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Biological Health Assessments of Lotic Waters by Biotic Integrity Indices and their Relations to Water Chemistry

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Received: 30 January 2019; Accepted: 26 February 2019; Published: 28 February 2019

Abstract: Biological health assessment (BHA) has developed as an imperative gauge in efficient management of freshwater resources and fish assemblages. The principal objective of this research is directed at the development and application of a new index under the umbrella of the famous index of biological integrity (IBI). Further, we intended to carry out comparative assessments of the new index with two existing indices and testified to their rational in Geum River watershed, which is the third largest river water basin in South Korea. We analyzed the biotic integrity of 149 different study sites in the streams and rivers of Geum River watershed, perusing fish assemblages and water quality data. The results revealed the newer index, i.e., multi-metric (mm) IBI $_{06}$ (mmIBI $_{06}$ metrics), as comparatively more efficient than previously used indices, i.e., mmIBI $_{08}$ and mmIBI $_{11}$. Furthermore, the linear regression and correlational analyses indicated harmonic relation of mmIBI $_{06}$ with mmIBI $_{08}$ (R^2 = 0.85) and mmIBI $_{11}$ (R^2 = 0.87). Nonetheless, linear regression modeling discovered a very strong positive relation between mmIBI $_{08}$ and mmIBI $_{11}$ (R^2 = 0.91), thereby implying previously used indices demonstrate better approximation. In significant contrast to both old indices, the newer index exhibited improved facility to better classify the study sites between the assortments of 'excellent to very poor' compared to old indices elucidated one-step lower, i.e., from 'excellent to poor'. For instance, the newer index categorized 60 sites as 'very poor', requiring immediate attention owing to biological degradation. Additionally, the newer index endures grander ability to indicate sites requiring immediate management or restoration attention with a plausible site classification, especially in relation to the invasive alien species (IAS). The water chemistry was mainly influenced by rampant anthropogenic actions compounded by intensive monsoon precipitation that may relinquish highly suitable grounds for invasive alien fish species. This may eventually lead to severe biological degradation and successive deterioration of habitat by the IAS. In conclusion, the newer index endures ample capacity to indicate the fish community disturbances in rivers and streams. Further, correlation, linear regression, as well as principal component analysis (PCA) analyses on biotic indices and water chemistry showed higher approximations. Therefore, our newer index would be a valuable BHA tool to classify and elucidate the streams and rivers by indicating sites necessitating preferred attention and restoration measures.

Keywords: biotic integrity; fish assemblages; water chemistry; invasive alien species; Geum River; biological degradation

1. Introduction

The biological health assessment (BHA) has evolved as an essential measure for effective management of freshwater resources [1,2] such as rivers, streams, and lakes. Among multi-metric indices (mmi), the index of biotic integrity (IBI) is the most widely applied approach to understand and assess the biological health status of rivers and streams [3]. Mostly, rivers, streams, and connected water basins are heterogeneous ecosystems subjected to a plethora of human activities and natural interactions at play that develop complex relationships within the lotic ecosystems [4,5]. Those activities, their effects, and consequential events collectively disrupt the aquatic environments in general and fish assemblages in particular.

The concept of 'integrity' was primarily conceived by Leopold [6] and referred to an ecosystem that was not transformed due to intensive anthropogenic activities. In other words, integrity is the sustainable characteristic and status of an ecosystem that has undergone an unremitting and rigorous evolutionary process [2,3,7–9]. In the wake of sustainable management of freshwater resources across the globe, several indices of biotic integrity were explored to evaluate biological health of lotic ecosystems, mainly perusing fish assemblages and have been extensively used to assess the fish and other aquatic organism populations [10–15]. The indices of biotic integrity developed in different countries have been designed to estimate the different biological communities and assemblages of complex ecological systems. That is why the primary phase in designing an index is the identification of measurable biological attributes (termed as metrics herein) that exhibit consistent changes along the gradient [5,16,17], as well as show the integral parts of biological systems. The extent of the gradient is recognized by considering the multiple environmental variables that could affect biodiversity. The metrics thus developed would be the ones that could illustrate a distinct relationship within the environmental gradient. Among the potential metrics, the species composition and their relative abundance, richness of taxa, and species attributes based on tolerance and feeding modes are included [11,18–20]. The study sites with none or the least disturbance would be labeled as ecologically integrated or biologically healthy and vice versa [14].

The fish assemblage assessment is normally based on traits such as habitat, origin, tolerance levels, feeding niches, and presence of endemic and exotic species in a specific ecosystem. Furthermore, the functional links between the trophic and tolerance levels are widely recognized as key factors that influence the local community structures and interactions [2,7,13]. The spatiotemporal dynamics of trophic guilds are meticulously connected with functional trophic levels and in support of this, quantitative and empirical models have been proposed to understand this dynamism [21–23]. Accordingly, the cascading trophic interactions concept has been recurrently applied for biological management of impaired ecosystems [13,23]. In cases where top predators are not present, a newer concept of bottom-up trophic dynamism comes into play, which suggests the interactions are mainly controlled by the ambient ratios or regimes of nutrient contributing factors such as total nitrogen (TN), total phosphorus (TP), TN:TP ambient ratios, and chlorophyll (CHL-a) [13]. Correspondingly, the tolerance guilds shift mainly because of environmental perturbations along the gradient. The enrichment in nutrient contributing factors especially TN and TP in rivers and streams drastic shifts and modifications in aquatic biodiversity [24,25]. Another important factor that has a proven effect on habitat and fish assemblage is the change of hydrologic flow in rivers and streams [26]. Changing habitat diversity as well as its watershed stability may particularly affect fish community structure and comparative role of different fish community metrics [27,28].

The foundation concept of IBI enlightens us the fact that different fish assemblages respond to modification in habitat in a predictable manner, for instance, loss in integrity will be displayed in the structure of total natives, tolerant and intolerant fish species, but also shift in specialized trophic and reproductive guilds [7,11]. How stream fish assemblages change in approximation with habitat degradation is anticipated from the underlying assumptions of IBI and shows that a total number of native and sensitive species would decline, whereas tolerant and trophic generalist fish species would increase along the gradient and invaders will also be imminent [2,5]. General fish abundance, as well as trophic specialists, will also show decline along with specialist breeders with an increase in hybrid individuals. Similarly, the proportion of exotic fish species, as well as diseased individuals with morphological anomalies, would show conspicuous abundance [17,29,30]. Furthermore, there is evidence of habitat stability as well as the diversity changes from the headwater to downstream of large rivers and the increasing number of none-native fish species sampled. Similarly, with increasing stream dimensions, there is an ostensible intensification of habitat, pool and riffle enlargement, and substrate stability.

Keeping above facts in mind, in this article, we studied the third largest river water basin, i.e., Geum River watershed and the study sites targeted are of national importance in South Korea. We applied and compared three different indices based on the concept of IBI. The indices include mmIBI $_{08}$ (the most widely used index in South Korea) [17], mmIBI $_{11}$ that was developed by An and Choi [31], and our newly developed mmIBI $_{06}$. The subscripts (08, 11, 06) denote the total number of metrics used. The newer index expected to assess the biotic integrity in terms of interplay between the invasive alien species (IAS) and native fish species while neglecting the morphological health status. There does not exist any novel approach mainly dealing with the IAS. The leading reason to develop the newer index was to reduce the number of candidate metrics and check if the new model could work where there are growing numbers of exotic fish species. Further, we compare and analyze the efficiency of these indices and their relationship to significant water chemistry parameters to estimate the index response to changing water chemistry.

2. Materials and Methods

2.1. Target Study Watershed

This study region is located in the third largest river watershed of South Korea, i.e., Geum River watershed (n = 149 sites), which is further subdivided into four distinct sub-watersheds viz. Geum river (n = 90), Mankyong river (n = 14), Dongjin river (n = 11), Sapkyo stream (n = 14), and some miscellaneous sites (n = 20) (Figure 1). The location coordinates are between the altitude of 35°34′47″–37°03′03″ and latitude of 126°40′25″–128°03′53″ [32]. The total watershed area of the Geum river inclusive of all the sub-watersheds is 17,537 km^2 [33]. The total reach length is 414 km, with a shape factor of 0.06 and the average slope was 16.74 percent, whereas average elevation was 85.31 m. The summer monsoon rainfall contributes most of the water resource during July–August and the annual rainfall in the watershed was 1342.5 mm approximately, which on average is greater than annual mean precipitation level of the whole country (1245 mm). The annual mean temperature was recorded as 11.5 °C, with the extent of evaporation fluctuating between 1070–1292 mm. All the sites included in this article were among the long-term national level monitoring in chemical and biological study over the past years by the government. The sampling sites included were from the 1st to 6th stream order according to Strahler's [34] classification of stream orders.

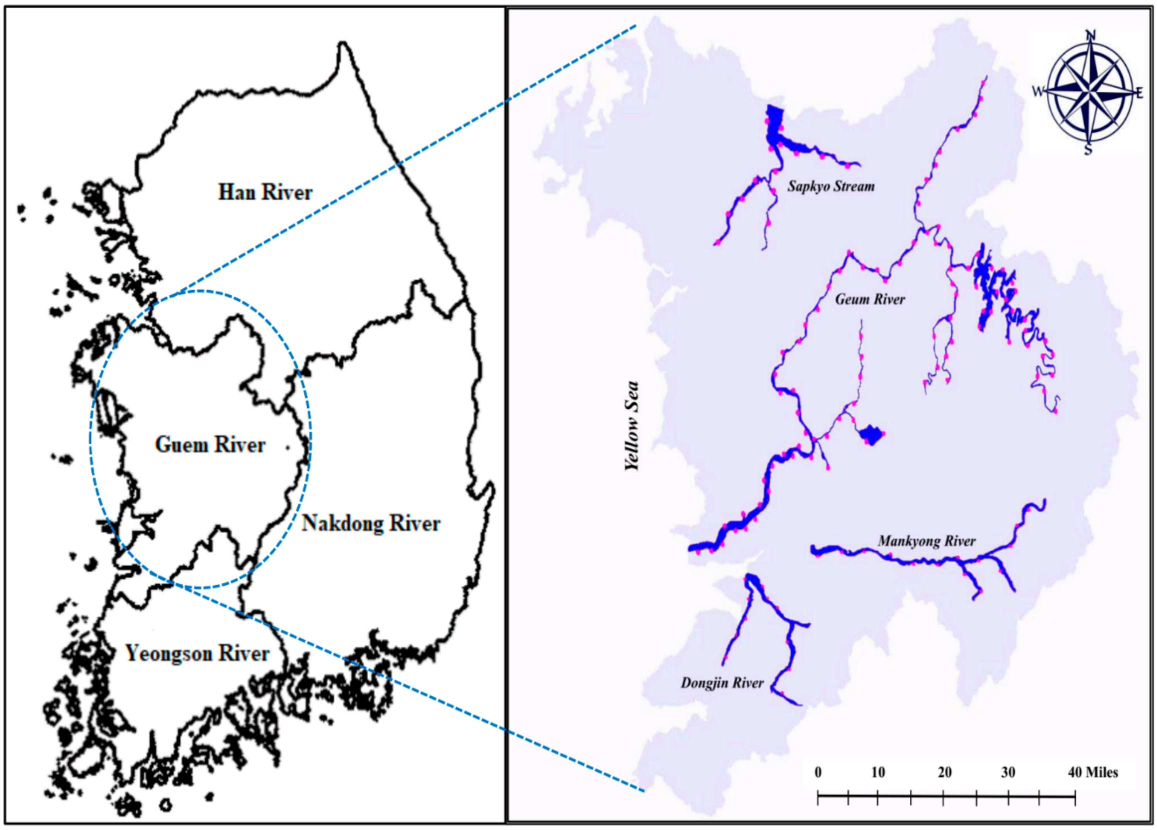

Figure 1. Arrangement of study sites in Geum River watershed and the sub-watersheds.

2.2. Fish Sampling and Collection Equipment

Fish data collection was performed twice at each study site during the pre-monsoon (May–June) and post-monsoon (September–October) months in 2010. During sampling, the hydrological flow was relatively stable and suitable for fish sampling. Typically characterized by summer monsoon rainfall, this watershed's streams hydrological flow fluctuates abruptly because of intensive precipitation, which is approximately 60% of annual precipitation.

Such a massive rainfall leads to drastic modifications in physicochemical factors, disturb habitat, as well as discompose aquatic populations. This was the compelling reason for not sampling during monsoon months (July–August), even though bio-assessment surveys during these months are quite in practice in North America and Europe. Fish sampling time at each site ranged between 50–60 min along the upstream and downstream extents covering all types of the micro-habitats such as pools, riffles, and run using the wading method. Fish sampling was carried out according to the modified sampling protocols of the US EPA [35] and South Korea [31]. The sampling gears included cast net (mesh size 5 × 5 mm) and kick net (mesh size 4 × 4 mm). In situ fish identification of each species then followed the immediate release. All the sampled fish were identified by their salient morphological features, as described by Kim and Park [36]. However, for systematic classifications, Nelson's [37] method was followed. Nevertheless, in case of any doubt of false identification, samples were preserved in 10% formalin solution for ex situ identification. The sampled fishes were also cautiously observed for any morphological anomalies such as deformities (D), erosion(s) (E), lesions (L), and/or tumors (T) (DELT) as an insight to the individual fish health [38].

2.3. Fish Guild Analysis

For the calculation of individual metric scores, the fish assemblages sampled at each site required being ascribed to different guilds according to habitat preference (water column or riffle benthic), origin (native or exotic), tolerance (sensitive, tolerant or intermediate), and trophic (omnivorous, carnivorous, herbivorous or insectivorous) guilds. The classification of tolerance and trophic guilds was accomplished according to the standard approaches [10,31]. Region-specific description of endemic fish species was followed from previous fish classifications [39,40]. This approach was derived from the principal that increase in the number of native and sensitive species will indicate better ecological health, whereas for omnivore, tolerant and exotic dominance would reflect biological degradation [41].

2.4. Water Quality Analysis

The water quality was monitored on a monthly basis from the 149 study sites, and significantly, eight parameters were selected. The parameters included biological oxygen demand (BOD), total nitrogen (TN), total dissolved nitrogen (TDN), ammonia nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), total phosphorus (TP), ortho-phosphorus (PO_4-P), and chlorophyll–a (CHL-a). The water samples were amounting to one liter in polyethylene sampling bottles for further chemical analysis placed in a dark icebox for transport from site to laboratory. The CHL-a was estimated with multi-parameter water quality sensor (YSI Sonde 6600, Environmental monitoring system, Yellow Springs, OH, USA). Total nitrogen was evaluated by the method of the second derivative followed by digestion in persulfate solution [42]. The NH_4-N and NO_3-N were analyzed by the phenate and ion chromatography methods, respectively, followed by filtration of an extract of the water source sample through GF/C filters (Whatman, Maidstone, UK). The TP and PO_4-P were assessed by an ascorbic acid method that was followed by persulfate oxidation [43,44]. BOD was measured by following standard methods [44]. Nutrient contributing factor analyses were performed in triplicates in order to ensure the rationality, while the estimation of BOD was performed in duplicate [44,45].

2.5. Indices of Biological Integrity (IBI)

Several multi-metric indices have been developed in South Korea and other countries. We used three indices based on the IBI model, out of which one is mmIBI $_{08}$ that is the standard and most widely used mmIBI model in South Korea and also adopted by the Korean government for ecological monitoring of rivers and streams at the national level. The second index used is mmIBI $_{11}$ primarily developed by An and Choi [31] and gained popularity being the basic IBI model which ultimately led to a most recent mmIBI $_{08}$. The third index was developed during this study and termed as mmIBI $_{06}$. The reason for the development of this model was to assess the study sites with the impact of growing invasion of IAS such as largemouth bass *(Micropterus salmoides)* and bluegill (*Lepomis macrochirus)* in Geum River watershed. We planned this study to assess the interaction between native and exotic invasive fish species.

Composed of eight, eleven, and six metrics, respectively, the three indices metric entailed of three key classes viz: (1) Species richness and composition, (2) trophic and tolerance guild compositions, and (3) the total fish abundance. The details of fish assemblage types used as metrics and their ascribed scoring criteria in three models are presented in Tables 1–3, respectively. The newer index metrics passed the redundancy test for eliminating the repetitive metrics to serve the desired purpose. The scores given to selected metrics were carefully evaluated and assigned on the basis of the MSRL (maximum species richness line) concept [46] vigilantly considering the stream order at each site. The given scores to each IBI metric were 5, 3, and 1 based on the criteria of Barbour et al. [35] and An and Choi [31]. This scoring critera was expected to reflect the biological health, either approximated, moderately deviated, or critically degraded in comparison to the pristine environments. The final scores obtained by the summation of all metrics in the three indices were classically anticipated to assess each study site into five categories of biotic integrity in terms of excellent (E), good (G), fair (F), poor (P), or very poor (VP). A detailed account of specified categories from 'excellent to very poor' along with their ranges in the respective index scores are mentioned in the bottom rows of each IBI model tables, i.e., Tables 1–3.

Table 1. Fish assemblages types, metrics and their ascribed scoring criteria in 08 metrics IBI model (mmIBI $_{08}$).

Category	Model Metric Components (M)	Scoring Criteria		
		5	3	1
Species Richness and Composition	M_1: Total Number of Native Fish Species *	>67%	33–67%	<33%
	M_2: Number of Riffle Benthic Species *	>67%	33–67%	<33%
	M_3: Number of Sensitive Species *	>67%	33–67%	<33%
	M_4: Proportion of Individuals as Tolerant Species	<5%	5–20%	>20%
Trophic Composition	M_5: Proportion of Individual as Omnivore Species	<20%	20–45%	>45%
	M_6: Proportion of Individuals as Native Insectivore Species	>45%	20–45%	<20%
Fish Abundance and Condition	M_7: Total Number of Native Individuals *	>67%	33–67%	<33%
	M_8: Percent Individuals with Anomalies	0	0–1%	>1%
Biological Health Criteria	A = Excellent (36–40); B = Good (28–34); C = Fair (20–26); D = Poor (14–18); E = Very poor (08–13)			

** = Expectations of metric values may vary with stream order and region.*

Table 2. Fish assemblages types, metrics and their ascribed scoring criteria in 11 metrics IBI model (mmIBI $_{11}$).

Category	Model Metric Components (M)	Scoring Criteria		
		5	3	1
Species Richness and Composition	M_1. Total number of native fish species *	>67%	33–67%	<33%
	M_2. Number of riffle benthic species *	>67%	33–67%	<33%
	M_3. Number of water column species *	>67%	33–67%	<33%
Tolerance Guild Composition	M_4. Number of sensitive species *	>67%	33–67%	<33%
	M_5. Proportion of individuals as tolerant species	<5%	5–20%	>20%
Trophic Composition	M_6. Proportion of individuals as omnivores	<20%	20–45%	>45%
	M_7. Proportion of individuals as native insectivores	>45%	20–45%	<20%
	M_8. Proportion of individuals as native carnivores	>5%	1–5%	<1%
Fish Abundance of Native and Exotics	M_9. Total number of native individuals *	>67%	33–67%	<33%
	M_{10}. Proportion of individuals as exotics	0	0–1%	>1%
Fish health status	M_{11}. Proportion of individuals with DELT	0	0–1%	>1%
Biological Health Criteria	A = Excellent (48–55); B = Good (40–47); C = Fair (30–39); D = Poor (18–29); E = Very poor (08–17)			

** = Expectations of metric values may vary with stream order and region.*

Table 3. Fish assemblages types, metrics and their ascribed scoring criteria in newer 06 metric IBI model (mmIBI $_{06}$).

Category	Model Metric Components (M)	Scoring Criteria		
		5	3	1
Species Richness and Composition	M_1: Total Number of Native Fish Species *	>67%	33–67%	<33%
	M_2: Number of Sensitive Species *	>67%	33–67%	<33%
Trophic Composition	M_3: Proportion of Individual as Omnivore Species	<20%	20–45%	>45%
	M_4: Proportion of Individuals as Native Insectivore Species	>45%	20–45%	<20%
Fish Abundance	M_5: Total Number of Native Individuals *	>67%	33–67%	<33%
	M_6: Proportion of individuals as exotics	0	0–1%	>1%
Biological Health Criteria	A = Excellent (26–30); B = Good (21–25); C = Fair (17–20); D = Poor (13–17); E = Very Poor (08–12)			

**= Expectations of metric values may vary with stream order and region.*

2.6. Statistical Analysis

We performed linear regression analyses on the log-transformed fish assemblages and water chemistry datasets in SigmaPlot version 10 [47]. Furthermore, means and standard deviations calculated by using the SPSS Statistics ver. 22 (IBI, Armonk, NY, USA). The principal component analysis (PCA) and Pearson's correlation was carried out by using the PAST software [48] to assess major factors influencing the indices used in this study, as well as their assortments in the Geum River water basin.

3. Results and Discussion

3.1. Water Quality Dynamics

The variation in different water chemistry parameters was directly contingent to the distinct watershed types in Geum river watershed (Table 4). In case of BOD, Sapkyo stream exhibited higher levels (6.9 ± 0.98, mg/L), whereas Geum River indicated lower BOD levels (2.55 ± 0.29, mg/L) among the studied sub-watersheds. However, whole Geum River BOD level was comparatively higher (4.17 ± 0.37, mg/L), which could be ascribed to the domestic and industrial effluents mainly in the Sapkyo Stream watershed and were the main culprits of high BOD level. On the other hand, Dongjin River also showed higher BOD levels (6.38 ± 1.34, mg/L), which is an indication of the watershed located near cities with relatively larger human populations as well as industrial activities. Similarly, in case of TN, Sapkyo Stream (6.71 ± 0.61, mg/L) followed by some of the miscellaneous sites (6.59 ± 0.78, mg/L) reflected higher inflow from this watershed. However, whole Geum River watershed TN level (4.99 ± 0.22, mg/L) was an indication of intensive agricultural activities in the watershed. Nevertheless, the TN level (4.99 ± 0.22, mg/L) in whole Geum River watershed clearly suggested that the watershed was nitrogen rich. The highest TP level was recorded in Sapkyo Stream watershed (1297.14 ± 353.3, µg/L), whereas the lowest was in the Geum River watershed (873.44 ± 136.35, µg/L). In case of sestonic CHL-a distribution in different watersheds, Dongjin River revealed higher sestonic CHL-a (8.28 ± 2.22, µg/L), followed by Sapkyo Stream (4.56 ± 0.68, µg/L). While in the case of whole Geum River watershed, mean sestonic CHL-a level was 4.59 ± 0.41, µg/L). Whole Geum River watershed exhibited an N, P co-limitation scenario except Mankyong River watershed that indicated P-limitation conditions (N:P = 28.27). Similarly, Spkyo stream and Mankyong River watershed sites showed a distinct increase in the allied nitrogen and phosphorus chemical species (NH_4-N, NO_3-N and PO_4-P). The TP level during premonsoon months was very low in comparison to post-monsoon duration. It clearly indicated that the precipitation amount and pattern mainly influenced the watershed water chemistry, especially the nutrient contributing factors, i.e., TN, TP, their ambient ratios, and sestonic CHL-a [2,24,31]. The ambient ratios of TN and TP are widely used to estimate the limitation scenario of nutrient contributing factors in water-bodies [13,42]. If the N:P mass ratio is >17 it indicates P-limitation and N:P mass ratios <4 show N-limitation, whereas N:P mass ratios <4 and >17 indicate N, P co-limitation [41,42,49]. Water quality plays decisive role in defining the physical activities, survival and ambient life of fish species in aquatic ecosystems [22,31,50–53]. The water quality parameters such as BOD, TP, N:P mass ratios, and CHL-a are studied to see how they affect the fish assemblages in rivers and streams. The sestonic CHL-a presence is linked to plethora of factors such as agricultural activities in the watershed, forest covers, land use patterns, elevation (>150 m), and other anthropogenic activities [17,22,42,54,55].

Table 4. Summary statistics of selected water chemistry parameters in Guem River watershed.

Watersheds	Summary Attributes	BOD (mg/L)	TN (mg/L)	TP (μg/L)	TN/TP Ratio	NH_4-N (mg/L)	NO_3-N (mg/L)	PO_4-P (μg/L)	CHL-a (μg/L)
Geum River (n = 90)	Mean	2.55	4.63	873.44	16.67	0.25	2.15	364	4.23
	SE	0.29	0.23	136.35	1.87	0.08	0.08	33.89	0.54
	Min	0.5	0.44	29	0.55	0	0.2	1	0.33
	Max	17.1	15.69	6891	84.36	5.48	4.3	1481	33.52
Dongjin River (n = 11)	Mean	6.38	3.65	888.27	7.39	0.68	1.63	511	8.28
	SE	1.34	0.36	191.63	2.03	0.16	0.19	95.69	2.22
	Min	1.7	1.87	95	1.7	0	1.2	81	0.87
	Max	15.1	5.46	2380	21.2	1.48	3.4	961	22.49
Mankyung River (n = 14)	Mean	4.91	4.29	876.57	28.27	1.28	1.55	813.86	4.15
	SE	2.38	1.14	693.76	6.26	0.94	0.19	661.64	1.30
	Min	0.6	1.82	41	1.91	0.01	0.5	11	0.3
	Max	35.2	18.86	9878	89.12	13.26	2.4	9401	17.25
Sapkyo Stream (n = 14)	Mean	6.9	6.71	1297.14	9.53	1.01	2.63	701	4.56
	SE	0.98	0.61	353.30	1.50	0.50	0.28	141.24	0.68
	Min	2.2	1.28	71	1.13	0	0.3	31	1.28
	Max	14.3	9.99	4698	18.1	7.4	4.4	1801	9.51
Miscellaneous (n = 20)	Mean	7.77	6.59	947.2	11.69	0.76	2.36	567	4.49
	SE	0.86	0.78	189.21	2.42	0.28	0.26	121.82	0.78
	Min	1.4	0.56	74	2.56	0	0.2	31	0.71
	Max	13.6	16.23	3168	49.97	5.48	5.3	2331	12.91
Whole Geum River Watershed (n = 149)	Mean	4.17	4.99	924.54	15.73	0.52	2.13	476.03	4.59
	SE	0.37	0.22	112.50	1.38	0.12	0.07	68.27	0.41
	Min	0.5	0.45	29	0.55	0	0.2	1	0.3
	Max	35.2	18.86	9878	89.12	13.26	5.3	9401	33.52

3.2. Nutrient and Chlorophyll Empirical Modeling

The regression modelling on log-transformed CHL-a, TN, TP, TN:TP mass ratios, and their empirical relationship indicated significant relationships between CHL-a, TP and TN:TP mass ratios with TP (R^2 = 0.14, p = < 0.001, F = 24.98) and (R^2 = 0.09, p = < 0.001, F = 15.81) (Figure 2). However, week relation on CHL-a and TN (R^2 = 0.08, p = < 0.001, F = 12.38) reflected on the possibility of N, P-co-limitation scenario. The empirical models of TN, TP and TN:TP mass ratios showed a positive linear relationship between TN and TP (R^2 = 0.34, p = < 0.001, F = 74.73). In case of the relationships between TN, TP, and TN:TP mass ratios, there was strong negative link between TN:TP mass ratios and TP (R^2 = 0.81, p = < 0.001, F = 617) and week negative relationships between TN:TP mass ratios and TN (R^2 = 0.05, p = < 0.001, F = 7.32). The linear modelling supported the earlier findings of TN and TP ambient ratios principal of designating the nutrient limitation criteria. However, increasing dependency of CHL-a on TP (14%) revealed that the whole Geum River is soon going to be P-limited like the majority of freshwater bodies [23]. The negative linear relationship between CHL-a and TP and positive linear relationships between CHL-a and TN again strongly indicated the water body was N and P-co-limited during the study duration. Therefore, the empirical modeling approach concluded that the primary nutrient contributing factor supporting the sestonic CHL-a productivity was P and both nutrient factors were originating from the agricultural activities and industrial effluents [2,13,31]. Previous studies have reported that in streams and rivers, limiting nutrients are also reinforced by the abiotic factors such as turbidity as well as geographical location of the water body, that indicate the water residence time and all these factors strongly influenced the light availability, which is a long-established catalyst for primary production [13,17,21,24,28]. Song et al. [56] also presented comparable conclusions from Yeongsan River. However, it is noticeable that such an occurrence merely manifested during the monsoon precipitation season. Therefore, TN:TP mass ratios could appear as very important and significant controlling factors in CHL-a production. Additionally, this concept strongly sustained by the previous conclusions by Downing and McCauley [57], who presented that TN:TP mass ratios are more reliant on the phosphorus, as compared to nitrogen if the water-body is under P limitation circumstances. However, the non-significant relationship between TN:TP mass ratios and nitrogen could be attributed towards the low variant ambient N level in comparison to the phosphorus [2,5,25,55,58].

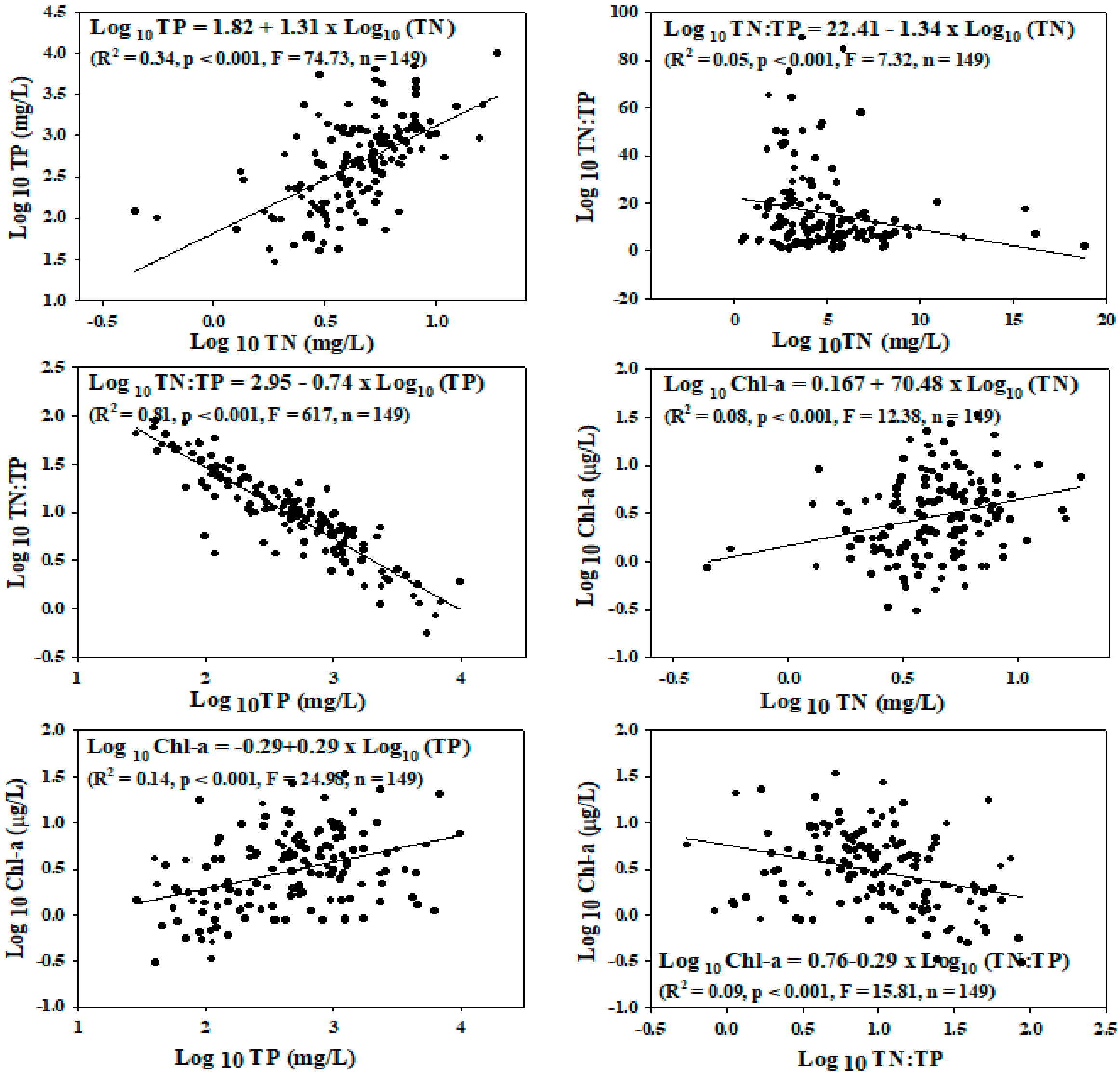

Figure 2. Regression relationship on CHL-a and nutrient contributing factors in Geum River watershed.

3.3. Watershed Assessment Based on the mmIBI $_{08}$ Model

The distribution of study sites in each sub-watershed in Geum River watershed based on mmIBI $_{08}$ model confirmed the majority of sites categorized under the fair (F) and poor (P) categories after obtaining the individual site scores (Table 5). Out of 149 sites, only three sites were categorized as excellent (A; 36–40), while 21 obtained the good (B; 28–34) categorization, which constituted only 16.10% of the total sites. The number of sites categorized under the fair (C; 20–26) was 73, which constituted 48.99% of the total study sites in whole Geum River watershed. Nevertheless, 52 sites obtained the poor (P) grade with mmIBI $_{08}$ model score range (14–18) and constituted 34.89%. In total, 83.90% of the study areas biological health status fell under the category of 'fair-poor' range, which calls for serious attention to sustainable management of Geum River watershed. It is noteworthy that there were no excellent graded sites in the sub-watersheds of Dongjin River, Mankyong River, Sapkyo Stream, and miscellaneous sites providing critical insight into the lack of pristine environment and it clearly pointed towards rapidly ongoing ecological degradation. Sapkyo Stream and Dongjin

River reflected severely deteriorating ecological health that also corresponded with degrading water chemistry in these watersheds. The majority of study sites displayed huge decline in the total number of native fish species in approximation to the decline of the total number of native individuals as well as the number of sensitive species (SS), which is an unambiguous indication of degrading biological health [35,41,50,59,60]. The proportion of omnivorous fish species showed an increase in the majority of sites and similar was the case with the proportion of individuals as native insectivores that yet again indicated the declining ecological health [2,5,17,35,59].

Table 5. Distribution of sites based on obtained mmIBI $_{08}$ values in Guem River watershed.

Sub-Watersheds	Scoring Criteria	Number of Study Sites According to Scoring Criteria								Sub-Watershed Site Status
		M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8	
Geum River (n = 90)	1	17	60	66	82	62	36	40	14	A = 03; B = 15; C = 50; D = 22
	3	30	21	19	05	17	24	23	02	
	5	43	09	05	03	11	30	27	74	
Dongjin River (n = 11)	1	02	11	09	10	09	07	07	03	B = 01; C= 03; D = 07
	3	08	00	02	00	01	02	04	00	
	5	01	00	00	01	01	02	00	08	
Mankyung River (n = 14)	1	04	09	09	13	06	03	10	03	B = 02; C = 09; D = 03
	3	07	03	04	00	05	03	04	02	
	5	03	02	01	01	03	08	00	09	
Sapkyo Stream (n = 14)	1	07	14	14	14	14	13	10	06	C = 03; D = 11
	3	05	00	00	00	00	01	03	01	
	5	02	00	00	00	00	00	01	07	
Miscellaneous (n = 20)	1	01	16	18	18	16	11	11	06	B = 03; C = 08; D = 09
	3	12	03	01	01	01	05	04	01	
	5	07	01	01	01	03	04	05	13	
Geum River Watershed Overall Health Status: A = 03; B = 21; C = 73; D = 52										

Note: For Metric (M) names please see the Table 1.

3.4. Watershed Assessment Based on the mmIBI $_{11}$ Model

The biological integrity class evaluation based on mmIBI $_{11}$ model and distribution of sites in the whole Geum River watershed showed that most sites were categorized under fair (F) and poor (P) categories (Table 6). In the whole Geum River watershed BHA, only three sites obtained the 'excellent' (E) score, whereas nine sites evaluated as 'good' (G), 54 as 'fair' (F), and 83 as biologically 'poor' (P). Based on the obtained results, 44.29% of the study sites were categorized into 'excellent to fair' in biological health status, whereas remaining 55.71% sites obtained poor biological health assortment. Like mmIBI $_{08}$, the mmIBI $_{11}$ led to categorize all the sites into four ecological health classes, i.e., from 'excellent' (E) to 'poor' (P). However, no sites obtained the very poor (VP) category score in this index criterion. According to the obtained results based on sub-watersheds, only Geum River sub-watershed harbored three sites with excellent biological health. In the Sapkyo stream, 13 out of 14 study sites were categorized under the poor (P) section, whereas, for the Dongjin River sub-watershed, 8 out of 11 sites also obtained the poor (P) biological health status. Mankyung River and Geum River sub-watersheds, however, they were uniformly distributed along the range of biological health categories based on the fish guilds compositions. This index result showed that it could not sufficiently explain the interplay between the native and exotic fish species. Further, its incapacity to categorize the study sites from excellent to very poor showed it to be an unfeasible index to explain the role of IAS. However, this index is very useful where we may find the species with diverse ecological guilds such as the entire range of trophic guild (omnivores, carnivores, insectivores) and the tolerance guild (sensitive and tolerant species). The predominance of poor biological health was in the downstream sites, as compared to upstream, which is an indirect indication of anthropogenic activities producing larger quantities of point-source pollutants from wastewater treatment plants (WWTPs) and mega-industries [61]. Such ecological disturbances were attributed to downstream ecosystem impairment, and such damages are evident on the values obtained by riffle benthic fish species, sensitive species (SS), insectivorous fish species, reduced native individuals, and increased DELT (percent of fish individuals with deformities (D), eroded fins (E), lesions (L), and tumors (T). The lower the values of these index metrics, the higher the chemical degradation and nutrient-rich effluents from WWTPs. Such downstream river health disturbances are richly supported by previous studies [2,24,28,41,62,63].

Table 6. Distribution of sites based on obtained mmIBI $_{11}$ values in Geum river watershed.

Sub-Watersheds	Scoring Criteria	Number of Study Sites According to Scoring Criteria											Sub-Watershed Site Status
		M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8	M_9	M_{10}	M_{11}	
Geum River (n = 90)	1	17	60	00	66	82	62	36	29	40	24	14	A = 03; B = 04; C = 41; D = 42
	3	30	21	09	19	05	17	24	37	23	04	02	
	5	43	09	81	05	03	11	30	24	27	62	74	
Dongjin River (n = 11)	1	02	11	00	09	10	09	07	01	07	06	03	B = 01; C = 02; D = 08
	3	08	00	01	02	00	01	02	02	04	00	00	
	5	01	00	10	00	01	01	02	08	00	05	08	
Mankyung River (n = 14)	1	04	09	00	09	13	06	03	03	10	07	03	B = 01; C = 06; D = 07
	3	07	03	04	04	00	05	03	06	04	00	02	
	5	03	02	10	01	01	03	08	05	00	07	09	
Sapkyo Stream (n = 14)	1	07	14	00	14	14	14	13	08	10	04	06	C = 01; D = 13
	3	05	00	00	00	00	00	01	01	03	00	01	
	5	02	00	14	00	00	00	00	05	01	10	07	
Miscellaneous (n = 20)	1	01	16	00	18	18	16	11	08	11	02	06	B = 03; C = 04; D = 13
	3	12	03	02	01	01	01	05	04	04	00	01	
	5	07	01	18	01	01	03	04	08	05	18	13	
Geum River Watershed Overall Health Status: A = 03; B = 09; C = 54; D = 83													

Note: For Metric (M) names please see the Table 2.

3.5. Watershed Assessment Based on the mmIBI $_{06}$ Model

The majority of study sites were assorted in the category of ‘poor (P)–very poor (VP)’ based on the mmIBI $_{06}$ model whether in the case of whole Geum River watershed or elsewhere in the sub-watersheds (Table 7). However, this model domino effect has revealed the scattering of study sites on a broader range from ‘excellent (E)–very poor (VP)’. It also displayed comparable index and logical distribution of study sites along the gradient into their ascribed biological health category. Contrasting the mmIBI $_{08}$ model, the $_{\text{mmI}}$BI $_{06}$ model indicated six sites in Geum River sub-watershed as excellent (E), 10 as good (G), 20 as fair (C), 26 as poor (P), and 28 in the very poor (VP) category. This showed a plausible study site classification unlike the previously used indices. The underlying assumption of development of this index was to evaluate interactions between the proportion of individuals as exotics and the total number of native fish individuals that is vividly clear and exhibited by this model of mmIBI $_{06}$. It has also revealed the Geum River sub-watershed is under the threat of exotic fish invasions and it was sufficiently evident by the fish data of this study as well. There were more exotic fish species and individuals that compete for food and shelter in the ecosystem [64,65]. The whole Geum River watershed distribution based on the mmIBI $_{06}$ showed that 34.89% of the total study sites were in the fair to excellent biological health, whereas the remaining 65.11% were in the range of poor to very poor biological health. Sapkyo stream and Dongjin River were among the sub-watersheds that displayed an overall very poor biological health status, and reflected that the anthropogenic activities in these watersheds were damaging the sustainable ecological health as well as rendering the ecosystem feasible to the invasive alien fish species. The unrestrained human perturbations resulted in the decline of sensitive fish species and paved the way for populations of tolerant and invasive fish species such as bluegill (*Lepomis macrochirus)* and largemouth bass

(*Micropterus salmoides*). Such invasive fish species are observed to have a relative impact potential [66] and huge ecological and economic impacts [64,65].

Table 7. Distribution of sites based on obtained mmIBI $_{06}$ values in Geum river watershed.

Sub-Watersheds	Scoring Criteria	Number of Study Sites According to Scoring Criteria						Sub-Watershed Site Status
		M_1	M_2	M_3	M_4	M_5	M_6	
Geum River (n = 90)	1 3 5	17 30 43	66 19 05	62 17 11	36 24 30	40 23 27	24 04 62	A = 06; B = 10; C = 20; D = 26; E = 28
Dongjin River (n = 11)	1 3 5	02 08 01	09 02 00	09 01 01	07 02 02	07 04 00	06 00 05	B = 01; C = 01; D = 02; E = 07
Mankyung River (n = 14)	1 3 5	04 07 03	09 04 01	06 05 03	03 03 08	10 04 00	07 00 07	B = 03; C = 04; D = 01; E = 06
Sapkyo Stream (n = 14)	1 3 5	07 05 02	14 00 00	14 00 00	13 01 00	10 03 01	04 00 10	C = 01; D = 02; E = 11
Miscellaneous (n = 20)	1 3	01 12	18 01	16 01	11 05	11 04	02 00	A = 01; B = 01; C = 04; D = 06; E = 08
Geum River Watershed Overall Health Status: A = 07; B = 15; C = 30; D = 37; E = 60								

Note: For Metric (M) names please see the Table 3.

3.6. Regression Analyses on Biotic Integrity Models

The regression relationship on three biotic integrity indices showed a consistently direct relationship to each other, indicating that indices could work as surrogates of each other in Korean streams and rivers (Figure 3). The regression analysis on mmIBI $_{08}$ and mmIBI $_{11}$ exhibited direct relationships ($R^2 = 0.91$, $p < 0.001$, $F = 1532$), which indicated that both indices could replace each other, however, the decision would be based on the fish assemblages under study. In comparison to mmIBI $_{08}$ and mmIBI $_{11}$, there showed a little less yet an equally strong relationship between mmIBI $_{08}$ and mmIBI $_{06}$ ($R^2 = 0.85$, $p < 0.001$, $F = 847.29$). These results indicated the biological health indices strongly responded to each other. In a similar fashion, the regression relation between the mmIBI $_{06}$ and mmIBI $_{11}$ showed a direct relationship ($R^2 = 0.87$, $p < 0.001$, $F = 996.55$). This indicated that instead of using mmIBI $_{08}$ and mmIBI $_{11}$, mmIBI $_{06}$ might sustainably be used for assessing the biological integrity where there are more invasive alien individuals as well as considering the other vital fish assemblages. Since these models differ from each other due to individual metric compositions, certain fish assemblages were to be ascribed an underlying role in the assumption of our newer mmIBI $_{06}$ index scores. For instance, the mmIBI $_{06}$ was designed to assess the role of invasive fish species such as largemouth bass and bluegill in the Geum River watershed in relation to other fish communities of paramount importance. This study confirmed the prevalent use of mmIBI $_{08}$ for the right reason, as it was enough to designate the Korean streams and rivers on the biological health chart, i.e., from excellent to poor. The IBI model is primarily an adjustable model to the changing fish communities and various studies have explored it [2,5,13,19,41,50,67].

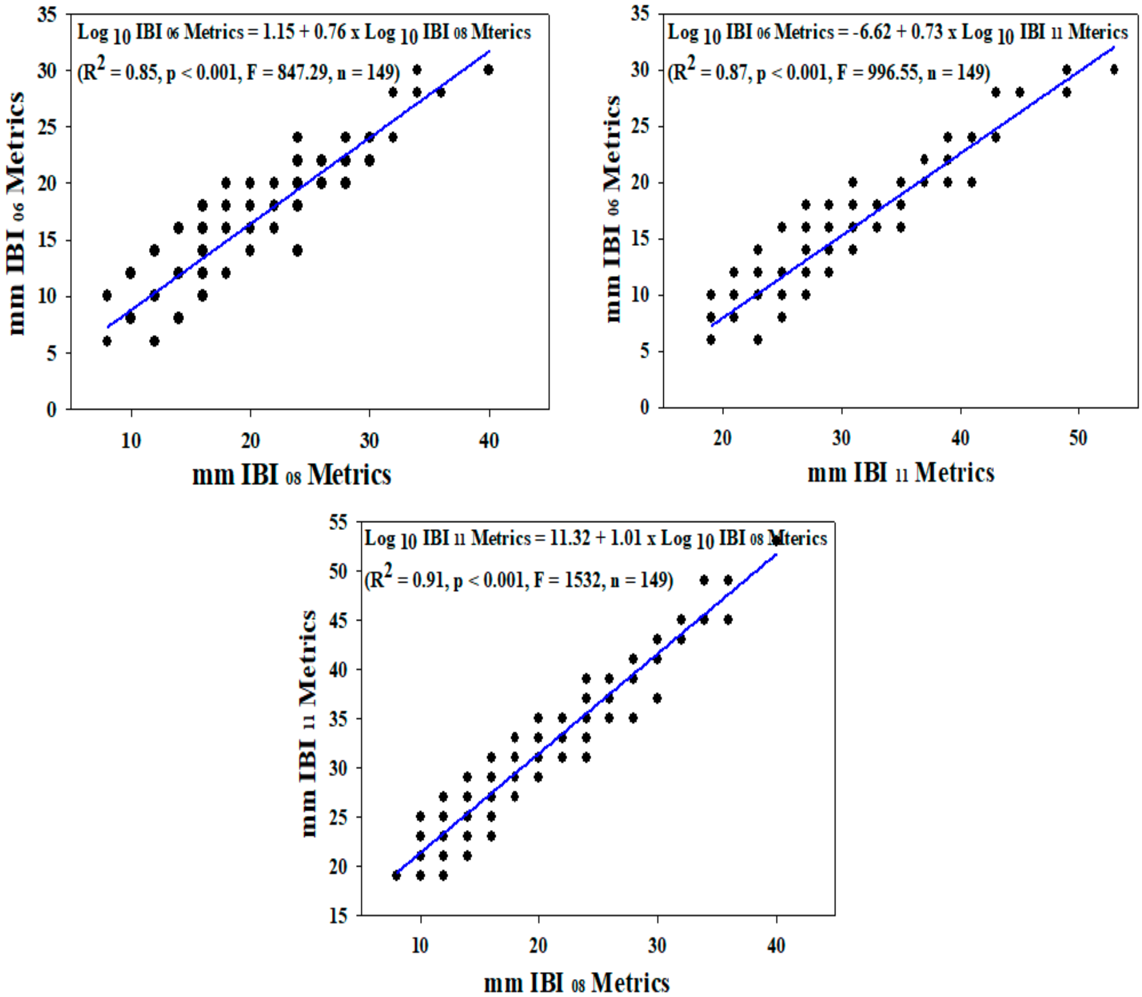

Figure 3. Regression analyses on the mmIBI models in Geum River watershed.

3.7. Multi-metric Fish Indices and Their Regression Analysis on Water Chemistry

The overall escalation of the values of chemical water quality parameters in the Geum River watershed negatively affected the biotic integrity indices assessments (Figure 4). The multi-metric integrity indices displayed identical responses to selected water chemistry factors such as BOD, TP, and CHL-a. The mmIBI $_{08}$ and mmIBI $_{11}$ responses to the water quality parameters were very overlapping in comparison with mmIBI $_{06}$. However, all the three multi-metric fish indices showed a distinctly negative relationship to water quality parameters. When the BOD was more than 6.0 mg/L, the TP level was above 700 µg/L, and CHL-a had more than 20 µg/L, the sites mostly fell into the category of 'poor to very poor' depending on the score range of multi-metric integrity indices applied. This showed a clear indication of strong relationship between the improvement of biological integrity and water quality. In addition, all the selected water quality parameters showed a significant negative relationship with the three IBI models applied in this study. Therefore, it could be concluded that degradation in water quality or negative change in water quality parameters have an incumbent deteriorative influence on fish biodiversity, individual fish wellbeing, species richness, composition, as well as simultaneously deciding the suitability of the aquatic ecosystem for the invasive fish species [13,22,28,31,41,59,68].

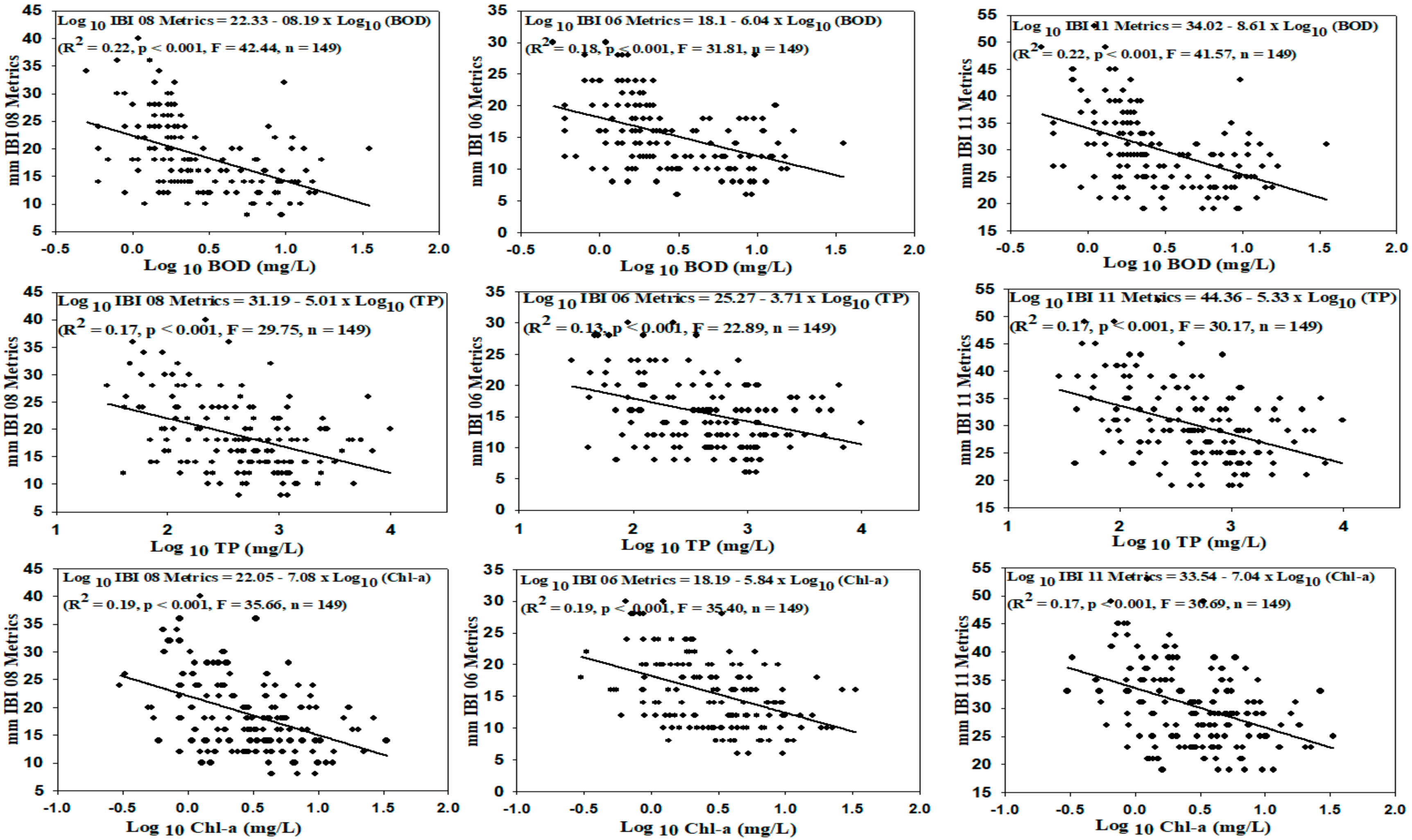

Figure 4. Regression relationship between the three IBI models and BOD, TP, and CHL-a in Geum River watershed.

3.8. Relationship between Multi-metric Integrity Models and Individual Metrics

The correlation analyses on multi-metric integrity models and their constituent metrics showed the indices to correlate each other as strong positively (Table 8). The analysis was performed on the total eleven metrics used in this study as well as their resultant IBI models. The mmIBI $_{08}$ showed strong positive linear relation with mmIBI $_{11}$ (r = 0.96) and mmIBI $_{06}$ (r = 0.92). This confirmed that mmIBI $_{08}$ model had a higher correlation with mmIBI $_{11}$ and a little less with mmIBI $_{06}$ index and mmIBI $_{06}$ showed a strong positive linear correlation with mmIBI $_{11}$ (r = 0.93). Instead, all the eleven individual metrics showed a positive correlation with the three multi-metric integrity models except M_3 and M_8 that showed very week negative correlation with the multi-metric integrity indices. The correlation coefficients of individual metrics, mmIBI $_{08}$ and mmIBI $_{06}$ models showed matching values, which is a reliable confirmation of our objectives that the newly proposed mmIBI $_{06}$ model is a very suitable surrogate of the mmIBI $_{08}$ model with special reference to invasive alien fish species. In comparison to the above-discussed scenario, the correlation coefficient values of individual metrics and mmIBI $_{11}$ were comparatively lower in their strength, which showed that mmIBI $_{06}$ was a better option for fish ecologists instead of mmIBI $_{11}$ [2,5]. The correlation coefficient value of individual metrics with each other showed the selection of these metrics and was confirmed to be useful because of very low or negative correlation among the metrics. It also showed that there is little or no conflict or repetition among the multi-metric integrity indices metrics, which were contributors of total IBI values [13,24,41]. Their usefulness was statistically proven in this study as well. There was no obvious correlation (r < 0.4) among most of individual metrics, which is another way to confirm that the metrics used were representative of fish assemblages in the Geum River watershed [31,41].

Table 8. Correlation matrix between the three mmIBI models and individual metrics applied during the study.

Parameters	08 Metric	06 Metric	11 Metric	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8	M_9	M_{10}	M_{11}
08 Metric	1.00													
06 Metric	0.92	1.00												
11 Metric	0.96	0.93	1.00											
M_1	0.51	0.53	0.49	1.00										
M_2	0.68	0.58	0.61	0.47	1.00									
M_3	−0.37	−0.29	−0.25	−0.04	−0.44	1.00								
M_4	0.71	0.70	0.69	0.34	0.55	−0.19	1.00							
M_5	0.56	0.49	0.53	0.06	0.20	−0.40	0.32	1.00						
M_6	0.60	0.58	0.57	−0.09	0.12	−0.30	0.29	0.62	1.00					
M_7	0.63	0.62	0.59	0.02	0.28	−0.32	0.42	0.38	0.70	1.00				
M_8	−0.14	−0.22	0.01	0.01	−0.22	0.19	−0.10	−0.08	−0.02	0.02	1.00			
M_9	0.63	0.64	0.58	0.64	0.49	−0.08	0.42	0.22	0.05	−0.01	−0.26	1.00		
M_{10}	0.29	0.53	0.44	0.04	0.23	−0.13	0.23	0.20	0.15	0.09	−0.39	0.21	1.00	
M_{11}	0.46	0.27	0.48	−0.06	0.18	−0.12	0.19	0.16	0.23	0.17	−0.07	0.16	0.26	1.00

Where M_1 = Total number of native fish species; M_2 = Number of riffle benthic species; M_3 = Number of water column species; M_4 = Number of sensitive species; M_5 = Proportion of individuals as tolerant species; M_6 = Proportion of individuals as omnivores; M_7 = Proportion of individuals as native insectivores; M_8 = Proportion of individuals as native carnivores; M_9 = Total number of native individuals; M_{10} = Proportion of individuals as exotics; and M_{11} = Proportion of individuals with DELT.

3.9. Principal Component Analysis (PCA)

PCA rendered an effective data reduction method with minimum loss of original dataset and helped in extracting the meaningful information between the parameters [2,5,69]. A powerful technique that explained the variance of a large dataset into small variables named principal components (PC), PCA analysis yielded five distinct components that explained 89.41% variance. The significance of the PC is evaluated by its eigenvalue; a highly significant PC has a greater eigenvalue obtained [5,25,28,70]. The variable loading plot showing all the parameters along with their correlation shown in Figure 5 and the loading values given in Table 9. The obtained loading value by each variable in PCs helped classify them into strong (>0.70), moderate (0.70–0.50), and week (0.50–0.30) and below 0.30 as very week in their variability [23,41,69]. The first PC accounted for 40.64% total variance (eigenvalue = 4.47) and showed strong negative variance between the three multi-metric integrity indices showing the indices could work independently with equal implications. On the other hand, BOD, TN and PO_4-P showed strong positive loading value and moderate loading values for TP, TN:TP mass ratios. At the same time, CHL-a produced week positive loading value, thereby implying TN appeared to be playing an insignificant role in the production of CHL-a. It also indicated huge variability of water quality factors and the multi-metric integrity indices. The second PC yielded 21.78% variance (eigenvalue = 2.40) and 63.42% cumulative variance. However, the multi-metric integrity indices showed moderate positive loading values and similar loading values for NH_4-N. The CHL-a showed very week negative loading value indicating thereby no reasonable influence of TN and TP in the CHL-a production in the watershed. It also indicated less agricultural activities in the watershed. The third axis accounted for 10.90% variance (eigenvalue = 1.20) and cumulative variance of 73.21%. The strong negative loading of NO_3-N in the third PC clearly pointed towards intensive industrial activities in the watershed. Similarly, the fourth and fifth components explained that CHL-a was strongly influenced by the combined action of TN:TP mass ratios, which are a confirmation of earlier described results in the case of most of the sub-watersheds. The PCA indicated that the sites that obtained poor and very poor classification mainly degraded due to the chemical pollution [5]. The positive loadings of CHL-a may also be an indication of increase in CHL-a production that may have favored the invasive fish species. The findings indicated the biological integrity was closely linked to the anthropogenic activities, nutrient contributing factor levels, organic matter regime, as well as trophic and tolerance guilds [2,5,17,23,54,55].

Table 9. Principal components and their loading values along with eigenvalue and percent variances in Guem River watershed.

Parameters	PC 1	PC 2	PC 3	PC 4	PC 5
08 Metric	−0.72	0.64	0.06	0.16	0.02
06 Metric	−0.69	0.65	0.16	0.15	0.04
11 Metric	−0.72	0.64	0.12	0.16	0.05
BOD	0.73	0.35	−0.17	−0.17	0.08
TN	0.74	0.35	0.42	−0.22	0.09
TP	0.64	0.42	0.05	0.35	−0.21
TN:TP	−0.53	0.16	0.00	−0.61	0.45
NH_4-N	0.65	0.59	−0.26	−0.20	0.10
NO_3-N	0.30	−0.11	0.93	−0.06	0.00
PO_4-P	0.73	0.55	−0.12	0.03	−0.02
CHL-a	0.37	−0.23	0.00	0.52	0.73
Eigenvalue	4.47	2.40	1.20	0.96	0.81
% variance	40.64	21.78	10.90	8.71	7.39
Cumulative Variance	40.64	62.42	73.31	82.02	89.41

Dark shade indicates strong loading strength (>0.70); light shade indicates moderate loading strength (0.70–0.50); Very light shade indicates week or very week loading strength (<0.50).

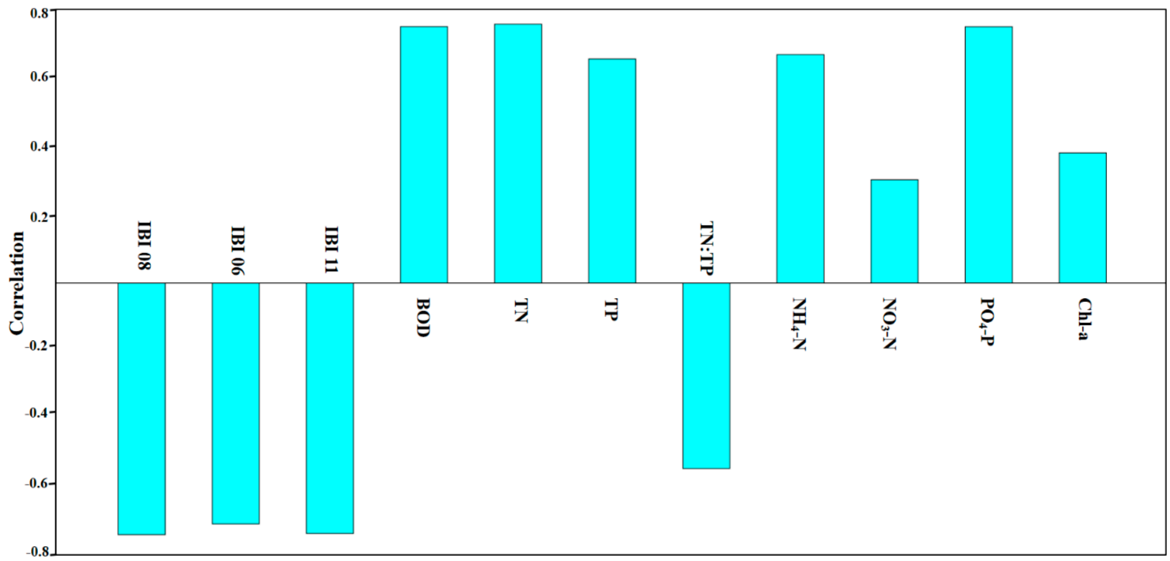

Figure 5. Loading plot for IBI models and selected water chemistry features in Geum River watershed.

3.10. Comparison of Fish Indices Used

During this study, we applied three different biotic integrity indices to fish assemblages and water chemistry datasets and the indices reflected the study sites distribution along the gradient (Figure 6). Two out of the three indices had already been used in previous studies and had been considered appropriate in explaining the fish assemblages [2,17,41,62]. However, none of these indices could effectively deal with the invading fish species, as well as declining native populations. The Geum River has become a favourite destination for IAS such as bluegill and largemouth bass. We therefore developed another mmIBI $_{06}$ that mainly dealt with invasive fish species as well as the population of native fish species (native individuals, native insectivore fish species and total number of native fish species). The mmIBI $_{11}$ also contained the one metric dealing with the invasive fish species but it could not effectively categorize the study sites along the range of biological health criteria because it is long ranged and more metrics. The mmIBI $_{06}$ is distinct and applicable IBI model because it sufficiently explained and classified the sites from excellent to very poor classification, whereas the other two IBI models could only categorize the study sites from excellent to poor. By the application of mmIBI $_{06}$ we could designate sites with critical biological health, problems as well as we could plan and manage those sites. Further, our newer index also indicated the use of DELT metric often manipulated the final IBI model score values as it gives an increase of straight five scores in the case of DELT absence. Normally, there is low percentage of fish individuals caught with possible DELT [5,41]. Our newer index could categorize the study sites more rationally on the biological health gradient, i.e., from excellent to very poor, which is a very strong point of success.

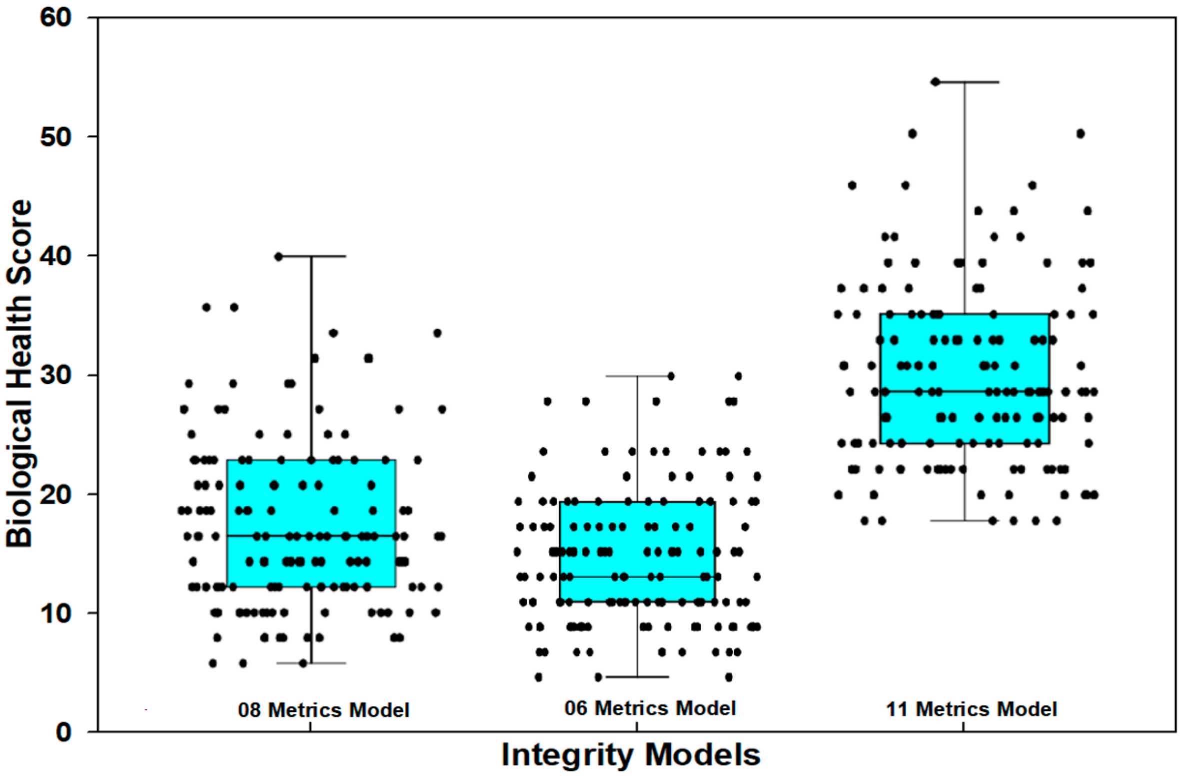

Figure 6. Box and jitter plot showing the spatial distribution of study sites based on biological health according to three models in Geum River watershed.

4. Conclusions

The multi-metric indices of biotic integrity (mmIBI) have evolved as the preferred BHA tool during recent decades and emerged as an influential tool for decision-making. In this study, we proposed a new model (mmIBI $_{06}$) and compared its efficiency with existing (mmIBI $_{08}$) and the first integrity model in South Korea (mmIBI $_{11}$). The results have shown very positive application of our newer index. It rationally classified the sites along the biotic integrity criteria, i.e., from 'excellent to very poor'. This implied greater meanings that our newer index could elucidate the sites requiring immediate attention and early warning detection of IAS. Furthermore, this index testified the results of regression and correlation analyses indicating it to be an effective one. The PCA results confirmed that the CHL-a production is mainly contingent to the TN:TP ambient ratios, while industrial effluents and anthropogenic activities played a substantial role in the ecosystem degradation. In conclusion, this study pointed out some serious drawbacks in the previously used IBI models and reflected that IAS early warning detection can be precluded with the help of IBI. That is why this new index successfully addressed the issue of IAS and clearly indicated the study sites that require immediate attention for restoration and conservation of the deteriorating lotic ecosystems.

Author Contributions: Conceptualization, U.A. and K.-G.A.; methodology, U.A.; software, U.A.; validation, U.A. and K.-G.A.; formal analysis, U.A.; investigation, U.A.; resources, B.L., J.Y. and K.G.A.; data curation, U.A. B.L., J.Y. and K.-G.A.; writing—original draft preparation, U.A.; writing—review and editing, U.A., B.L., J.Y. and K.-G.A.; visualization, U.A.; supervision, K.-G.A.; project administration, K.-G.A.; funding acquisition, K.-G.A.

Funding: This research was supported by Korea Environment Industry & Technology Institute (KEITI) through "Exotic Invasive Species Management Program" (Grant # RE201807019), funded by Korean Ministry of Environment (MOE).

Acknowledgments: This study was supported by Basic Environmental Research Program of the Geum River System (2010), the Ministry of Environment (MOE), Republic of Korea and the Research Development Program (Year 2017), Daejeon Green Environment Center). The authors are thankful for the support.

Conflicts of Interest: The authors declare no conflict of interest.

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