Article

Title : Relationship between watershed scale macroinvertebrate community and environmental factors in the Japanese archipelago

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Abstract

The conservation and restoration of freshwater ecosystems require the understanding of potential biota of the target area. My ultimate study goal was to clarify the potential fauna of the watershed unit of the Japanese archipelago, a hotspot of biodiversity. Here, I attempted to classify the macroinvertebrate community of the major rivers within the Japanese archipelago, thereby elucidating its biogeography, and to investigate the extent to which environmental factors drive the watershed's macroinvertebrate community. I classified the rivers located in the northern region of the Japanese archipelago geographically, but did not group the geographically adjacent rivers in the western region together. Differences in watershed size, geological history (including river conflict), and paleo-drainage systems seem to affect the classification results. Moreover, Indicator Species Analysis results suggest that river groups in the northern part of the Japanese archipelago had highly endemic species, whereas, the river groups in the western part of the Japanese archipelago had few highly endemic species. The result of the canonical correspondence analysis indicated that topographic factors, the flow regime, geology, water quality, and anthropogenic factors were significantly correlated with macroinvertebrate classification and distribution. The results of the decision tree model indicated that water temperature and maximum specific discharge were explanatory factors in the classification of the macroinvertebrate community. Further, my results also suggest that environmental factors at a smaller scale than that of the watershed were needed to explain further subdivisions in classification of the macroinvertebrate community.

Keywords: *macroinvertebrate community; environmental factors; watershed scale; ecological region*；*potential biota*

1. Introduction

The freshwater area occupies only 0.8% of the earth's surface; nevertheless, it accounts for 100,000 species (6 % of all recorded species) (Dudgeon et al., 2006). The freshwater ecosystems of many regions have been degraded by anthropogenic impact (Gleick, 2003; Giller, 2005). Stream ecosystems provide humans with critical services such as flood protection, food, water filtration, and carbon sequestration, and are important conservation targets (Durance et al., 2016; Boulton et al., 2016). The setting of ecological regions as the basic unit of conservation (Illies, 1978; Zogaris et al., 2009; Omernik and Griffith, 2014), the evaluation of ecosystem integrity (Karr, 1993; Nel et al., 2009; Kuehne et al., 2017), and the determination of the reference condition (Karr and Chu, 1999; Stoddard et al., 2006) are essential aspects in preserving and restoring stream ecosystems. In some regions, based on these researches, collective efforts toward the conservation and restoration of freshwater ecosystems have been conducted for many taxonomic groups (Feld et al., 2010; Langhans et al., 2019; Pander and Geist, 2013; Geist, 2011).

The accumulation of knowledge regarding basic ecology including natural and anthropogenic factors controlling biota distribution serves as a framework underpinning ecosystem conservation and management. Biota distribution patterns, long a major theme in ecology, have prompted numerous studies in the past (e.g. Levin, 1992; Hawkins et al., 2000; Collen et al., 2013). The controlling environmental factors of community structure have been investigated in many regions and at various spatial scales (e.g. Allan et al., 1997; Marchetti et al., 2006; Feld and Hering, 2007). The controlling factors of various spatial scales greatly influence the distribution pattern of the biota, and the degree of the effect is thought to depend on the size of the taxonomic group (Johnson et al., 2007). For example, fish fauna are strongly affected by relatively large scale factors such as land use, whereas macroinvertebrates and the diatom community are influenced by changes in the environmental factors of habitat scale and water quality (e.g. Snyder et al., 2003; Rosenberg and Resh, 1993; Heatherly et al., 2007; Funnell et al., 2020). However, various environmental factors influence each other spatially, and the response of biota to environmental factors is thought to differ locally. Therefore, accumulated knowledge in various climatic zones and taxa is vital in planning the local biota-specific conservation of ecosystems.

Among freshwater ecosystems, the macroinvertebrate community is important in gaining perspective on nutrient cycles, primary productivity, decomposition, and translocation of materials (Wallace and Webster, 1996). Due to its sensitivity to environmental change (Péru and Dolédec, 2010), the macroinvertebrate community has long been used as an assessment criteria for water quality, nutrient enrichment, and the detection of contaminated material (e.g. Metcalfe, 1989; Barbour et al., 1999; Maul et al., 2004; Lock et al., 2011). Additionally, the macroinvertebrate community has also been utilized in the evaluation of a habitat's hydraulic conditions, including river bed material and flow characteristics (Mažeika et al., 2004; McGoff and Irvine, 2009; Rempel et al., 2000; Extence et al., 2013), in the assessment of flow regime modification by water intake structures (Morgan et al., 1991; Carlisle et al., 2014 ； Marchetti et al., 2011), detection of anthropogenic impact such as land use change (Martínez et al., 2016; Fu et al., 2016), and for environmental monitoring of river revitalization projects (Carlson et al., 2018; dos Reis Oliveira et al., 2019; Itsukushima et al., 2019). Recent research has investigated the response of the macroinvertebrate community to climate change (Durance and Ormerod, 2007; Chiu et al., 2017; Lencioni, 2018). In addition to these studies, much basic ecological research has focused on the relationship between the macroinvertebrate community and environmental factors at various spatial scales (e.g. Richards et al., 1997;

Shearer and Young, 2011; Wang and Tan, 2017; Jonsson et al., 2017; Calabrese et al., 2020).

Knowledge of the endemism and universality of an ecosystem's biota in the area is essential for its conservation, as is an understanding of the relationships between biota and environmental factors, anthropogenic impacts, and factors that give rise to a region's endemism. The Japanese archipelago targeted in this study is recognized as a "global hotspot of biodiversity" (Gerardo and Brown, 1995; Marchese, 2015; Conservation International, 2016), and the fauna of the Japanese archipelago is characterized by a high level of diversity and endemism (Motokawa and Kajihara, 2017). Several factors are hypothesized to have created this high level of biodiversity. First, the archipelago is long from north to south, and stretches 3,000 km from the sub-tropics in the south to the sub-Arctic in the north, thus traversing multiple climate categories and biomes to create a high degree of diversity. Second, the archipelago is influenced by an Asian monsoon climate; increased humidity results in an increased diversity of plants and insects (Hayashi et al., 2017; Kubota et al., 2016), and the frequent floods also help maintain high biodiversity levels (Wilkinson, 1999). Finally, geological history is also a factor that causes high biodiversity. The Japanese archipelago is located at the boundary of four tectonic plates (the Pacific, Philippine Sea, Asian, and North American plates). The collision of these plates forms a high backbone range in the central part of the archipelago, which led to geological isolation (high altitude and steep geography), genetic differentiation, and speciation (Ohnishi et al., 2009; Miyazaki et al., 2011; Suzuki et al., 2014; Tojo et al., 2017). Furthermore, plate tectonics also contributes to increased marine biodiversity (Leprieur et al., 2016). Various ocean currents, including cold and warm currents, flow along the coast of the Japanese archipelago, and these diverse currents play an important role in improving estuary biodiversity (Itsukushima, 2019). My ultimate goal in this study is to reveal the potential fauna at watershed scale by (1) classifying the macroinvertebrate communities of the major rivers belonging to the Japanese archipelago to contribute to the elucidation of its biogeography and (2) clarifying the extent to which environmental factors explain the macroinvertebrate community of the watershed.

2. Material and methods

2.1. Study area and data collection

2.1.1. Study area

My study focused on the macroinvertebrate communities of the 109 main watersheds of the Japanese archipelago (Fig. 1). The total catchment area of these rivers accounts for 63% of the land area of Japan.

2.1.2. Data collection of macroinvertebrate community

I used presence–absence macroinvertebrate community data compiled by the Ministry of Land, Infrastructure, Transport and Tourism (MLIT; The National Census on River Environments from 1992 to 2015, http://www.nilim.go.jp/lab/fbg/ksnkankyo/). A total of 1,137 sampling sites in 109 rivers were used to sample macroinvertebrate fauna, and 10.4 ± 10.4 (mean ± standard deviation) sites per river were investigated longitudinally. The minimum elevation value of the investigation sites was -0.6 m, while the maximum was 1,361 m; the average value was 51.8 ± 106.3 m, which included the area from the estuary to the upstream area (Appendix 1). The investigation was conducted in each river at least once every five years, in summer and winter. In the freshwater areas, sampling was conducted in each habitat (rapid, pool, spring, fluvial lagoon, reservoir area, and river bank) using Surber nets (25 cm × 25 cm, mesh size of 0.493 mm), scoop nets, and scrape nets. In the river estuary, in areas where the bottom surface dried out at low tide, a 30 cm square quadrat was installed, and the bottom sediments in that area were sampled up to a depth of 10 cm using a shovel and rake. Macroinvertebrate organisms were collected by filtering the bottom sediments with a 0.5 mm mesh sieve. In areas that were very deep at low tide, macroinvertebrate organisms were sampled using a Ekman-Birge grab (15 cm × 15 cm), and were collected by filtering the bottom sediments with 0.5 mm mesh sieve. Collected macroinvertebrate organisms were sorted using sieves with a combined mesh size of 2.8 mm and 0.5 mm (JIS standard, JIS Z 8801), and were identified based on Kawai and Tanida (2005) (Ministry of Land, Infrastructure, Transport and Tourism, 2016). Where a species was confirmed at least once, I added that species to those inhabiting the watershed. In this study, the analysis was conducted using genus level data, to ensure that the analysis results would not be affected by issues in species-level identification, the presence of cryptic species that are difficult to identify by morphology, or any differences occurring due to new species.

2.2.2. Data collection of environmental factors

I employed 32 environmental indicators classified into location, topographic factors, flow regime, anthropogenic factors, geology, and water quality (Table 1).

I used the latitude (Lat) and longitude (Long) of each river mouth as the location. Under topographic factors I included the watershed area (WA), stream length (SL), altitude of the riverhead (AH), terrain gradient (TG), form ratio (FR), and drainage density (DD). I used the value of the WA and SL obtained by the Japan River association (2006). I investigated the AH using the electronic topographic map 25000 released by Geographical Survey Institute of Japan. I calculated the TG by dividing the AH by the length of the main stream. I obtained the FR by dividing the WA by the square of the length of the main stream. The FR is an index that approaches 1.0 as the basin becomes closer to a square or circle (Horton, 1932). DD indicates the degree of development of a drainage network calculated by dividing the SL by the WA (Schumm, 1956). I used various specific discharge (SD) values, including the maximum (SDmax), 75-day (SD75), ordinary (SDo), 275-day (SD275), 355-day (SD355), and minimum (SDm) specific discharge as indicators. In addition, I adopted the coefficient of river regime (CR) as the indicator of disturbance, calculated by dividing SDmax by SDmin. I used the average of each year's data of flow discharge at the observation points at the reference points in each watershed, obtained from the Water Information System managed by the Ministry of Land, Infrastructure, Transport and Tourism (MLIT) (http://www1.river.go.jp/). The average period that water discharge had been measured for the 109 rivers was 27.8 ± 19.0 years. The gaging station's elevation, where the flow regime index was obtained, was 35.7 ± 41.4 m (Appendix 1). I considered anthropogenic factors to include the number of dams (ND), obtained from Japan Dam Foundation (2019), population density (PD) in the watershed, and land use (percentage of mountain area: MO, percentage of mountain agriculture area: AG, percentage of urban area: UR). I obtained PD and land use from the published data of the MLIT. Under the geological factors, I used the surface geology of each watershed obtained from the subsurface geological map with a scale of 1 to 250,000. Based on the generation process, geology is roughly classified into sedimentary rock (SR), igneous rock (IR), and metamorphic rock (MR). A detailed geological map is given in Appendix 2 (The National Institute of Advanced Industrial Science and Technology https://gbank.gsj.jp/geonavi/?lang=en). I classified water quality using average water temperature (WT), potential of hydrogen (PH), biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids (SS), dissolved oxygen (DO), total nitrogen (TN), total phosphorus (TP), and number of colitis germ legions (CGL). I obtained these data from the Water Information System. The average period of water quality measurements for the 109 rivers was 18.9 ± 8.4 years. Water quality data was acquired at 7.3 ± 17.8 m (Appendix 1).

2.2. Statistical analysis

2.2.1. Classification of macroinvertebrate community

I classified the macroinvertebrate community data for each watershed based on similarity by TWINSPAN (Two-Way Indicator Species Analysis, Hill, 1979). The more commonly used cluster analysis is an intensive way to pool similar data together; TWINSPAN, conversely, is geared toward dividing a set of data into small groups. TWINSPAN is the most appropriate statistical approach as a method for dividing points as it differentiates particular species from component species at each point, and Hill applies this method to divide the biotic community. I used PC-ORD ver. 4 (MjM Software Design) to calculate TWINSPAN, defining the pseudo-species cut-off level as 0 (that is, presence–absence), and setting the maximum number of indicator species for a division at five.

Following TWINSPAN, I executed Indicator Species Analysis (IndVal) (Dufrêne and Legendre, 1997) to determine indicator species of each group divided by TWINSPAN, in which index values range from 0 to 100% and indicate the degree of concentration in specific groups.

2.2.2. Analysis of the relationship between macroinvertebrate community and environmental factors

To investigate the relationship between the macroinvertebrate community and environmental factors, I conducted canonical correspondence analysis (CCA) for species that appeared in over 10 rivers, to exclude the influence of species appearing at a low frequency. Moreover, to eliminate the occurrence of multicollinearity of environmental factors as explanatory variables, I narrowed down environmental factors from 32 to 19 variables so that the variance inflation factor (VIF) was less than 10 based on the correlation between variables. I found large variations in the 32 environmental factors between the watersheds, and some of these variations were detected to be correlated (Table 1). Reflecting the large climatic variation of the Japanese archipelago, I confirmed correlations between Lat and Long, and both SDmax and WT. In addition, I confirmed correlations between PD and UR, as well as TN and TP. In addition, I selected environmental factors that showed significant effects on CCA axes ($p < 0.05$) by the Monte Carlo permutation test. Next, I conducted the multiple comparison tests for the environmental factors that significantly related to the result of CCA. First, I performed the Bartlett test, the Kruskal-Wallis test for the environmental factors with unequal variance, and the one-way analysis of variance for the environmental factors with equal variance. Next, I conducted the Steel-Dwass test if the significant difference between groups was confirmed by the Kruskal-Wallis test, or the Tukey-HSD test if the significant difference between groups was confirmed by the one-way analysis of variance. I considered a significance level of $p < 0.05$.

Finally, I used the decision tree model (Classification and Regression Tree: CART) to identify the environmental factors that contributed to the classification of the macroinvertebrate community. The 32 environmental factors were used as explanatory variables, and the classification result of TWINSPAN was used as the objective variable. I adopted the Gini index as judgment criteria, and calculated the optimal ramification number by cross-validation (De'Ath and Fabricius, 2000). All analyses were conducted using the statistical software “R” and package ‘rpart’ (Therneau and Atkinson, 1997).

3. Results

3.1. Classification result of macroinvertebrate community in the Japanese archipelago

As a result of the investigation, 57 orders, 203 families, and 504 genera were confirmed in the targeted rivers. Majority of the 504 genera were in family Diptera (110 genera), followed by Trichoptera (48 genera), Ephemeroptera (46 genera), Odonata (39 genera), and Coleoptera (35 genera). Among the confirmed genera, four genera belonged to Chironomidae (*Chironomus*, *Microtendipes*, *Polypedilum*, and *Stictochironomus*), *Baetis*, *Cheumatopsyche*, *Palaemon* were confirmed in all rivers. In addition, the largest number of genera was confirmed in the Tone River, which has the largest basin area in the Japanese archipelago, with 406 genera, followed by the Kiso River with 371 genera and the Yodo River with 333 genera. The number of genera tended to be larger in the large rivers. The smallest number of genera was confirmed in the Shiribetsu River with 87 genera. The number of genera tended to be small in the northern part of the Japanese archipelago.

A correlation was found between the index related to the location (Lat and Log) and the index, such as WT, SDmax, or DD. This reflects the climatic factors that cause northern Japan to have less rainfall than southwestern Japan. In addition, a positive correlation between indicators was confirmed among the flow regime indicators. Furthermore, PD, which indicates anthropogenic impacts, was correlated with UR, TN, and TP (Table 1).

TWINSPAN classified the 109 rivers into eight groups based on macroinvertebrate community (Fig. 1). In the first step, the 109 rivers were divided into northern and southwestern groups. The northern rivers were further divided into Hokkaido (Group A), rivers located in the northern part of Tohoku (Group B), rivers located on the Sea of Japan side of Tohoku and Hokuriku (Group C), and the remaining 10 rivers (Group D). The western rivers were divided into the rivers located on the Pacific Ocean side of Tohoku and Kanto (Group E), rivers mainly flowing into the Seto Inland Sea (Group H) and so on. While I grouped the rivers in the northern part of the Japanese archipelago geographically, I did not necessarily group geographically adjacent rivers together in the western region. The species contributing to the eight classifications identified during each step are as follows: ① *Fistulob*, *Neocarid*, *Assimine*, *Helice*, *Xenostro*, *Stenelmi*, and *Ligia*; ② *Chiroma* and *Ischnura*; ③ *Saetheri*, *Monodiam*, *Pseudove*, *Pisidium*, *Dryopomo*, *Bibiocep*, and *Cladopel*; ④ *Nuttalli*; ⑤ *Littorar*, *Patelloi*, *Paratya*, *Reishia*, *Laomedia*, *Paratany*, *Pagurus*, *Athanas*, *Rapana*, *Arcother*, *Varuna*, *Prionoce*, *Leptocer*, and *Mediomas*; ⑥ *Eubasili*, *Neocarid*, *Niponiel*, and *Protonem*; and ⑦ *Armandia*, *Pagurus*, *Rhynchos*, and *Ruditape*.

In Table 2 I list the macroinvertebrate species with the top five IndVal values in each group. Indicator species of Group A—such as *Dicosmoecus*—showed high values only in Group A, indicating a high endemism. In addition, in Groups B, C, and D, although the IndVal values of the species were smaller than those of Group A, these species did not overlap with other regions. In contrast, the top five IndVal values for species in Groups E, F, G, and H—such as *Stylaria*—had low values, and were among the top species in multiple groups. The groups in the northern part of the Japanese archipelago, and classified by geographical area, had highly endemic species, whereas the groups in the western part of the Japanese archipelago, where rivers were not clearly classified by geographical areas, had few highly endemic species.

3.2. Relationship between macroinvertebrate community and environmental factors

The permutation test selected WA, AH, SDmax, SDo, DD, ND, IR, WT, and PH as the significant variables which influenced the classification of macroinvertebrate communities ($p < 0.05$). Axis 1 was positively correlated with WT, PH, SDmax, DD, and IR and negatively correlated with the remaining five variables. Axis 2 was positively correlated with PH, SDmax, DD, and WT, and was negatively correlated with the remaining five variables. In comparing these results with the classification results, I found that Group A, comprising the rivers located mainly in Hokkaido, was plotted in the second quadrant; Groups B and C, comprising the rivers located in northern Tohoku and the Sea of Japan side of Tohoku and Hokuriku, were plotted in the third quadrant; and group E, comprising the rivers located on the Pacific side of Tohoku and Kanto, was plotted in the fourth quadrant. The rivers belonging to group D were plotted near the origin point (Fig.2 (a)).

In Figure 2 (b) I show the relationship between species distribution pattern and environmental factors. Genera of *Pomacea*, *Gammarus*, and *Sinotaia* were plotted in the first quadrant, where WT, SDmax, and AG values were high. Species of *Dicosmoecus* and *Hydatophylax* were plotted in the second quadrant, where WT and SDmax were low. Species including *Oreodytes*, and *Laccophilus* were plotted in the third quadrant, where WA and AH were large. Species including *Urnatella*, and *Benthalia* were plotted in the fourth quadrant, where IR was high.

3.3. Environmental characteristics of each group

In Fig. 3 (a)-(j) I indicate the environmental factors significant in relation to the classification results for the macroinvertebrate community. Among 10 environmental factors, I confirmed significant differences among groups for six factors, excluding WA, ND, IR, and SS. In addition, among these factors, I confirmed SDmax, WT, and DD as having significant differences between many groups. Conversely, I confirmed only a small number of groups with significant differences for AH and PH.

In Figure 4, I show the result of the decision tree model using the classification results of fish fauna by TWINSPAN as an objective variable and environmental factors as explanatory variables. I found the optimal ramification number after cross-validation to be four, and the total false classification rate to be 46.2%. Among the four ramifications, Lat, WT, SDmax, and Log were selected as the variables for classifying the macroinvertebrate fauna of the Japanese archipelago. In cases where the Lat was larger than 41.73° (at ramification 1), rivers were predicted as groups from Hokkaido (Group A). In cases where Lat was less than 41.73° (at ramification 1) and WT was less than 15.69 ℃ (at ramification 3), rivers were predicted as groups in the northeastern part of Japan (mainly Group C). At ramification 5, in cases where SDmax was less than 101.03 m3/s/km2, rivers were predicted as those on the Pacific side of Tohoku and Kanto (Group E). In addition, in cases where SDmax was larger than 101.03 $m^3/s/km^2$ at ramification 5, rivers were predicted to be those on the Pacific side of Tohoku and Kanto (Group E). In cases where the SDmax at ramification 5 was larger than 101.03 $m^3/s/km^2$, rivers were predicted as those on the southwestern part of the Japanese archipelago (mainly Groups F, G, and H); however, there were multiple groups of rivers, indicating a relatively high misclassification rate.

4. Discussion

4.1. Macroinvertebrate genera and environmental factors affecting the classification results

As a result of IndVal, groups in the northern part of the Japanese archipelago had highly endemic species, whereas, groups in the western part of the Japanese archipelago had few highly endemic species (Table 2). In Group A, *Hydatophylax* and *Dicosmoecus* were confirmed. These genera inhabit from Hokkaido northwards (Ito et al., 2007), indicating a highly endemic macroinvertebrate fauna. The lowest sea level in the Tsugaru Strait, which separated the Honshu and Hokkaido rivers after it was formed during the glacial stage, was −80 m and the maximum depth was −140 m (Ohshima, 1980); therefore, freshwater macroinvertebrate species could not migrate between the Honshu and Hokkaido rivers, and the unique separation is assumed to form the endemic macroinvertebrate fauna in Hokkaido. In Group E, *Naididae*, *Chironomidae*, and *Aeshnidae* were found. These three genera are strongly related to indicators of watershed scale and anthropogenic impacts and were marked in CCA as having high IndVal values. Species belonging to these genera are often used as indicators of degraded water quality (Kawai et al., 1989; Iyama et al., 1984). In rivers with large watersheds, plains tend to develop and are highly impacted by humans, thus, many genera with a strong resistance to water pollution were found in this group. In Group F, unlike the other groups, Alpheus and *Xenostrobus* which inhabit the estuarine area indicated high IndVal value. Since Group F composed of mainly rivers affected by warm currents on the Pacific Ocean side, the macroinvertebrate fauna of the estuarine area seemed to differ from the other groups. In Groups G and H, high IndVal genera overlapped, and no highly endemic species were confirmed. In addition, these groups were classified in southwestern Japan, and no clear geographical boundary was confirmed (Fig. 1). Since the rivers belonging to these groups were smaller than those in the north, and watershed boundaries were frequently changed by stream capture, it is probable that the genetic exchange of species between the basins is active, since no clear division of macroinvertebrate fauna was confirmed. Classification using only macroinvertebrate fauna is difficult in this area where high amounts of misclassification were confirmed by the decision tree model (Fig.4). The longitudinal distribution of the indicator genera *Arctopsyche*, *Dicosmoecus*, *Niponiella* and *Limnocentropus* in the northern part of the Japanese archipelago inhabit mainly in the mountainous stream. Conversely, the indicative genera in the southwestern group, such as *Stenelmis*, *Gammarus*, or *Stylaria*, inhabit from midstream to downstream. The fact that the longitudinal position of highly indicative genera varies from region to region is important when formulating conservation and management plans for river environments.

As a result of the decision tree model using the classification result of macroinvertebrate fauna by TWINSPAN as an objective variable and environmental factors as explanatory variables, Lat, Log, WT, and SDmax were selected as the important variables for the classification of macroinvertebrate fauna. The northern and southwestern parts of the Japanese archipelago were classified by Lat and WT, indicating that geographical location is an important factor in considering macroinvertebrate classification of the Japanese archipelago. SDmax classified the southwestern part of the Japanese archipelago into areas with relatively high rainfall and other areas suggesting that the climatic zone is also important for macroinvertebrate classification. In the final step, the classification is done by Log; however, some misclassification was included, unlike the northern part of the Japanese archipelago. The ambiguous classification of biotic communities in southwestern Japan has also been confirmed in fish fauna, and it has been pointed out that complex geological history, such as river capture and the connection of paleo-water systems, are factors (Itsukushima, 2019). Geological position, WT, and SDmax, are the environmental factors responsible for macroinvertebrate classification of the Japanese Archipelago, but smaller scale environmental factors seem to be needed to explain the differences in the subdivided biota.

4.2. Watershed scale Macroinvertebrate community and environmental factors

The results of the CCA suggest that, for investigations into the relationship between the macroinvertebrate community and environmental factors, topographic factors, flow regime, anthropogenic factors, geology, and water quality are significantly correlated with the CCA axes (Table 3).

Regarding the topographic factors, WA, HA, and DD were significantly correlated with the CCA axes. A phenomenon in which species increase with the increase of catchment area has been confirmed in various taxa (Horner-Devine et al., 2004; Drakare et al., 2005; Allan and Castillo, 2007). In addition, I found a positive correlation between basin area and habitat for both Groups E and G. Further, I considered that the high elevation of the riverhead generates the various WTs and altitude distribution, which in turn provide a large variety of habitats. Since the macroinvertebrate community structure varies with the longitudinal direction (Grubaugh et al., 1996; Maiolini and Lencioni, 2001; Tomanova et al., 2007), the altitude of the riverhead is important in defining the macroinvertebrate community at the watershed scale. In addition, my results selected DD as a significant factor. The northern part of the Japanese archipelago has low DD (Fig. 3(C)) due to the undeveloped drainage network and low precipitation. This factor seems to be selected as an index showing the characteristics of the river.

Among flow regimes, there was a significant relationship between SDmax and SDo classification results and indicator species in CCA. Previously, many studies related to the flow regime focused mainly on the relationship between macroinvertebrate fauna and the impact–response of catastrophic drought and flood (Fritz and Dodds, 2004; Snyder and Johnson, 2006; Kim et al., 2014; Bae and Park, 2016). In addition, some researchers reported a change in the macroinvertebrate fauna due to the increase in ordinary flow discharge that impacts the inflow of organic material and coarser grain sizes (Cabria et al., 2011; Chen et al., 2013; Pan et al., 2015). In this study, in the CCA, *Alpheus* and *Xenostrobus*, which inhabit the river estuary, were confirmed in the high SDmax area. Further, *Aulodrilus* and *Benthalia* were confirmed in the high SDo area (Fig. 2(b)). No report details the dependence of these species on flow discharge, yet I considered that some environmental changes caused by the large flow discharge brought about such results. To elucidate the relationship between the flow regime and macroinvertebrate community, it is necessary to accumulate studies not only at the large scale but also at a smaller scale.

Based on my CCA results, I selected ND as an important factor of anthropogenic impact, but found no significant relationship between AG or UR and CCA axes. The influence of land use change appears to occur at a smaller scale of environmental change, including habitat degradation and water quality change, both of which alter the macroinvertebrate community (Zhang et al., 2010; Jonsson et al., 2017; Damanik-Ambarita et al., 2018; dos Reis Oliveira et al., 2020). Therefore, it is necessary to introduce smaller scale environmental factors in the prediction of basin scale benthic fauna.

4.3. Comparison of classification result with other taxonomic groups

My results for the macroinvertebrate community classified the 109 main watersheds belonging to the Japanese archipelago into eight groups. The groups in the northern part of the Japanese archipelago tended to be grouped geographically, whereas the those in the western part of the Japanese archipelago did not necessarily result in geographically adjacent rivers being grouped in the same classification (Fig. 1(b)). In this section, I compare of classification results of this study with those of fish fauna of 181 rivers of the Japanese archipelago (Appendix 3). In the classification of fish fauna, although Hokkaido was divided into two groups, the classification results for northern Tohoku, the Pacific side of Tohoku, and the Sea of Japan side of Tohoku and Hokuriku corresponds well with my classification results for the macroinvertebrate community. The classification of the fish fauna of the western part of the Japanese archipelago reflects its biogeography and geohistory well, in contrast with that of the macroinvertebrate fauna. This may be explained by the ability of some macroinvertebrate species to move between watersheds in the western part of the Japanese archipelago, because rivers in the western Japan are smaller than those in the northern regions. Among the macroinvertebrate communities included in this study, *Ephemeroptera*, *Plecoptera*, and *Trichoptera* are able to fly during the adult stage (Nishimura, 1987; Hayashi and Tanida, 2008). Migration between watersheds is therefore relatively easy for these groups, compared with fish species. Furthermore, in the Chugoku region situated in the western part of the Japanese archipelago, there is much evidence of river conflicts (Inami, 1951; Yamanouchi and Shiraishi, 2009; 2010), and some of the geological factors ensure similarity between the macroinvertebrate communities of these watersheds.

The classification result of the macroinvertebrate community in the first step, which distinguish the northern part and western parts, is closely similar to that of the fish fauna. The Japanese archipelago is said to have been formed from the eastern margin of Asia and independently separated eastern Japan from western Japan. Approximately 5 million years ago, these boundary areas (Fossa Magna) were below sea level and contained caves (Otofuji and Matsuda, 1984; Otofuji et al., 1985). The classification results of my analysis suggest the importance of Fossa Magna in the formation history of the Japanese archipelago, as the accepted boundary that divides the north and south of the Japanese archipelago in many taxa. Furthermore, the boundaries of Hokkaido and Tohoku are common to both fish fauna and the macroinvertebrate community. This suggests that the zoogeographical boundary called the “Blakiston line”—located between Honshu and Hokkaido (Blakiston, 1883)—established for mammals and birds, might also be adopted for macroinvertebrate fauna.

The ecological region has been widely used to evaluate ecosystem integrity and to plan conservation (Omernik, 1987; Urbanic, 2008; Omernik and Griffith, 2014). I used the classification of fauna such as phytoplankton (Beaver et al., 2012), diatomaceous forms (Chen et al., 2008), and fish (Krause et al., 2013, Ferreira et al., 2007; Ellender et al., 2017; Mehner et al., 2007) to determine this ecological region. However, considering the results of this study, the macroinvertebrate community—including flying species with high migration ability—may not be suitable when performing ecoregion classification in areas that include watersheds with relatively small catchment areas.

5. Conclusions

The ultimate goal of tour study was to clarify the potential fauna of the watershed unit of the Japanese archipelago, which is a hotspot of biodiversity. In this paper, I classified the macroinvertebrate community of major rivers belonging to the Japanese archipelago to contribute to the elucidation of its biogeography, and investigate the extent to which environmental factors explained the macroinvertebrate community of the watershed. Major conclusions and recommendations of this study include the following.

The results of my classification of the macroinvertebrate community suggested that rivers located in the northern part of the Japanese archipelago were classified geographically, whereas the rivers in the western part did not necessarily result in geographically adjacent rivers being grouped into the same classification. This is because the scale of the rivers in western Japan is finer than that in northern Japan, and macroinvertebrate species can migrate between watersheds due to historical river conflict and the connection of paleo-drainage systems. The results of the CCA indicated that topographic factors, flow regime, anthropogenic factors, geology, and water quality were significantly correlated with the macroinvertebrate classification and distribution. Further, the results of the decision tree model indicated that water temperature and maximum specific discharge were important factors which could explain the classification of the macroinvertebrate community. However, the results also suggested that environmental factors at a smaller scale than that of the watershed are needed to explain further subdivided classification of the macroinvertebrate community.

Future research should, by revealing the relationship with environmental factors, including habitat scale, and by predicting the emergence of individual species, be able to predict the potential biota in the watershed and contribute to the conservation and restoration of stream ecosystems.

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Tables

Table 1 Environmental factors used for statistical analysis

Environmental factors	Explanation	Average	±	Standard deviation	Correlation factors (|r| > 0.4)
Location					
Lat (°)	Latitude of the river mouth	36.23	±	3.32	Log+, DD-, SDmax-, WT-, DO+
Log (°)	Longitude of the river mouth	136.38	±	4.00	Lat+, DD-, SDmax-, WT-, DO+
Topographic factor					
WA (km^2)	Watershed area	2203.05	±	2869.58	SL+, TG-, SDmax-, ND+
SL (km)	Stream length	813.83	±	1045.41	WA+, TG-, ND+
AH (m)	Altitude of the riverhead	1405.11	±	638.97	
TG	Terrain gradient	0.02	±	0.01	WA-, SL-, ND-
FR	Form ratio	0.17	±	0.08	
DD (km^{-1})	Drainage density	0.40	±	0.15	Lat-, Log-, SDmax+, WT+, DO-
Flow regime					
SDmax (m^3/s/km^2)	Maximum specific discharge	180.66	±	129.61	Lat-, Log-, WA-, DD+, CR+, WT+, DO-
SD75 (m^3/s/km^2)	75-day specific discharge	5.14	±	2.54	SDo+, SD275+, SD355+, PH-
SDo (m^3/s/km^2)	Ordinary specific water discharge	3.10	±	1.66	SD75+, SD275+, SD355+, SDmin+,
SD275 (m^3/s/km^2)	275-day specific discharge	2.08	±	1.20	SD75+, SD275+, SD355+, SDmin+,
SD355 (m^3/s/km^2)	355-day specific discharge	1.26	±	0.90	SD75+, SDo+, SD275+, SDmin+,
Sdmin (m^3/s/km^2)	Minimum specific discharge	0.86	±	0.72	SDo+, SD275+, SD355+,
CR	Coefficient of river regime	465.96	±	793.90	SDmax+
Anthropogenic factor					
ND	Number of dams	13.95	±	17.22	WA+, SL+, TG-,
PD (people per km^2)	Population density	384.23	±	1009.02	MO-, UR+ DO-, TN+, TP+
MO (%)	Percentage of mountain area	76.44	±	17.15	PD-, AG-, UR-, COD-, TN-, TP-, CGL-
AG (%)	Percentage of agricultural area	16.16	±	10.64	MO-, COD+, TN+
UR (%)	Percentage of urban area	6.49	±	9.87	PD+, MO-, COD+, DO-, TN+, TP+
Geology					
SR (%)	Percentage of sedimentary rock	65.14	±	25.43	IR-, MR-
IR (%)	Percentage of igneous rock	29.32	±	23.23	SR-
MR (%)	Percentage of metamorphic rock	5.54	±	11.66	SR-
Water quality					
WT (°C)	Average water temperature	15.35	±	3.22	Lat-, Log-, DD+, SDmax+, PH+, DO-
PH	Potential of hydrogen	7.58	±	0.32	SD75-, WT+
BOD (mg/L)	Biochemical oxygen demand	1.15	±	0.65	COD+, TN+, TP+, CGL+
COD (mg/L)	Chemical Oxygen Demand	2.99	±	1.36	MO-, AG+, UR+, BOD+, TN+, TP+, CGL+
SS (mg/L)	Suspended Solids	10.34	±	11.50	
DO (mg/L)	Dissolved Oxygen	9.87	±	1.13	Lat+, Log+, DD-, SDmax-, PD-, UR-, WT-, TN-, TP-
TN (mg/L)	Total nitrogen	1.15	±	0.99	PD+, MO-, AG+, UR+, BOD+, COD+, DO-, TP+, CGL+
TP (mg/L)	Total phosphorus	0.07	±	0.07	PD+, MO-, UR+, BOD+, COD+, DO-, TN+, CGL+
CGL (pieces/mL)	Number of colitis germ legions	11443.31	±	13374.79	MO-, BOD+, COD+, TN+, TP+,

Table 2 IndVal index of macroinvertebrate genus in each group

Family	Genus	A	B	C	D	E	F	G	H
Alpheidae	*Alpheus*	0.0	0.0	0.0	0.0	11.4	31.8	3.0	9.9
Hydropsychidae	*Arctopsyche*	47.8	0.0	3.2	0.0	0.4	0.0	0.0	0.0
Naididae	*Aulodrilus*	0.0	0.0	5.8	0.0	29.4	0.6	0.0	0.0
Chironomidae	*Benthalia*	0.0	0.0	1.1	3.5	27.2	1.8	0.4	0.3
Sphaeromatidae	*Chitonosphaera*	0.0	0.0	0.0	0.0	1.6	28.7	4.6	9.8
Dytiscidae	*Copelatus*	0.2	41.8	4.6	1.7	3.2	3.4	0.0	0.0
Limnephilidae	*Dicosmoecus*	85.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Psephenidae	*Ectopria*	0.0	0.0	12.7	14.8	16.3	13.4	18.3	17.2
Erpobdellidae	*Erpobdella*	0.9	2.1	7.2	19.3	15.2	8.0	9.8	10.7
Phryganeidae	*Eubasilissa*	28.2	0.0	30.4	0.0	7.6	1.8	0.8	0.3
Gammaridae	*Gammarus*	0.1	0.0	6.5	9.4	11.6	4.8	19.1	16.1
Potamidae	*Geothelphusa*	0.1	0.0	9.8	22.0	9.8	9.1	11.2	12.2
Limnephilidae	*Hydatophylax*	82.9	0.0	1.2	0.0	0.0	0.0	0.0	0.0
Dytiscidae	*Laccophilus*	0.2	45.0	8.9	4.1	5.0	0.2	0.0	0.0
Limnocentropodidae	*Limnocentropus*	0.0	0.0	28.4	2.2	13.6	1.1	2.5	0.6
Erirhinidae	*Lissorhoptrus*	0.0	8.0	31.9	0.7	3.5	0.0	0.4	0.0
Perlodidae	*Megarcys*	47.8	0.0	3.2	0.0	0.4	0.0	0.0	0.0
Chironomidae	*Microchironomus*	0.7	35.2	1.7	0.0	10.9	4.5	2.9	2.8
Chironomidae	*Monodiamesa*	0.8	4.3	7.7	3.5	26.9	3.2	0.2	0.5
Atyidae	*Neocaridina*	0.0	0.0	0.6	20.4	16.1	20.4	15.0	17.4
Perlidae	*Niponiella*	0.4	0.0	38.1	0.7	5.6	0.4	0.4	0.3
Dytiscidae	*Oreodytes*	17.6	0.0	29.5	6.8	2.1	0.9	0.0	0.0
Dytiscidae	*Platambus*	5.0	0.0	13.7	18.6	15.4	20.1	9.5	11.4
Aeshnidae	*Polycanthagyna*	0.0	52.3	4.0	0.0	0.2	4.3	0.3	0.8
Ampullariidae	*Pomacea*	0.0	0.0	0.0	9.6	3.0	9.6	19.6	16.8
Hydrobiidae	*Potamopyrgus*	0.0	34.9	18.2	5.6	5.3	1.6	0.7	0.0
Viviparidae	*Sinotaia*	0.0	2.7	3.7	19.9	10.9	10.1	10.1	7.9
Perlodidae	*Skwala*	74.9	0.0	4.3	0.0	0.0	0.0	0.0	0.0
Sphaeromatidae	*Sphaeroma*	0.2	0.0	0.0	2.8	19.1	27.2	5.8	9.0
Elmidae	*Stenelmis*	0.0	0.0	2.6	3.7	23.4	14.4	20.1	18.1
Naididae	*Stylaria*	0.9	5.1	10.2	13.8	27.6	16.8	25.7	25.7
Leptophlebiidae	*Thraulus*	0.0	0.0	2.2	1.8	4.9	27.5	8.2	5.3
Urnatella	*Urnatella*	0.0	0.0	0.3	1.1	28.0	0.6	0.0	0.0
Mytilidae	*Xenostrobus*	0.0	0.0	0.1	0.0	15.9	30.5	7.6	15.3

Table 3 Relationship between environmental factors and CCA axis

	Classification result of TWINSPAN vs environmental factors				Macroinvertebrate genera that appeared in over 10 rivers vs environmental factors			
	CCA1	CCA2	R2		CCA1	CCA2	R2	
WA	-0.460	-0.888	0.395	***	0.0528	4.1679	0.002	**
AH	-0.744	-0.668	0.073	*	0.0299	2.3585	0.008	**
DD	0.986	0.164	0.307	***	0.1338	10.5633	0.001	***
SDmax	0.818	0.575	0.387	***	0.0440	3.4737	0.002	**
SDo					0.0311	2.4522	0.010	**
SDmin	-0.280	-0.960	0.088	**				
ND	-0.163	-0.987	0.398	***	0.0545	4.2982	0.001	***
AG					0.0263	2.0721	0.020	*
UR								
IR	0.626	-0.780	0.071	*	0.0307	2.4251	0.006	**
WT	0.980	0.200	0.796	***	0.1735	13.6898	0.001	***
PH	0.822	0.570	0.228	***				
CGL					0.0286	2.2549	0.019	*

Signif. Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

Figure Captions.

Fig.1 TWINSPAN dendrogram and distribution of the classification result of macroinvertebrate community in the Japanese archipelago

Fig. 2(a) Canonical correspondence analysis (CCA) of the classification of the macroinvertebrate community and environmental factors

Fig. 2(b) Canonical correspondence analysis (CCA) of the macroinvertebrate genera that appeared in over 10 rivers and environmental factors. Abbreviated genus names are the first two or three letters. Al *Alpheus*, Ar *Arctopsyche*, Au *Aulodrilus*, Be *Benthalia*, Ch *Chitonosphaera*, Co *Copelatus*, Di *Dicosmoecus*, Ec *Ectopria*, Er *Erpobdella*, Eu *Eubasilissa*, Ga *Gammarus*, Ge *Geothelphusa*, Hy *Hydatophylax*, La *Laccophilus*, Lim *Limnocentropus*, Lis *Lissorhoptrus*, Me *Megarcys*, Mi *Microchironomus*, Mo *Monodiamesa*, Ne *Neocaridina*, Ni *Niponiella*, Or *Oreodytes*, Pl *Platambus*, Pol *Polycanthagyna*, Pom *Pomacea*, Pot *Potamopyrgus*, Si *Sinotaia*, Sk *Skwala*, Sp *Sphaeroma*, Ste *Stenelmis*, Sty *Stylaria*, Th *Thraulus*, Ur *Urnatella*, Xe *Xenostrobus*.

Fig. 3(a) Water temperature among classification groups of macroinvertebrate community

Fig. 3(b) Altitude of the riverhead among classification groups of macroinvertebrate community

Fig. 3(c) Drainage density among classification groups of macroinvertebrate community

Fig. 3(d) Maximum specific discharge among classification groups of macroinvertebrate community

Fig. 3(e) Ordinary specific water discharge among classification groups of macroinvertebrate community

Fig. 3(f) Number of dams among classification groups of macroinvertebrate community

Fig. 3(g) Percentage of igneous rock among classification groups of macroinvertebrate community

Fig. 3(h) Average water temperature among classification groups of macroinvertebrate community

Fig. 3(i) Potential of hydrogen among classification groups of macroinvertebrate community

Fig. 3(j) Suspended solids among classification groups of macroinvertebrate community

Fig. 4 Result of decision tree model using classification result of macroinvertebrate community by TWINSPAN as the objective variable and environmental factors as the explanatory variables

Appendix 1 Location of the data collection sites.

Appendix 2 Geological base map of the Japanse archipelago. Detailed legend of geology is shown in the website (https://gbank.gsj.jp/seamless/legend.html).

Appendix 3 Classification result of the fish fauna of 181 rivers located in the Japanese archipelago (Modified Itsukshima 2019)