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Ecological health assessments using multiple parameters of fish blood tissues to community along with water chemistry in urban streams

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Abstract

The objectives of this study were to identify multi-level stressors from blood biomarkers to community-level bioindicators and diagnose the stream ecosystem health in polluted streams. Blood chemistry such as total protein (T_{Pro}), blood urea nitrogen (B_{UN}), total cholesterol (T_{Cho}) and albumin (A_{lb}) were analyzed from sentinel fish tissues; the functions of kidney, gill and liver were significantly decreased in the impacted zone (I_z), compared to the control zone (C_z). Histopathological analysis showed that fish liver tissues were normal in the C_z. Fish liver tissues in the I_z, however, showed large cell necrosis and degeneration and also had moderate lobular inflammation and inflammatory cell infiltration of lymphocytic histocytes. Species biotic index (SBI) at species level and stream health assessment (SHA) at community level indicated that chemical impacts were evident in the I_z (ecological health; poor - very poor), and this was matched with the blood tissue analysis and histopathological analysis. The impairments of the streams were supported by water chemistry analysis (nitrogen, phosphorus). Tolerance guild analysis and trophic guild analysis of fish were showed significant differences ($P < 0.01$) between C_z and I_z. Overall, multiple parameter analysis from biomarker level (blood tissues) to bioindicator level (community health) showed significantly greater impacts in the I_z than C_z. This approach may be effective as a monitoring tool in identifying the multilateral and forthcoming problems related to chemical pollution and habitat degradation of stream ecosystems.

Keywords: blood chemistry, health assessment, histopathological analysis, species biotic index

INTRODUCTION

Ecological health/risk assessments in stream ecosystems have been partially conducted by several research approaches from micro-level biomarkers of DNA (Singh et al. 1988), cellular (Adams and Greeley 2000), physiological (Barbour et al. 1999) and histopathological assays (Adams 2002) to macro-level bioindicators of individual (Goede and Barton 1990, Anderson and Neumann 1996), population (Munkittrick et al. 2000) and community levels (Barbour et al. 1999). For these researches, three trophic levels of key organisms such as periphyton (primary producer), macroinvertebrate (primary consumer) and fish (top consumer) were used for the assessments,

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and fish indicators were most widely used in the world (Lang et al. 1989, Plafkin et al. 1989, Kelly and Whitton 1995, Lang and Reymond 1995, Kelly et al. 1998). Major limitation of these studies is that single indicator or single model was applied in most studies (Adams and Greeley 2000). The health assessments, thus, were partially evaluated in extreme levels of DNA level or community level rather than a general wide-range levels in the ecosystems.

Standardization and protocols of stream health assessments have nationally been adopted in United States Environmental Protection Agency (US EPA) and European Union (EU). US EPA developed a new methodology of stream health assessments using various biota including fish, which is Rapid Bioassessment Protocol (RBP) based on the high-levels of biological organization approach of fish community (Barbour et al. 1999, US EPA 2002). Similarly, Water Framework Directive (WFD) in the European Union (EU) also adopted the multi-metric bioassessment models using fish assemblages for an effective management and conservation (Water Framework Directive 2000). These bioassessment models become a worldwide methodology for health assessments of aquatic environments including Canada, France, United Kingdom, Australia, Japan and China, and so on (Karr and Dionne 1991, Oberdorff and Porcher 1994, Koizumi and Matsumiya 1997, Karr and Chu 2000). These studies of US EPA and EU were mainly based on macro-level researches of community-levels.

In the meantime, Adams (2002) pointed out that community-level assessment models show slow response against water pollution and can only identify the condition which pollution proceed over some serious levels as ecological significance. Thus, numerous stream health assessments have been fulfilled in the population (Munkittrick et al. 2000) and community levels (Barbour et al. 1999). Munkittrick and Dixon (1989) developed a biomonitoring framework using the population level responses and reviewed the responses of a wide variety of fish population changes in eutrophication, acidification, predation pressure and industrial wastes using published data. Particularly, fish population can be used to assess long-term damage with environmental modification, population growth, obesity and fish conditions using the indices such as length-weight relationship and condition factor (C_F) for the understanding of population dynamics (Munkittrick and Dixon 1989). These indicators were applied to provide information on size distributions and energy flow (expenditure and storage), which can be used to assess the health of the fish populations (Munkittrick et al. 2000). Therefore, fish populations and communities were known as good indicators of stream ecosystem health assessment because they reflect varied anthropogenic disturbances, including nutrient enrichment (Finney et al. 2000, Attrill and Power 2002), and toxic pollution (Robinet and Feunteun 2002).

Despite of such numerous advantages in population and community-level assessments, still micro-level researches were required in the health assessments. The uses of only population or community-level could not identify the potential effects on physiological, cellular, and molecular levels of organisms. Adams and Greeley (2000) pointed out that inputs of industrial and urban wastewater had an effect on wide-range biological responses from low-levels of biological organization such as cells, tissues, organs, and individuals to high-levels such as populations and communities (Peakall and Walker 1994). Hence, biological responses of cellular, physiological, and molecular levels may not directly affect the health of population and community levels, but are potentially influenced on chemical pollutants and disturbance (Schmitt and Dethloff 2000, Triebskorn et al. 2001). Micro-level biomarkers have developed and proved to be a useful tool in identifying early biological changes caused by environmental pollutions. These biomarkers are defined as sensitive, measurable, xenobiotically induced in biochemical processes such as enzyme activity (Peakall and Shugart 1992). In addition, the use of biomarkers provides various information that cannot be obtained from chemical analysis of pollutants and reflects impacts of chemical mixtures for long exposure periods (Lam and Gray 2003). Also, this approach discerns pollutant impacts in early time after emission has started, and responds to low concentration value of pollutants (Van Gestel and Van Brummelen 1996). Therefore, the low-level approach has been often used as a pre-warning or early-warning tool for environmental supervision in aquatic ecosystems and applied extensively to polluted urban streams (Lam and Gray 2003).

These biological responses of low-level biomarkers may not directly affect the health of population and community (Schmitt and Dethloff 2000, Triebskorn et al. 2001). Therefore, for stream health assessment, it is necessary to develop the integrated stream health assessment approach from the DNA-level biomarkers to community-level bioindicators in aquatic ecosystem assessment (Karr 1981, Gore 1985, Brookes and Shields, 1996). Because these integrative health assessment includes biomarkers and bioindicators, it has various advantages such as worthwhile information that cannot be obtained from chemical analysis of pollutants, effects of chemical mix-

tures over long periods (Lam and Gray 2003), and the integrated effects of various environmental stressors on the health of individuals, populations, and communities (McCarty et al. 2002, Van der Oost et al. 2003). Moreover, these assessments reflected from the long-term response of bioindicators such as individual, population and community level parameters to the short-term responses of various biomarkers such as molecular, cellular, physiological, histopathological parameters. Therefore, it is possible to provide the pre-warning alarm systems for the aquatic ecosystem health assessments (Lam and Gray 2003, Wepener et al. 2005). In other worlds, this new integrative methodology can be used as a warning tool for predicting and diagnosing the overall ecological health of urban streams, which are largely influenced by toxic chemicals and nutrient enrichment.

The objectives of this research were to assess integrative ecological stream health using both biomarkers of blood tissue and histopathological parameters and bioindicators of individual, population and community parameters in two different streams.

MATERIALS AND METHODS

Sampling sites and point sources

The sampling was conducted every month at two urban streams of Gap Stream (GS) and Miho Stream (MS) during 2013 to 2014 (Fig. 1). Gap Stream is one of main tributaries in Geum River watershed and located in the middle of Daejeon city. Two sampling zones of upstream (control zone, C_z) and downstream regions (impacted zone, I_z) were selected depending on the locations of point sources within the watershed. The C_z is mainly surrounded by forest (> about 65 %), whereas the I_z is largely influenced by Daejeon industrial complex and wastewater disposal plant (WDP). The effluents from the WDP discharged 9×10^5 m^3/day and went to the I_z of Gap Stream directly. Miho Stream is also one of main tributaries in Geum River watershed and is located in the Cheongju city of North Chungcheong province. In the Miho Stream, the C_z is surrounded by forest (> 55.5%), whereas the I_z is largely influenced by the complex for agriculture industry complex, WDP with effluents of 2.8×10^4 and 8×10^3 m^3/day from Cheongju and Naesu, respectively.

Sampling methods and sampling gears

Fish sampling, based on the catch per unit effort (CPUE; Ohio EPA 1989), was conducted at the sampling sites and all habitats of riffle, run and pool were included in the sampling zones. Stream distance sampled was 200 m and the sampling time passed was 60 min. The fish was captured using sampling gears such as cast net (mesh size: 7 × 7 mm) and kick net (mesh size: 4 × 4 mm). Fish species were identified using keys of Nelson (1994) and Kim and Park (2002), examined for external abnormalities (deformities, erosion, lesion and tumors) based on the criteria of Sanders et al. (1999), and classified into the order of trophic guild as omnivores, insectivores, carnivores and herbivores and tolerance guild as tolerant, intermediate and sensitive species, based on the previous regional studies (An et al. 2004). Sampled fishes returned to their natural habitats except two habitat-type species, *Zacco platypus* (Pelagic species) and *Pseudogobio esocinus* (Benthic species) for obtaining samples. Two sentinel

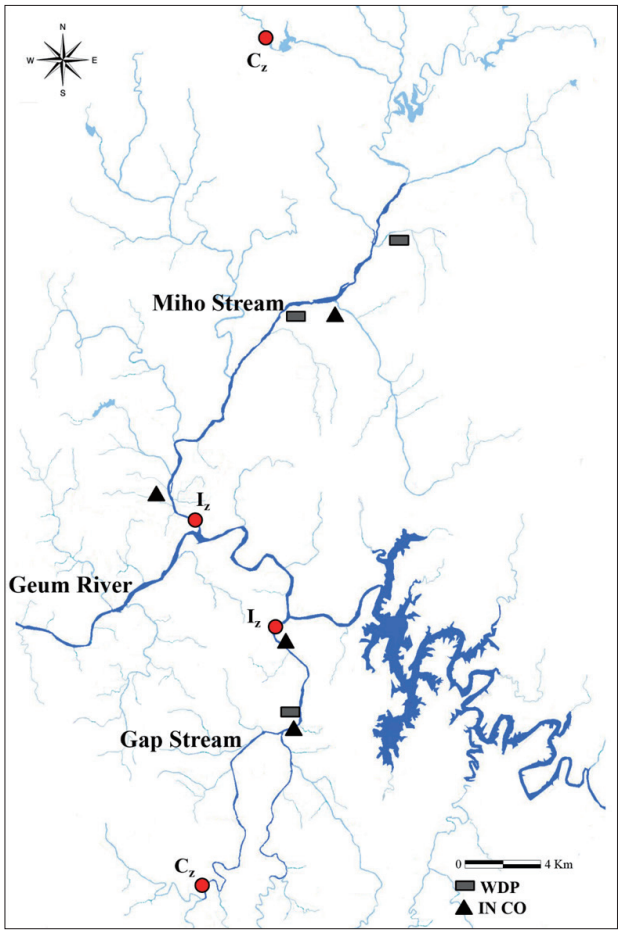

Fig. 1. The sampling sites in the Gap Stream and Miho Stream. C_z the control zone; I_z impacted zone; WDP, wastewater disposal plants; IN CO, an industrial complex.

species, the pelagic species (*Z. platypus*) and the benthic species (*P. esocinus*), were used as the indicator species for the analysis of biomarkers and bioindicators. These species were representative species which were most widely distributed in streams and rivers.

Blood chemistry analysis

After the fish sampling, the fishes were befuddled in the ice, total length and weight were measured, and then blood samples for analysis of blood chemistry were prepared. Blood samples (30 μL) were obtained from caudal artery of two sentinel habitat-type fishes by incision of the caudal peduncle using capillary tubes. The blood samples were analyzed by Automated Biochemical Analyzer (Spotchem™ EZ, ARKRAY, model SP-4430, Japan), and the analyzed parameters were total protein (T_{Pro}), blood urea nitrogen (B_{UN}), total cholesterol (T_{Cho}), and albumin (A_{lb}).

Total mercury analysis of fish tissues

Concentrations of total mercury (total [Hg]) were analyzed from fish tissues (gill, intestine, liver, spine and muscle) by Direct Mercury Analyzer (DMA-80; Milestone Inc., Italy, US EPA Method 7473). The samples of gill (Gi), intestine (In), liver (Li), spine (Sp) and muscle (Mu) were initially dried in the oxygen stream passing through a quartz tube located inside a controlled heating coil under the condition of oxygen supplies as a carrier gas to each cylinder. The combustion gases were further decomposed on a catalytic column at 750°C. Mercury vapor was collected on gold amalgamation traps and subsequently desorbed for quantification. Thus, the mercury levels were determined using a single beam spectrophotometer with two sequential, flow-through measurement cells under the condition of 254 nm. The light source for the spectrophotometer was a low pressure mercury vapor lamp and the silicon UV photo-detector was used for the analysis.

Histopathological analysis

Histopathological analysis of fish liver tissue was conducted by the paraffin methods of Mela et al. (2007). After the liver tissue samples were fixed with 4% paraformaldehyde solution, the samples were treated with ethanol, xylene and paraffin, in turn. And then, liver tissue in paraffin wax was cut into a thickness of 4 μm. The cross section of liver tissue was fixed on slide glass, and each section was stained with hematoxylin-eosin (H&E).

Water chemistry analysis

Spatial patterns of total nitrogen (TN), total phosphorus (TP), biological oxygen demand (BOD), and chemical oxygen demand (COD) during 2012 to 2014 were analyzed using the data obtained from the Ministry of Environment, Korea (MEK).

Species Biotic Index (SBI)

Species Biotic Index (SBI) was based on a fish assemblage and the tolerance score of each fish species. Tolerance ranks on each fish species were obtained from the approach of Species Biotic Index (An and Kim 2007). The fish tolerant scores (FTSs) for all other species distributed in Korea were used for analysis. The equation of SBI is as follows;

$$\text{Values of SBI} = \Sigma\, ni * ti / N$$

[N = total number of individual; ni = total number of each species; ti = tolerance value of each species]

Each value was categorized as 7 criteria, which is based on the rank values of Hilsenhoff (1988). The ranks of 0.00 - 3.75, 3.76 - 4.25, 4.26 - 5.00, 5.01 - 5.75, 5.76 - 6.50, 6.51 - 7.25, and 7.26 - 10.00 were classified as “Excellent”, “Very good”, “Good”, “Fair”, “Fairly poor”, “Poor” and “Very poor”, respectively.

Population recruitment analysis

Frequency of size distribution of two habitat-type species was analyzed and categorized using a total length of fish. Two species of *Zacco platypus* and *Pseudogobio esocinus* were used for the study. The size intervals from I to XV were used for the population analysis. The size intervals (I - XV, unit = mm) are as follows;

Water-column species (*Z. platypus*)
I : 21 - 30, II : 31 - 40, III : 41 - 50, IV : 51 - 60, V : 61 - 70, VI : 71 - 80, VII : 81 - 90, VIII : 91 - 100, IX : 101 - 110, X : 111 - 120, XI : 121 - 130, XII : 131 - 140, XIII : 141 - 150, XIV : 151 - 160, XV : 161 - 170

Benthic species (*P. esocinus*)
I : 41 - 50, II : 51 - 60, III : 61 - 70, IV : 71 - 80, V : 81 - 90, VI : 91 - 100 VII : 101 - 110, VIII: 111 - 120, IX : 121 - 130, X : 131 - 140, XI : 141 - 150, XII : 151 - 160, XIII : 161 - 170, XIV : 171 - 180, XV : 181 - 190

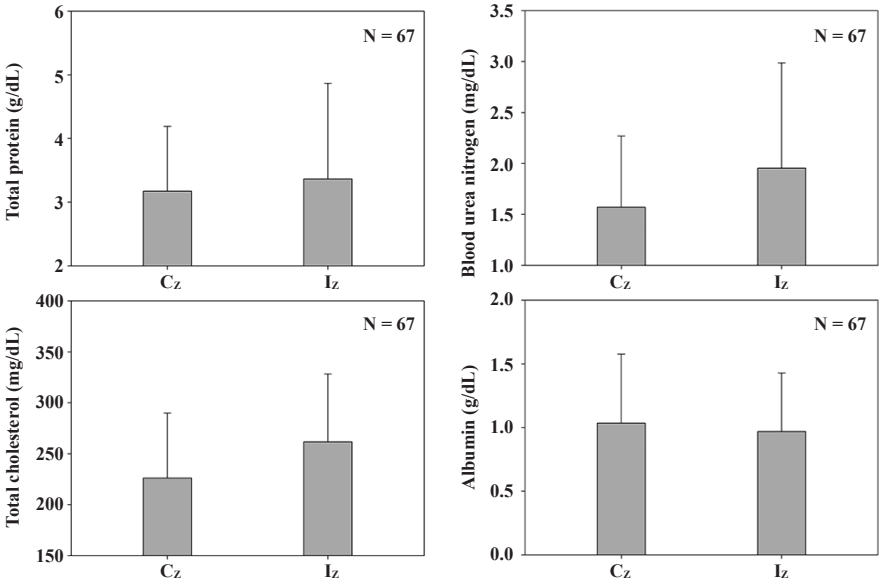

Fig. 2. Blood chemistry parameters from the control zone (C_z) and impacted zone (I_z) in the sampling streams.

Stream Health Assessment Index

Stream Health Assessment (SHA) index, based on the Index of Biological Integrity (IBI; Karr 1981, Barbour et al. 1999), was developed on the basis of regional application (An et al. 2006) and used for community-level health assessment. The metrics attributes (M) were consisted in three major groups as ecological characteristics by species richness and composition (M_1 - M_4), trophic composition (M_5 - M_6), and fish abundance and conditions (M_7 - M_8). Each metric were scored 1, 3 or 5 point, respectively. These scores were then summed to obtain a site-specific model value that ranged from 8 to 40, and judged as 5 categories; excellent (36 - 40), good (28 - 34), fair (20 - 26), poor (14 - 18) and very poor (≤ 13).

Statistical analysis

For the data analysis of biomarkers and bioindicators, independent two sample t-test was conducted for sample comparison between sampling zones or two habitat-type species. Simple linear regression analysis was conducted to examine the relationships among the environmental factors. Statistical analyses were performed at the significant level of $P < 0.05$ using the SPSS ver. 21.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Blood chemistry analysis

Total protein (T_{Pro}), blood urea nitrogen (B_{UN}), total cholesterol (T_{Cho}), and albumin (A_{lb}) were measured for blood chemistry analysis as shown in Fig. 2. Content of T_{Pro} was 3.17 ± 1.0 g/dL in the C_z and 3.36 ± 1.5 g/dL in the I_z. B_{UN} level was 1.57 ± 0.7 mg/dL in the C_z vs. 1.95 ± 1.0 mg/dL in the I_z. In the meantime, content of T_{Cho} was 226.3 ± 63.7 mg/dL in the C_z, 261.8 ± 66.6 mg/dL in I_z, while the content of A_{lb} was 1.03 ± 0.5 g/dL in the C_z and 0.97 ± 0.5 g/dL in the I_z. The content of T_{Pro} is a good indicator for total amount of proteins in the fish blood, and the content of A_{lb} indicates total amount of proteins synthesized in liver, thus, both two blood parameters generally show identical patterns which are low value in the blood during malnutrition (Gopal et al. 1997). However, in this study, T_{Pro} appeared higher in the I_z than the C_z and lower A_{lb} in the I_z. Generally, both indices were expected to show identical patterns, but they showed different trends and this can be analyzed as better nutrition and better liver function because of higher value of I_z in the parameter of T_{Pro} (Rosenoer et al. 1977). Nonetheless, it does not seem that eutrophication of I_z brings positive influence on all the nourishment and liver function, according to the blood chemistry values of A_{lb}. The case of B_{UN}, is also used frequently as a good indicator for kidney malfunction and liver function, indirectly. B_{UN} is urea nitrogen in the blood,

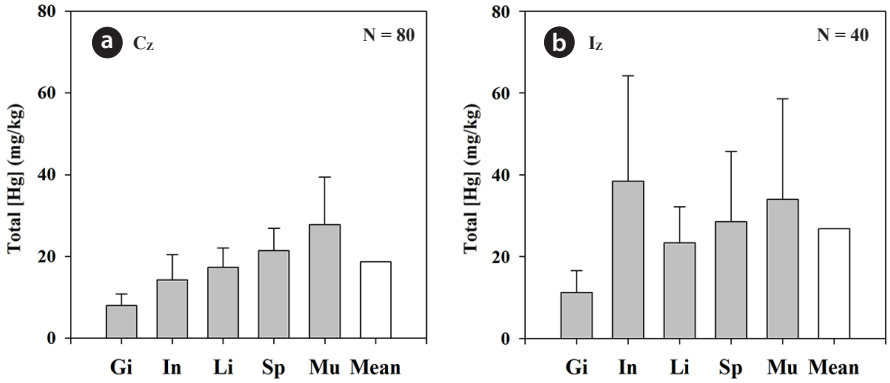

Fig. 3. Measurements of total Hg concentration (total [Hg]) in each tissue in the sampled streams. Gi, gill; In, intestine; Li, liver; Sp, spine; Mu, muscle.

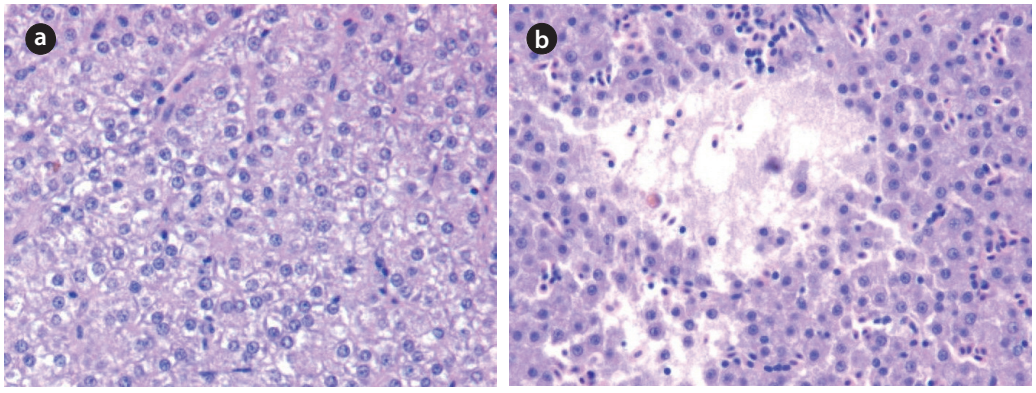

Fig. 4. Histopathological appearance of liver tissue in *Pseudogobio esocinus* in the control zone (a) and impacted zone (b) in the Gap Stream. Liver cells on a liver tissue section were stained with hematoxylin-eosin (optical microscope 400 × magnification).

which is produced from protein decomposition, and it indicates health of kidney and liver which are related to nitrogen waste elimination of fish. Higher values of B_{UN} in the I_z than C_z indicate increases of B_{UN} due to decreased amount of nitrogen eliminated from the reduced function of kidney and liver compared to the C_z as well (Adams et al. 1996). Also, T_{Cho} is used as an index of nutrition intake and metabolism (Adams and Tremblay 2003). T_{Cho} appeared higher in the I_z than C_z which indicates high fat content in the blood cells, active nutrition intake and high metabolism. Nevertheless, there were no clear differences in blood chemistry between the C_z and I_z.

Total mercury contamination of fish tissue and the histopathological analysis

Heavy metal concentrations of total mercury (total [Hg]) were analyzed from five different tissues of gill (Gi), intestine (In), liver (Li), spine (Sp) and muscle (Mu). Total mercury, based on the mean values of the five different tissues, was 1.4 fold higher in the I_z than in the C_z (Fig. 3). Especially, statistically significant differences between C_z and I_z were observed in the tissues of intestine (In; $P < 0.01$) and liver (Li; $P < 0.05$). This result showed a similar pattern, like previous analysis of comet assay (Lee and Steinert 2003, Olive and Banáth 2006, Kang et al. 2014). It is well known that heavy metals influence the damage of deoxyribonucleic acid in aquatic organisms (Lee and Steinert 2003). These heavy metals may directly influence the histopathological damages as shown in Fig. 4. The left panel (C_z) showed normal liver, which had no remarkable histopathological changes. The right panel (I_z), however, showed moderate lobular inflammations and some histocytes. Also, cell necrosis and degeneration in the liver were evident in the I_z. The area of cell necrosis was 21.4% of the total cell area. Our result indicates that such histopathological responses in the I_z are due to chemical pollution, and this was supported by the previous studies of Adams (2002) that cell necrosis and degeneration are closely associated with chemical toxicants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and agricultural pesticides.

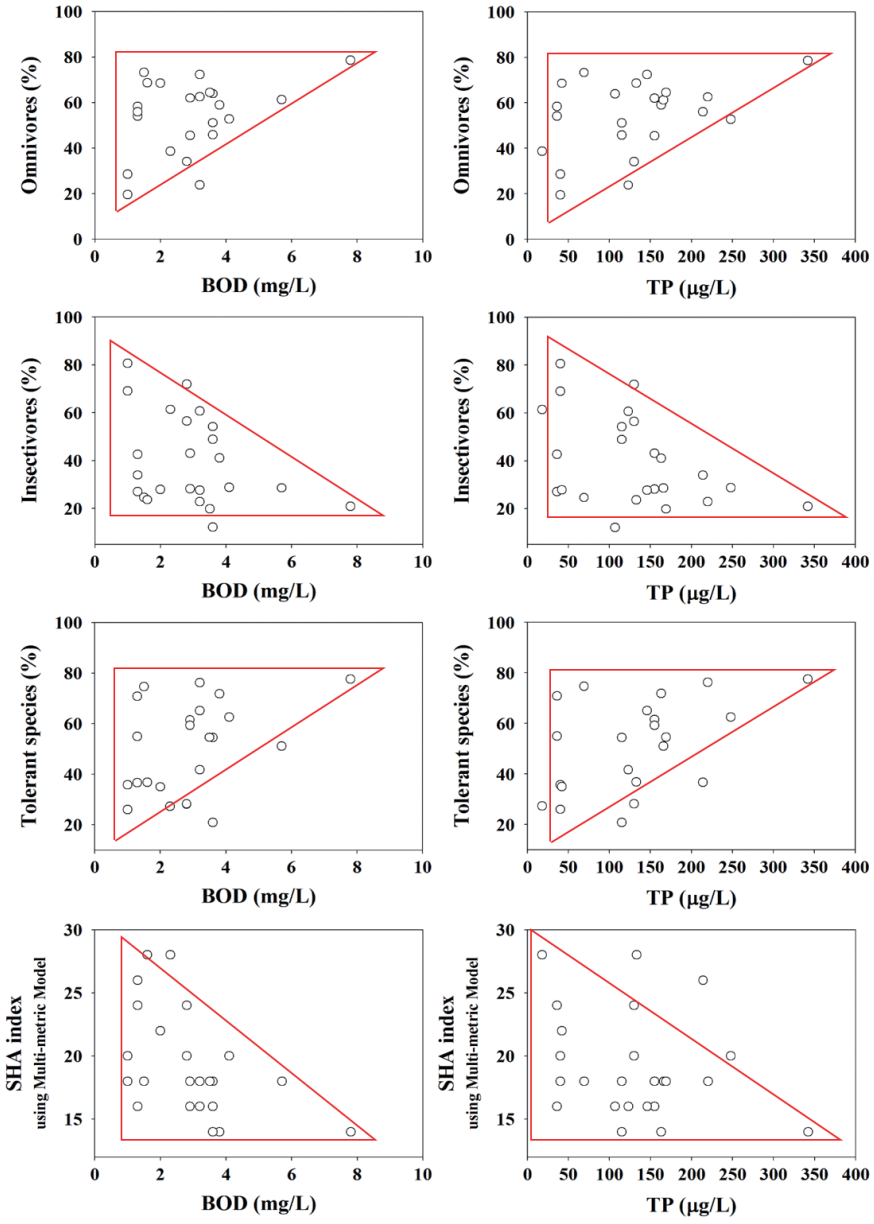

Fig. 5. The relationships among trophic guild, tolerance guild, Stream Health Assessment (SHA) index using the multi-metric model and chemical water quality variables in the sampled streams.

Water chemistry

Water chemistry directly influenced the trophic compositions and fish tolerance. As water quality in different variables get worse, the relative proportions of omnivores and tolerant species tended to increase, but the relative proportions of insectivores and sensitive species tended to decrease in the urban streams (Fig. 5). Total phosphorus (TP) increased from 0 to 350 µg/L, and the proportions of omnivores and tolerant species approximately increased from 20% to 80% depending on the TP concentrations. Especially, the highest TP values were observed in the I_z, which had high proportions of omnivores and tolerant species. Previous studies (Karr 1981, US EPA 1991, Barbour et al. 1999) reported that nutrient enrichment (N, P) and organic matter pollutions cause large increases in total number and species of omnivores and tolerant species. Nitrogen and phosphorus enrichments were evident in the I_z of both Gap Stream and Miho Stream. The increase of BOD from 0 in the C_z to 8 mg/L in the I_z caused increases of omnivores from 20% in the C_z to 80 % in the I_z.

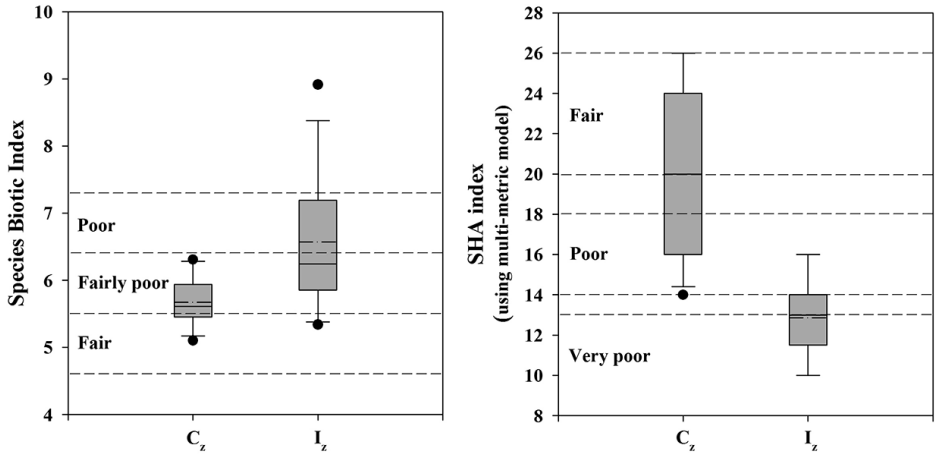

Fig. 6. The assessment of fish population and community level using the Species Biotic Index (SBI) and Stream Health Assessment (SHA) index from the control zone (C_z) to the impacted zone (I_z) in the sampled streams.

Species Biotic Index as a species-level indicator

Stream ecosystem health was analyzed in the species-level using a SBI. Values of SBI were 5.67 ± 0.35 in the C_z and 6.57 ± 1.02 in the I_z (Fig. 6). Thus, ecological health, calculated as a sum of each species' tolerance value, was ranked as "fair condition" in the C_z and "poor condition" in the I_z. SHA index, based on multimetric IBI model, showed that values of SHA index were 20 in the C_z and 13 in the I_z. This species-level result of SBI was consistent with the community-level result of stream health assessments of IBI (Table 1 and Fig. 6). Both SBI value and SHA index showed significant difference ($P < 0.01$) in the value of C_z and I_z; the SBI in the two zones of C_z and I_z was evaluated as "fair" and "poor", respectively, while the SHA index in the two zones of C_z and I_z was evaluated as "fair" and "very poor". These results indicate that impairments in the health condition were evident in the I_z, which was influenced by the industrial complex and wastewater disposal plants (WDP) in Gap Stream and Miho Stream. The metric evaluations of SHA index (Table 1) showed that model values of M_1, M_2, M_7 and M_8 were greater in the I_z than the C_z. Our results are supported by previous studies of health assessments that biological health is impaired in the point source region than the control of the upstream (Hugueny et al. 1996, An et al. 2001, Bae and An 2006).

Population recruitment analysis and community-level stream health

Population recruitment analysis of water-column species and benthic species indicated that population size frequency showed normal distribution in the C_z but skewed distributions in the I_z. Both species showed normal distribution in all size frequency intervals except for two classes (II and III) in the C_z (Fig. 7). In contrast, skewed distribution was in evident in the I_z, so the mid-size classes of VI or VII were the highest. In the water-column species, the maximum value of C_z was 18.1% in the VI class and the minimum value was 0.3% in the III class, while the maximum of I_z was 35.6% in the II class, and there were no individuals in the interval III and XV. In benthic species, the maximum value of C_z was the 20.2% in the VI class, while the maximum of I_z was 27.4% in the VII class and there were no individuals in the intervals from I to III class. Our results suggest that chemical degradations of organic matter pollution and nutrient enrichments resulted in recruitment failure of young-stage individuals from the class I to III (Gibbons and Munkittrick 1994). Stream health assessment (SHA) index, based on multiple fish metrics, showed that several metric values of the model were lower in the I_z, compared to the C_z (Table 1). Mean model values (M_v) was 20 in the C_z, which was diagnosed as a "fair condition" in the health. In contrast, the mean of M_v was 12 in the I_z, indicating a "very poor condition". Thus, five model metrics of native fish species (M_1), riffle benthic species (M_2), total number of native individual (M_7) and the abnormality (M_8) were higher in the I_z than in the C_z. These results agree with the population recruitment analysis, indicating that ecological health was impaired due to the chemical pollution and habitat degradations (Klemm et al. 1993, An et al. 2001, Bae and An 2006).

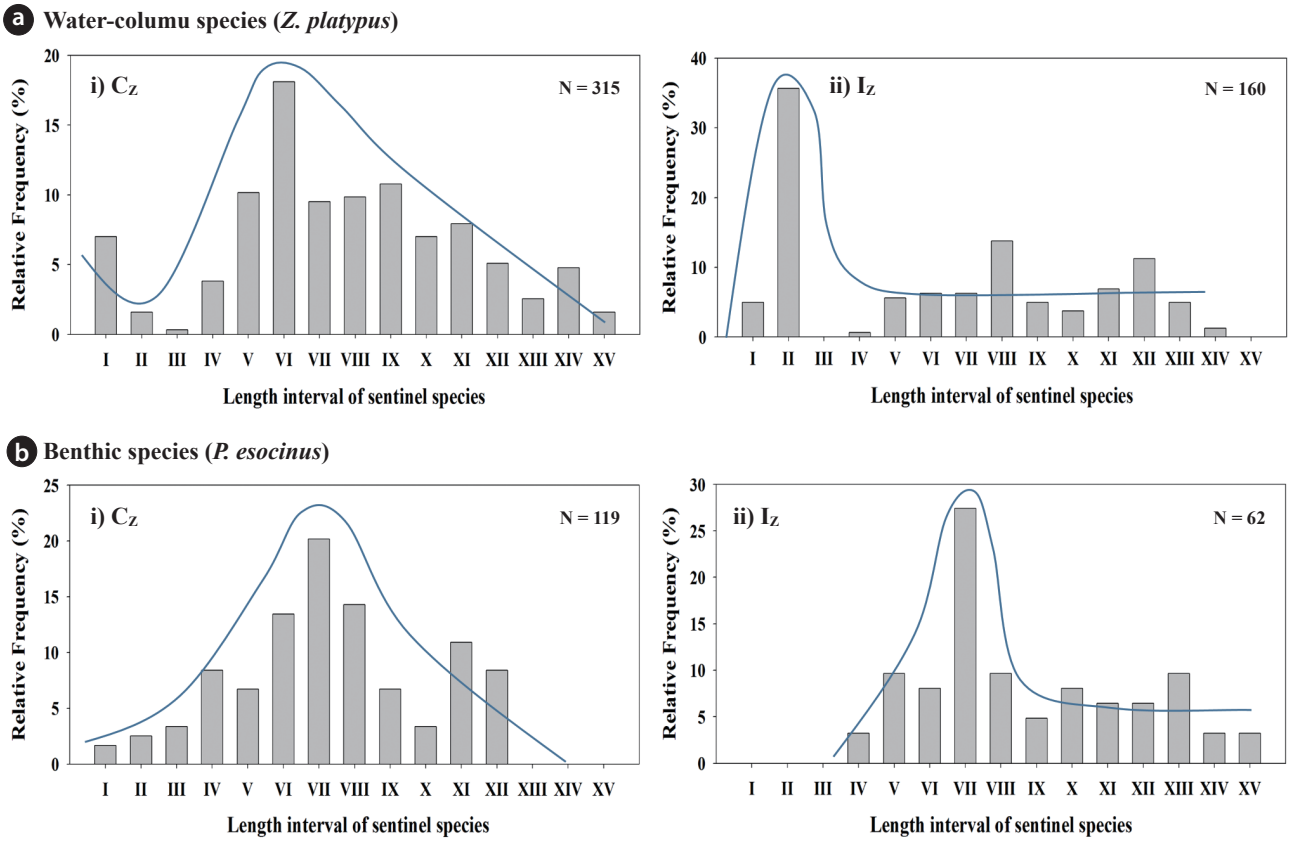

Fig. 7. The relative age distribution of *Zacco platypus* (a) and *Pseudogobio esocinus* (b) in relation to 15 total length intervals from the control zone (C_z) to the impacted zone (I_z) in the sampled streams. (a) I (21 - 30 mm) - XV (161 - 170 mm), (b) I (41 - 50 mm) - XV (181 - 190 mm).

Table 1. Stream Health Assessment (SHA) index* based on fish assembly

Category	Metric attributes	Scoring criteria			Sampling sites			
					C_z		I_z	
		5	3	1	O_v	M_v	O_v	M_v
Species richness & compositions	M_1 Total number of native fish species	Expectations of M_1-M_3 vary with stream size and region.			11.8 ± 5.84	5	11.0 ± 4.56	3
	M_2 Number of riffle benthic species				2.55 ± 1.86	3	0.43 ± 0.76	1
	M_3 Number of sensitive species				1.64 ± 1.62	1	0.57 ± 0.65	1
	M_4 Proportion of individuals as tolerant species	< 5%	5-20%	> 20%	47.4 ± 19.2	1	56.9 ± 21.6	1
Trophic compositions	M_5 Proportion of individuals as omnivore species	< 20%	20-45%	> 45%	54.2 ± 20.5	1	51.9 ± 17.7	1
	M_6 Proportion of individuals as native insectivore species	> 45%	45-20%	< 20%	37.6 ± 17.8	3	40.5 ± 20.0	3
Fish abundance & conditions	M_7 Total number of native individuals	Expectations of M_7 vary with stream size and region.			115.4 ± 53.8	3	135.2 ± 80.0	1
	M_8 Proportion of individuals with deformities, erosion, lesion, and tumors (DELT)	0	0-1%	> 1%	0.27 ± 0.67	3	1.71 ± 4.73	1

*Each value was expressed as a mean ± standard deviation.
C_z, control zone; I_z, impacted zone, N = 25; O_v, observation values; M_v, SHA metric values.

CONCLUSIONS

In this study, stream impact assessments were conducted in urban aquatic ecosystems using multiple biomarkers and bioindicators of blood chemistry, histopathological tissue, species-level, population-level and community-level parameters. Blood chemistry analysis of fish cells indicated that contents of total protein, blood urea nitrogen, and total cholesterol were higher in the impacted zone than the control. Also, heavy metal analysis of total mercury, based on five different tissues of gill, intestine, liver, spine and muscle, were 1.4 fold higher in the impacted zone than in the control and histopathological changes were not shown in the control. In contrast, cell necrosis and degeneration in the histopathological liver analysis were evident in the impacted zone. Previous studies of Adams (2002) reported that cell necrosis and degeneration are closely associated with chemical toxicants such as PAHs, PCBs, and agricultural pesticides. Species-level analysis, based on species biotic index, showed that high values of SBI were evident in the impacted zone, indicating chemical influences by nutrients, organic pollutants and xenotoxicants. Population-level bioindicator, based on size-fraction, showed a reproductive failure of small size population (I - III), especially in the impacted zone. Similarly, community-level bioindicator using the multimetric model of SHA indicated that urban pollution has a negative relationship with biological integrity of streams. These biomarkers and bioindicators reflected the chemical degradations in the impacted zone. Our approaches using multiple biomarkers and bioindicators may be used as a key tool for identifying the effects of urban streams in terms of physical habitat health, chemical health, and biological health and provide important suggestions for stream restoration in the impacted streams.

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