



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

Han-il Kang<sup>1</sup>, Ji-Woong Choi<sup>1</sup>, Seock-Yeon Hwang<sup>2</sup> and Kwang-Guk An<sup>1,\*</sup>

<sup>1</sup>Department of Biological Science, College of Biosciences and Biotechnology, Chungnam National University, Daejeon 305-764, South Korea

<sup>2</sup>Department of Biomedical Laboratory Science, College of Applied Science and Industry, Daejeon University, Daejeon 300-716, South Korea

## 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,

<http://dx.doi.org/10.5141/ecoenv.2015.031>



This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Received 6 April 2015, Accepted 21 May 2015

\*Corresponding Author

E-mail: [kgan@cnu.ac.kr](mailto:kgan@cnu.ac.kr)

Tel: +82-42-821-6408

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 ( $C_p$ ) 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, Triebkorn 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, Triebkorn 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 words, 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.

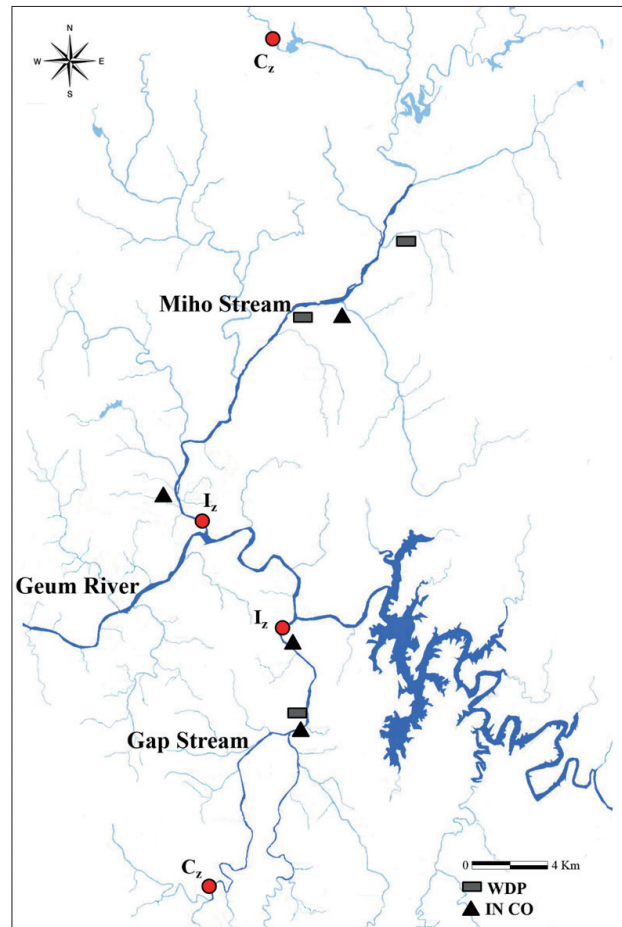
## MATERIALS AND METHODS

### 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  $9 \times 10^5 \text{ m}^3/\text{day}$  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  $2.8 \times 10^4$  and  $8 \times 10^3 \text{ m}^3/\text{day}$  from Cheongju and Naesu, respectively.

### Sampling methods and sampling gears

Fish sampling, based on the catch per unit effort



**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.

(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:  $7 \times 7 \text{ mm}$ ) and kick net (mesh size:  $4 \times 4 \text{ mm}$ ). 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

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  $\mu$ L) 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  $\mu$ m. 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;

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

[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

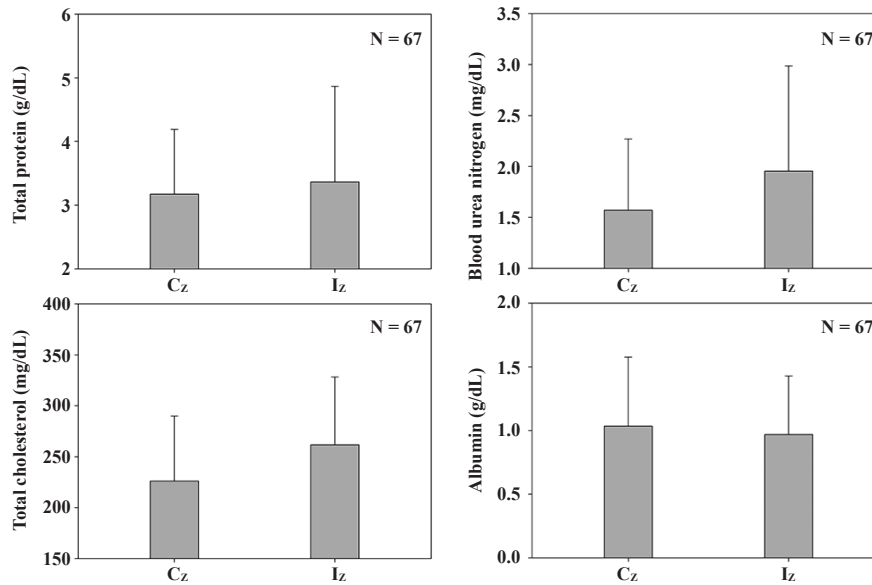


Fig. 2. Blood chemistry parameters from the control zone (C<sub>z</sub>) and impacted zone (I<sub>z</sub>) in the sampling streams.

## 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<sub>1</sub> - M<sub>4</sub>), trophic composition (M<sub>5</sub> - M<sub>6</sub>), and fish abundance and conditions (M<sub>7</sub> - M<sub>8</sub>). 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 ( $\leq 13$ ).

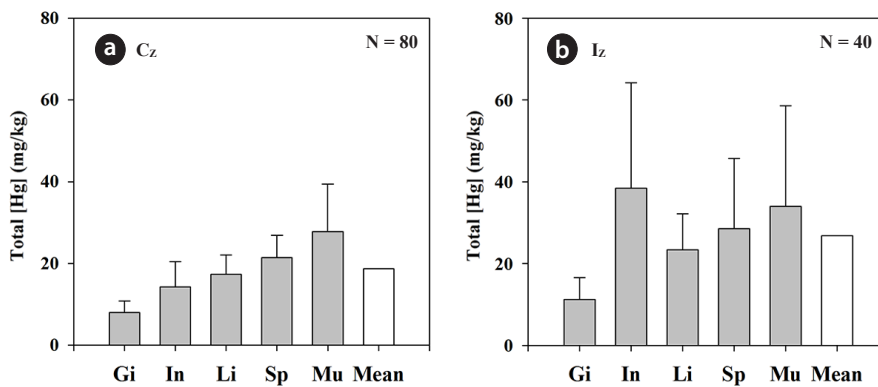
## Statistical analysis

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).

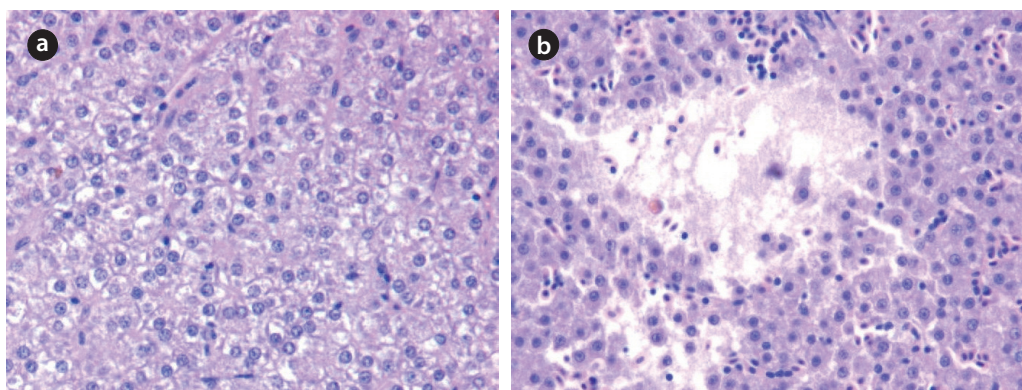
## RESULTS AND DISCUSSION

### Blood chemistry analysis

Total protein (T<sub>Pro</sub>), blood urea nitrogen (B<sub>UN</sub>), total cholesterol (T<sub>Cho</sub>), and albumin (A<sub>ib</sub>) were measured for blood chemistry analysis as shown in Fig. 2. Content of T<sub>Pro</sub> was  $3.17 \pm 1.0$  g/dL in the C<sub>z</sub> and  $3.36 \pm 1.5$  g/dL in the I<sub>z</sub>. B<sub>UN</sub> level was  $1.57 \pm 0.7$  mg/dL in the C<sub>z</sub> vs.  $1.95 \pm 1.0$  mg/dL in the I<sub>z</sub>. In the meantime, content of T<sub>Cho</sub> was  $226.3 \pm 63.7$  mg/dL in the C<sub>z</sub>,  $261.8 \pm 66.6$  mg/dL in I<sub>z</sub>, while the content of A<sub>ib</sub> was  $1.03 \pm 0.5$  g/dL in the C<sub>z</sub> and  $0.97 \pm 0.5$  g/dL in the I<sub>z</sub>. The content of T<sub>Pro</sub> is a good indicator for total amount of proteins in the fish blood, and the content of A<sub>ib</sub> 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<sub>Pro</sub> appeared higher in the I<sub>z</sub> than the C<sub>z</sub> and lower A<sub>ib</sub> in the I<sub>z</sub>. 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<sub>z</sub> in the parameter of T<sub>Pro</sub> (Rosenoer et al. 1977). Nonetheless, it does not seem that eutrophication of I<sub>z</sub> brings positive influence on all the nourishment and liver function, according to the blood chemistry values of A<sub>ib</sub>. The case of B<sub>UN</sub>, is also used frequently as a good indicator for kidney malfunction and liver function, indirectly. B<sub>UN</sub> 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.



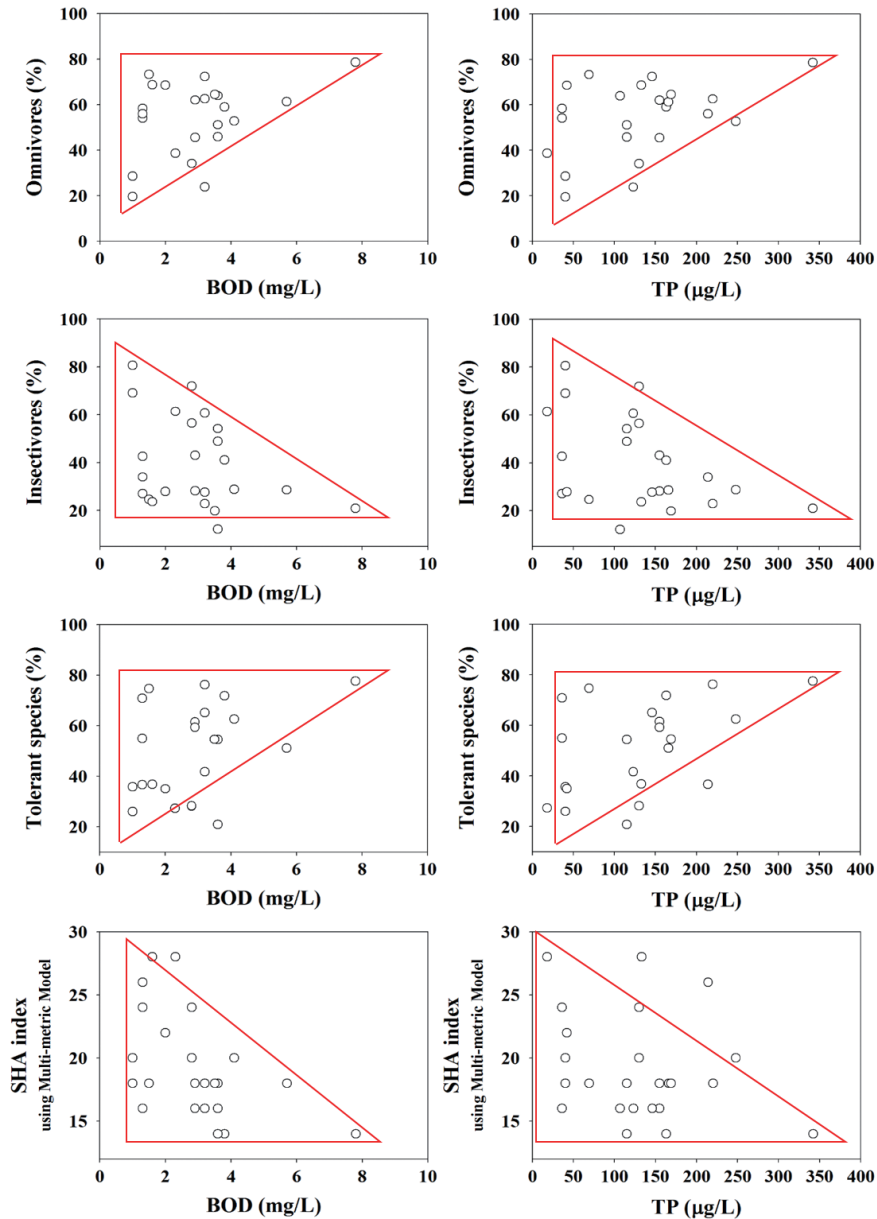
**Fig. 4.** Histopathological appearance of liver tissue in *Pseudogobio esocinus* in the control zone (a) and impacted zone (b) in the Gap Stream. Liver cells on a liver tissue section were stained with hematoxylin-eosin (optical microscope 400 × magnification).

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.

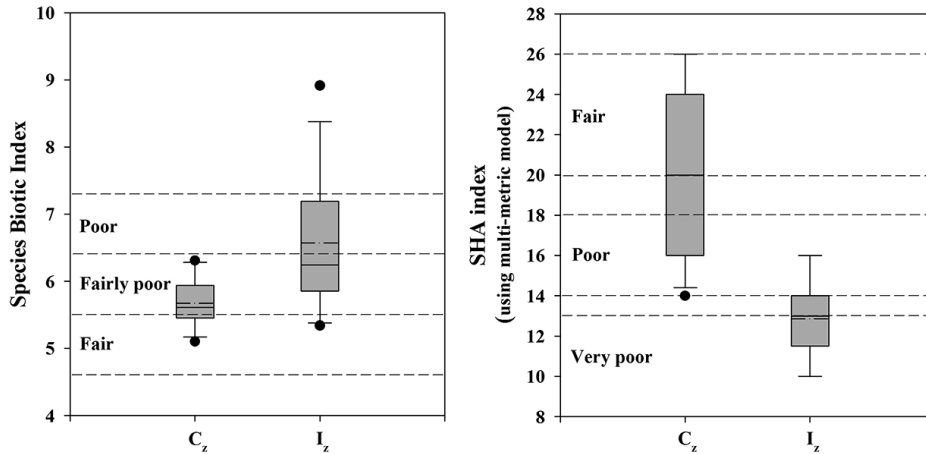


**Fig. 5.** The relationships among trophic guild, tolerance guild, Stream Health Assessment (SHA) index using the multi-metric model and chemical water quality variables in the sampled streams.

## 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  $\mu\text{g/L}$ , and the proportions of omnivores and tolerant species approximately increased from 20% to 80% depending on the TP concen-

trations. 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<sub>z</sub>) to the impacted zone (I<sub>z</sub>) in the sampled streams.

### Species Biotic Index as a species-level indicator

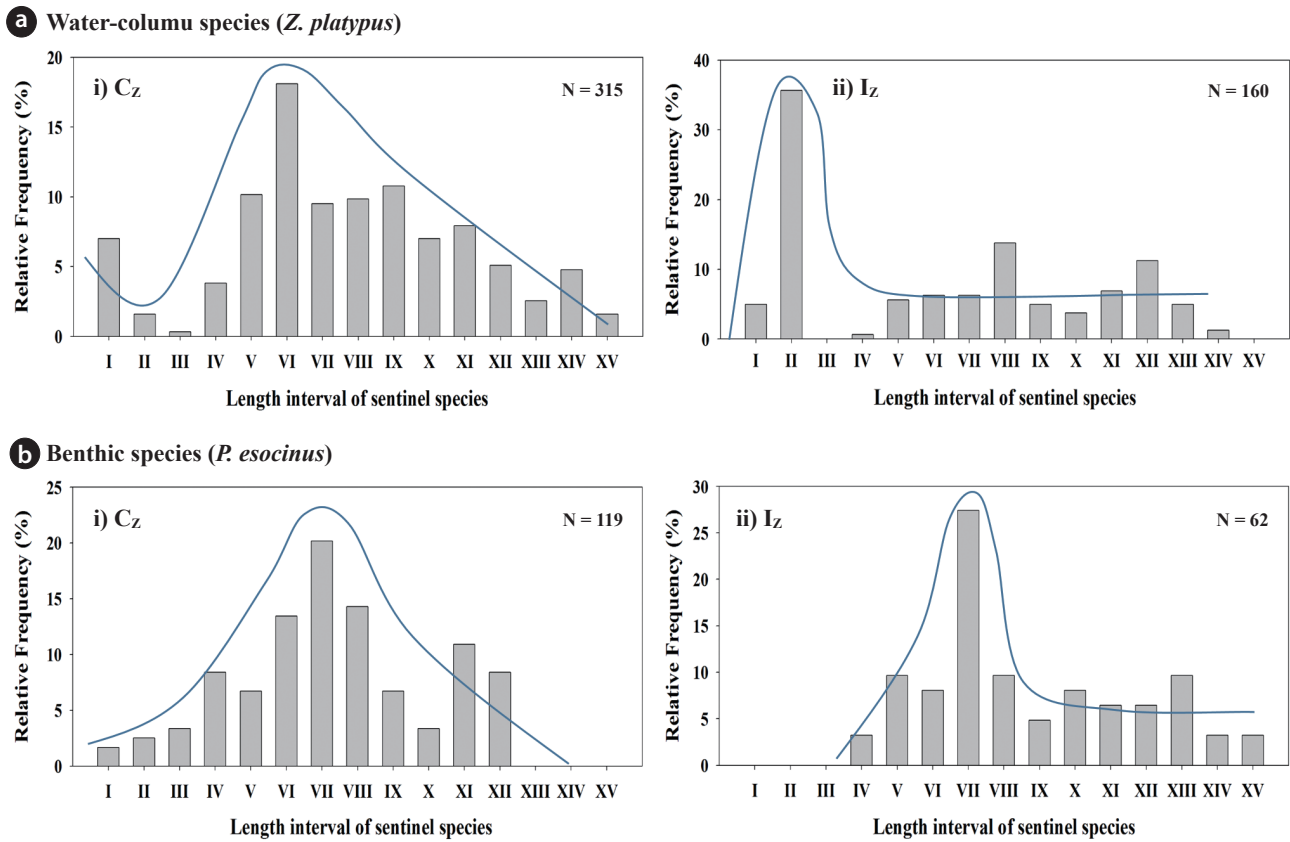
Stream ecosystem health was analyzed in the species-level using a SBI. Values of SBI were  $5.67 \pm 0.35$  in the C<sub>z</sub> and  $6.57 \pm 1.02$  in the I<sub>z</sub> (Fig. 6). Thus, ecological health, calculated as a sum of each species' tolerance value, was ranked as "fair condition" in the C<sub>z</sub> and "poor condition" in the I<sub>z</sub>. SHA index, based on multimetric IBI model, showed that values of SHA index were 20 in the C<sub>z</sub> and 13 in the I<sub>z</sub>. 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<sub>z</sub> and I<sub>z</sub>; the SBI in the two zones of C<sub>z</sub> and I<sub>z</sub> was evaluated as "fair" and "poor", respectively, while the SHA index in the two zones of C<sub>z</sub> and I<sub>z</sub> was evaluated as "fair" and "very poor". These results indicate that impairments in the health condition were evident in the I<sub>z</sub>, 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<sub>1</sub>, M<sub>2</sub>, M<sub>7</sub> and M<sub>8</sub> were greater in the I<sub>z</sub> than the C<sub>z</sub>. 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<sub>z</sub> but skewed distributions in the I<sub>z</sub>. Both species showed normal distribution in all size frequency intervals except for two classes (II and III) in the C<sub>z</sub> (Fig. 7). In contrast, skewed distribution was evident in the I<sub>z</sub>, so the mid-size classes of VI or VII were the highest. In the water-column species, the maximum value of C<sub>z</sub> was 18.1% in the VI class and the minimum value was 0.3% in the III class, while the maximum of I<sub>z</sub> 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<sub>z</sub> was the 20.2% in the VI class, while the maximum of I<sub>z</sub> 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<sub>z</sub>, compared to the C<sub>z</sub> (Table 1). Mean model values (M<sub>v</sub>) was 20 in the C<sub>z</sub>, which was diagnosed as a "fair condition" in the health. In contrast, the mean of M<sub>v</sub> was 12 in the I<sub>z</sub>, indicating a "very poor condition". Thus, five model metrics of native fish species (M<sub>1</sub>), riffle benthic species (M<sub>2</sub>), total number of native individual (M<sub>7</sub>) and the abnormality (M<sub>8</sub>) were higher in the I<sub>z</sub> than in the C<sub>z</sub>. 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<sub>z</sub>) to the impacted zone (I<sub>z</sub>) 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			
		5	3	1	C <sub>z</sub>		I <sub>z</sub>	
					O <sub>v</sub>	M <sub>v</sub>	O <sub>v</sub>	M <sub>v</sub>
Species richness & compositions	M <sub>1</sub> Total number of native fish species				11.8 ± 5.84	5	11.0 ± 4.56	3
	M <sub>2</sub> Number of riffle benthic species	Expectations of M <sub>1</sub> -M <sub>3</sub> vary with stream size and region.			2.55 ± 1.86	3	0.43 ± 0.76	1
	M <sub>3</sub> Number of sensitive species				1.64 ± 1.62	1	0.57 ± 0.65	1
	M <sub>4</sub> Proportion of individuals as tolerant species	< 5%	5-20%	> 20%	47.4 ± 19.2	1	56.9 ± 21.6	1
Trophic compositions	M <sub>5</sub> Proportion of individuals as omnivore species	< 20%	20-45%	> 45%	54.2 ± 20.5	1	51.9 ± 17.7	1
	M <sub>6</sub> 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 <sub>7</sub> Total number of native individuals	Expectations of M <sub>7</sub> vary with stream size and region.			115.4 ± 53.8	3	135.2 ± 80.0	1
	M <sub>8</sub> 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<sub>z</sub> control zone; I<sub>z</sub> impacted zone, N = 25; O<sub>v</sub>, observation values; M<sub>v</sub>, 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.

## ACKNOWLEDGEMENTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (No. 2013R1A1A4A01012939).

## LITERATURE CITED

Adams SM. 2002. Biological indicators of aquatic ecosystem stress: introduction and overview. In: *Biological Indicators of Aquatic Ecosystem Stress* (Adams SM, ed). Amer-

- ican Fisheries Society, Bethesda, MD, pp 1-11.
- Adams SM, Greeley MS. 2000. Ecotoxicological indicators of water quality: using multi-response indicators to assess the health of aquatic ecosystems. *Water Air Soil Pollut* 123: 103-115.
- Adams SM, Ham KD, Greeley MS, LeHew RF, Hinton DE, Saylor CF. 1996. Downstream gradients in bioindicator responses: Point source contaminant effects on fish health. *Can J Fish Aquat Sci* 53: 2177-2187.
- Adams SM, Tremblay LA. 2003. Integration of chemical and biological tools in environmental management and regulation. *Australas J Ecotoxicol* 9: 157-164.
- An KG, Kim DS, Kong DS, Kim SD. 2004. Integrative assessments of a temperate stream based on a multimetric determination of biological integrity, physical habitat evaluations, and toxicity tests. *Bull Environ Contam Toxicol* 73: 471-478.
- An KG, Kim JK. 2007. Ecological impact analysis of a stream on the dam construction using species biotic index (SBI) as a tool of ecosystem health assessment. *Korean J Limnol* 40: 495-502.
- An KG, Lee JY, Bae DY, Kim JH, Hwang SJ, Won DH, Lee JK, Kim CS. 2006. Ecological assessments of aquatic environment using multi-metric model in major nationwide stream watersheds. *J Korean Soc Water Qual* 22: 796-804.
- An KG, Yeom DH, Lee SK. 2001. Rapid bioassessments of Kap Stream using the index of biological integrity. *Korean J Environ Biol* 19: 261-269.
- Anderson RO, Neumann RM. 1996. Length, weight, and associated structural indices. In: *Fisheries Techniques* (Murphy BR, Willis DW, eds). American Fisheries Society, Bethesda, MD, pp 447-482.
- Attrill MJ, Power M. 2002. Climatic influence on a marine fish assemblage. *Nature* 417: 275-278.
- Bae DY, An KG. 2006. Stream ecosystem Assessments, based on a biological multimetric parameter model and water chemistry analysis. *Korean J Limnol* 39: 198-208.
- Barbour MT, Gerritsen J, Snyder BD, Stribling JB. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish. 2nd Ed. EPA-841-B-99-002. US. EPA Office of Water, Washington, DC.
- Brookes A, Shields FD. 1996. River channel restoration: guiding principles for sustainable projects. John Wiley, Chichester.
- Finney BP, Gregory-Eaves I, Sweetman J, Douglas MSV, Smol JP. 2000. Impacts of climatic change and fishing on Pacific salmon abundance over the past 300 years. *Science* 290: 795-799.

- Gibbons WN, Munkittrick KR. 1994. A sentinel monitoring framework for identifying fish population responses to industrial discharges. *J Aquat Ecosyst Health* 3: 227-237.
- Goede RW, Barton BA. 1990. Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. In: *Biological indicator of stress in fish* (Adams SM, ed). American Fisheries Society Symposium, Bethesda, MD, pp 93-108.
- Gopal V, Parvathy S, Balasubramanian PR. 1997. Effect of heavy metals on the blood protein biochemistry of the fish *Cyprinus carpio* and its use as a bio-indicator of pollution stress. *Environ Monit Assess* 48: 117-124.
- Gore JA. 1985. *The restoration of rivers and streams*. Butterworth Publishers, Boston, MA.
- Hilsenhoff WL. 1988. Rapid field assessment of organic pollution with a family-level biotic index. *J North Am Benthol Soc* 7: 65-68.
- Hugueny B, Camara S, Samoura B, Magassouba M. 1996. Applying an index of biotic integrity based on fish assemblages in a West African river. *Hydrobiologia* 331: 71-78.
- Kang N, Kang H, An KG. 2014. Analysis of fish DNA biomarkers as a molecular-level approach for ecological health assessments in an urban stream. *Bull Environ Contam Toxicol* 93: 555-560.
- Karr JR. 1981. Assessment of biotic integrity using fish communities. *Fisheries* 6: 21-27.
- Karr JR, Chu EW. 2000. Introduction: sustaining living rivers. In: *Assessing the Ecological Integrity of Running Waters* (Jungwirth M, Muhar S, Schmutz S, eds). Springer, Dordrecht, pp 1-14.
- Karr JR, Dionne M. 1991. Designing surveys to assess biological integrity in lakes and reservoirs. In: *Biological criteria: Research and Regulation-Proceedings of a symposium*. EPA-44015-91-005. Office of Waters, US EPA, Washington, DC, pp 62-72.
- Kelly MG, Cazaubon A, Coring E, Dell'Uomo A, Ector L, Goldsmith B, Guasch H, Hurlimann J, Jarlman A, Kawecka B, Kwandrans J, Laugaste R, Lindstrom EA, Leitao M, Marvan P, Padisak J, Pipp E, Prygiel J, Rott E, Sabater S, van Dam H, Vizinet J. 1998. Recommendations for the routine sampling of diatoms for water quality assessments in Europe. *J Appl Phycol* 10: 215-224.
- Kelly MG, Whitton BA. 1995. The trophic diatom index: a new index for monitoring eutrophication in rivers. *J Appl Phycol* 7: 433-444.
- Kim IS, Park JY. 2002. *Freshwater fish of Korea*. Kyo-Hak Publishing Co., Seoul.
- Klemm DJ, Stober QJ, Lazorchak JM. 1993. *Fish field and laboratory methods for evaluating the biological integrity of surface waters*. US Environmental Protection Agency. EPA/600/R-92/111, Cincinnati, OH.
- Koizumi N, Matsumiya Y. 1997. Assessment of stream fish habitat based on index of biotic integrity. *Bull Jpn Soc Fish Oceanogr* 61: 144-156.
- Lam PK, Gray JS. 2003. The use of biomarkers in environmental monitoring programmes. *Mar Poll Bull* 46: 182-186.
- Lang C, l'Eplattenier G, Reymond O. 1989. Water quality in rivers of Western Switzerland: Application of an adaptable index based on benthic invertebrates. *Aquat Sci* 51: 224-234.
- Lang C, Reymond O. 1995. An improved index of environmental quality for Swiss rivers based on benthic invertebrates. *Aquat Sci* 57: 172-180.
- Lee RF, Steinert S. 2003. Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. *Mutat Res* 544: 43-64.
- McCarty LS, Power M, Munkittrick KR. 2002. Bioindicators versus biomarkers in ecological risk assessment. *Hum Ecol Risk Assess* 8: 159-164.
- Mela M, Randi MAF, Ventura DF, Carvalho CEV, Pelletier E, Oliveira Ribeiro CA. 2007. Effects of dietary methylmercury on liver and kidney histology in the neotropical fish *Hoplias malabaricus*. *Ecotox Environ Safe* 68: 426-435.
- Munkittrick KR, Dixon DG. 1989. A holistic approach to ecosystem health assessment using fish population characteristics. In: *Environmental Bioassay Techniques and their Application* (Munawar M, Dixon G, Mayfield CI, Reynoldson T, Sadar MH, eds). *Hydrobiologia* 188/189: 123-135.
- Munkittrick KR, McMaster ME, Van Der Kraak G, Portt C, Gibbons WN, Farwell A, Gray M. 2000. Development of methods for effects-driven cumulative effects assessment using fish populations: Moose River project. A Technical Publication of SETAC, Pensacola, FL.
- Nelson JS. 1994. *Fishes of the world*, 3rd Ed. John Wiley & Sons, New York, NY.
- Oberdorff T, Porcher JP. 1994. An index of biotic integrity to assess biological impacts of salmonid farm effluents on receiving waters. *Aquaculture* 119: 219-235.
- Ohio EPA. 1989. *Biological criteria for the protection of aquatic life: Vol. III. Standardized biological field sampling and laboratory method for assessing fish and macroinvertebrate communities*. Technical Report. Columbus, OH.
- Olive PL, Banáth JP. 2006. The comet assay: a method to measure DNA damage in individual cells. *Nat Protoc* 1: 23-29.
- Peakall DB, Shugart LR. 1992. *Biomarkers: Research and application in the assessment of environmental health*.

- NATO ASI Series H: Cell Biology 68, Springer-Verlag, Berlin and Heidelberg.
- Peakall DW, Walker CH. 1994. The role of biomarkers in environmental assessment (3). *Vertebrates. Ecotoxicology* 3: 173-179.
- Plafkin JL, Barbour MT, Porter KD, Gross SK, Hughes RM. 1989. Rapid bioassessment protocols for use in streams and rivers: Benthic macroinvertebrates and fish. EPA-444-4-89-001. Office of Water Regulations and Standards, United States Environmental Protection Agency, Washington, DC.
- Robinet TT, Feunteun EE. 2002. Sublethal effects of exposure to chemical compounds: a cause for the decline in Atlantic eels. *Ecotoxicology* 11: 265-277.
- Rosenoer VM, Oratz M, Rothschild MA. 1977. Albumin structure, function and uses. Pergamon Press, Oxford.
- Sanders RE, Miltner RJ, Yoder CO, Rankin ET. 1999. The use of external deformities, erosion, lesions, tumors (DELT anomalies) in fish assemblages for characterizing aquatic resources: a case study of seven Ohio streams. In: *Assessing the Sustainability and Biological Integrity of Water Resources using Fish Communities* (Simon TP, ed). CRC Press, Boca Raton, Florida, pp 225-246.
- Schmitt CJ, Dethloff GM. 2000. Biomonitoring of environmental status and trends (BEST) program: selected methods for monitoring chemical contaminants and their effects in aquatic ecosystems (No. USGS/BRD/ITR-2000-0005). US Geological Survey, Biological Resources Division, Columbia, MO.
- Singh NP, McCoy MT, Tice RR, Schneider EL. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res* 175: 184-191.
- Triebskorn R, Böhmer J, Braunbeck T, Honnen W, Köhler HR, Lehmann R, Oberemm A, Schwaiger J, Segner H, Schüürmann G, Traunspurger W. 2001. The project VALIMAR (VALIdation of bioMARKers for the assessment of small stream pollution): objectives, experimental design, summary of results, and recommendations for the application of biomarkers in risk assessment. *J Aquat Ecosyst Stress Recovery* 8: 161-178.
- US EPA. 1991. Technical support document for water quality-based toxic control. EPA-505-2-90-001. US EPA, Office of Water, Washington, DC.
- US EPA. 2002. Summary of biological assessment programs and biocriteria development for states, tribes, territories, and interstate commissions: streams and wadable river. EPA-822-R-02-048. Office of Environmental Information and Office of Water, Washington, DC.
- Van der Oost R, Beyer J, Vermeulen NP. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ Toxicol Pharmacol* 13: 57-149.
- Van Gestel CAM, Van Brummelen TC. 1996. Incorporation of the biomarker concept in ecotoxicology calls for a redefinition of terms. *Ecotoxicology* 5: 217-225.
- Water Framework Directive (EU WFD). 2000. Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy, *Off J Eur Com* 22: 12.
- Wepener V, Van Vuren JHJ, Chatiza FP, Mbizi Z, Slabbert L, Masola B. 2005. Active biomonitoring in freshwater environments: early warning signals from biomarkers in assessing biological effects of diffuse sources of pollutants. *Phys Chem Earth Pt A/B/C* 30: 751-761.