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# Evaluating the Restoration of a Stream in an Abandoned Mine Land via Biomass Calculation of Benthic Macroinvertebrates

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**Abstract** It is essential that continual assessments of the impact of mine-derived water as a long-lasting burden on freshwater environments. Abundance-based evaluations of benthic macroinvertebrates have been conducted to evaluate anthropogenic disturbances and devise policies to reduce their impact. In this study, the status of a stream habitat was evaluated based on the body length and biomass weight of benthic macroinvertebrates of the family Baetidae. Following the renewal of the mining water treatment plant, the abundance of Baetidae assemblages recovered to a level comparable to that of a reference site. However, relatively low values were found for both body length and biomass weight in Baetidae species inhabiting the reddened streambed area, suggesting that the habitat has not yet been completely recovered despite the recovery of the abundance of the Baetidae assemblages. Therefore, continuous investigation and evaluation of this disturbed stream are necessary until their growth conditions of the habitat have functionally recovered.

**Key words:** stream habitat, anthropogenic disturbance, abandoned mine lands, benthic macroinvertebrate, biomass

## INTRODUCTION

The diversity of benthic macroinvertebrate communities has been used as an important bioindicator for evaluating the status of running water ecosystems in past decades as it can accurately reflect environmental changes in freshwater ecosystems (e.g., water quality and land use change) (Trigal *et al.*, 2007). When assessing ecosystems, numerical data such as the density or abundance of macroinvertebrates have been used for rapid bioassessments. Numerical approaches can also be applied to substitute biomass when estimating macroinvertebrate production (Benke and Huryn, 2010, 2017). However, biomass data are essential for measuring ecosystem structure and dynamics, including carbon cycle, secondary production, and trophic structure (Nakano *et al.*, 2007). In energy studies, biomass data are necessary to determine caloric values, and are either measured directly or estimated by conversion from tables of calories per unit biomass (Di Sabatino *et al.*, 2014). Biomass plays an important role in identifying the structural position of target organisms in the aquatic ecosystem food web and in evaluating the function of the water body in terms of material circulation. Biomass can be measured directly (weighing organisms via ash-dry mass and biovolume determination) or indirectly (using length-dry mass conversion) (Burgherr and Meyer, 1997; Benke *et al.*, 1999). Among them, length-mass conversions have been

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widely applied because they are faster than the other two direct measuring methods (e.g., Burgherr and Meyer, 1997).

Freshwater ecosystems are subject to various anthropogenic disturbances. Among them, mining activities cause long-lasting disturbances in streams and rivers (Marqués *et al.*, 2001). The abundance or species richness of macroinvertebrates usually decreases with physical and chemical deterioration (e.g., increased conductivity, sediment contamination) (Bae *et al.*, 2021a). Even though mining operations have ceased for several decades, it is difficult to completely remove heavy metals accumulated in streambeds from past mining activities, resulting in long-term damage to freshwater ecosystems. Therefore, benthic macroinvertebrate assemblages in streams near mining areas are frequently disturbed, which can severely disrupt the functional connectivity of ecological networks and reduce species diversity (Bae *et al.*, 2021a; Rico-Sánchez *et al.*, 2022). Therefore, continuous and comprehensive observations of biodiversity changes in freshwater ecosystems near mining areas are required.

Since 2017, we have continuously conducted biodiversity assessments in Hwangjicheon stream, which is affected by abandoned mine lands and is the source of the Nakdonggang River, a major river in South Korea. Biodiversity assessment based on benthic macroinvertebrate assemblages in this area (Bae *et al.*, 2021a) and a DNA metabarcoding-based biodiversity survey using environmental DNA (eDNA) was performed (Bae *et al.*, 2021b) to confirm whether the biodiversity in Hwangjicheon stream had recovered from the impact of abandoned mine lands in a section of the streambed that had reddened due to iron precipitation. Here, we expanded upon previous research further to examine the recovery of biodiversity in a stream near mining area from a quantitative perspective based on the body length of benthic macroinvertebrates and biomass based on mass-conversion equations.

## MATERIALS AND METHODS

### 1. Ecological data

In 2020, benthic macroinvertebrates were collected at six sites in Hwangjicheon stream of the Nakdonggang River basin (NHJ) using a Surber net (30 cm × 30 cm, 250 μm mesh) (Fig. 1). Hwangjicheon stream is located near 16 currently operating and abandoned mines in Taebaek-si, South Korea. The streambed was acid-sulfated in the regions from NHJ2 to NHJ4, and the mining water treatment plant near NHJ2 was renewed in 2019 (Bae *et al.*, 2021a).

We sampled the macroinvertebrates three times at riffles within a 50-m range at each site. We identified macroinvertebrates at the lowest levels and only used the species belonging to Baetidae because only these species were present from NHJ1 to NHJ6 (except at NHJ4). However, as no individuals were observed in NHJ4, we excluded this site from further analysis. After determining the abundance of each species in

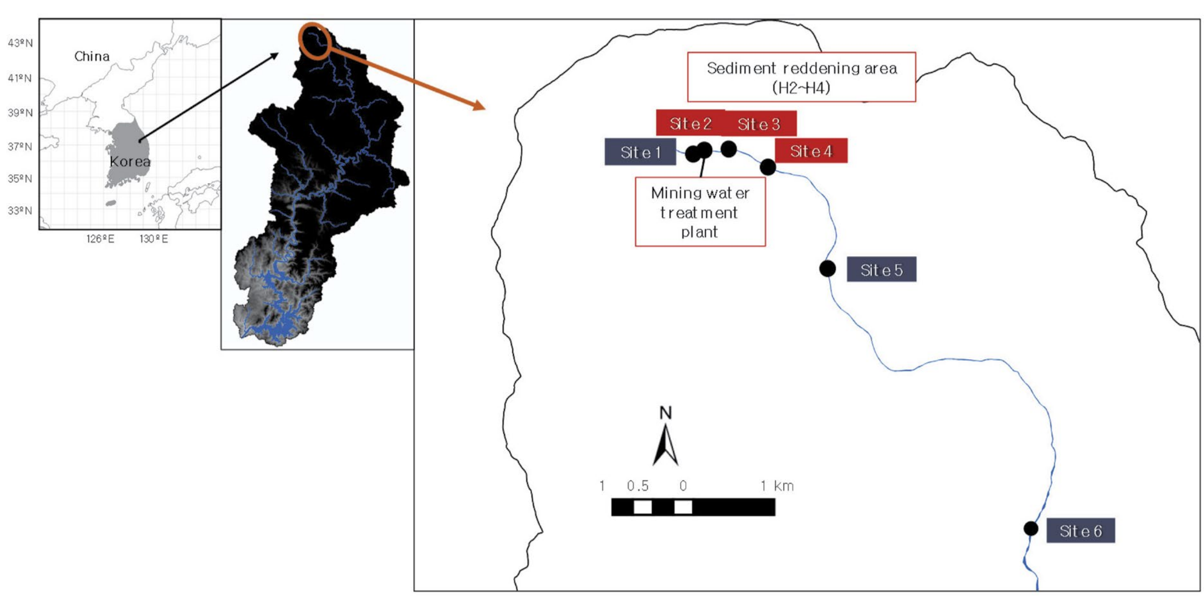


**Fig. 1.** Sampling sites in Hwangjicheon stream.

Baetidae, we measured the body length of each individual to the nearest 0.1 mm using a microscope (SMZ10, Nikon, Japan) (293 individuals in NHJ1, 413 in NHJ2, 148 in NHJ3, 0 in NHJ4, 180 in NHJ5, and 327 in NHJ6). We only measured body length which maintain the whole body structure. Body length was measured as the distance between the head and the posterior of the last abdominal segment (Von Schiller and Solimini, 2005).

### 2. Data analysis

To determine whether the Baetidae had recovered (i.e., abundance, body length, and biomass) from the impact of mining after the renewal of the mining water treatment plant, we compared the abundance (individuals $m^{-2}$), length (mm), and biomass (mg) of Baetidae among the six sampling sites. To calculate biomass, we applied length-mass conversion methods, which have been preferred by many researchers because of their easy computation (Von Schiller and Solimini, 2005), and a power regression model (ln dry-mass = ln a + b*ln Body Length) was fitted (e.g., Burgherr & Meyer, 1997; Benke *et al.*, 1999). The fitted parameter estimates a (0.0071) and b (2.832) were obtained from Benke and Hiryn (2007).

Subsequently, we applied three approaches: First, we compared the abundance of Baetidae assemblages between 2018 (before the renewal of the mining water treatment plant) and 2020 (after the renewal). Second, we compared the length and biomass of Baetidae individuals among the six sampling sites in 2020 using the Kruskal-Wallis (K-W) Test. Dunn's multiple comparison test was applied only when a significant difference was observed. Finally, the body length or biomass was divided at regular intervals and compared at each interval among sites.

The K-W test and Dunn's multiple comparison tests were analyzed using R software (the package 'dplyr': K-W test and 'FAS': Dunn's multiple comparison test) (Wickham *et al.*, 2020; Derek and Wheeler, 2020),

## RESULTS AND DISCUSSION

In the streambed substrate compositions, the ratio of large substrates, such as cobble (64~256 mm), was the highest, ranging from 77.6% (NHJ3 in 2020) to 88.7% (NHJ1 in 2018), regardless of the sampling year and survey sites (Table 1). In water quality variables, the DO, pH, and conductivity were similar in both years (Table 2). DO and pH values were also similar among the six sites; however, the conductivity values increased from NHJ3 (567.0 μS $cm^{-1}$) and then became lower up to NHJ6 (302.1 μS $cm^{-1}$).

In 2020, six Baetidae species (*Acentrella sibirica*, *Baetiella tuberculata*, *Baetis fuscatus*, *B. silvaticus*, *B. ursinus*, and *Nigrobaetis bacillus*) were observed in the NHJ. Although the environmental factors did not differ between 2018 and 2020, the abundance of Baetidae showed different patterns before and after the renewal of the mining water treatment plant. In 2018, the abundances at NHJ1 and NHJ2 were 440 and 550 individuals $m^{-2}$, respectively. However, at NHJ3 and NHJ4, where streambed reddening was observed, the abundance

**Table 1.** Comparison of substrate composition (%) at six sites between 2018 and 2020.

| Year | Sites | 0.063 mm | 0.063~2 mm | 2~4 mm | 4~64 mm | 64~256 mm | 256 mm |
|---|---|---|---|---|---|---|---|
| 2018 | NHJ1 | 0.09 | 0.94 | 0.94 | 6.56 | 88.68 | 2.8 |
| | NHJ2 | 0.11 | 1.05 | 1.05 | 10.5 | 87.29 | 0 |
| | NHJ3 | 0.11 | 1.06 | 1.06 | 9.83 | 84.67 | 3.26 |
| | NHJ4 | 0.09 | 0.89 | 0.89 | 11.93 | 86.19 | 0 |
| | NHJ5 | 0.77 | 1.16 | 1.16 | 15.49 | 81.41 | 0 |
| | NHJ6 | 0.08 | 0.85 | 0.85 | 9.51 | 77.61 | 11.11 |
| 2020 | NHJ1 | 0.09 | 0.91 | 0.91 | 12.2 | 84.48 | 1.4 |
| | NHJ2 | 0.1 | 0.77 | 1.02 | 9.66 | 86.08 | 2.37 |
| | NHJ3 | 0.1 | 0.84 | 0.84 | 12.68 | 77.75 | 7.79 |
| | NHJ4 | 0.1 | 0.5 | 1.03 | 12.4 | 85.96 | 0 |
| | NHJ5 | 0.44 | 1.09 | 1.09 | 13.36 | 84.02 | 0 |
| | NHJ6 | 0.09 | 0.65 | 0.89 | 9.39 | 83.43 | 5.55 |

**Table 2.** Characteristics of the water quality variables at six sites between 2018 and 2020.

| Year | Sites | DO (mg $L^{-1}$) | pH | Conductivity (μS $cm^{-1}$) | TN (mg $L^{-1}$) | T-P (mg $L^{-1}$) |
|---|---|---|---|---|---|---|
| 2018 | NHJ1 | 9.35 | 8.77 | 312.0 | 2.4 | 0.007 |
| | NHJ2 | 8.82 | 8.70 | 310.9 | 0.9 | 0.005 |
| | NHJ3 | 8.82 | 8.44 | 567.0 | 0.8 | 0.005 |
| | NHJ4 | 8.40 | 8.45 | 457.2 | 1.8 | 0.009 |
| | NHJ5 | 10.22 | 8.84 | 374.1 | 1.7 | 0.009 |
| | NHJ6 | 9.79 | 8.58 | 302.1 | 2.1 | 0.012 |
| 2020 | NHJ1 | 9.11 | 8.94 | 248.2 | 1.1 | 0.009 |
| | NHJ2 | 9.08 | 8.63 | 249.8 | 1.0 | 0.030 |
| | NHJ3 | 8.81 | 8.78 | 471.2 | 0.9 | 0.008 |
| | NHJ4 | 9.03 | 8.33 | 501.0 | 2.8 | 0.010 |
| | NHJ5 | 9.55 | 8.73 | 380.4 | 2.5 | 0.010 |
| | NHJ6 | 8.15 | 8.73 | 307.8 | 2.5 | 0.006 |

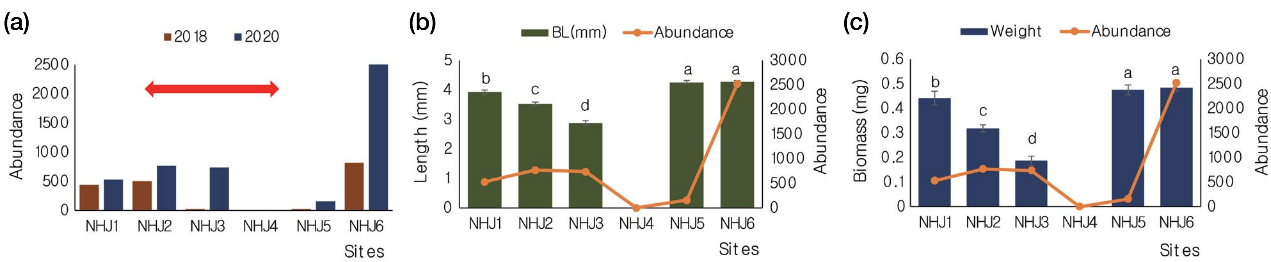


**Fig. 2.** Comparison of abundance (a), body length (b) and biomass (c) of Baetidae among the survey sites in Hwangjicheon stream.

decreased sharply to 22 and 4 individuals $m^{-2}$, respectively. However, comparison of the abundance of Baetidae at the six sites between 2018 and 2020 revealed that the abundance was higher in NHJ3 (737 individuals $m^{-2}$). Additionally, abundance in NHJ3 was higher in 2021 than in 2018 (377 individuals $m^{-2}$) and 2022 (254 individuals $m^{-2}$) (Bae *et al.* submitted).

Even though the abundance of Baetidae assemblages from NHJ1 to NHJ3 was similar in 2020, the body length significantly decreased from NHJ1 (3.92 mm) to NHJ3 (2.88 mm). The length was highest in NHJ5 (4.25 mm) and NHJ6 (4.26 mm). With regard to the body length of Baetidae individuals in 2020, the average length tended to decrease from NHJ1 to NHJ3, but was highest at NHJ5 and NHJ6 (Fig. 2B). In the case of biomass converted by the calculation formula based on the measurement results of body length, the degree of decline from NHJ1 (0.44 mg) to NHJ3 (0.19 mg) was larger than that of body length (Fig. 2C). However, although the abundance of Baetidae assemblages was higher in NHJ6 than in NHJ5, the body length and biomass of Baetidae individuals were similar.

When the individual sizes of Baetidae assemblages were classified into 0.1 mm intervals, the length of Baetidae in NHJ5 and NHJ6 was the highest at 4~5 mm sections, followed by 6~7 mm and 3~4 mm sections (Fig. 3A). In the case of NHJ1 and NHJ2, which are upstream areas, the length of Baetidae was the highest at the 3~4 mm sections, followed by the 4~5 mm and 2~3 mm sections. In contrast, in NHJ3, where the disturbance was the most severe, the length of Baetidae was the highest at the 2~3 mm sections, followed by the 3~4 mm sections, indicating a relatively small body size compared with those from NHJ5, NHJ6, NHJ1, and NHJ2.

Even when the measurement results of the body length of Baetidae individuals at each site were converted into biomass, the differences between each site remained similar (Fig. 3B). However, in contrast to the body length measurements, the number of individuals in the 0.1~0.2 mg and 0.3~0.4 mg sections was higher than that in the 0.2~0.3 mg section in NHJ1~NHJ3. In NHJ3, most individuals had very low body

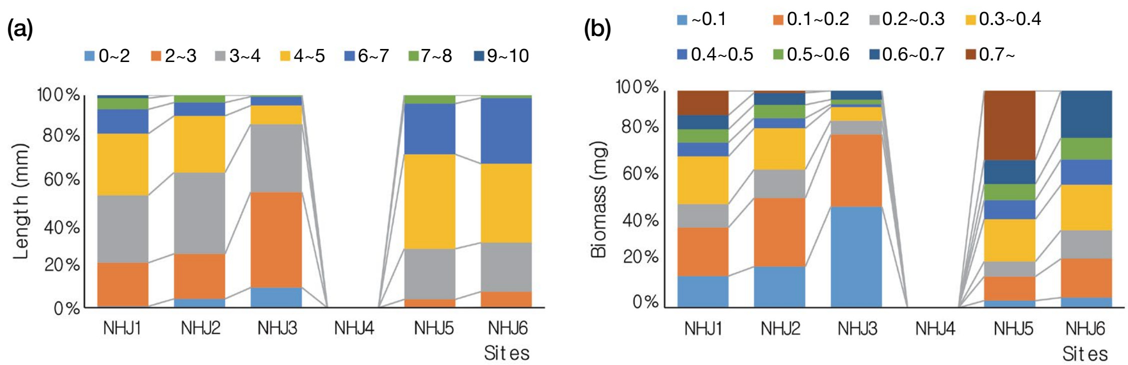


**Fig. 3.** Body length (a) and biomass spectra (b) for Baetidae in Hwangjicheon stream.

weight (less than 0.1 mg).

Most research on the effects of anthropogenic disturbances on macroinvertebrate communities has focused on numerical approaches (e.g., species richness or abundance) (Kasangaki *et al.*, 2006; Maloney *et al.*, 2011; Ligeiro *et al.*, 2020). However, even though efforts to eradicate the disturbance sources (e.g., the mining water treatment plant) resulted in the abundance of macroinvertebrates becoming similar between the reference and disturbed sites, we cannot conclude that the disturbed streams have structurally and ecologically recovered. The size of organisms can still be affected by unrecovered basal resources, resulting in changes in feeding rates and the growth or maximum body size of organisms (Albariño and Balseiro, 2002; Larrañaga *et al.*, 2016).

All the Baetidae species in our study were collector-gatherers in functional feeding groups. Thus, the reduction in resources (i.e., organic matter) can affect the size of organisms (Ferreiro *et al.*, 2011). The streambed in some sections (approximately 1 km) of Hwangjicheon stream has been covered by iron precipitates from the mining area instead of organic matter precipitates for a long period of time. This indicates that even though the abundance of Baetidae recovered due to the drifting of Baetidae from upstream, the optimal growth of the organisms is restricted by iron precipitates. Long-term monitoring of the length, mass, and abundance of macroinvertebrates should be conducted until the growth conditions of the habitat are completely restored.

## CONCLUSIONS

The purpose of this study was to examine the process of biodiversity recovery from the impact of the mining industry, a representative anthropogenic disturbance that frequently occurs in small streams (e.g., headwater) and can cause semi-permanent loss of biodiversity in ecosystems. Unlike the 2018 survey results, in the 2020 survey, which was conducted after the mining water treatment plant was established, the abundance of the Baetidae assemblages, a benthic macroinvertebrate family, had largely recovered at the survey site (i.e., NHJ3) where the streambed reddening occurred. However, Baetidae individuals inhabiting the reddened area exhibited low body length and biomass weight, suggesting that the habitat has not yet recovered completely despite the recovery of the abundance of the Baetidae assemblages. Therefore, it is necessary to conduct continuous surveys and evaluations, even after the recovery of the abundance of the Baetidae assemblages, until the growth conditions of the habitat are functionally restored.

**Author Contributions** Conceptualization: M.-J.B.; Field survey: M.-J.B., H.-C.S., and S.-N.H.; Literature survey: M.-J. B., H.-C.S., E.-J.K.; Writing, Review and Editing: M.-J.B. and E.-J.K. All authors have read and agreed to the published version of the manuscript.

**Conflicts of 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.

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