Comparing impacts of metal contamination on macroinvertebrate and fish assemblages in a northern Japanese river (#53178)

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Comparing impacts of metal contamination on macroinvertebrate and fish assemblages in a northern Japanese river

Hiroki Namba [1] , **Yuichi Iwasaki** [Corresp., 2] , **Kentaro Morita** [3] , **Tagiru Ogino** [4] , **Hiroyuki Mano** [2] , **Naohide Shinohara** [2] , **Tetsuo Yasutaka** [5] , **Hiroyuki Matsuda** [1] , **Masashi Kamo** [2]

[1] Graduate School of Environment and Information Sciences, Yokohama National University, Yokohama, Kanagawa, Japan

[2] Research Institute of Science for Safety and Sustainability, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki, Japan

[3] Japan Fisheries Research and Education Agency, Sapporo, Hokkaido, Japan

[4] Geological Survey of Hokkaido, Hokkaido Research Organization, Sapporo, Hokkaido, Japan

[5] Research Institute for Geo-Resources and Environment, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki, Japan

Corresponding Author: Yuichi Iwasaki
Email address: yuichiwsk@gmail.com

Researchers have long assessed the ecological impacts of metals in running waters, but few such studies investigated multiple biological groups. Our goals in this study were to assess the ecological impacts of metal contamination on macroinvertebrates and fishes in a northern Japanese river receiving treated mine discharge and to evaluate whether there was any difference between the metrics based on macroinvertebrates and those based on fishes in assessing these impacts. Macroinvertebrate communities and fish populations were little affected at the downstream contaminated sites where concentrations of Cu, Zn, Pb, and Cd were 0.1–1.5 times higher than water-quality criteria established by the U.S. Environmental Protection Agency. At the two upstream contaminated sites with metal concentrations 0.8–3.7 times higher than the water-quality criteria, we detected a significant reduction in a few macroinvertebrate metrics such as mayfly richness and the abundance of heptageniid mayflies. There were, however, no remarkable effects on the abundance or condition factor of the four dominant fishes, including masu salmon. These results suggest that the richness and abundance of macroinvertebrates are more sensitive to metal contamination than abundance and condition factor of fishes in the studied river. Because the sensitivity to metal contamination can depend on the biological metrics used, and fish-based metrics in this study were limited, it would be valuable to accumulate empirical evidence for ecological indicators sensitive to metal contamination within and among biological groups to help in choosing which groups to survey for general environmental impact assessments in metal-contaminated rivers.

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[1]Graduate School of Environment and Information Sciences, Yokohama National University, Yokohama, Kanagawa, Japan

[2]Research Institute of Science for Safety and Sustainability, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki, Japan

[3]Japan Fisheries Research and Education Agency, Sapporo Hokkaido, Japan

[4]Geological Survey of Hokkaido, Hokkaido Research Organization, Sapporo, Hokkaido, Japan

[5] Research Institute for Geo-Resources and Environment, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki, Japan

Corresponding Author:

Yuichi Iwasaki[2]

16-1 Onogawa, Tsukuba, Ibaraki 305-8569, Japan

Email address: yuichiwsk@gmail.com

Abstract

Researchers have long assessed the ecological impacts of metals in running waters, but few such studies investigated multiple biological groups. Our goals in this study were to assess the ecological impacts of metal contamination on macroinvertebrates and fishes in a northern Japanese river receiving treated mine discharge and to evaluate whether there was any difference between the metrics based on macroinvertebrates and those based on fishes in assessing these impacts. Macroinvertebrate communities and fish populations were little affected at the downstream contaminated sites where concentrations of Cu, Zn, Pb, and Cd were 0.1–1.5 times higher than water-quality criteria established by the U.S. Environmental Protection Agency. At the two upstream contaminated sites with metal concentrations 0.8–3.7 times higher than the water-quality criteria, we detected a significant reduction in a few macroinvertebrate metrics such as mayfly richness and the abundance of heptageniid mayflies. There were, however, no remarkable effects on the abundance or condition factor of the four dominant fishes, including masu salmon. These results suggest that the richness and abundance of macroinvertebrates are more sensitive to metal contamination than abundance and condition factor of fishes in the studied river. Because the sensitivity to metal contamination can depend on the biological metrics used, and fish-based metrics in this study were limited, it would be valuable to accumulate empirical evidence for ecological indicators sensitive to metal contamination within and among biological groups to help in choosing which groups to survey for general environmental impact assessments in metal-contaminated rivers.

Keywords: Aquatic insects, fish, trace metals, abandoned mines, legacy mines, cross taxon congruence, environmental assessment, ecological risk assessment

Introduction

The impact of trace metals on aquatic ecosystems is an important issue in many regions of the world (Iwasaki & Ormerod 2012; Nriagu & Pacyna 1988). Laboratory toxicity tests of surrogate species are routinely used to assess the potential effects of metals on aquatic organisms and to provide a first step in inferring the effects on ecosystems. Responses of surrogate species in the laboratory, however, are not necessarily a good indicator for predicting responses of natural populations and communities (Clements, Cadmus & Brinkman 2013; Hickey & Clements 1998; Kimball & Levin 1985; Niederlehner et al. 1990). Thus, biological assessments of natural aquatic populations and communities that likely reflect time-integrated effects can provide useful information for evaluating ecological impairments in actual environments (Barbour et al. 1999).

In conducting the biological assessments in natural environments, the first question to answer is which aquatic organisms are to be investigated. For example, benthic macroinvertebrates have a wide range of sensitivities to contamination by metals (Iwasaki, Schmidt & Clements 2018). Also, macroinvertebrates have been the most frequently used in assessing the ecological impacts of metals in streams and rivers (Namba et al. 2020). Studies have indicated, however, that in aquatic ecosystems there are generally low correlations between changes in different biological groups (de Morais et al. 2018; Heino 2010; Namba et al. 2020). Despite this observation, surprisingly a limited number of studies published in peer-reviewed journals have investigated multiple biological groups in metal-contaminated rivers (Freund & Petty 2007; Namba et al. 2020). Therefore, to provide a more comprehensive assessment for overall ecosystem protection, it is important to investigate responses of not only macroinvertebrates but also other biological groups in metal-contaminated rivers.

The closed Motokura mine is located in the upstream area of the Tokushibetsu River in northern Japan (Figure 1). The mine mainly produced Cu, Pb, and Zn. In 1962, there were mass mortalities of Pacific salmon (*Oncorhynchus* spp.) in the river and Takayasu et al. (1964) concluded that mine drainage discharged into the river was likely a major cause. The mine was closed in 1967, and discharge from the mine is currently treated by using artificial wetlands. A bioassessment in 2017 using only macroinvertebrates showed that the abundance and richness of macroinvertebrates were little affected at downstream sites in the Tokushibetsu River (Iwasaki et al. 2020). Given that hatchery-reared masu salmon (*Oncorhynchus masou*) are released into the river system, it is important to evaluate the effects of mine drainage on not only macroinvertebrates as food resources for fish, but also on fish communities. However, no recent studies have evaluated the effects of mine discharge on fish in the river (but see Takayasu et al. 1964). We thus aimed to assess whether there are ecological impacts in the contaminated river by investigating macroinvertebrates and fishes. By doing so, we also evaluated whether there were any differences between metrics based on macroinvertebrates and those using fishes in detecting effects of metal contamination.

Materials & Methods

Study site

Field sampling of macroinvertebrates, fishes, and physicochemical characteristics was performed at nine sites in the Tokushibetsu River system in Hokkaido Island, northern Japan (Figure 1) from 26 to 28 June 2018. Five of the nine sites (sites S1a–S4) were in the Ofuntarumanai River, a metal-contaminated stream receiving treated mine discharge, and four reference sites (R1–R4) were in the main stream of the Tokushibetsu River. The reference sites were established at similar elevations as the contaminated sites, and study sites with the same numbers had similar elevation levels, for example, S1 (a and b) and R1. Sites S1a and S1b were upstream and downstream of the inflow of treated mine discharge, respectively (Figure 1). Permits for field sampling in the river were obtained from the local municipal office and Hokkaido government.

Water-quality parameters

During field sampling, three water samples (50 ml) were filtered from each study site for dissolved metals analysis (0.45 µm pore-size) and refrigerated in the field. Ultrapure nitric acid was added to those water samples on the day of sampling so that the pH was less than 2. Concentrations of dissolved Cu, Zn, Cd, and Pb were measured by using an inductively coupled plasma mass spectrometer (Element XR, Thermo Fisher Scientific, Tokyo, Japan) according to method 200.8 of the U.S. Environmental Protection Agency (U. S. EPA 1994). The limits of quantification were 0.001 µg/L for Cu, 0.06 µg/L for Zn, and 0.005 µg/L for both Cd and Pb.

Water temperature, dissolved oxygen, pH, and electrical conductivity were measured by using multi-parameter portable meters (Multi 3630IDS, Xylem Analytics Germany, Weilheim, Germany). Filtered water samples were also collected for measuring concentrations of dissolved organic carbon (DOC) and major ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-}). DOC was measured with a total organic carbon analyzer (TOC-L CPH, Shimadzu, Kyoto, Japan). Concentrations of major ions were measured with an ion chromatograph (Dionex ICS-1100/2100, Thermo Fisher Scientific). We calculated water hardness as $2.497 \times [Ca^{2+}] + 4.118 \times [Mg^{2+}]$.

As an index of contamination by multiple metals, we calculated the cumulative criterion unit (CCU; Clements et al. 2000) as the sum of the ratios of measured concentrations of four metals to the U.S. EPA hardness-adjusted water-quality criteria (WQC; U. S. EPA 2002):

$$\mathrm{CCU} = \sum \left({}^{m_i}/_{c_i} \right), \qquad (1)$$

where m_i is the concentration of dissolved metal i and c_i is the corresponding WQC. Hardness-adjusted WQC for Cu, Zn, Cd, and Pb were calculated at a water hardness of 10 mg/L based on the observed range of water hardness in this study (Table 1) and a previous study of the same river (Iwasaki et al. 2020). Note that, because the hardness of 10 mg/L is below the lower end of the hardness range of toxicity data used in the WQC development (20 mg/L; U. S. EPA 2002), caution is required for the interpretation of the calculated CCU values. Also, we did not consider water quality variables other than water hardness (e.g., pH and DOC) in this calculation (Iwasaki et al. 2020). This is because these variables varied little among study sites (Table 1), and U.S. EPA WQCs based on biotic ligand models that can consider the influence of water chemistry on metal toxicity were available only for Cu (U. S. EPA 2007).

Physical parameters

Average channel width (surface-water width measured at run) and riffle width were measured at each study site. Riffle width was averaged if benthic macroinvertebrates were collected at multiple riffles within individual sites. The catchment area of each site was quantified using a digital elevation model (50-m grid; Geographical Survey Institute of Japan, www.gsi.go.jp/ENGLISH/index.html) and a geographic information system (ArcGIS 10.2 for Desktop, Esri Japan, Tokyo, Japan). Maximum water velocity and depth were evaluated on the basis of measurements at multiple places in riffles that macroinvertebrates were collected at each study site. Current velocity was measured at 60% of water depth using an electromagnetic velocity meter (VR-301; Kenek, Tokyo, Japan).

Macroinvertebrates

At riffles at each site, we collected macroinvertebrates from five randomly chosen stones (maximum diameter, 14–27 cm) using a Surber net (mesh size, 0.355 mm). Samples were preserved in the field in 99.5% ethanol and washed through a 0.5-mm sieve in the laboratory. Macroinvertebrates remaining on the sieve were preserved in 70% ethanol and identified generally to genus or species level. For each stone from which macroinvertebrates were collected, water depth and current velocity (at 60% depth) were measured above its upper surface before collecting macroinvertebrates. The relative surface area of each stone was estimated as the product of its maximum diameter and maximum boundary length.

We analyzed eight community metrics for abundance (the number of individuals per stone) and richness (the number of taxa per stone): total abundance, total taxon richness, and the abundance and richness of three major aquatic insect orders in the benthic samples collected: Ephemeroptera (mayflies), Trichoptera (caddisflies), and Diptera (true flies). We also determined the abundance of the dominant families (i.e., Ephemerellidae, Baetidae, Heptageniidae, Hydropsychidae, Chironomidae, and Simuliidae) of the three major aquatic groups, which were defined as those families that accounted for more than 5% of the total abundance at each sampled stone and that were collected at more than 30% of the sampled stones (i.e., more than 14 stones of a total of 45 stones collected). For all macroinvertebrate metrics, the means and standard errors (as indicators for the uncertainty in site mean) of five stones at each site were calculated and used for further analyses. Macroinvertebrate abundances were $\log_{10}$-transformed (x + 1) before calculation of the site means to satisfy the assumptions of further analyses.

Fishes

At each site, we established five fish-sampling areas of approximately 5 m × 10 m to cover all of the habitats available (e.g., run, riffle, pool, and backwater) as much as possible. The distance between sampling areas was set to be >20 m. Fishes were collected from the downstream to the upstream end of each sampling area by using a backpack electrofishing unit (200–300 VDC; LR-20B, Smith-Root, Inc., Vancouver, WA, USA) and by throwing a cast-net. After one pass electrofishing, we used a cast-net four or five times within each sampling area to catch fishes in places where the pool was too deep for electrofishing to work. The captured fishes were anesthetized with phenoxyethanol and identified to species level if possible. The fork length was measured to the nearest 1 mm and body weight was measured to the nearest 0.1 g onsite.

A total of five fish species were collected: *Oncorhynchus masou* (masu salmon; Salmonidae), *Salvelinus leucomaenis* (white-spotted char; Salmonidae), *Barbatula oreas* (stone loach; Nemacheilidae), *Lethenteron* spp. (lamprey; Petromyzontidae), and *Tribolodon* spp. (Cyprinidae). We excluded *Tribolodon* spp. from the analyses because of their very limited abundance in our samples (only two individuals collected at R4) and determined the abundance (the number of individuals per sampling area) and condition factor of the other four species. The abundances of fishes were $\log_{10}$-transformed (x + 1), and the means and standard errors of the five replicate samplings at each site were used for later analyses. Also, the condition factor (CF) was calculated as an indicator representing the health status of individual fish by using the following equation:

$$\mathrm{CF} = \text{body weight (g)}/[\text{fork length (cm)}]^3 \times 1000. \qquad (2)$$

The condition factor is relatively easy to measure in the field and is a sensitive measure to detect the population-level consequences (Environment and Climate Change Canada 2015; Munkittrick & Dixon 1989a). Condition factor data were pooled at individual sites and used in later analyses.

Approximately 128,000 individual hatchery-reared masu salmon fry (*O. masou*; mean fork length: 5.6 cm) were released at a location between S2 and S3 on the contaminated river (44°41′49″N, 142°30′20″E; Figure 1) on 6 June 2018. Masu salmon were also released at three other locations including a tributary between R1 and R2 in the Tokushibetsu River basin in April and June 2018 (not shown). All released fry have thermally induced otolith marks (Volk, Schroder & Grimm 1999). To estimate the proportion of wild (natural-origin) and hatchery fish at each site, we sampled and checked the otolith marks of 20–27 masu salmon captured from each site in the laboratory. We then tested whether the inclusion of hatchery fish affected the results of our analyses.

Data analysis

All statistical tests were performed using R version 3.6.1 (R Core Team 2019). A significance level (α) of 0.05 was used. All the data used are available in the Supplementary File. In order to evaluate any effects at the five contaminated sites in the river receiving the mine discharge (i.e., S1a–S4), we first evaluated whether the site mean for each biological metric was within the 90% confidence interval for the four reference sites calculated on the basis of the standard deviation of the reference site means. We refer to the 90% confidence intervals as “reference ranges” that are assumed as likely observed ranges at reference sites. We then examined whether there were statistically significant differences in biological metrics between each contaminated site and the corresponding reference site with a similar elevation (R1 vs. S1a, R1 vs. S1b, R2 vs. S2, R3 vs.

S3, R4 vs. S4) by using a multiple comparison test (the single-step *P*-value adjustment; Bretz, Hothorn & Westfall 2010) followed by analysis of variance.

We used the results of these two analyses to operationally interpret the findings in three ways. If the mean of a given biological metric at a contaminated site was lower or higher than the corresponding reference range and was significantly lower or higher than that of the corresponding reference site by the multiple comparison test, we report that as an "adverse effect". If either one of these two results was observed we report that as "some effect of concern" and if neither was observed, we conclude that there was "no effect of concern."

Results

Physicochemical parameters

Concentrations of the four trace metals (Cu, Zn, Cd, and Pb) at the contaminated sites (S1a–S4) were approximately 2 to 190 times higher than the concentrations at the corresponding reference sites at similar elevations, except for the concentration of Zn (25 µg/L) at reference site R1, which was similar to the concentrations at S1a and S1b (Table 1). Concentrations of the metals excluding Cu at many contaminated sites were higher than the values of the U.S. EPA WQC, with higher concentrations and CCU values at the upstream sites. As previously observed (Iwasaki et al. 2020), there was little difference in metal concentrations between the site just upstream (S1a) and just downstream (S1b) of the inflow of treated discharge. This was most likely due to the high concentrations of metals in an upstream tributary draining the mining area (Iwasaki et al., unpublished data; Note that this is beyond the scope of the present study). CCU values were greater than 1 at all of the contaminated sites except for S4, indicating potential ecological risks based solely on the concentrations of the trace metals measured.

There were marginally lower values of pH, DOC, and water hardness at the metal-contaminated sites compared with reference sites (Table 1), all of which generally increase the bioavailability of metals (Adams et al. 2020). The estimated catchment areas of the metal-contaminated sites were generally larger than those of the corresponding reference sites with similar elevations (particularly between S2 and R2 and S3 and R3; Table 2), but other physical parameters were similar at those sites.

Macroinvertebrates and fishes

All eight community metrics for macroinvertebrates at S3 and S4 were within the reference ranges and were not significantly different from those at the corresponding reference sites (Figure 2), indicating that there were no effects of concern at those contaminated sites. On the other hand, there were adverse effects or some effects of concern for several of the community metrics at the upstream contaminated sites (S1a, S1b, and S2). For example, the mayfly richness at S2 (46% lower than at R2), the mayfly abundance at S1b (58% lower than at R1), and the caddisfly abundance at S1b (83% lower that at R1), were lower than the reference ranges and significantly lower than at the corresponding reference sites.

As with the metrics for the macroinvertebrate community, there were no effects of concern for the abundances of any of the six dominant macroinvertebrate families at S3 and S4 (Figure 3**)**. Although the variations within individual sites (i.e., the 90% confidence intervals of site means) were relatively large, the abundances of heptageniid mayflies at S1a and S1b (68% lower than R1) and the abundance of hydropsychid caddisflies at S1a (84% lower than R1) were lower than the reference ranges and significantly lower than at the corresponding reference sites, indicating adverse effects. Furthermore, there were some effects of concern for the abundances of Simuliidae and Chironomidae at some of the upstream contaminated sites (S1a, S1b, and S2).

No adverse effects were detected for the abundances or condition factor of the four fish species sampled, except for the abundance of *O. masou* at S3. Although there were some occasional effects of concern (e.g., the abundances of *B. oreas* at S2–S4; Figure 4), the sites where significant differences were observed or the mean value was higher or lower than the reference range varied depending on species. An adverse effect was detected for the abundance of *O. masou* at S3, whereas there were no effects of concern for this metric at other contaminated sites. The estimated proportions of released hatchery masu salmon at three of the reference sites (R1, R3, R4) and two of the contaminated sites (S1a, S1b) were 0%, whereas at R2, S2, S3, and S4 the proportions were 9% (2 of 23), 48% (13 of 27), 5% (1 of 21), and 18% (4 of 22), respectively. We estimated the abundances of wild *O. masou* at each site using these proportions and reran the two analyses. The reanalysis did not change the conclusions on the effects of mine contamination on the abundance of *O. masou* at contaminated sites.

Discussion

Our results suggest that macroinvertebrate communities and fish populations at the two downstream sites in the contaminated river in northern Japan, with CCU values <4, were little affected by metal contamination. This is consistent with the results of a previous study in 2017 sampling benthic macroinvertebrates (see Iwasaki et al. 2020 for the detailed discussion about the relationship between CCUs and effects on macroinvertebrate richness and abundance). Although we observed a significant decrease in the abundance of *O. masou* at S3, this is unlikely due to metal contamination because no such decrease was observed at the contaminated sites farther upstream with higher metal concentrations (Figure 4).

The concentration of dissolved Zn at the most upstream reference site (R1; Table 1) was relatively high compared with other reference sites and the U.S. EPA WQC (the CCU value was

2.1 at this site). The relative standard deviation for Zn based on three replicate water samples was small (2%) at R1. Although there were no measurements before the sampling campaign, the Zn concentration at R1 was comparable to other reference sites in the sampling conducted in September 2018 (1.0 μg/L; Table S1). It is impossible to determine the underlying reasons for the relatively high Zn concentration at R1, but it is reasonable to regard R1 as a reference site given that we detected no effects on macroinvertebrates and fishes at S3 and S4 with CCUs <4.

At the two upstream sites (S1a and S1b) with CCU values of approximately 9, we detected adverse effects with some macroinvertebrate metrics, such as the mayfly abundance and the abundance of heptageniid mayflies. Similar results were obtained in the benthic macroinvertebrate sampling in September 2018 (Figures S1 and S2). Among the macroinvertebrate metrics, mayfly richness and abundance are relatively sensitive to changes in metal contamination levels (Carlisle & Clements 1999; Clements, Vieira & Church 2010) and heptageniid mayflies are also well known as one of the families most sensitive to metal contamination (Clements et al. 2000; Iwasaki, Schmidt & Clements 2018). These results suggest that the metal contamination levels at sites S1a and S1b might have been close to the threshold where some adverse effects on sensitive macroinvertebrates would be detected.

We observed several significantly lower values for some macroinvertebrate metrics at S2 compared with the corresponding reference site (R2), but few effects were observed at S2 in a previous study (Iwasaki et al. 2020) or in the field sampling in September 2018 (Figures S1 and S2). The lower values at S2 could have been attributable to factors other than metal contamination, given that such lower values in the macroinvertebrate metrics were not often observed at the more upstream sites (S1a and S1b). One possible factor is the presence of stenopsychid caddisflies (3.4 individuals/stone at R2; they were absent at S2). The biomass of macroinvertebrates can increase following colonization of the riverbed by net-spinning stream caddisfly larvae, which construct fixed "retreats" that increase riverbed stability and modify the microhabitat structure (Nunokawa et al. 2008; Statzner 2012; Takao et al. 2006; Tumolo et al. 2019). Thus, we speculate that the differences in macroinvertebrate metrics between S2 and R2 might have been associated with the presence of stenopsychid caddisflies at R2. While biological assessments like this study are useful to detect ecological impairments in the field (Barbour et al. 1999), diagnostic tests of metal exposure and biomarkers may be valuable to further examine the causes (Forbes, Palmqvist & Bach 2006; Miller et al. 2015).

With the exception of *S. leucomaenis*, there were no effects of concern for fish abundances or condition factor, even at the two most contaminated sites (S1a and S1b). Although the abundance and condition factor of *S. leucomaenis* at S1a and S1b were significantly lower than at the corresponding reference site, they were still within the reference ranges. Given the relatively large variation and the limited number of individuals collected (a total of 13), further study is likely required to reach a more firm conclusion for this species as well as for *Lethenteron* spp. Results from fish sampling in September 2018 were generally similar to our results (Figure S3), but there are inconsistencies; the contaminated sites showing significant differences from reference sites varied between the two sampling periods. However, these results at least suggest that there is little need for concern about the effects of metal contamination on the abundance and condition factor of *O. masou*, for which there is a local stocking program.

Conclusions

Overall, the results from our field study suggest that the richness and abundance of macroinvertebrates (e.g., mayfly richness and abundance of heptageniid mayflies) are more sensitive to metal contamination than the abundance and condition factor of fishes in the river studied. These differences in responses to metal contamination have been reported in several studies, and metrics based on fishes are generally less responsive to metal contamination than those based on macroinvertebrates (Clements, Vieira & Church 2010; Freund & Petty 2007; Namba et al. 2020), which is consistent with our results. Although it is difficult to determine the underlying reasons for these differences, spatial–temporal characteristics of organisms' responses to metal contamination should have an important role; macroinvertebrates tend to reflect local and more recent conditions than fishes, which are more mobile and relatively longer-lived. Compared with macroinvertebrates, however, the number of fishes captured and the associated metrics were limited in our study. For instance, benthic fishes such as sculpins can be more responsive to metals than salmonids (Maret & MacCoy 2002; Munkittrick & Dixon 1989b), and physiological and biochemical responses of fishes have been employed as early warnings for the population level effects (Forbes, Palmqvist & Bach 2006; Hanson 2009). It would therefore be valuable to accumulate empirical evidence for ecological indicators sensitive to metal contamination within and among biological groups to choose which groups to survey for general environmental impact assessments in contaminated rivers.

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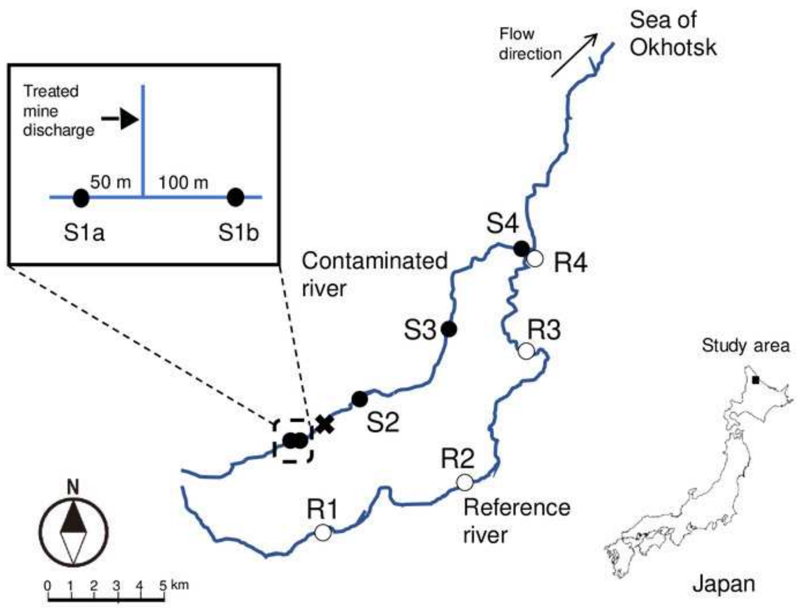
Figure 1

Map showing location of the study area and sampling sites.

The cross mark indicates the location where hatchery-reared masu salmon were released (see text for details). Map was created using Quantum Geographic Information System (QGIS version 3.10; http://qgis.osgeo.org) based on National Land Numerical Information provided by Geospatial Information Authority of Japan (http://nlftp.mlit.go.jp/ksj/).



Sea of Okhotsk
Flow direction
Treated mine discharge
50 m
100 m
S1a
S1b
S4
R4
Contaminated river
S3
R3
S2
R2
R1
Reference river
N
0 1 2 3 4 5 km
Study area
Japan

1

Figure 2

Abundance (number of individuals) and taxon richness (number of taxa) of macroinvertebrates at reference (R1–R4) and contaminated (S1a–S4) sites.

The same symbols indicate sites with similar elevations. Error bars indicate 90% confidence intervals of site means. Horizontal lines and gray areas are the means and 90% confidence intervals calculated from means for the four reference sites, respectively. Asterisks indicate contaminated sites with values significantly lower or higher than the corresponding reference sites with similar elevation ($P < 0.05$).

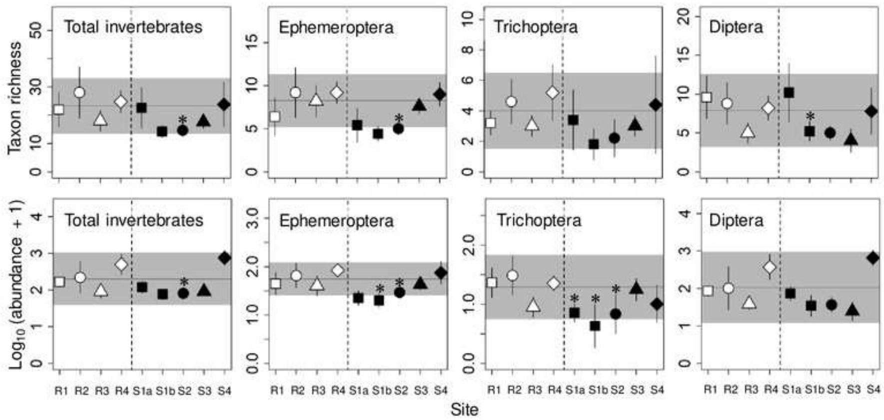

Total invertebrates
Ephemeroptera
Trichoptera
Diptera
Taxon richness
Log10 (abundance + 1)
R1 R2 R3 R4 S1a S1b S2 S3 S4
Site

Figure 3

Abundance (number of individuals per stone) of dominant families of macroinvertebrates at reference (R1–R4) and contaminated (S1a–S4) sites.

The same symbols indicate sites with similar elevations. Error bars indicate 90% confidence intervals of site means. Horizontal lines and gray areas are the means and 90% confidence intervals calculated from means for the four reference sites, respectively. Asterisks indicate contaminated sites with values significantly lower or higher than the corresponding reference sites with similar elevations ($P < 0.05$).

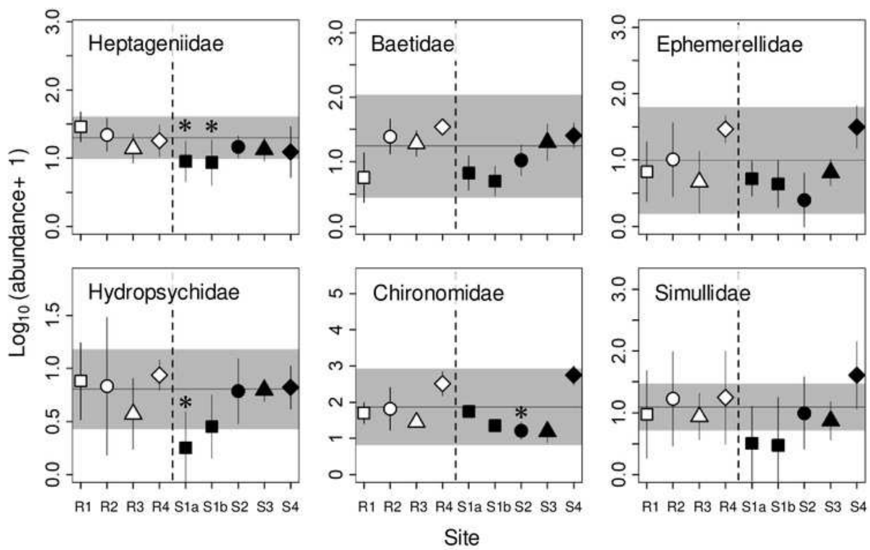

Heptageniidae
Baetidae
Ephemerellidae
Hydropsychidae
Chironomidae
Simullidae
Log10 (abundance+ 1)
R1 R2 R3 R4 S1a S1b S2 S3 S4
Site

Figure 4

Abundance (number of individuals per 50 m^2) and condition factor of fishes at reference (R1–R4) and contaminated (S1a–S4) sites.

The same symbols indicate sites with similar elevations. Error bars indicate 90% confidence intervals of site means. Horizontal lines and gray areas are the means and 90% confidence intervals calculated from means for the four reference sites, respectively. Asterisks indicate contaminated sites with values significantly lower or higher than the corresponding reference sites with similar elevations ($P < 0.05$). For *S. leucomaenis*, the 90% confidence interval was not calculated from reference site means because this species was only captured at one reference site (R1).

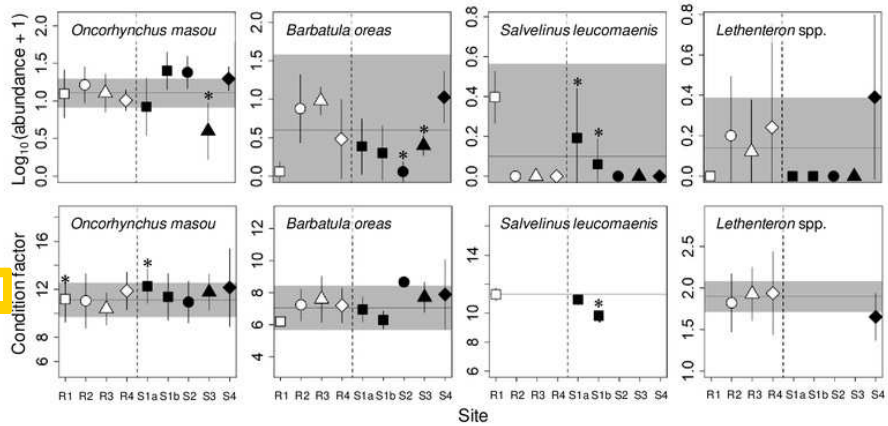

Oncorhynchus masou
Barbatula oreas
Salvelinus leucomaenis
Lethenteron spp.
Log10 (abundance + 1)
Condition factor
R1 R2 R3 R4 S1a S1b S2 S3 S4
Site

Table 1 (on next page)

Water-quality measurements at study sites in the Tokushibetsu River system, northern Japan (26–28 June 2018)

Table 1. Water-quality measurements at study sites in the Tokushibetsu River system, northern Japan (26–28 June 2018)

Site	Cu	Cd	Pb	Zn	CCU	Temp	pH	DO	DOC	Conductivity	Hardness
	Dissolved (μg/L)					(℃)		(mg/L)	(mg/L)	(μs/cm)	(mg/L)
Contaminated sites											
S1a	1.0	0.13	0.69	24.0	8.4	9.1	7.1	11	0.3	54	13
S1b	1.1	0.16	0.71	27.5	9.4	9.3	7.0	11	0.4	52	13
S2	0.8	0.17	0.25	25.9	6.8	9.4	7.2	11	0.3	57	14
S3	0.5	0.07	0.23	11.5	3.8	11.5	7.4	11	0.4	56	13
S4	0.3	<0.005	0.05	4.8	0.9	10.2	7.5	11	0.7	60	14
Reference sites											
R1	0.1	<0.005	0.09	25.3	2.1	10.6	7.5	10	0.8	41	10
R2	0.1	<0.005	<0.005	0.1	0.1	10.2	7.5	11	0.7	46	11
R3	0.1	<0.005	0.04	0.1	0.3	11.7	7.7	11	0.6	48	11
R4	0.1	<0.005	0.03	0.3	0.3	9.7	8.0	12	0.7	50	12
WQC	1.3	0.05	0.19	16.8							

DO, dissolved oxygen; DOC, dissolved organic carbon; CCU, cumulative criterion unit (see text for details); Temp, temperature; WQC, U.S. EPA chronic water-quality criterion at a water hardness of 10 mg/L (U. S. EPA 2002). Limits of quantification for Cu, Zn, Cd, and Pb were 0.001, 0.06, 0.005, and 0.005 μg/L, respectively.

Table 2 (on next page)

Physical parameters at the study sites in the Tokushibetsu River system, northern Japan

Table 2. Physical parameters at the study sites in the Tokushibetsu River system, northern Japan

Site	Elevation (m a.s.l.)	Catchment area (km^2)	Channel width (m)	Studied riffles			Sampled stones		
				Width (m)	Maximum depth (cm)	Maximum velocity (cm/s)	Depth (cm)	Velocity (cm/s)	Relative surface area (cm^2)
Contaminated sites									
S1a	330	18	11	4.5	27	170	6.9 (3.5)	102 (33)	1026 (403)
S1b	330	19	10	8.6	28	170	6.3 (3.2)	73 (27)	851 (169)
S2	230	29	9	9	25	165	7.6 (3.6)	98 (25)	1003 (260)
S3	130	46	11	14	25	200	6.6 (2.5)	98 (31)	1192 (404)
S4	30	117	21	5.1	25	230	6.5 (2.8)	89 (24)	1114 (396)
Reference sites									
R1	285	27	11	11	26	180	7.4 (1.2)	87 (47)	1016 (278)
R2	170	77	14	11	25	170	4.2 (1.6)	91 (40)	931 (311)
R3	75	107	21	16	23	170	6.4 (3.3)	100 (36)	1007 (280)
R4	35	127	24	7.7	24	170	5.5 (1.7)	86 (24)	1039 (322)

Depth, velocity, and relative surface area for sampled stones are the means (and standard deviations) of five stones sampled.