7	56 d	94
	(J)	Reduced alarm response	2.0	120	7 d	149
	(J)	Competitive impairment	2.0	120	24 hr	154
	LkC					
	(A)	Reduced prey capture	0.5	81	106-112 d	84
	BkT					
	(J)	Reduced prey capture	0.5	156	30 d	126
	(J)	Reduced condition	0.5	156	30 d	127
	BnT					
	(J)	Reduced biomass	0.9	31	30 d	10
		Direct lethality				
	RbT					
	(J)	Death	0.4-0.5*	30	120 hr	59
	(A)	Death	5.2*	54	17 d	23
	BlT					

	(J)	Death	0.8-0.9*	30	120 hr	59
	ArG					
	(J)	Death	4.0*	41	96 hr	17
	BnT					
	(J)	Death	1.2*	29	96 hr	10
	CkS					
	(J)	Death	1.8-3.5*	23	96 hr	21
	CoS					
	(J)	Death	2.0*	22	9 d	23
	(A)	Death	3.7*	22	9 d	32
	BkT	Death	2.4*	44	96 hr	20
	SkS	Death	30.0*	84	160 hr	150
	(J)					
Cu		**Sub-lethal effects**				
	CkS					
	(J)	Habitat avoidance	0.7	25	20 min	57
	(J)	Reduced avoidance	2.0	25	21 d	57
	RbT					
	(A)	Habitat avoidance	1.0	284		167
	(J)	Habitat avoidance	1.6	25	20 min	57
	(J)	Reduced growth	4.6	25	20 d	100
	(J)	Impaired olfaction	5.0	58	3 hr	3
	(J)	Reduced swim speed	6.0	30	120 hr	179
	CoS					
	(J)	Impaired olfaction and alarm response	2.0	24-32	3 hr	137
	(J)	Reduced alarm response and survival	5.0	56	3 hr	105
	(J)	Impaired migration	5.0	89-99	144 hr	95
	(J)	Reduced stress resistence	13.8	90	8 d	146
	(J)	Reduced disease resistence	13.9	20-83	30 d	165
	AtS					
	(J)	Habitat avoidance	2.4	20	20 min	161
	(A)	Impaired migration	20.0	20	Indefinite	161
	CmS					
	(J)	Impaired olfaction	3.0	75	4 hr	136
	BkT					
	(J)	Reduced growth	3.4	45	1-23 wk	106
	(J)	Increased cough frequency	6.0	44-46	24 hr	35
	(J)	Reduced feeding	6.0	44-46	2 hr	35
	BnT					
	(A)	Reduced spawning	10.0	10	4 d	79

	SkS					
	(J)	Impaired migration and survival	30.0	36-46	144 hr	29
		Direct lethality				
	ArG					
	(J)	Death	2.6-49.3*	41	96 hr	17
	RbT					
	(J)	Death	9-17*	24-25	96 hr	21, 101
	(J)	Death	14-36*	41	96 hr	17
	CoS					
	(J)	Death	15-32*	41	96 hr	17
	(J)	Death	21-22	24-32	60 d	115
	CkS					
	(J)	Death	19*	24	96 hr	21
	CtT					
	(J)	Death	37*	18	96 hr	170
	SkS					
	(J)	Death	103-240*	36-46	96 hr	29
Pb		**Sub-lethal effects**				
	RbT					
	(J)	Physical deformity	7.6	28	42 d	27
	(J)	Reduced enzyme activity	10	135	14 d	88
	(A)	Reduced oocycte growth	10	121-125	12 d	132
		Direct lethality				
	RbT					
	(J)	Death	1000*	120	96 hr	128
	BkT					
	(J)	Death	3362*	44	96 hr	75
Ni		**Sub-lethal effects**				
	RbT					
	(J)	Attraction behaviour	6^t	28	20 min	51
	(J)	Avoidance behaviour	$>19^t$	28	20 min	51
	(J)	Reduced swim speed	384	140	12 d	121
	(J)	Reduced aerobic capacity	394	140	34 d	121
		Direct lethality				
	RbT					
	(J)	Death	8100*	33	96 hr	118
Ag		**Sub-lethal effects**				
	RbT					
	(J)	Reduced weight and length	0.1	36	60 d	117
	(J)	Reduced growth and swim speed	5	120	5-10 d	48
		Direct lethality				
	RbT					
	(J)	Death	0.5	36	21 d	117

	(J)	Death	6.5*	26	96 hr	28
Zn		**Sub-lethal effects**				
	RbT					
	(J)	Habitat avoidance	8.6	13-15	20 min	159
	(J)	Reduced immune response	10	-	30 d	134
	(J)	Increased stress	81	6-6.5	1 d	177
	AtS					
	(J)	Habitat avoidance	53	18	20 min	157
		Direct lethality				
	RbT					
	(J)	Death	93*	24	96 hr	21
	CkS					
	(J)	Death	97*	24	96 hr	21
	BnT					
	(J)	Death	140*	10	96 hr	42
	SkS					
	(J)	Death	749*	22	96 hr	22