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Study of aquatic ecological regions using fish fauna and geographic archipelago factors

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Abstract

An evaluation of ecological integrity is required for ecosystem conservation and restoration. The ecological region, or “ecoregion”, has been adopted as a unit of geological area to enable a comparison of the ecological integrity of different regions. The delineation of an ecological region is difficult in countries in East Asia, including Japan because of complex topographies (i.e., several peninsulas and islands) and fauna that are very finely delineated based on climate or geology. Therefore, it is important to appropriately determine the ecoregions when determining their biological integrity and comparing it among that of other ecoregions. I attempted to delineate an ecological region of the Japanese archipelago based on the similarities among fish fauna by integrating the information on fish fauna that was collected by the researchers and the national government and local governments. In addition, quantitative analyses to investigate the

relationship between fish fauna classification and meteorological and geographical factors were conducted to discuss the factors that influence fish fauna classifications. The archipelago was classified into 15 fish fauna groups, and the results of these grouped classifications were closely related to the process by which the Japanese archipelago was formed, the ocean current in its coastal waters, and the connection of the water system to the glacial age. Our findings suggest that rivers within geographical areas that are different from those within the Japanese archipelago might have different fish fauna classifications based on our results and potential fish fauna depending on the characteristics of the watershed, such as the scale of the floodplain, river conflicts, or river formation process. By applying the results of our fish fauna classification, we are able to make a comparison of the biological integrity of fish fauna among different watersheds for managing the river environments or establishing conservation policies.

Keywords: ecoregion; fresh water; fish fauna; archipelago; geographic factor

1. Introduction

Freshwater habitats cover only ~0.8% of the Earth's surface; however, approximately 100,000 species account for 6% of all recorded species living in these habitats (Gleick, 1996; Hawksworth and Kalin-Arroyo, 1995; Dudgeon et al., 2006). An inventory of freshwater animals (Leveque et al., 2005) or freshwater "ecoregions" of the world (Abell et al., 2008) was provided to enable scientists to better understand integrative conservation of aquatic biodiversity. The evaluation of ecological integrity is necessary to better conserve and restore our ecosystems, and the ecoregion was adopted as a unit of geological or climatic area by which to compare ecological integrity across geographic areas. Ecoregions are areas in which ecosystems, including the type, quality, and quantity of environmental resources, are generally similar (Omernik, 1987; Bailey, 2004; McDonald et al., 2005). They serve as a spatial framework for studying, assessing, managing, and monitoring ecosystems and their components (Omernik, 1987). In North America, ecoregions were established as levels I to V according to the geographical scale, and have been used to, among other applications, develop regional biological criteria and water quality standards, set management goals for nonpoint-source pollution, assess land cover trends, report on ecosystem carbon sequestration, and frame wildlife conservation research (Omernik and Griffith, 2014). For Europe, Illies (1978) classified 25 ecoregions using the endism of freshwater fish and benthic invertebrates, and subsequently, on the basis of this classification, more ecoregion subdivisions were established in Slovenia (Urbanic, 2008), northern Europe (Ecoregion form Nordic Council of Ministers 1984), and the southern Balkans (Zogaris et al., 2009). In recent years, ecoregions have been established in China using fish fauna or environmental factors of individual catchment areas (Kong et al., 2013; Gao Y et al., 2011; Wang et al., 2015). In contrast to ecoregion research on a continental scale, as mentioned, research is also being conducted on relatively small islands, and the effectiveness of delineating ecoregions in these smaller areas has been confirmed. The South Island of New Zealand is an ecoregion classified using the following six indicators: climatic region, rainfall, relief vegetation, soils, and geology. The classification results were found to be similar to those of the ecoregion classification using terrestrial Oligochaeta (Lee, 1959; Harding and Winterbourn, 1997).

Many researches evaluating ecological integrity or analyzing relationships between biota and the physical environment were conducted based on the ecoregion concept. Studies have been conducted using phytoplankton (Beaver et al., 2012), diatomaceous (Chen et al., 2008) and benthic animals (King and Richardson, 2003; Ogren and Huckins, 2014; Feld and Hering, 2007; Butcher et al., 2003), fish (Krause et al., 2013, Ferreira et al., 2007; Ellender et al., 2017; Mehner et al., 2007), and multiple taxonomic groups (Pace et al., 2012; Wang et al., 2007; Simboura et al., 2005, Johnson et al., 2007). The ecoregion is also used in studies as an indicator by which the spatial scale affecting community structure can be identified (Johnson and Goedkoop, 2002; Uzarski et al., 2005; Sandin and Johnson, 2004). The concept of the ecoregion was adopted to evaluate abiotic factors, and a reference nutrient condition was determined for lakes within the same ecoregion in China (Huo et al., 2015; Huo et al., 2013; Zhang et al., 2014). In addition, the ecoregion has been used for analyzing the invasion route of non-native species (Bajer et al., 2015). Hering et al. (2009) analyzed the sensitivity of European Trichoptera species to climate change and revealed that there was a high percentage of potentially endangered species in southern European ecoregions. Furthermore, the concept of the ecoregion was applied not only to land and freshwater areas but also to coastal and marine areas, and studies have been conducted to determine reference conditions (Lucena-Moya et al., 2009), evaluate biodiversity (Simboura and Reizopoulou, 2008; Barnes et al., 2011; Easton et al., 2017), and assess conservation plans (Giakoumi et al., 2013).Most of the research on how to determine ecoregions were conducted in North American or Europe; whereas, despite the abundant biodiversity in East Asia, including Japan (Allen 2008; De Silva et al., 2007; Lopes-Lima et al., 2014), the concept of an ecological region within these areas is rarely clear. The delineation of an ecological region is difficult in countries within the Asian monsoon region because of complex topographies (i.e., several peninsulas and islands) and fauna that are very finely delineated based on climate. In particular, the Japanese archipelago is a biodiversity hotspot because of its location and complex geological history, including that it traverses multiple biomes and comprises an intense diastrophism formed by the collision of four large tectonic plates (i.e., the Pacific, Philippine Sea, Asian, and North American). On the other hand, the biota is regionally subdivided; therefore, it is important to appropriately determine the ecoregions when determining their biological integrity and comparing it among that of other ecoregions.

Research on fish fauna within the Japanese archipelago has been conducted from the perspective of phylogeny or biology. Research on the geographical distribution pattern of fish fauna has been conducted based on their similarities (Lindberg, 1972; Nakajima et al., 2006; Yodo et al., 2001; Hirayama and Nakagoshi, 2003) or the mechanism by which the distribution area was formed from the molecular phylogenetic tree (Yokoyama & Goto, 2002, Takahashi et al., 2001; Yamazaki et al., 2003; Yamamoto et al., 2004, Mukai et al., 2004., Watanabe & Uyeno, 1999). These research results have contributed greatly to understanding the derivation of Japanese fish fauna or the transition of the distribution pattern; however, the creation of a river environmental management or conservation plan was not conducted on a watershed scale based on the genetic information that resulted from these recent researches for the following reasons: 1) genetic information is difficult to use on a basinwide scale, which is the basic unit of river environmental conservation; 2) acquiring genetic information on each species is difficult from the perspective of cost and technical in-river surveys conducted by administrators; and 3) genetic information is difficult to understand and limited to fish ecologists and evolutionists.

On the other hand, the administrative agency or researchers have stored information on fish fauna, although the information is not centrally managed. In addition, information on fish fauna is expected to be added by environmental assessment or periodic environmental research; therefore, I attempted to delineate an ecological region based on the similarities among fish fauna by integrating the information on fish fauna that was collected by the researchers and the national government and local governments. In addition, as the influencing factors that contribute to fish distribution, geographic factors, such as distribution boundaries or the geological environment, were qualitatively discussed in previous research. In this study, quantitative analyses to investigate the relationship between fish fauna classification and meteorological and geographical factors were conducted to discuss the factors that influence fish fauna classifications. The results of this classification define the geographical unit in which ecological integrity is comparable, and will contribute to the management of the river environment and establishment of conservation plans.

2. Materials and Methods

2.1. Study area

There is a logarithmic relationship between river size and number of fish species (Angermeier & Schlosser, 1989; Nakajima et al., 2006; Reyjol et al., 2007), and small rivers are not suitable for delineating an ecological region because fish fauna in them is poor compared with that in large rivers, regardless of geographical factors; therefore, this study focused on 181 rivers within the Japanese archipelago with a river basin area of ≥150 km2 (except for the basin areas of the relatively small rivers of the Amami-Oshima and Okinawa Islands) encompassing the rivers where data on resident fish have been compiled and published. The targeted 181 rivers are located evenly within each region of Japan and the total value of the catchment area of these rivers accounts for 71% of total land area. Furthermore, primary freshwater fish confirmed in these rivers accounted for approximately 84% of that confirmed throughout Japan. Based on these facts, these rivers were sufficiently large and had enough fish fauna to enable ecoregion delineations within the Japanese aquatic areas.

2.2. Fish fauna data

I used fish fauna data on the presence–absence of fish species that were investigated by the Ministry of Land, Infrastructure, Transport and Tourism (MLIT; The National Census on River Environments from 1992 to 2015) and the Ministry of Environment (MOE; National Survey on the Natural Environment 1978 and 1994). In addition, I conducted a literature search to include any additional fish fauna information. 118 species of 84 rivers were added by the literature survey. The literatures used for addition were described in supplementary data. Non-native species were excluded from the analysis based on the information from the invasive species database released by the National Institute for Environmental Studies (National Institute for Environmental Studies, 2015). I used the data on both freshwater and migratory fish. Migratory fish living in brackish water and freshwater have infiltrated into the Japanese archipelago using ocean currents (Aoyagi, 1957); therefore, I added these migratory fish species to the analysis because they appear to be an important factor in delineating the ecoregion. Presence–absence data on each species were used for analysis.

2.3. Environmental data

I conducted the statistical analysis to investigate the relationship between results of fish fauna classification and environmental factors. I adopted the meteorological factors (annual average of seawater temperature [ST], air temperature [AT], and average-rainfall over watershed [R]) and topographic factors (a reciprocal of the channel slope gradient [G] and form ratio [F]) as the environmental factors.

Annual average ST was calculated using the value of temperature data at definite points obtained from the Japan Oceanographic Data Center (http://jdoss1.jodc.go.jp/vpage/coastal_j.html). To represent ST at the rivers studied, I adopted the recorded value from the nearest observation point. For annual AT, I adopted the average value of 10 years' data obtained from the meteorological observatory and local meteorological station managed by the Japan Meteorological Agency and extracted R from the Rivers Handbook (Land, Infrastructure and Development Committee 2006). For other rivers, I adopted R from rainfall data from each meteorological observatory and local meteorological station that was within the watershed of each river. G and F were used as topographic factors, the values of which were obtained using an electronic map from the Geospatial Information Authority of Japan and published data from the Ministry of Land, Infrastructure, Transport and Tourism. F was calculated by dividing the basin area by the square of the length of the river channel (Horton 1932).

As a result of confirming the correlation coefficient among these variables, I found a high correlation value between AT and ST; therefore, four indicators (ST, R, G, and F) were adopted for statistical analyses.

2.4. Statistical analyses

The fish fauna data for each site were classified using two-way indicator species analysis (TWINSPAN; Hill, 1979) based on dividing a reciprocal averaging ordination space. Because TWINSPAN covers groups of all samples from the beginning, it is difficult for the results to be affected by accidental fluctuations in individual small units and more correctly reflects the character of the community than the intensive method, such as cluster analysis (Kobayashi, 1995). PC-ORD ver. 4 (MjM Software Design) (McCune & Mefford 1999) was used to calculate TWINSPAN. The pseudospecies cutoff levels were defined as 0 (i.e., presence–absence), and the maximum number of indicator species for a division was set at five.

Following TWINSPAN, an indicator species analysis (IndVal) was executed to determine the indicator species. IndVal was proposed by Dufrêne and Legendre (1997) to determine whether some species are characteristic of specific groups of samples (intervals), and might reflect the environmental conditions inherent in these groups of samples (Trindade & Carvalho 2018). IndVal was applied to select the representative species for the environmental impact assessment (Niwa et al., 2009; Penczak, 2009, Takahashi et al., 2011; Itsukushima et al., 2017). In this study, because species that represent each ecological region can be subject to environmental evaluation and conservation, I used IndVal to extract the representative species for each group classification. In addition, the similarity of the fish fauna within each group was calculated using Jaccard's Index (Jaccard, 1912). The Jaccard Index is applied to presence–absence data and has been most frequently used in conjunction with the

Sorensen index since 1990 (Doi & Okamura, 2011). Furthermore, Cordoso et al. (2009) conducted the performance evaluation of similarity indices by simulation and recommended the Jaccard Index.

Further, decision tree model was executed to identify an environmental factor that contributes to classifying fish fauna of the Japanese archipelago. The four environmental factors (ST, R, G, and F) were used as explanatory variable and the classification result of TWINSPAN used as objective variable. Gini index was adopted for judgment criteria and optimal ramification number was calculated by cross-validation (De'Ath and Fabricius, 2000) Analysis was conducted by statistical software “R”.

3. Results

3.1. Classifications of fish fauna

As the result of TWINSPAN, 181 rivers were classified by the similarity of fish fauna (Fig. 1 (a)). The map reflects the classification results as indicated in Fig.1 (b). First, the 181 rivers were divided into 31 rivers located in the Hokkaido and northern Tohoku districts (groups A, B, and C) and other rivers. This result indicates the specificity of fish fauna of the rivers located in Hokkaido. In addition, although the northern Tohoku district is part of Honshu and accessible by land, fish fauna of northern Tohoku was similar to that of Hokkaido. Second, the remaining 150 rivers were divided into eastern and western Japan. The rivers in eastern Japan was classified into rivers flowing into the Pacific Ocean (groups D and E) and on the Sea of Japan (groups F and G). Among the rivers located in western Japan, those located in the Nansei islands were divided first (groups O and P). The remaining rivers were classified into groups on the Sea of Japan and Pacific Ocean (group H), flowing into Seto Inland Sea (groups J and K), flowing into Ise Bay (group I), and flowing into the Ariake Sea (group L). From these classification results, it was revealed that the fish fauna in each river was influenced by coastal water body in addition to geographical position. In addition, because western Japan comprises islands (Kyushu, Shikoku, and Nansei) within the Inland Sea, the rivers in the region were more finely classified than those in eastern Japan. In conclusion, the 181 rivers were classified into 15 groups using seven steps. The species that contributed to the 15 classifications identified during each step are as follow: ①*Zacco platypus* or *Tribolodon sachalinensis*, ②*Barbatula*, ③*Silurus asotus* or *Nipponocypris temminckii*,④*Cottus hangiongensis, Phoxinus percnurus sachalinensis,* and *Pungitius* sp., ⑤*Lethenteron japonicum* or *Cottus* sp. ME, ⑥*Rhinogobius* sp. MO, ⑦*Z. platypus*, ⑧*R. giurinus* or *Tanakia lanceolata*, ⑨*Sicyopterus japonicus* or *Acheilognathus rhombeus,* ⑩*Entosphenus tridentatus*, ⑪ *Cobitis matsubarae* or *Rhynchocypris lagowskii*, ⑫*P. lagowskii* *steindachneri* or *Gnathopogon elongatus elongatus*, ⑬*L. japonicum* or *Oncorhynchus keta*, ⑭*Rhodeus ocellatus kurumeus*, and ⑮*Pseudobagrus nudiceps*.

3.2. Characteristics of each fish fauna group

In this section, characteristics of fish fauna of each classification groups was described based on the result of TWISPAN. Table 1 shows the average number of fish species belonging to each family, which was representative of Japanese fish fauna (confirmed to be more than 10 species). Group I contained the largest number species (noted as the average ± standard deviation; 52.4± 9.2), followed by group J (48.6± 8.5), group I, and group L (46.9±7.1) from Kyushu Island. Moreover, the number of species from group I tended to decline in both north and south directions. The number of *Cyprinidae* was highest in group J (22.1± 4.9), followed by group L (21.9± 2.5) and group I (20.2

± 4.9). *Cyprinidae* species were not confirmed in group O at Amami Oshima Island. *Salmonidae* species were frequently confirmed in northern Japan and in the rivers flowing into the Sea of Japan; the number of *Salmonidae* species was largest in group A (6.3 ± 1.9), followed by group C (5.3 ± 2.2) and group F (5.4 ± 1.4). *Salmonidae* species were not confirmed in group O or group P. *Gobiidae* species are different from *Salmonidae* species, and there were only few in northern Japan with numbers that tended to increase toward the south. The number of *Gobiidae* species was largest in group O, followed by group I (12.8 ± 3.2), which belonged to rivers flowing into the Pacific Ocean that are affected by the Japanese currents. *Cobitidae* was confirmed in all groups except for groups O and P in the Nansei islands; the number of *Cobitidae* species did not differ among the groups.

Table 2 lists the fish species with IndVal ≥30 in each group. In the rivers of Hokkaido (groups A and B), several species had high IndVal scores, particularly *R. percnurus sachalinensis* and *P. pungitius* (group A) and *C. hangiongensis* and *C. nozawae* (group B). No species had IndVal ≥30 in groups located on the Pacific Ocean side of eastern Japan (groups C, D, and E); however, in the groups on the Sea of Japan side, *A. tabira tohokuensis* and *T. nakamurai* (group F), *Cottus sp.* (group G), and *Salvelinus leucomaenis imbrius* (group H) had higher IndVal values than other species within these groups. *Coreobagrus ichikawai* and *Cobitis* sp. 2 subsp. had high IndVal values in group I, which is composed of rivers flowing to Ise Bay. *Hemibarbus barbus* and *Cobitis* sp. 3 subsp. 1 had high IndVal values in group J. In addition, many species showed high IndVal values in group L, which is composed of rivers flowing into the Ariake Sea. In particular, *Acheilognathinae* had high IndVal values. No species had IndVal values ≥30 in the groups located on the Pacific Ocean side of western Japan (groups M and N), which was similar to those in eastern Japan. In group O located in the waters of Amam-Oshima, the IndVal values of *Gobiidae* species included those of *Sicyopterus. Entosphenus tridentatus* also had high IndVal values in group P.

3.3. Environmental factor contribute to classifying fish fauna

Figure 2 shows the results of the decision tree model using the results of the fish fauna classifications with TWINSPAN as the objective variable and environmental factors ST, R, G, and F as explanatory variables. The number of rivers classified into each node is indicated in Table 3. As a result of cross validation, the optimal ramification number was 12, and the total false classification rate was 40.9%. Among the 12 ramifications, the most selected index was OT, followed by R and G. If OT was >19.15℃ at ramification 1, the rivers were predicted to be a group on the Pacific side of western Japan (groups M, N, O, and P). If OT was <19.15℃ (ramification 1) and <15.15℃ (ramification 5), the rivers were predicted to be groups within Hokkaido and the Pacific side of Tohoku (groups A, B, C, and D). At ramification 4, the rivers were predicted to be within the Sea of Japan side of Tohoku and Hokuriku (groups F and G) and eastern Setouchi (group J) when OT was <17.75℃. When OT was >17.75℃ (ramification 4) and <17.95℃ (ramification 7), the rivers were predicted to be the group flowing into Ise Bay (group I). In addition, when OT was >17.95° C and G was >220.555 at ramification 7, the rivers were predicted to be those flowing into Kanto Plain (group E). If G was <220.555 at ramification 7, the rivers were predicted to be those in western Setouchi and northeastern Kyushu (group K) or the Sanin region (group H); however, the false classification rate was relatively high at ramifications 11 and 12. The groups located within northern Japan (A, C, and D) and within southern Japan (N, O, and P) had high predictive value, whereas the misclassification rate was higher in groups within the Seto Inland Sea or Sanin region (K or H).

4. Discussion

4.1. Environmental factors affecting the fish classification

As a result of the decision tree model using results of fish fauna classification with TWINSPAN as the objective variable and environmental factors ST, R, G, and F as explanatory variables, OT was selected by 9 out of 12 ramifications as the factors contributing to fish fauna classification. I considered migratory fish as an important factor in the fish community structure in the Japanese archipelago, and the ocean currents have a large influence on the invasion of migratory fish to the area. At ramifications 6 and 8, R was selected as a classification factor; therefore, the rainfall amount was a factor that affected fish fauna classifications in areas of low rainfall, such as Hokkaido or eastern Setouchi. Because flow regime characteristics influence fish community structure (Warfe et al., 2014; Weliange et al., 2017; Sago & Nagai, 2003), differences in rainfall appear to create a peculiar flow regime that influences fish fauna within these areas; whereas, G was selected only at ramification 9, and the rivers belonging to the Kanto Plain (group E) were extracted. The Backbone Mountains are connected at the north and south of the Japanese archipelago; therefore, high-gradient rivers were minant, although relatively large rivers were targeted in this study. On the other hand, the Kanto is the largest plain in the Japanese archipelago, and contains many rivers with moderate gradients. It is considered that a longitudinal gradient different from that of other rivers affects the fish fauna of these groups. Rivers with $G < 220.555$ at ramification 9 were mostly those belonging to Setouchi and Sanin (groups H, J, and K); however, these rivers were classified in multiple nodes and difficult to predict using the environmental factors adopted in this research. In the area to which these rivers belong, there is a complicated geological history, such as river conflicts and connected river systems from the glacial period; therefore, I will explain the characteristics of the fish fauna and the geographical factors in the next section.

4.2. Fish fauna classifications and geographical factors

In this section, I discuss the relationship between fish fauna classification results and geographical factors. First, the fish fauna of the rivers belonging to the Hokkaido region (groups A and B) were remarkably different from that of other regions. This revealed that the zoogeographical boundary called the “Blakiston line” located between Honshu and Hokkaido (Blakiston, 1883), which was established for mammals and birds, might also be adopted for fish fauna. The lowest sea level in the Tsugaru Strait after it was formed during the glacial stage was -80 m and the maximum depth was -140 m (Ohshima 1980); therefore, freshwater fish could not migrate between Honshu and Hokkaido, and the endemic fish fauna became established in the waters of Hokkaido. On the other hand, group C was classified within the same group of rivers belonging to Hokkaido (group A and B) during the first step. The Jaccard similarity index indicated that the similarity between groups C and A was 0.36 for freshwater fish and 0.45 for migratory fish, and between groups C and B was 0.33 for freshwater fish and 0.65 for migratory fish (Table 4). The similarity between migratory fish of group C and Hokkaido was also similar; therefore, these species were classified into the same group during first step. In addition, the fish species in the Pacific Ocean and the Sea of Japan sides were classified into different groups in Honshu (Fig. 1[b]); whereas, those in the Iwaki River located farthest north of Honshu on the Sea of Japan side were classified into the same group as those on the Pacific Ocean side. These results indicated that the migratory fish in the rivers belonging to group C are believed to be influenced by the Tsugaru Warm Current (Conlon, 1982) flowing from the Sea of Japan to the south along the Sanriku Coast; therefore, the Iwaki River was classified into the same group as the rivers on the Pacific Ocean side. During the third step of TWINSPAN, the Japanese archipelago was divided into groups D–G located in eastern Japan and groups H–P located in western Japan. The Japanese archipelago is said to have been formed from the eastern margin of Asia and independently separated eastern Japan from western Japan. These boundary areas (Fossa Magna) were caved and below sea level approximately 5 million years ago (Otofuji and Matsuda, 1984; Otofuji et al., 1985). Among the species distributed across the Fossa Magna, *Lefua echigonia* and *C. biwae* populations have been noted as having different genetic structures across the Fossa Magna boundaries (Kitagawa et al., 2003; Saka et al., 2003; Sakai et al., 2003; Mihara et al., 2005). In addition, Watanabe (1998) explained that the most dramatic change in fish fauna occurred east and west of the Fossa Magna area. In this study, the fish fauna of the Japanese archipelago were divided into eastern Japan and western Japan as bounded by the Fossa Magna area by the distribution of *S. asotus* or *N. temminckii*, and an influence by the Fossa Magna area on the fish fauna was suggested.

The Sea of Japan is an inland lake and provides an aquatic route through which Asian continental fish can invade the Japanese archipelago. Furthermore, the invasion of *Salmonidae* species was influenced by northern ocean currents, while the invasion of *Gobiidae* species was influenced by southern ocean currents. This reflects the abundance of *Gobiidae* species in southeastern Japan and that of *Salmonidae* species in northeastern

Japan, and also the large number of invasive species in the northwestern part of Kyushu and Kinki into the Japanese archipelago

The rivers on the Pacific Ocean side of Japan were classified from the north in the following order: groups C, D, E, M, and N. The Jaccard's similarity index of migratory fish among groups C, D, and E was higher (>0.70) than the similarity index between groups E and M (0.53) (Table 4). In addition, the fish fauna of the migratory species within groups M and N appears to be similar given that the Jaccard's similarity index was higher (0.73) than that between groups E and M (Table 4); therefore, the migratory fish fauna within group E and northward were different from that within group M and southward. This reflects that the ocean current influenced the invasion route of the migratory fish. Figure 1(b) shows the ocean currents near Japan. The Kurile Current flows adjacent to the sea of group E and northward, whereas, the Japan Current flows adjacent to the sea of group M and southward; therefore, the ocean current is considered to have a significant effect on Japanese aquatic ecoregions.

The rivers located in western Japan and flowing into the Seto Inland Sea were divided into group J (eastern Setouchi), group K (western Setouchi and northeastern Kyushu), and group H (Sanin region). Kuwashiro (1959) revealed that the three riverine systems, which are divided by the catchment boundary of the Bisan and Kanmon Straits, existed during the last glacial period (70,000～10,000 BP) using the geography of the submerged valley of the Seto Inland Sea. The east river system flowed into the Kii Channel, and the central river and west river systems were joined at Hoyo Strait and flowed into the Pacific Ocean through the Bungo Channel (Fig. 2). As a result of the classifications, the Ashida River and the rivers to the east belong in group J, and the Shimada River and the rivers to the west were classified into group K. This classification suggests that the previous river systems influenced the existing fish fauna, although the current location of the classification boundaries are different from those of the previous river systems. The influence of the river systems during the glacial age on the present fish fauna has been reported for the islands within the Seto Inland Sea (Hirayama and Touyama, 2011).

4.3. Fish fauna classifications and the geographical position

The rivers located within Hokkaido were divided into groups A and B. The Rumoi, Syokotsu, and Mu Rivers are located in eastern Hokkaido; however, they were classified into group B, which is composed of the rivers located in western Hokkaido. Table 3 shows the average value of the Jaccard's similarity index for each river in their respective groups within Hokkaido. In the Mu and Syokotsu Rivers, the average values of the similarity index among the groups were nearly the same. In addition, as a result of the one-way analysis of variance, significant differences in the similarity index were not confirmed among the groups for these two rivers. The fish fauna of these rivers were intermediate between groups A and B, therefore, the potential fish fauna of these rivers are believed to be more similar to those of group A, considering their geographical position.

The rivers located in the Tohoku region of the Sea of Japan side and the Hokuriku region were divided into groups F and G. The Ara and Kaji Rivers were classified into group G (Hokuriku Distinct) from the similarity of their fish fauna, even though they were located within the Tohoku region on the Sea of Japan side. In these rivers, a high occurrence of species in group F, such as *Carassius auratus* subsp. 1, *A. tabira*, *A. tabira tohokuensis*, *A. typus*, and *Pseudorasbora pumila*, were not confirmed; therefore, these rivers were classified into other groups. Figure 3 shows the longitudinal gradient of the middle and lower areas of the Ara River, the Kaji River, and neighboring rivers. The longitudinal gradient of the Ara and the Kaji Rivers was steeper than that of other rivers in the section ~10 km from the river mouth, and the floodplain area of these rivers was revealed to be smaller than that of other rivers. Because fish species such as *A. tabira*, which live in the floodplain, were not confirmed in these two rivers, it is highly probable that these species could not inhabit these rivers given their relatively steep gradients and poor floodplain environment. The Ara and Kaji Rivers were classified as group G based on their topographical factors; however, the potential fish fauna was considered homologous to group F.

Among the rivers flowing into the Seto Inland Sea, the Kurose, Ota, and Nishiki Rivers were classified into group H, which comprised rivers located within the Sanin region. In the Chugoku region was formed in the middle Miocene age when the backbone of the Chugoku region became a peneplane (low-relief plain formed by protracted erosion); therefore, there are many traces of river conflicts in these areas (Nishimura, 1962). In these areas, river conflicts over the Ota River system and the Gono river system (Inami, 1951), the Oze River system and the Nishiki River system (Yamanouchi and Shiraishi, 2009), and the Oze River system and Takatsu River system (Yamanouchi and Shiraishi, 2010) have been reported; therefore, fish fauna of the Kurose, Ota, and Nishiki Rivers could be similar to those within the rivers flowing into the Sea of Japan because freshwater fish migrate beyond the boundary of river systems because of river conflicts. Table 4 shows the Jaccard's similarity index for all species, including freshwater and migratory fish, among these rivers and adjacent groups (groups K and H). In these three rivers, the Jaccard's similarity index was higher in group H than in group K, particularly with regard to freshwater fish. These rivers were classified into group H because the highly indicative *S. leucomaenisimbrius* and *Leucopsarion petersii* were confirmed.

In addition, the classification results for the Shira and Kuma Rivers did not correspond to their geographical positions. In these rivers, the number of species of *Acheilognathinae*, specifically in the northern Kyushu region, was low because of the establishing process and timing of these rivers (Itsukushima et al., 2013); however, fish fauna, with the exception of *Acheilognathina*, was similar to that of other rivers flowing into the Ariake Sea. Therefore, the potential fish fauna was considered similar to that of group L.

Based on the classification results of the TWINSPAN analysis and our discussion, I suggest the aquatic ecoregions of the Japanese archipelago as designated in Fig. 4; however, the classification boundaries shown here need further verification.

4.4. Future prospects for establishing aquatic ecological regions

In this study, I attempted to define ecological regions based on the similarities within the fish fauna in relation to environmental factors and geological history. The use of fish fauna as a suitable environmental indicator is widely accepted, because they require a wide range of habitat conditions and reflect the accumulation of disturbances caused through anthropogenic activity (Karr 1991, Gansan & Hughes 1998). On this basis, fish fauna have been utilized for the delineation of ecoregions in a number of areas (Krause et al., 2013, Ferreira et al., 2007; Ellender et al., 2017; Mehner et al., 2007). On the other hand, ecological regions are commonly defined as areas in which ecosystems, including the type, quality, and quantity of environmental resources, are generally similar; therefore, the assessment of other taxonomic groups, such as benthos or phytoplankton, is necessary to confirm the robustness of the proposed ecological regions.

In addition, I attempted to delineate ecological regions based on the similarities among fish fauna by integrating the information on fish fauna collected by researchers and national and local governments. Recent studies include those focusing on the effectiveness of modelling techniques, such as artificial neural networks, for the interpretation of distribution patterns of biotic communities (Kruk and Penczak, 2013; Kruk et al., 2017), a contemporary real-life case study (Sefeedpari et al., 2016), and those including the prediction of hydrological phenomena (Olyaie et al., 2015; Chau 2017; Chen et al 2016; Wang et al, 2014). Modelling technologies will considerably improve the accuracy of delineating ecological regions and their boundaries by enabling the prediction of fish fauna in rivers whose data have not been collected.

5. Conclusion

The delineation of an ecological region is difficult in East Asia, including Japan because of complex topographies (i.e., several peninsulas and islands) and fauna that are very finely delineated based on climate. I attempted to delineate an ecological region based on the similarities among fish fauna by integrating the information on fish fauna that was collected by the researchers and the national government and local governments. Further, quantitative analyses to investigate the relationship between fish fauna classification and meteorological and geographical factors were conducted to discuss the factors that influence fish fauna classifications. The major conclusion and recommendations of this study include as following:

- The Japanese archipelago was classified into 15 groups according to fish fauna.
- The number of species and *Cyprinidae* was largest in the central region of the Japanese archipelago and tends to decline from the central area of the Japanese archipelago toward the north and south, except for northwest Kyushu. In addition, *Salmonidae* species were frequently confirmed in northern Japan and in the rivers flowing into the Sea of Japan,

with the number of *Salmonidae* species declining toward the south. *Gobiidae* species were scarce in northern Japan, but the number of species tended to increase toward the south.

・The results of classification using fish fauna are closely related to the process of the formation of the Japanese archipelago, the ocean currents in Japanese coastal waters, and the connections of the water system during the glacial age.

・It was suggested that rivers within geographical locations different from those within our classifications might contain different fish fauna resulting from potential migrating fish species depending on the characteristics of the watershed, such as the scale of the floodplain, river conflicts, or the river formation process.

・We can apply the results of the classifications system used in this study to enable a comparison of the biological integrity of fish fauna among watersheds for managing river environments or establishing conservation policies.

・Fish fauna within the Japanese archipelago was revealed to be an effective measure by which to delineate an ecological region; however, the boundaries of each ecological region or the applicability to other taxon should also be considered in future research.

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Table 1. Average number of fish species in each family which was representative of Japanese fish fauna (Mean ± SD).

Groups	All species	*Cyprinidae*	*Salmonidae*	*Gobiidae*	*Cobitidae*
A	27.2 ± 3.7	4.7 ± 0.8	2.6 ± 0.7	6.3 ± 1.9	4.8 ± 1.0
B	25.4 ± 3.2	4.3 ± 1.0	2.4 ± 0.7	4.1 ± 0.6	6.1 ± 1.4
C	29.0 ± 4.1	6.9 ± 1.9	1.9 ± 0.5	5.3 ± 2.2	6.2 ± 1.0
D	30.7 ± 5.6	8.8 ± 2.3	2.7 ± 0.7	4.2 ± 1.0	7.0 ± 1.5
E	32.1 ± 8.0	11.7 ± 2.4	2.9 ± 0.3	2.1 ± 2.1	7.6 ± 2.4
F	40.8 ± 9.2	12.4 ± 4.8	2.4 ± 0.5	5.4 ± 1.4	9.1 ± 1.9
G	38.9 ± 7.0	11.8 ± 2.7	2.5 ± 0.8	4.2 ± 1.6	9.4 ± 2.3
H	45.5 ± 9.1	13.9 ± 4.5	3.4 ± 0.7	3.8 ± 1.4	10.6 ± 2.5
I	52.4 ± 9.2	20.2 ± 4.9	4.7 ± 1.3	2.2 ± 1.2	12.8 ± 3.2
J	48.6 ± 8.5	22.1 ± 4.9	4.1 ± 1.0	1.8 ± 1.0	9.5 ± 2.5
K	34.5 ± 6.4	12.9 ± 2.5	2.6 ± 1.0	1.1 ± 1.3	8.9 ± 2.2
L	46.9 ± 7.1	21.9 ± 2.5	3.1 ± 0.7	1.1 ± 1.1	8.3 ± 2.8
M	35.9 ± 6.3	10.7 ± 3.1	2.8 ± 0.8	2.2 ± 1.0	11.9 ± 1.8
N	31.8 ± 6.5	9.0 ± 2.6	1.6 ± 0.9	0.9 ± 0.6	12.0 ± 2.2
O	22.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	16.0 ± 0.0
P	7.0 ± 0.0	3.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 0.0

Table 2. Result of IndVal analysis for each group.

Species	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Petromyzontidae																
Entosphenus tridentatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
Lethenteron kessleri	42	6	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Anguillidae																
Anguilla marmorata	0	0	0	0	0	0	0	0	2	0	0	0	3	3	35	35
Cyprinidae																
Acheilognathus tabira nakamurae	0	0	0	0	0	0	0	0	0	0	0	71	0	0	0	0
Acheilognathus rhombeus	0	0	0	0	0	0	0	4	12	21	0	37	0	0	0	0
Acheilognathus cyanostigma	0	0	0	0	0	0	0	0	39	14	0	0	0	0	0	0
Acheilognathus tabira tabira	0	0	0	0	0	0	0	0	12	33	0	0	0	0	0	0
Acheilognathus tabira	0	0	0	0	4	32	9	2	0	0	0	0	0	0	0	0
Acheilognathus tabira tohokuensis	0	0	0	0	0	55	0	0	0	0	0	0	0	0	0	0
Rhodeus ocellatus kurumeus	0	0	0	0	0	0	0	0	3	7	0	61	0	0	0	0
Rhodeus atremius	0	0	0	0	0	0	0	0	0	0	0	94	0	0	0	0
Aphyocypris chinensis	0	0	0	0	0	0	0	0	0	0	0	43	0	0	0	0
Rhynchocypris percnurus sachalinensis	83	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tribolodon nakamurai	0	0	0	0	0	55	0	0	0	0	0	0	0	0	0	0
Pseudorasbora pumila pumila	0	0	0	2	1	33	0	0	0	0	0	0	0	0	0	0
Sarcocheilichthys variegatus variegatus	0	0	0	0	0	0	0	1	20	14	1	32	0	1	0	0
Abbottina rivularis	0	0	0	0	0	0	0	0	3	6	0	51	0	0	0	0
Hemibarbus longirostris	0	0	0	0	0	0	0	5	10	30	7	0	0	0	0	0
Hemibarbus barbus	0	0	0	0	0	0	0	2	4	57	0	0	1	0	0	0
Squalidus chankaensis tsuchigae	0	0	0	0	0	0	0	5	28	31	0	6	0	0	0	0
Cobitidae																
Parabotia curtus	0	0	0	0	0	0	0	0	0	30	0	0	0	0	0	0
Cobitis matsubarae	0	0	0	0	0	0	0	0	1	0	24	43	0	9	0	0
Cobitis sp. 3 subsp. 1	0	0	0	0	0	0	0	3	1	54	3	0	0	0	0	0
Cobitis sp. 2 subsp. 3	0	0	0	0	0	0	0	0	51	0	0	0	5	0	0	0
Cobitis sp. 2 subsp. 4	0	0	0	0	0	0	0	15	0	0	0	57	0	0	0	0
Barbatula barbatula	50	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lefua nikkonis	38	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bagridae																
Pelteobagrus nudiceps	0	0	0	0	0	0	0	11	0	36	14	3	0	1	0	0
Coreobagrus ichikawai	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0
Pseudobagrus aurantiacus	0	0	0	0	0	0	0	0	0	0	1	42	0	2	0	0
Osmeridae																
Plecoglossus altivelis ryukyuensis	0	0	0	0	0	0	0	0	1	0	0	0	0	0	90	0
Salangidae																
Salanx ariakensis	0	0	0	0	0	0	0	0	0	0	1	52	0	0	0	0
Neosalanx reganius	0	0	0	0	0	0	0	0	0	0	0	43	0	0	0	0
Salmonidae																
Parahucho perryi	42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Salvelinus leucomaenis imbrius	0	0	0	0	0	0	0	32	0	0	1	0	0	0	0	0
Salvelinus malma	40	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oncorhynchus gorbuscha	31	4	9	0	0	1	0	0	0	0	0	0	0	0	0	0
Gasterosteidae																
Pungitius tymensis	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pungitius pungitius	49	8	4	0	0	0	0	0	0	0	0	0	0	0	0	0
Cottidae																
Trachidermus fasciatus	0	0	0	0	0	0	1	0	0	0	0	74	0	0	0	0
Cottus sp.	0	1	0	0	0	21	35	8	1	0	0	0	0	0	0	0
Cottus hangiongensis	0	31	7	1	0	2	5	0	1	0	0	0	0	0	0	0
Cottus nozawae	28	49	0	1	0	2	0	0	0	0	0	0	0	0	0	0
Cottus amblystomopsis	36	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kuhliidae																
Kuhlia rupestris	0	0	0	0	0	0	0	0	1	0	0	0	1	1	74	0
Kuhlia marginata	0	0	0	0	0	0	0	0	1	0	0	0	7	23	44	0
Gobiidae																
Butis amboinensis	0	0	0	0	0	0	0	0	0	0	0	0	0	1	92	0
Eleotris fusca	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0
Eleotris acanthopoma	0	0	0	0	0	0	0	0	0	0	0	0	2	27	51	0
Lentipes armatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0
Sicyopus zosterophorus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0
Sicyopterus macrostetholepis	0	0	0	0	0	0	0	0	0	0	0	0	1	0	91	0
Stiphodon percnopterygionus	0	0	0	0	0	0	0	0	1	0	0	0	0	1	83	0
Stiphodon atropurpureus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0
Rhinogobius sp.DL	0	0	0	0	0	0	0	0	1	0	0	0	0	0	90	0
Rhinogobius sp.MO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	50
Rhinogobius brunneus	0	0	0	0	0	0	0	0	2	0	1	0	2	7	45	0
Rhinogobius sp.TO	0	0	0	0	0	0	0	0	33	0	0	0	0	0	0	0
Tridentiger kuroiwae	0	0	0	0	0	0	0	0	1	0	0	0	0	0	90	0
Tridentiger barbatus	0	0	0	0	0	0	0	0	1	0	0	68	0	0	0	0
Parioglossus dotui	0	0	0	0	0	0	0	0	2	0	0	1	2	10	47	0
Parioglossus philippinus	0	0	0	0	0	0	0	0	4	0	0	0	0	0	82	0

Table.3 the number of rivers classified into each node in decision tree analysis

node	A		B		C		D		E		F		G		H		I		J		K		L		M		N		O		P	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
4	12	75	4	25	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
6	0	-	0	-	8	89	1	11	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
7	0	-	4	22	3	17	8	44	2	11	1	6	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
11	0	-	0	-	0	-	0	-	0	-	7	88	1	12	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
12	0	-	0	-	0	-	0	-	1	5	2	10	12	60	1	5	1	5	2	10	1	5	0	-	0	-	0	-	0	-	0	-
13	0	-	0	-	0	-	0	-	3	21	1	7	0	-	0	-	0	-	10	71	0	-	0	-	0	-	0	-	0	-	0	-
16	0	-	0	-	0	-	0	-	6	86	0	-	0	-	0	-	0	-	1	14	0	-	0	-	0	-	0	-	0	-	0	-
18	0	-	0	-	0	-	0	-	2	12	0	-	0	-	9	56	0	-	3	19	1	6	0	-	0	-	1	6	0	-	0	-
20	0	-	0	-	0	-	0	-	1	12	0	-	0	-	5	62	0	-	2	25	0	-	0	-	0	-	0	-	0	-	0	-
21	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	7	47	6	40	2	13	0	-	0	-	0	-
22	0	-	0	-	0	-	0	-	0	-	0	-	0	-	1	8	8	62	0	-	4	31	0	-	0	-	0	-	0	-	0	-
24	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	2	17	1	8	6	50	3	25	0	-	0	-
25	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	2	8	1	4	0	-	2	8	18	72	1	4	1	4

Table 4. Jaccard's similarity index between group A, B, C, D, E M, and N (upper: all species, middle: freshwater fish, lower: migratory fish).

	A	B	C	D	E	M
B	0.78					
	0.86					
	0.74					
C	0.48	0.52				
	0.36	0.33				
	0.55	0.65				
D	0.40	0.47	0.74			
	0.25	0.27	0.71			
	0.51	0.62	0.76			
E	0.34	0.38	0.67	0.69		
	0.20	0.18	0.60	0.63		
	0.46	0.55	0.72	0.74		
M	0.16	0.19	0.36	0.36	0.51	
	0.14	0.12	0.33	0.33	0.50	
	0.18	0.25	0.38	0.40	0.53	
N	0.12	0.14	0.29	0.27	0.44	0.60
	0.10	0.08	0.24	0.24	0.40	0.51
	0.14	0.20	0.33	0.31	0.47	0.73

Table 5. Average value of the Jaccard's similarity index for each river in their respective group and other groups in Hokkaido (Mean ± SD).

No	River name	group	all rivers belonged Hokkaido	Index with the group which the river belongs	Index with the group which the river not belongs
1	Koetoi	A	0.57 ± 0.06	0.60 ± 0.06	0.53 ± 0.04
2	Tonbetsu	A	0.64 ± 0.08	0.69 ± 0.07	0.59 ± 0.03
3	Shokotsu	B	0.54 ± 0.05	0.55 ± 0.04	0.54 ± 0.05
4	Yubetsu	A	0.64 ± 0.08	0.69 ± 0.07	0.57 ± 0.04
5	Tokoro	A	0.62 ± 0.09	0.67 ± 0.07	0.55 ± 0.03
6	Abashiri	A	0.67 ± 0.08	0.72 ± 0.07	0.61 ± 0.04
7	Shari	A	0.55 ± 0.09	0.60 ± 0.07	0.48 ± 0.07
8	Kushiro	A	0.64 ± 0.10	0.71 ± 0.07	0.54 ± 0.05
9	Tokachi	A	0.63 ± 0.08	0.66 ± 0.08	0.58 ± 0.06
10	Saru	A	0.62 ± 0.09	0.64 ± 0.06	0.60 ± 0.13
11	Mu	B	0.66 ± 0.08	0.65 ± 0.07	0.66 ± 0.10
12	Abira	A	0.63 ± 0.07	0.65 ± 0.06	0.60 ± 0.08
13	Yurappu	B	0.57 ± 0.06	0.61 ± 0.05	0.54 ± 0.04
14	Shiribeshitoshibetsu	B	0.63 ± 0.08	0.70 ± 0.07	0.60 ± 0.06
15	Shubuto	B	0.61 ± 0.10	0.68 ± 0.09	0.56 ± 0.07
16	Shiribetsu	B	0.62 ± 0.11	0.73 ± 0.09	0.56 ± 0.07
17	Yoichi	B	0.63 ± 0.10	0.70 ± 0.09	0.58 ± 0.08
18	Ishikari	A	0.67 ± 0.08	0.68 ± 0.08	0.65 ± 0.09
19	Rumoi	B	0.60 ± 0.09	0.66 ± 0.10	0.56 ± 0.06
20	Teshio	A	0.67 ± 0.07	0.70 ± 0.07	0.63 ± 0.04

Table 6. Jaccard's similarity index of all species, including freshwater and migratory fish, among these rivers and adjacent groups (groups K and H) (upper: all species, middle: freshwater fish, lower: migratory fish).

	Kurose. R	Ota. R	Nishiki. R	Oze. R	group K
Ota. R	0.40 0.29 0.63				
Nishiki. R	0.45 0.38 0.60	0.25 0.24 0.28			
Oze. R	0.47 0.36 0.69	0.27 0.26 0.28	0.25 0.26 0.24		
group K	0.56 0.44 0.76	0.41 0.36 0.48	0.42 0.36 0.52	0.45 0.45 0.46	
group H	0.63 0.55 0.79	0.45 0.45 0.45	0.44 0.42 0.48	0.51 0.52 0.48	0.35 0.34 0.36