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*Article*

# Integrated Ecological River Health Assessments, Based on Water Chemistry, Physical Habitat Quality and Biological Integrity

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**Abstract:** This study evaluated integrative river ecosystem health using stressor-based models of physical habitat health, chemical water health, and biological health of fish and identified multiple-stressor indicators influencing the ecosystem health. Integrated health responses (IHRs), based on star-plot approach, were calculated from qualitative habitat evaluation index (QHEI), nutrient pollution index (NPI), and index of biological integrity (IBI) in four different longitudinal regions (Groups I–IV). For the calculations of IHRs values, multi-metric QHEI, NPI, and IBI models were developed and their criteria for the diagnosis of the health were determined. The longitudinal patterns of the river were analyzed by a self-organizing map (SOM) model and the key major stressors in the river were identified by principal component analysis (PCA). Our model scores of integrated health responses (IHRs) suggested that mid-stream and downstream regions were impaired, and the key stressors were closely associated with nutrient enrichment (N and P) and organic matter pollutions from domestic wastewater disposal plants and urban sewage. This modeling approach of IHRs may be used as an effective tool for evaluations of integrative ecological river health.

**Keywords:** integrative health responses (IHRs) model; river ecosystem health; stressor identification; chemical pollution

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## 1. Introduction

Recent studies of river ecosystems [1–3] pointed out that integrated ecological health assessment is one of the key issues for efficient river management and is frequently used as a tool for the identification of major factors in impaired ecosystems. The degradation of river ecosystem health is largely associated with chemical pollution and physical habitat alterations due to rapid industrialization and urbanization [4–7]. Especially, stream ecosystems are rapidly disturbed by heavy sources of pollution such as industrial effluents [8], municipal wastewater discharges [9] and intense agricultural activities [10]. These sources of pollution may modify longitudinal patterns in nutrients (N and P) and physical habitat from headwaters to downstream near estuaries, and these directly or indirectly influence ecological functions of trophic compositions and tolerance species in aquatic biota [11–13]. Thus, comprehensive indicator analysis of each component in river ecosystems are necessary for assessing and diagnosing the river health, but still little is known about the integrated approach in river health assessments.

Earlier studies on stream/river health have traditionally focused on chemical monitoring due to analytical easiness of chemical condition [14]. Recent paradigms of stream health assessments, however, pointed out that chemical monitoring alone may not be enough for assessing the status of integrative ecological health and thus further biological and ecological health assessments of aquatic systems are necessary for effective management [15–17]. Complex outcomes on habitat modifications arising from channelization, barriers, and altered flow regimes [18] demonstrated partially some reasons why ecological health is modified in the assessments. An integrative ecological health approach is required to identify key factors influencing chemical water quality, physical habitat and biological conditions [15,19,20]. Despite these facts, stream monitoring and assessments for broad goals and management objectives were largely demonstrated by each chemical, physical, and biological criteria, respectively [21].

The assessments of stream and river health were conducted by multi-metic models based on different trophic-level taxa of aquatic organisms along with physical habitat models of Habitat Quality Index (HQIs; [22]). Early studies of Winget and Mangum [23] and Platts *et al.* [24] used Biotic Condition Index (BCI; [23]) for the health assessments, and later biological integrity concepts have been widely applied for evaluating the ecological health of river ecosystems. The concept of "Rapid Bioassessment Protocol" (RBP), developed by US EPA [25], was largely applied to many other countries. This concept, based on the index of biological integrity (IBI) using fish assemblage, was originally developed by Karr [15], and the concept was used with qualitative habitat evaluation index (QHEI; [26]) as an important factor of numerous physical parameters in the health assessments.

The key biota used most frequently in the assessments of river ecosystem health are periphyton or aquatic plants, as an indicator of primary production [25,27,28], macroinvertebrates as an indicator of primary consumers [25,26], and fish as an indicator of primary and top consumers [29,30]. Fish indicator among the biota was most widely used in other Asian [31] and European countries [32,33] as well as in North America (USA [34] and Canada [35]). The biological integrity models using fish assemblages have been regionally developed [36,37] and adapted by many countries in North America [38,39], Europe [40], South America [41,42] and Africa [43–45]. These studies suggested that fish is one of the best indicators for health assessments of aquatic ecosystems due to following characteristics of easiness to collect and identify in the field, longevity in the water during their entire life, and sensitive response to change of water chemistry and physical habitat. Fish taxa are effectively used in assessing long-term damage with environmental modification, the population growth, obesity and fish health conditions [46]. For these reasons, fish was used in various research approaches from micro-level biomarkers of DNA [47], cellular [48], physiological [25], histopathological assays [49] to macro-level bioindicators of organism, population and community [50,51], and these studies inferred the river/stream health using the different organization of the fish. Low-level health response could identify the potential effects on DNA, cellular and physiological levels of organisms, thus diagnosed the impaired health influenced on chemical pollutants and disturbance [52,53]. Major problem of these studies, however, were short-term response and ecological relevance is low [54]. Thus, Adams and Greeley [48] pointed out that integrative multi-metric modeling, based on population or community-levels is required for actual assessments of ecological health assessments [54].

The objective of this study was to evaluate the ecological health of Nakdong River in Korea using an integrated health responses (IHRs) model based on chemical water quality, physical habitat and biological parameters. We developed an original national model of index of biological integrity (IBI) using fish assemblages in 2006 and the model was applied to more than 1000 wadable streams and rivers in Korea. However, it was not enough to diagnose the stream health using only fish variables. Thus, the government required a new methodology for "integrative stream health assessments" and this research was part of that. Our hypothesis was that the single IBI model might not assess the overall ecological health in Korean stream ecosystems because the model did not cover the physical habitat conditions of the stream and also did not include chemical pollution (nutrient pollution). The integrative health assessments, based on the overall parameters of physical, chemical and biological variables, were required in the national health assessments. Our integrative stream health assessments provide key identification of key factors in a problem in the stream health degradations to the Ministry of Environment, Korea, so our research suggests which factor (physical, chemical or biological components) should be restored in the Korean stream ecosystem. Under the hypothesis, we developed an integrative health assessment methods to evaluate (1) the overall ecological health condition of a specific watershed (Nakdong River) of Korean stream ecosystems using biological assessments; (2) nutrient pollution (N, P, biochemical oxygen demand (BOD), and chemical oxygen demand (COD)); and (3) physical habitat health (QHEI model), which is one of the key stressors damaging the Korean stream ecosystems. The outcomes of this research were intended to serve as a starting point for Korean government to eventually establish overall assessment approaches and ecological health criteria, and specifically a new integrative modeled for Korean stream ecosystem. The comprehensive approach has been used to demonstrate the river ecosystems health. Chemical health was evaluated using the nutrient pollution index (NPI) developed in this study. Physical habitat health was determined using the qualitative habitat evaluation index (QHEI) and biological health was determined using the index of biological integrity (IBI). Based on these models, integrative ecological health was compared using a star-plot approach. This IHRs approach can be used as a key tool for the integrative ecological health assessments of river ecosystems. In addition, these approaches provide valuable results for effective management and restoration of river ecosystems in the future.

## 2. Materials and Methods

### *2.1. Study Sites*

This study was conducted in the Nakdong River watershed with the length of 525 km and basin area of 23,860 km$^2$, South Korea (Figure 1), which is located in the southeast of the Korean Peninsula (35°–36° N, 128° E). Nakdong River watershed is influenced by various point/non point sources of pollution such as wastewater disposal plants and urban sewage from several tributaries of Yeong River, Geumho River, Hwang River, Nam River, and Miryang River. Most largest point sources are located on the Geumho River, with a large industrial complex and wastewater disposal plants, so the water quality downstream is rapidly degraded, as suggested in numerous previous studies [8–10]. Intensive agricultural activities, as non-point sources, are concentrated in the zone of Sites 5–8. The total number of sampling sites, consisting of sixth order streams [55], was 21, with 5 reference sites and 16 sites in the main stream. The selection of reference site followed the approach of Hughes *et al.* [56] and U.S. EPA [25].

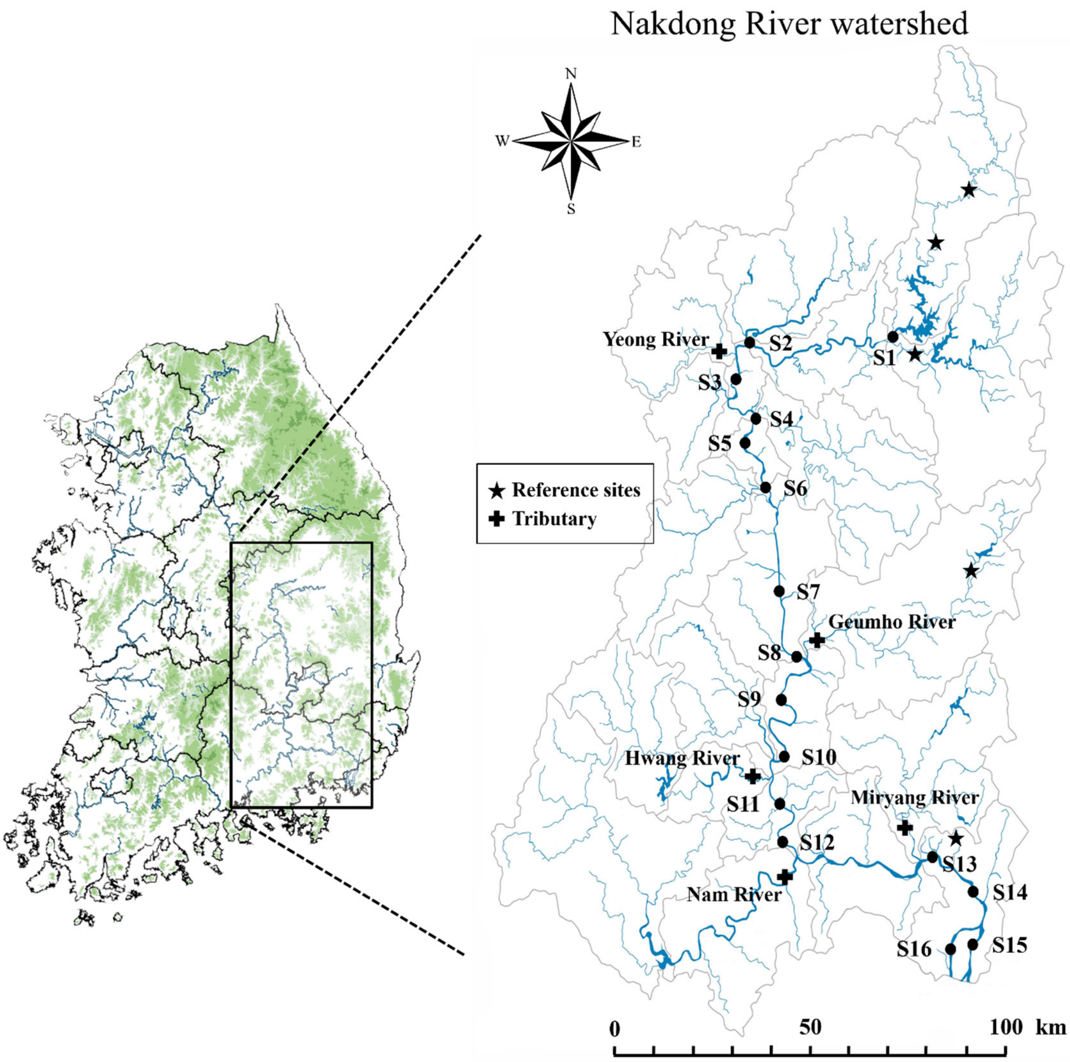



**Figure 1.** Map showing sampling sites in Nakdong River watershed.

The reference streams in the watershed were originally designated in 2006 by the Ministry of the Environment, Korea for efficient watershed management. The reference site was defined as a least-disturbed stream with low impact from human activities such as farming, urban development, and forest management. The selection of reference streams in this region was based on overall ecological conditions of chemical water quality (N, P, or organic pollutants), physical habitat conditions, and biota (periphyton, macroinvertebrate, and fish taxa) in Korea.

### *2.2. Fish Sampling*

Field sampling for fish and water chemistry was conducted twice in the premonsoon (May) and postmonsoon (September) seasons during 2008–2009. Stream flows were relatively stable in both seasons. Fish samplings were conducted by a modified wading method [57,58] to evaluate the Korean aquatic ecosystem health based on the Ohio EPA method [59]. For the fish sampling, we considered all habitat types, such as riffle, run, and pool, in the same site and directed in an upstream to downstream reach for at least 200 m distance during 50 min for the catch per unit efforts (CPUE). Casting-net (7 × 7 mm, CN) and kick-net (4 × 4 mm, KN), the most popular fish sampling gears in Korea, were applied to sample. All fishes were identified *in situ* and released immediately. All specimens were identified according to the key characteristics of Kim and Park [60] and the classification system of Nelson [61]. However, some ambiguous specimens hard to identify were preserved in 10% formalin solution and then brought to the laboratory for further research. All sampled fishes were examined for anomalous external characteristics such as deformities (D), erosion (E), lesion (L), and tumors (T) (DELT) based on the concept of Sanders *et al.* [62]. Tolerance and trophic species analysis were based on the previous regional studies [63].

### *2.3. Analysis of Water Quality Parameters*

Sampling for water quality was conducted twice at the same time as for fish sampling per watershed in 2008–2009. Ten water chemistry parameters analyzed in this study are as follows: biological oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen (TN), ammonia nitrogen ($NH_4$–N), nitrate nitrogen ($NO_3$–N), total phosphorus (TP), ortho phosphorus ($PO_4$–P), total suspended solids (TSS), electrical conductivity (EC) and chlorophyll-a (Chl-*a*). TSS, EC and Chl-*a* were measured at the time of sample collection with the YSI sonde 6600. TN, total dissolved nitrogen (TDN) and total particle nitrogen (TPN) were measured by second derivative method after a persulfate digestion [64]. TP was determined using the ascorbic acid method after persulfate oxidation [65]. TSS, BOD and COD were measured by the standard methods [66]. Nutrient analyses were performed thrice, and BOD, COD and SS were measured twice [66].

### *2.4. Nutrient Pollution Index (NPI) for Chemical Health Analysis*

To develop chemical health assessment model, multi-metric model of nutrient pollution index (NPI), followed methods used by Dodds *et al.* [67] and Lee and An [68]. The metrics were composed as following; $M_1$: total nitrogen (TN, $mg \cdot L^{-1}$), $M_2$: total phosphorus (TP, $\mu g \cdot L^{-1}$), $M_3$: TN:TP ratio, $M_4$: BOD ($mg \cdot L^{-1}$), $M_5$: total suspended solids (TSS, $mg \cdot L^{-1}$), $M_6$: electrical conductivity ($\mu S \cdot cm^{-1}$), and $M_7$: chlorophyll-*a* ($\mu g \cdot L^{-1}$). We established the criteria for boundaries and boundary was defined by the third of the observed distribution of the values. Each metric was scored 5, 3 or 1 point, respectively. The health conditions of the chemistry were evaluated by summing the scores obtained from the seven parameters and then categorizing the system as excellent (Ex; 31–35), good (G; 25–29), fair (F; 19–23), poor (P; 13–17), and very poor (VP; 7–11).

### *2.5. Qualitative Habitat Evaluation Index (QHEI) for Physical Habitat Health Analysis*

Physical habitat health, based on the multi-metric model of the qualitative habitat evaluation index (QHEI), was evaluated at the sampling sites. The original 11-metric QHEI model [25,26] was modified as a six metric model for regional application [58,69]. The metric attributes were as follows: $M_1$: epifaunal substrate/available cover, $M_2$: pool substrate characterization, $M_3$: channel flow status, $M_4$: existence of small-scale dams, $M_5$: channel alteration, and $M_6$: sediment deposition. Habitat health conditions were evaluated by summing the scores obtained from the six parameters and divided into 4 categories of excellent (Ex; score 96–120), good (G; 66–80), fair (F; 36–60) or poor (P; 6–30) conditions [58].

### *2.6. Index of Biological Integrity (IBI) for Biological Health Analysis*

Multi-metric fish model was developed for the diagnosis of the ecosystem health in the Nakdong River. Our model, IBI, which was based on the IBI concept [15,70], was modified from the original U.S. EPA [71] model and the regional model of An *et al.* [72]. The metrics (M) were consisted in three major groups as ecological characteristics by species richness and composition, trophic composition, and fish abundance with health condition. The individual metrics were: $M_1$: total number of native species, $M_2$: number of riffle-benthic species, $M_3$: number of sensitive species, $M_4$: proportion of individuals as tolerant species, $M_5$: proportion of individuals as omnivore species, $M_6$: proportion of individuals as insectivore species, $M_7$: total number of native individuals, and $M_8$: percent individuals with anomalies. Four of the eight metrics ($M_1$, $M_2$, $M_3$, and $M_7$) were evaluated by the maximum species richness line (MSRL, [73]) with the stream orders. Each metric was scored 5, 3 or 1 and community-level health conditions were judged using the criteria of Barbour *et al.* [25]. The IBI scores were judged as five categories, excellent (Ex; 36–40), good (G; 28–34), fair (F; 20–26), poor (P; 14–18) and very poor (VP; 8–13). Detailed descriptions of specific metric characteristics and scoring criteria for the model are available in An *et al.* [72].

### *2.7. Integrated Health Responses (IHRs) Model Using Star-Plot Analysis*

The integrated health responses (IHRs) model was developed in this research to enable the multi-metric assessment of ecological health. The IHRs model was composed of multiple functional metrics and was based on the integration of all parameters derived from biological, chemical and physical health parameters. Data-processing step was to generate the assessment scores (*i.e.*, standardized data) followed methods used by Yeom and Adams [74], Lee *et al.* [75] and Lee and An [3]. The area score enclosed by each star-plot was used to compare the assessment results for the difference among sampling sites relative to their ecological health response to environmental conditions at each site. The area score of star-plot was calculated according to methods described by Beliaeff and Burgeot [76] and Kim *et al.* [77]. The integrated health, IHRs model values, was judged as five ranks of excellent (Ex; >90% of reference), good (G; 75%–90% of reference), fair (F; 55%–75% of reference), poor (P; 35%–55% of reference), and very poor conditions (VP; <35% of reference).

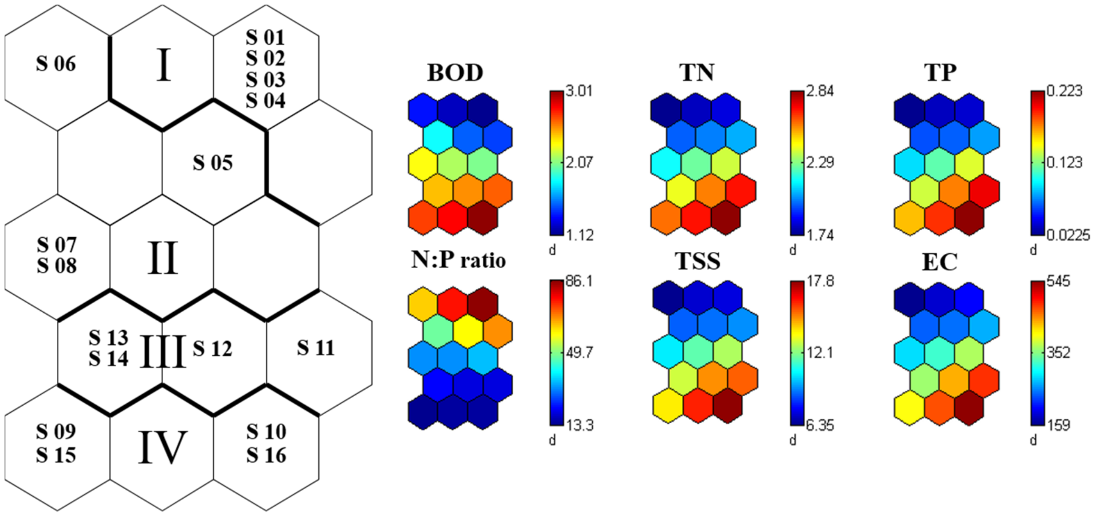
*2.8. Statistical Analysis*

Self-organizing map (SOM) was used to analyze the longitudinal patterns of fish composition and water chemistry parameters at the 16 sampling sites. The SOM approach is based on a learning algorithm in an artificial neural network and approximates the probability density function of the input data [78,79]. It has a wide range of engineering applications for handling complex ecological data (e.g., non-linear modeling or optimization) and is typically used for classification, clustering, prediction, modeling, and data mining [80]. The learning process of the SOM was applied using the SOM Toolbox package developed by the Laboratory of Information and Computer Science in the Helsinki University of Technology for Matlab ver. 6.1, and we adopted the initialization and training methods suggested by the authors of the SOM Toolbox that allow the algorithm to be optimized [81]. In addition, the PC-Ord statistical package (Ver. 4.25 for Windows; [82]) was used for principal components analysis (PCA) to identify the major environmental factors influencing ecological parameters on clustered by SOM.

## 3. Results and Discussion

*3.1. Cluster Analysis of Sampling Regions Using a Model of Self-Organizing Maps (SOMs)*

The relations between the chemical water quality and biological variables of fish (tolerance and trophic compositions) were analyzed using the modeling approach of Self-Organizing Maps (SOMs). As shown in Figure 2, each variable of BOD, TN, TP, TSS, electrical conductivity (EC) and N:P ratios were patterned according to the similarity of community compositions through training with the SOM. To evaluate the relations between chemical and biological parameters, the classified variables were visualized by the color on the map (Figure 2). Red color regions in the map indicated high values, whereas blue color regions indicated low values. Through the learning process of the SOMs, the clusters were divided into four groups, I–IV along the longitudinal gradients of ecological factors. Undisturbed sites were grouped in Group I of the SOM map, while polluted sites appeared Group IV. Group I, which was located in the headwater region, occurred in the pristine regions with low organic matter (BOD: 0.96 ± 0.15 mg·L$^{-1}$) and nutrient levels (TN: 1.79 ± 0.12 mg·L$^{-1}$, TP: 19 ± 0 μg·L$^{-1}$). In contrast, Group IV, which was located in the downstream region, occurred in the polluted regions with high organic matter (BOD: 2.9 ± 1.33 mg·L$^{-1}$) and nutrient levels (TN: 2.66 ± 0.48 mg·L$^{-1}$, TP: 195 ± 80 μg·L$^{-1}$). Chemical parameters were clearly differentiated between Group I and Group IV. This pattern of downstream degradations was similar to other parameters of BOD, TN and TP. Likewise, the proportion of sensitive species (SS) was higher in the Group I and the proportion of tolerant species (TS) was relatively higher toward the downstream region (Group IV). In the case of omnivores (O), they were widely distributed in every site of the stream of Nakdong River, but the proportion of omnivores was relatively lower in downstream than upstream due to high dominance of carnivorous species (55%). Our results of SOMs model suggest that the clustering of the trained SOMs units reflected the regional differences of water chemistry (chemical parameters) and biological compositions (biological parameters) from the upstream to downstream.

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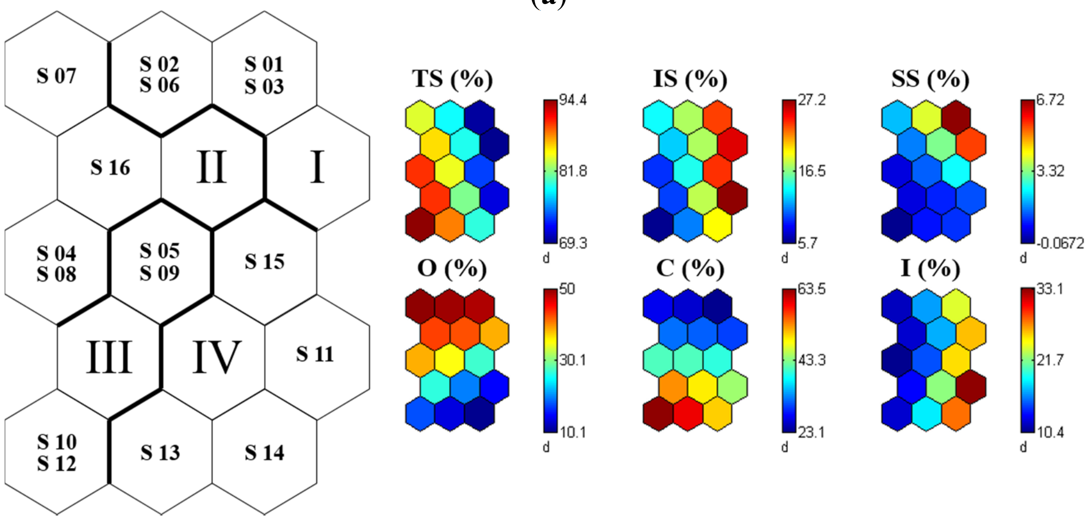



(**b**)

**Figure 2.** Clustering of the trained self-organizing maps (SOMs) units for chemical parameters and biological parameters. The four groups (I–IV) indicate different clusters of ecological characteristics, and the code in each unit of the map refers to the sampling site. The mean value of each variable was calculated from each output neuron of the trained SOM. The red and blue colors indicate a high and low value, respectively, for each environmental parameter: (**a**) Chemical parameters and (**b**) biological components.

### *3.2. Ecological Factor Identification Using a Principal Component Analysis* (*PCA*)

Principal component analysis (PCA) was used to analyze key factors influencing biological components and chemical parameters (Figure 3). Results indicated that the groups of river regions could be divided into Group I, Group II, Group III and Group IV by eigenvalues of >1.0. Results of PCA indicated that three axes explained 80.8% of the variation in our data matrix (eigenvalues of >1.0). The axis-1 on BOD, TN, and TP could explain about 47.4% of the total and the axis-2 on %

omnivores, % insectivores, and IBI model values explained 21.6% of the total. Also, the axis-3 on the proportions of tolerant species and sensitive species explained 11.9% of the total (Table 1). The PCA analysis indicated that axis-1 was mainly influenced by organic matter (BOD; −0.3955) and nutrient levels (TN; −0.4420, TP; −0.4131), which had negative responses. The biological responses of % omnivores, % insectivores and IBI model values were useful indicators in the axis-2 analysis. The eigenvalue of omnivore species was –0.5207, which indicated a negative response, while % insectivores and IBI model values of >0.5 were showed positive responses. In the meantime, axis-3 was weakly influenced by tolerant species and sensitive species. Thus, Group I, which is located in the upstream, was directly influenced with mass ratios of N:P and NPI values, and Group IV, which is located in downstream, was directly influenced by organic matter (BOD), high nutrient levels (TN and TP) and suspended solids (TSS). Overall, the results of PCA suggested that greater impacts of chemical pollution were evident in the downstream regions.

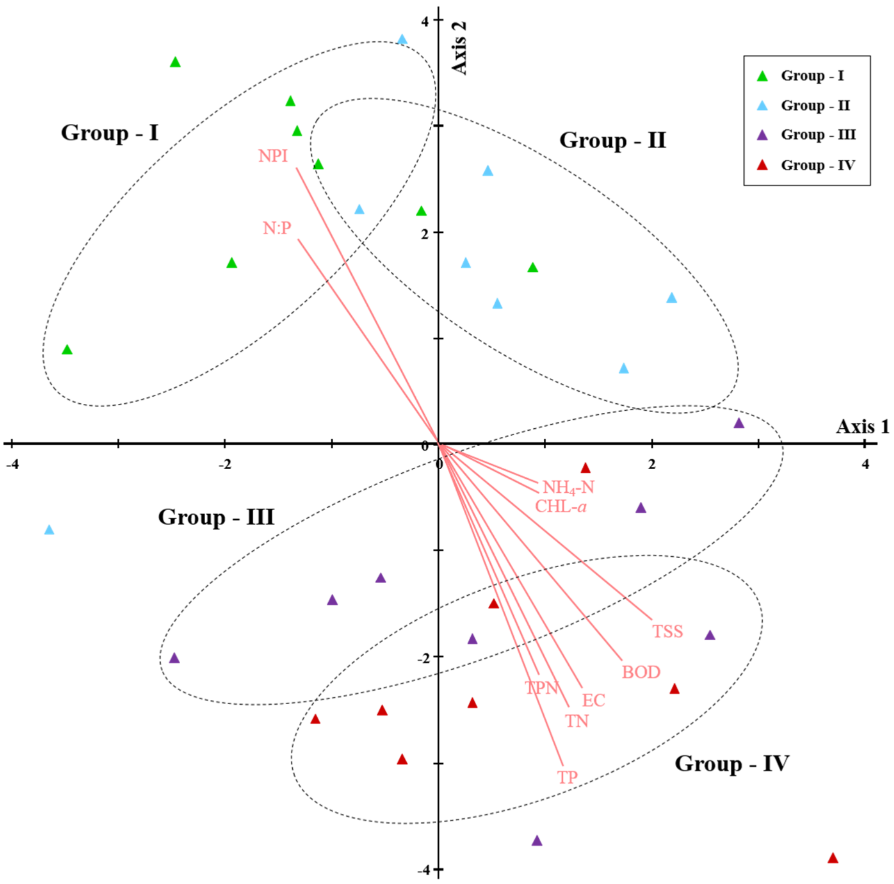



**Figure 3.** Principal component analysis (PCA) based on biological components (% tolerant species, % sensitive species, % omnivores, % insectivores, IBI value) and chemical factors (BOD = biological oxygen demand, COD = chemical oxygen demand, TN = total nitrogen, $NH_4$–N = ammonium nitrogen, $NO_3$–N = nitric nitrogen, TDN= total dissolved nitrogen, TPN = total particle nitrogen, TP = total phosphorus, $PO_4$–P = ortho phosphorus, N:P = N:P ratio, TSS = total suspended solids, EC = electrical conductivity, CHL-*a* = chlorophyll-*a*, NPI = nutrient pollution index score).

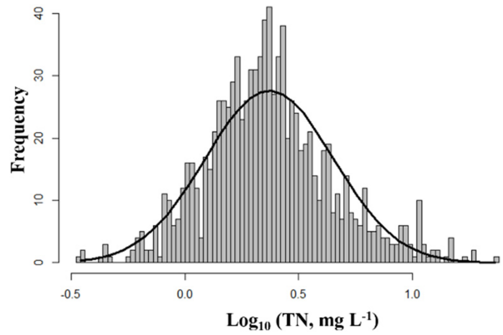
**Table 1.** Principal component analysis (PCA) based on biological and chemical variables. Bold values indicate statistically significant in the level of <0.05.

| **Principal Component Analysis/Eigenvalue >1.0** | | | |
|---|---|---|---|
| **Structure Metrics** | **Axis 1** | **Axis 2** | **Axis 3** |
| % Tolerant species | −0.2508 | −0.0625 | **0.7282** |
| % Sensitive species | 0.2932 | 0.0293 | **−0.4934** |
| % Omnivores | 0.2854 | **−0.5207** | −0.0887 |
| % Insectivores | 0.2392 | **0.5115** | −0.0667 |
| BOD | **−0.3955** | −0.1632 | −0.1394 |
| TN | **−0.4420** | −0.0142 | −0.2790 |
| TP | **−0.4131** | −0.0689 | −0.3019 |
| IBI score | −0.1209 | **0.6550** | 0.0112 |
| Eigenvalue: | 4.264 | 1.941 | 1.070 |
| Proportion of variance | 47.380 | 21.562 | 11.890 |

### *3.3. Chemical Model of Water Quality Index and Its Evaluation*

Multi-metric model of nutrient pollution index (NPI) was developed and applied to the model Nakdong River watershed (Figure 4). The metrics of NPI model was composed of seven ($M_1$–$M_7$) and were categorized as four groups of nutrient compositions (N and P), organic matter (BOD), inorganic contents/solids, and primary production indicators (Table 2). For variables of the NPI model, we selected total nitrogen (TN) and total phosphorus (TP) along with N:P mass ratios, which are known as key determinants regulating the river water quality and eutrophication [83–86].

Chemical criteria, based on ambient nutrient metrics of TN, were categorized as oligotrophic (<1.5 $mg \cdot L^{-1}$), mesotrophic (1.5–3 $mg \cdot L^{-1}$) and eutrophic (>3 $mg \cdot L^{-1}$), respectively, and these criteria differed from the previous criteria in North America [87–89] and Europe [90,91]. Mean value of TN in Group III and Group IV regions were significantly higher ($p < 0.05$) than those at the regions of Group I and Group II as well as the reference sites (1.82 ± 1.3 $mg \cdot L^{-1}$). The values of TN, however, were categorized as mesotrophic ("3" in the metric score) in the analysis, indicating no large spatial variations in the model score. In the meantime, TP had large longitudinal variations along the main axis of the headwaters to the downstream, by the criteria of TP (<30, 30–100, >100 $\mu g \cdot L^{-1}$); TP was oligotrophic (mean: 12 ± 5 $\mu g \cdot L^{-1}$) in the region of Group I, and this condition was similar to the reference sites. In contrast, the mean TP of Group III and Group IV was >10 fold than the reference and Group I streams, indicating severe phosphorus enrichment in the downstream regions. The metric indicator of N:P applied in this study also showed the similar pattern with TP rather than TN (Table 2). Based on the mass ratio metric of TN:TP, reference and Group I streams had high ratios of >100, whereas Group III and Group IV streams had low ratios of <20. Previous studies pointed out that N:P ratio in the ambient stream and river waters is an indirect indicator of limiting nutrient for algal biomass or primary production/growth [86,92,93] and is lower in polluted streams or eutrophic waterbodies [67]. Our outcome of N:P ratios in this watersheds was supported by previous research. In fact, the contents of chlorophyll-a (CHL), as good indicators of primary productivity, were directly determined by mass nutrient ratios (N:P) and TP.

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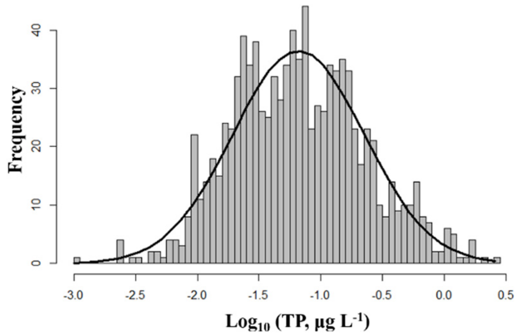


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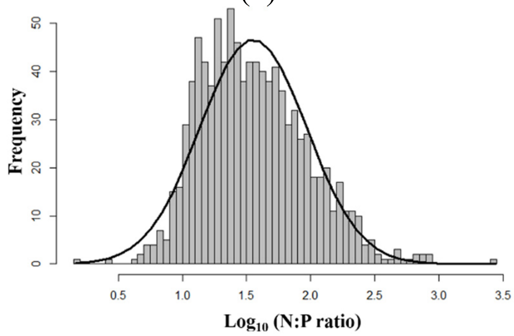


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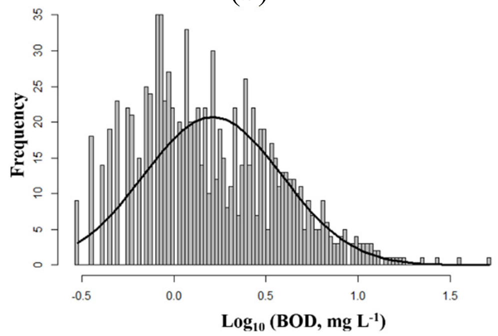


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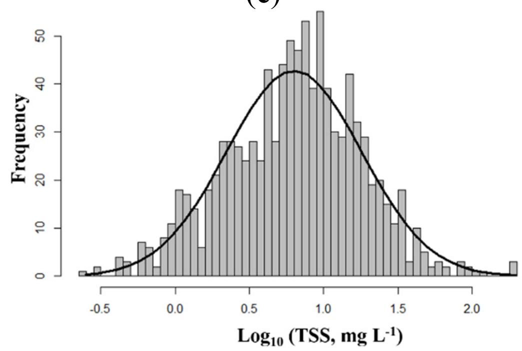


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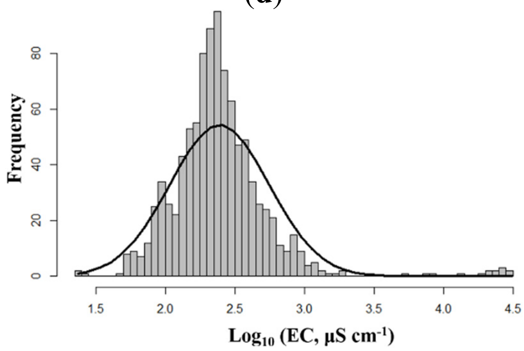


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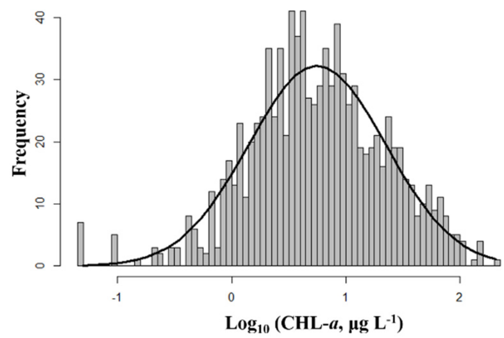



(**g**)

**Figure 4.** Observed frequency diagram (total nitrogen: $n = 974$, total phosphorus: $n = 973$, TN:TP ratio: $n = 973$, BOD: $n = 974$, total suspended solids: $n = 974$, electrical conductivity: $n = 974$, chlorophyll-a: $n = 969$). (**a**) $\mathbf{M_1}$: Total Nitrogen (mg·L$^{-1}$); (**b**) $\mathbf{M_2}$: Total Phosphorus (μg·L$^{-1}$); (**c**) $\mathbf{M_3}$: TN: TP ratio; (**d**) $\mathbf{M_4}$: BOD (mg·L$^{-1}$); (**e**) $\mathbf{M_5}$: Total Suspended Solids (mg·L$^{-1}$); (**f**) $\mathbf{M_6}$: Electrical Conductivity (μS·cm$^{-1}$); and (**g**) $\mathbf{M_7}$: Chlorophyll-*a* (μg·L$^{-1}$).

**Table 2.** Chemical health assessment model, based on the Nutrient Pollution Index (NPI), suggested scoring criteria and evaluated score in the watershed of Nakdong River.

| Category | Metric | Scoring Criteria | | | Mean ± SD (Score) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 5 | 3 | 1 | $R_f$ | Group I | Group II | Group III | Group IV |
| Nutrient regime | $M_1$: Total Nitrogen ($mg \cdot L^{-1}$) | <1.5 | 1.5–3 | >3 | 1.82 ± 1.33 (3) | 1.80 ± 0.1 (3) | 1.85 ± 0.33 (3) | 2.82 ± 0.42 (2.5) | 2.66 ± 0.49 (2) |
| | $M_2$: Total Phosphorus ($\mu g \cdot L^{-1}$) | <30 | 30–100 | >100 | 12 ± 5 (5) | 19 ± 6 (5) | 46 ± 18 (3.5) | 197 ± 37 (1) | 195 ± 95 (1) |
| | $M_3$: TN: TP ratio | >50 | 20–50 | <20 | 188 ± 189 (5) | 100 ± 33 (5) | 46 ± 21 (3) | 14 ± 2 (1) | 14 ± 3 (1) |
| Organic matter | $M_4$: BOD ($mg \cdot L^{-1}$) | <1 | 1–2.5 | >2.5 | 0.72 ± 0.22 (5) | 0.96 ± 0.20 (4) | 1.87 ± 0.80 (3) | 2.37 ± 0.56 (2) | 2.9 ± 1.55 (2) |
| Ionic contents and solids | $M_5$: Total Suspended Solid ($mg \cdot L^{-1}$) | <4 | 4–10 | >10 | 1.87 ± 1.31 (5) | 6.1 ± 5.5 (4) | 9.0 ± 2.3 (2) | 14.3 ± 2.9 (1.5) | 15.8 ± 7.2 (1.5) |
| | $M_6$: Electrical conductivity ($\mu S \cdot cm^{-1}$) | <180 | 180–300 | >300 | 179 ± 94 (5) | 170 ± 28 (4) | 231 ± 67 (3) | 434 ± 110 (1) | 460 ± 245 (2) |
| Primary production indicator | $M_7$: Chlorophyll-a ($\mu g \cdot L^{-1}$) | <3 | 3–10 | >10 | 2.6 ± 1.2 (5) | 7.1 ± 3.4 (3) | 36.6 ± 18.7 (1) | 37.7 ± 25.0 (1) | 32.4 ± 12.3 (1) |
| Scores (model criteria of NPI) | | | | | 33 (Ex) | 28 (G) | 19.5 (F) | 10 (VP) | 10.5 (VP) |

Mean values of CHL in the Group III and Group IV regions were >30 $\mu g \cdot L^{-1}$, at high TP and low N:P ratios, and these values were significantly higher ($p < 0.05$) than those at the regions of Group I (7.1 ± 3.4 $\mu g \cdot L^{-1}$) and reference (2.6 ± 1.2 $\mu g \cdot L^{-1}$) with low TP and high N:P ratios. These results indicate that the high CHL values in our watershed were closely associated with high P and low N:P ratios. The ratio of N:P, thus, is a key chemical health parameter controlling cyanobacterial blooms in the aquatic environment. In the meantime, the metric of biological oxygen demand (BOD), as an indicator for organic matter pollution, showed the distinct differences between the headwaters (Group I) or reference and the downstream regions (Groups III/IV); the metric values were 4–5 in the reference and headwater streams but were 1.5 in the downstream regions (Groups III/IV). Thus, metric values of BOD showed similar spatial patterns with total suspended solids (TSS) as well as the parameter metrics of N:P ratios and TP.

Chemical health, based on seven multi-metric model of Nutrient Pollution Index (NP index), showed distinct spatial differences between the regions of the watershed. Index model values of NP in the region of Group I was 28 and this value was similar to the reference sites (33 score). This indicates that the chemical health was judged as “good condition (G)” in the headwater region (Group I) by the health criteria of five classes. In contrast, the model values of NP in the downstream regions (Groups III and IV) ranged between 10.0 and 10.5, which were judged as “very poor (VP) condition” (most impaired level) among the five classes. The impaired chemical health in the downstream regions was mainly due to effluents from the massive point/non point sources of municipal wastewater disposal plants and urban runoff, which are come from tributary of Geumho River. The degradation of chemical health in the downstream is supported by previous research on chemical water quality [39,94,95].

*3.4. Responses of Biological Indicators on Water Chemistry*

Responses of biological indicators, as fish tolerance and trophic species, on water chemistry are shown in Figure 5. The proportions of tolerant species (TS), sensitive species (SS) and insectivore species (I) in the watershed were directly determined by chemical water quality parameter. When chemical concentrations of TP, BOD, and electrical conductivity (EC) are low, these three environmental factors showed a wide variation in biological responses between the maximum and minimum values (Figure 5). The responses in the proportion of tolerant species, sensitive species, and insectivore species, however, had direct functional relations with chemical conditions. When values of TP were >200 μg·L$^{-1}$, the proportions of tolerant species and insectivore species had positive functional responses to increased TP, but the proportions of sensitive species had negative functional responses to TP (Figure 5). Similar functional responses in the proportions of tolerant species, sensitive species, and insectivore species were shown in BOD, as an indicator for organic matter pollution, and the EC as an indicator of ionic pollution, when BOD and EC values were >2.0 mg·L$^{-1}$ and 270 μS·cm$^{-1}$, respectively (Figure 5). Such nutrients of phosphorus directly determined concentrations of sestonic chlorophyll-a (CHL), and the CHL values, in turn, influenced the fish compositions of tolerant species, sensitive species and insectivore species. These responses are supported by findings of US EPA [39] that the proportions of sensitive and/or insectivore fish species decrease with nutrient enrichment and organic matter pollution, and vise verse in tolerant species. In the meantime, the responses on the levels of TN were not shown in this study due to high concentrations of N regardless of location and season. The high nitrogen was more attributed to stream geology rather than degree of nutrient pollution, thus nitrogen contents were high in the pristine regions with 100% forest stream.

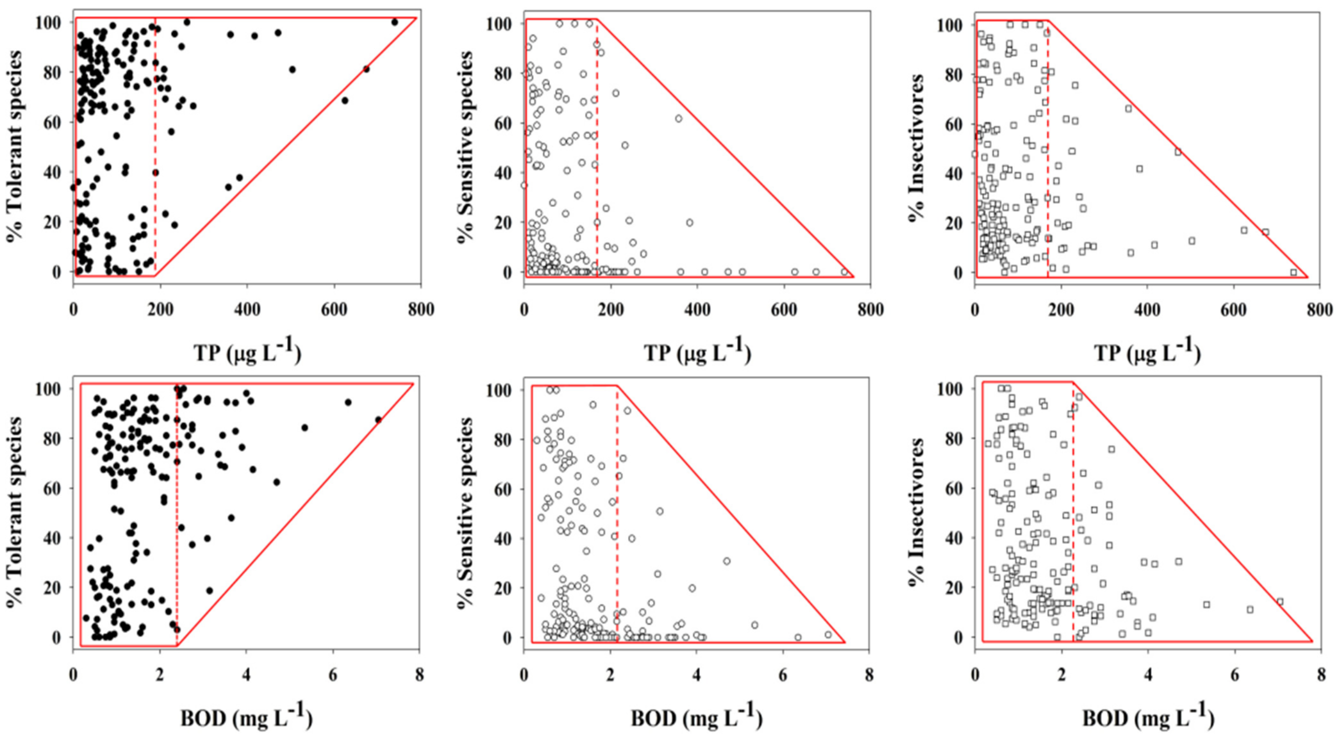



**Figure 5.** *Cont.*

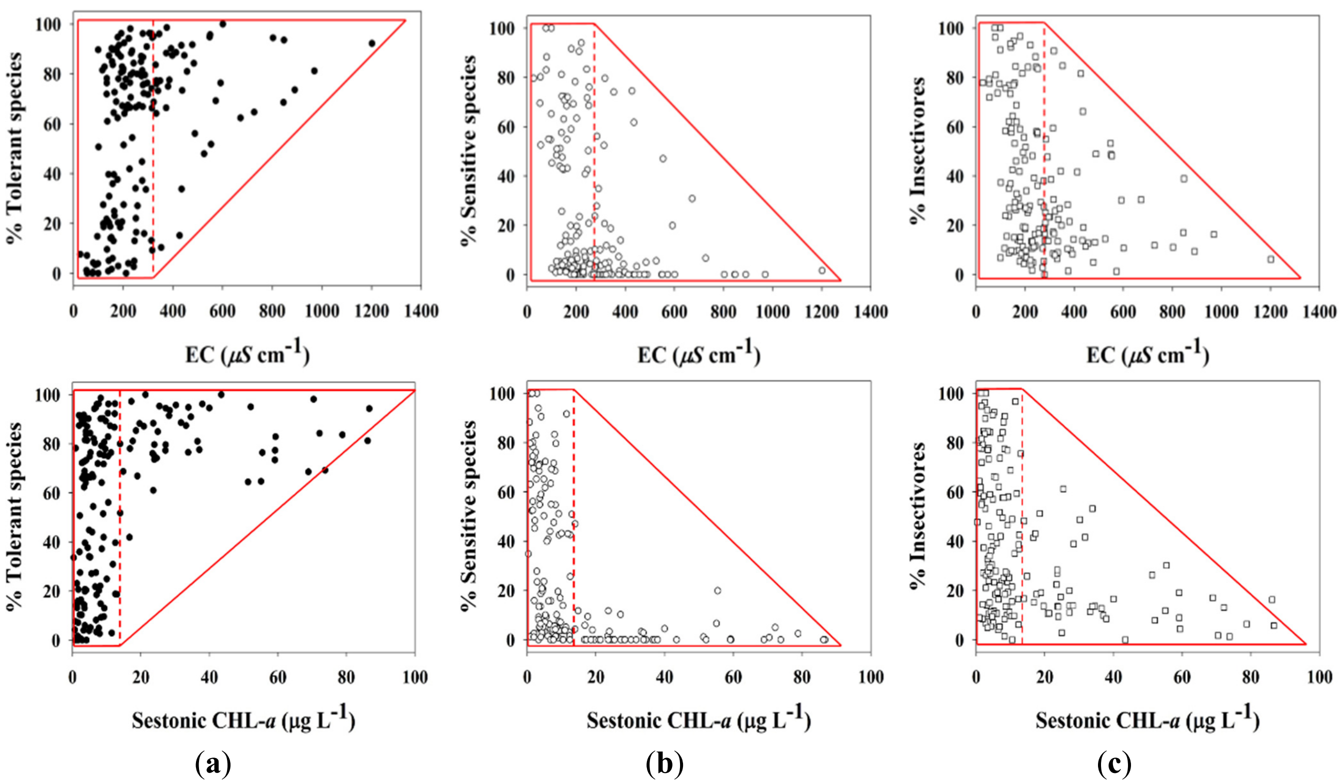



**Figure 5.** The relation of fish indicators (tolerance and trophic species) to water quality parameters (total phosphorus (TP), biochemical oxygen demand (BOD), electrical conductivity (EC), sestonic chlorophyll-a (Chl-*a*)): (**a**) % Tolerant Sp.; (**b**) % Sensitive Sp.; and (**c**) % Insectivores Sp.

### *3.5. Physical Habitat Health Using a Multi-metric Model*

Qualitative Habitat Evaluation Index (QHEI), based on a six metric model, was used for the evaluation of physical habitat health in the watershed of Nakdong River. Values of QHEI averaged 67 in the watershed regions of Groups I–IV and ranged between 60 and 75. Thus, physical habitat health in all regions was judged as a "good condition" (G) by the criteria of An *et al.* [96] (Table 3). As shown in Table 3, spatial variations from the headwaters to the downstream were not high, unlike other watersheds in Korea [97,98]. Physical habitat health of Groups I and II showed 30% more degradation, compared to the reference regions, thus some sites were not suitable for fish habitats and this was mainly influenced by human disturbances. Habitat health of Groups III and IV in downstream regions, was better than the regions of Groups I and II. The health impairments in the upstream were mainly due to poor epifaunal substrate/available cover ($M_1$) and poor pool substrate conditions ($M_2$) throughout the habitat simplification by sand accumulations. In addition, partial channel alterations and sediment depositions were found in the impaired habitats.

### *3.6. Biological River Health Using a IBI-Multimetric Community Model and Fish Compositions*

Biological river health assessments, based on multi-metric Index of Biological Integrity (IBI) are shown in Table 4. The river health in the regions of Groups I–IV was compared with the regions of reference sites. The values of IBI model averaged 30 in the reference sites with ranges of each metric value from 3 to 5 (Table 4). The river health, thus, was judged as a "good condition (G)" by the criteria of An *et al.* [72]. Such IBI values in these reference sites were not so high as shown in reference regions of other countries [15,39]. In the meantime, the model values of IBI in all regions of Groups I–IV ranged from 12 to 18, which is corresponding to poor (P) to very poor (VP), respectively. The IBI values averaged 12 in regions of Groups III and IV, and this value was lower than the IBI values of Groups I and II (mean IBI = 16) as well as reference regions (IBI = 30; Table 4). Biological river health in this watershed was more impaired downstream than upstream, and the impairment was mainly attributed to reduced metric values of riffle-benthic species, sensitive species, insectivore species and native species and anomalies. The low values in the metrics were due to chemical degradations of the downstream and the degradations in the main river downstream was closely associated with nutrient-rich effluents of wastewater disposal plants from tributary streams. Such impairments of the river health in the downstream are similar in previous studies [39,97,98], which are directly influenced by large point-source pollutions of wastewater treatment plants and industrial complex.

**Table 3.** Physical habitat health assessment, based on the Qualitative Habitat Evaluation Index (QHEI), in the watershed of Nakdong River.

| Metric | Study Areas | | | | |
|---|---|---|---|---|---|
| | $R_f$ | Group I | Group II | Group III | Group IV |
| $M_1$: Epifaunal substrate/Available cover | 15.3 | 4.8 | 6.6 | 9.4 | 13.1 |
| $M_2$: Pool substrate characterization | 14.2 | 7.5 | 8.8 | 8.6 | 10.8 |
| $M_3$: Channel flow status | 8.3 | 12.8 | 11.1 | 13.4 | 14.8 |
| $M_4$: Existence of small-scale dams | 13.4 | 12.3 | 13.0 | 15.4 | 13.6 |
| $M_5$: Channel alteration | 11.9 | 11.5 | 10.6 | 13.9 | 11.1 |
| $M_6$: Sediment deposition | 12.5 | 13.1 | 10.3 | 11.9 | 11.1 |
| Scores (model criteria of QHEI) | 75.6 (G) | 61.9 (G-F) | 60.4 (G-F) | 72.5 (G) | 74.5 (G) |

Notes: $R_f$: Reference sites; Group I: 4 site (S1, S2, S3, S4), Group II: 4 site (S5, S6, S7, S8), Group III: 4 site (S9, S10, S11, S12), Group IV: 4 site (S13, S14, S15, S16), Ex: excellent, G: good, F: fair.

In addition, the river health was closely associated with community structures, based on fish compositions of tolerance species and trophic species. In this study, total 45 species and 4610 individuals were collected from the watershed of Nakdong River. The dominant fishes with greater than 5% in relative abundance are shown in Table 5 in Nakdong River. The highest dominant species was *Opsarichthys uncirostris*, which composed about 30% of the total, and then followed by *Zacco platypus* (28%), *Micropterus salmoides* (5%), and *Squalidus chankaensis tsuchigae* (5%). The fish fauna suggest that the dominant species are composed of more tolerant species on the water chemistry or physical habitat. Meanwhile, key dominant species in the reference streams were *Zacco koreanus* and *Coreoleuciscus splendidus*, which made up 58% of the total and are sensitive species and insectivore species (Table 5). Thus, the reference region was designated as *Zacco-Coreoleuciscus* community, and differed largely from the regions of Groups I and IV, indicating a distinct difference in species composition in the structural aspects of the community (Figure 6). The community of the redions of Group I, with *Zacco-Opsarichthys* domination, showed tolerant species at >70% of the total, while the regions of Group IV, with a *Opsarichthys-Micropterus* community, were composed of a community of only tolerant species.

**Table 4.** Biological river health assessment, based on the multi-metric fish model of Index of Biological Integrity (IBI), in the watershed of Nakdong River.

| Category | Metric | Scoring Criteria | | | Mean ± SD (Score) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 5 | 3 | 1 | $R_f$ | Group I | Group II | Group III | Group IV |
| Species richness & compositions | $M_1$: Total number of native species | Expectations of $M_1$ vary with stream order | | | 11.4 ± 3.2 (3) | 11 ± 2.2 (3) | 11.8 ± 4.3 (3) | 10.5 ± 1.9 (3) | 9.8 ± 2.4 (1) |
| | $M_2$: Number of riffle-benthic species | Expectations of $M_2$ vary with stream order | | | 3.2 ± 1.3 (3) | 1.5 ± 0.6 (1) | 1.5 ± 1.7 (1) | 1.3 ± 0.5 (1) | 0.8 ± 0.5 (1) |
| | $M_3$: Number of sensitive species | Expectations of $M_3$ vary with stream order | | | 6.4 ± 1.1 (3) | 2.8 ± 1.0 (1) | 1.5 ± 1 (1) | 0.8 ± 1.0 (1) | 0.3 ± 0.5 (1) |
| | $M_4$: Proportion of individuals as tolerant species | <5 | 5–20 | >20 | 11 ± 7 (3) | 74 ± 9 (1) | 77 ± 10 (1) | 83 ± 5 (1) | 86 ± 7 (1) |
| Trophic compositions | $M_5$: Proportion of individuals as omnivore species | <20 | 20–45 | >45 | 19 ± 12 (5) | 54 ± 4 (1) | 47 ± 10 (1) | 27 ± 18 (3) | 18 ± 9 (5) |
| | $M_6$: Proportion of individuals as insectivore species | >45 | 45–20 | <20 | 73 ± 12 (5) | 27 ± 5 (3) | 17 ± 5 (1) | 12 ± 8 (1) | 14 ± 5 (1) |
| Fish abundance & conditions | $M_7$: Total number of native individuals | Expectations of $M_7$ vary with stream order | | | 226 ±82 (3) | 213.5 ± 59.6 (3) | 327.5 ± 118.4 (3) | 196 ± 23 (1) | 81.5 ± 36.7 (1) |
| | $M_8$: Percent individuals with anomalies | 0 | 0–1 | >1 | 0 (5) | 0 ± 0 (5) | 0.2 ± 0.2 (3) | 1.3 ± 2.5 (1) | 1.8 ± 3.6 (1) |
| Scores (model criteria of IBI) | | | | | 30 (G) | 18 (P) | 14 (P) | 12 (VP) | 12 (VP) |

**Table 5.** Fish fauna and dominant species for collected fish population in Nakdong River.

| Sample | Fish Community | Dominant Species | To. | Tr. | RA (%) |
|---|---|---|---|---|---|
| Reference Sites | *Zacco-Coreoleuciscus* Community | *Zacco koreanus* † | SS | I | 47.8 |
| | | *Coreoleuciscus splendidus* † | SS | I | 10.6 |
| | | *Zacco platypus* | TS | O | 10.4 |
| | | *Pungtungia herzi* | IS | I | 9.2 |
| | | *Niwaella multifasciata* † | SS | O | 4.4 |
| Group I | *Zacco-Opsarichthys* Community | *Zacco platypus* | TS | O | 46.7 |
| | | *Opsarichthys uncirostris amurensis* | TS | C | 15.6 |
| | | *Pseudogobio esocinus* | IS | I | 7.8 |
| | | *Hemibarbus labeo* | TS | I | 6.3 |
| | | *Pungtungia herzi* | IS | I | 5.7 |
| Group II | *Zacco-Opsarichthys* Community | *Zacco platypus* | TS | O | 38.5 |
| | | *Opsarichthys uncirostris amurensis* | TS | C | 31.3 |
| | | *Rhinogobius brunneus* | IS | I | 6.1 |
| | | *Pseudogobio esocinus* | IS | I | 5.8 |
| | | *Squalidus chankaensis tsuchigae* † | IS | O | 5.8 |
| Group III | *Opsarichthys-Zacco* Community | *Opsarichthys uncirostris amurensis* | TS | C | 47.7 |
| | | *Zacco platypus* | TS | O | 13.7 |
| | | *Micropterus salmoides* ‡ | TS | C | 8.9 |
| | | *Squalidus chankaensis tsuchigae* † | IS | O | 7.2 |
| | | *Carassius auratus* | TS | O | 3.5 |

**Table 5.** *Cont.*

| Sample | Fish Community | Dominant Species | To. | Tr. | RA (%) |
|---|---|---|---|---|---|
| Group IV | *Opsarichthys-Micropterus* Community | *Opsarichthys uncirostris amurensis* | TS | C | 29.8 |
| | | *Micropterus salmoides* ‡ | TS | C | 21.0 |
| | | *Lepomis macrochirus* ‡ | TS | I | 9.2 |
| | | *Tridentiger obscurus* | TS | I | 6.2 |
| | | *Mugil cephalus* | TS | H | 5.8 |

Notes: To.: tolerance species (TS: tolerant species, IS: intermediate species, SS: sensitive species), Tr.: trophic species (C: carnivores, O: omnivores, I: insectivores, H: herbivores), RA: relative abundance, †: Korean endemic species, ‡: exotic species.

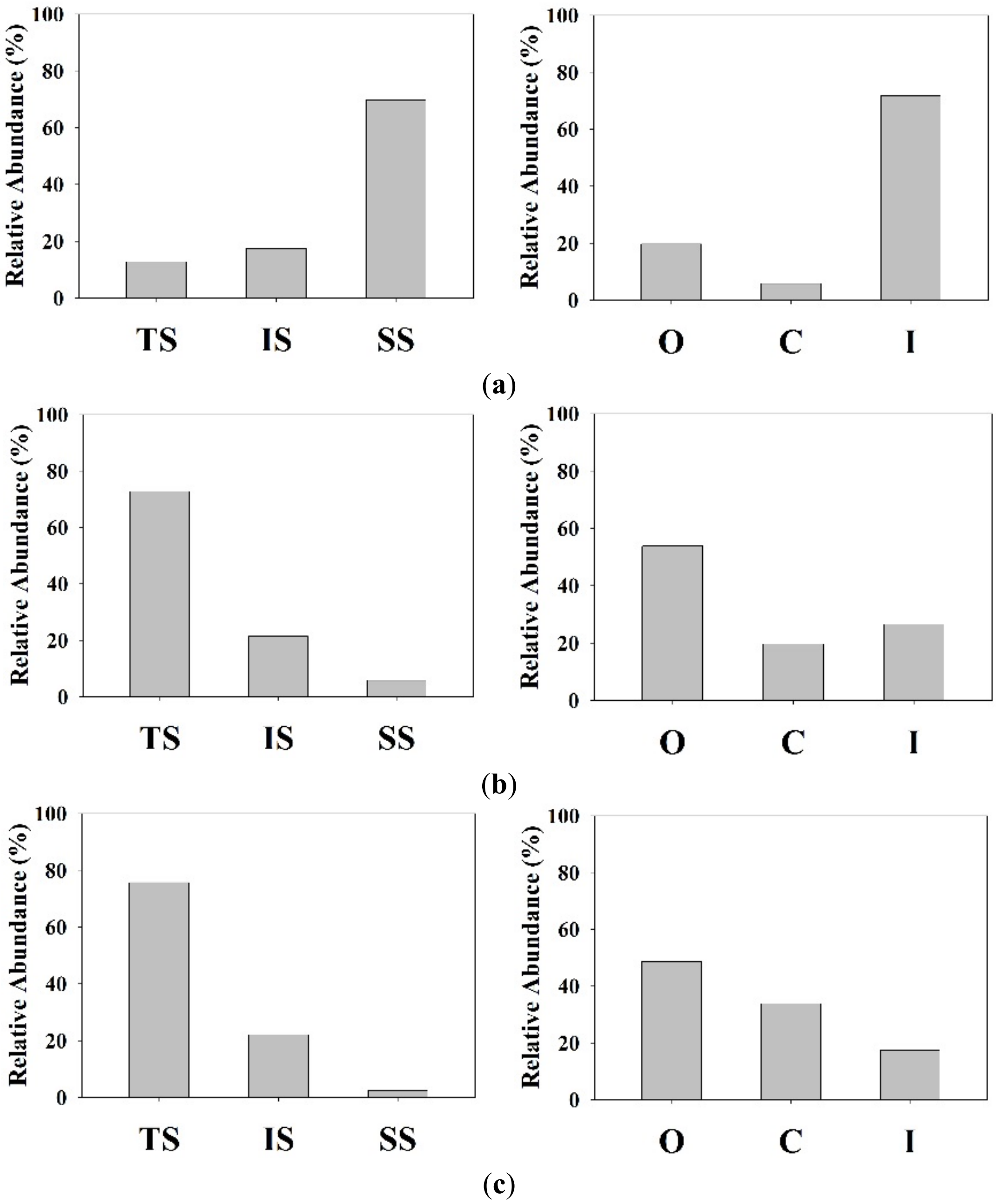
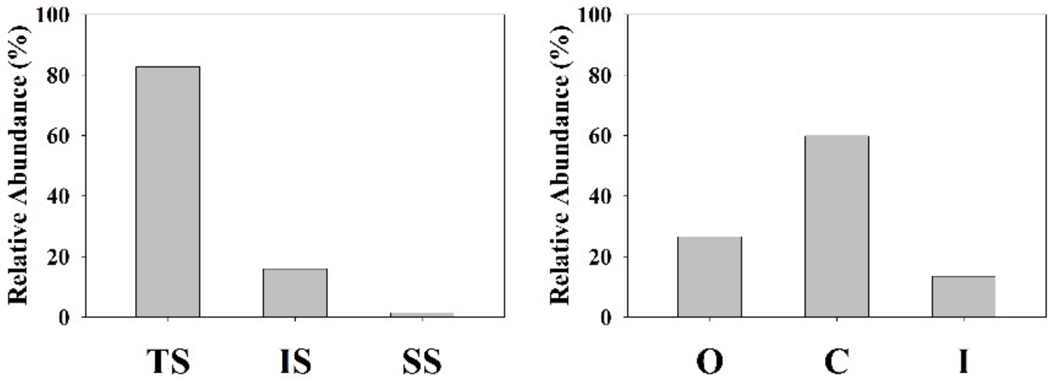
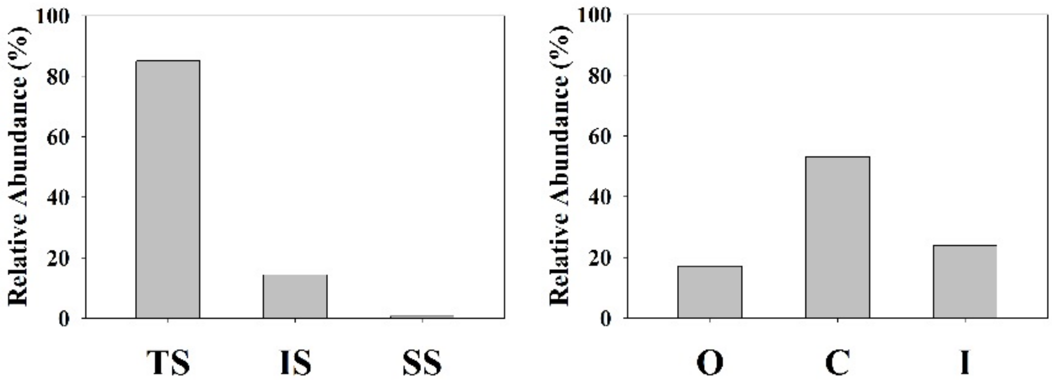



**Figure 6.** *Cont.*




(**d**)




(**e**)

**Figure 6.** Comparison of tolerance and trophic species analysis in Nakdong River (TS: tolerant species, IS: intermediate species, SS: sensitive species, O: omnivores, C: carnivores, I: insectivores): (**a**) Reference: *Zacco-Coreoleuciscus* Community; (**b**) Group I: *Zacco-Opsarichthys* Community; (**c**) Group II: *Zacco-Opsarichthys* Community; (**d**) Group III: *Opsarichthys-Zacco* Community; and (**e**) Group IV: *Opsarichthys-Micropterus* Community.

### *3.7. Integrated Health Responses (IHRs), Based on Physical, Chemical, and Biological Parameters*

The model of Integrated Health Responses (IHRs), based on the star-plot approach of Beliaeff and Burgeot [76], was used for a diagnosis of overall ecological river health. Mean values of IHRs model for the upstream to downstream were derived by integrating the physical habitat health (Qualitative Habitat Evaluation Index, QHEI), chemical health (nutrient pollution index, NPI), and biological health parameters (Index of Biological Integrity, IBI; Figure 7). Area score of Group I in upstream regions was 0.37, which was less than twice of the reference regions (0.62). In the Group I, the axis values of biological health (0.45) and physical health (0.52) were lower than the value (0.8) of chemical health, and the area of Group I was 62% of the reference condition, indicating a “fair” condition. The axis values of Group II were 0.35, 0.56 and 0.5, respectively, for the three variables of NPI, QHEI, and IBI. The area score of Group II was 2.6 times lower, compared to the values of the reference condition, indicating a “poor” condition, and also was lower than the regions of Group I (0.37). The lowest area score (0.17) in the star-plot were found in the Group III and this area value was similar to the regions of Group IV (area score = 0.18). The integrated health in Group III was judged as a “very poor” condition, and was same as Group IV. The star-plot analysis indicated that integrated river health, based on Integrated Health Responses (IHRs), was more impaired in the downstream regions (Group III and IV) than in the upstream (Group I and II) and reference regions. The impaired river health was due to greater impacts in biological health and chemical health than the physical health. Physical habitat health did not largely differ among the four regions, indicating not so significant in the health gradients of regions. In contrast, chemical health was most pronounced in the downstream of Group III and IV (axis value of Group III = 0.29, Group IV = 0.3) due to nutrient enrichment and organic matter pollutions of tributary river (*i.e.*, Geumho River), which is directly influenced by domestic wastewater disposal plants and the urban sewage.

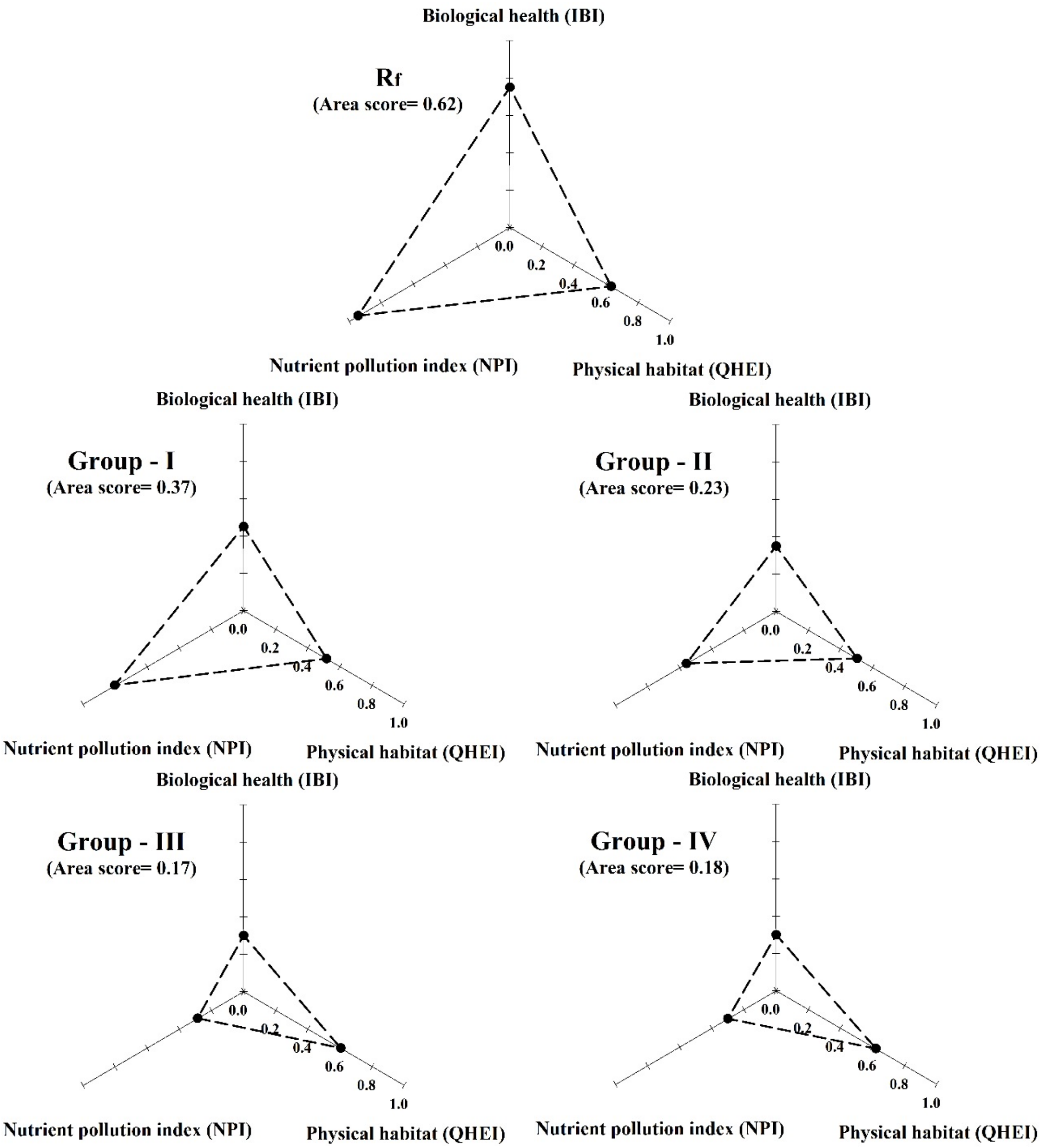



**Figure 7.** Integrated health responses (IHRs) using the star-plot area analysis in Nakdong River.

## 4. Conclusions

Integrated Health Responses (IHRs) in this study were determined by the integration of three multi-metric models of chemical water health (NPI), physical habitat health (QHEI) and biological health (IBI). Each metric model was developed separately for the application of IHRs model using a star-plot approach, and then the health conditions were determined by the comparison of the five reference sites. In the data analysis, the integrated ecological health, based on the mean of IHRs, was more impaired downstream than upstream, and this was mainly attributed to influences of point-sources and urban developments downstream. Thus, longitudinal gradients in the health from the upstream to downstream were evident in the three model, NPI, QHEI, and IBI. The model of Self-Organizing Map (SOM) at 16 sampling streams was matched to the longitudinal patterns of chemical and biological parameters from headwater to downstream. Statistical tests of principle component analysis (PCA) indicated that Group I was located in the region of the upstream and was closely associated with high N:P ratios in the ambient water. In contrast, Group IV was located in the downstream with nutrient enrichment and organic matter pollution. These results of PCA were also supported by spatial pattern analysis using the SOM model. Overall, this approach of the IHRs may be used as a key tool for the quantification of integrated ecological river health in the river ecosystems.

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**Author Contributions**

Ji Yoon Kim collected the field data, and performed the data analysis and manuscript writing under the supervise of Kwang-Guk An who is Ji Yoon Kim's M.S. thesis advisor.

**Conflicts of Interest**

The authors declare no conflict of interest.

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