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The Use of Molluscan Fauna as Model Taxon for the Ecological Classification of River Estuaries

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Abstract: River estuaries are important aquatic environments characterized by large environmental gradients in their water quality, riverbed material, and microtopography in the longitudinal and transverse directions. The geography or habitats in river estuaries differ depending on the energy from the tide, waves, and river; therefore, the biota inhabiting river estuaries vary depending on the river estuary type. In view of this, for effective conservation in river estuaries, there is a need for information about potential habitats and biota based on objective data about the river estuary type. The objective of this study thus was to classify river estuaries by their molluscan fauna and physical indicators to reveal the relationship between molluscan fauna and the physical environment. The classification results using physical indicators indicated three types of river estuaries (wave energy-dominated group, tide energy-dominated group, and low tide and wave energy group). This classification result was similar to the classification of molluscan fauna. Therefore, it was suggested that molluscan fauna is extremely useful as a variable representing the river estuary environment. From the comparison between molluscan fauna and the physical environment, some rivers were not classified into the same group in the classification of molluscan fauna, despite them having similar physical environments. Some of these rivers with a molluscan fauna that diverged from expectations had undergone channel modification, which is expected to have caused a shift in this fauna group. These results suggest that this approach could be used to identify rivers that have been degraded by human activities.

Keywords: river estuary; molluscan fauna; habitat; riverbed material; wave; tide; and river energy

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1. Introduction

Estuaries are located at the boundary between land and sea, and are a particular environment influenced by periodic tides and waves [1,2]. Estuaries provide multiple ecological services, such as nutrient cycling, climate change adaptation, and function as habitats and spaces for recreation [3]. Costanza et al. (1997) estimated that the total value of annual ecosystem services of estuaries is \$22,832 $ha^{-1} \cdot yr^{-1}$, which is among the highest of 21 biomes [4]. In contrast, estuaries were reported to be highly degraded by human activities because of their rich biodiversity, high levels of nutrients in the land, and abundant natural resources [5,6]. Approximately four billion people now inhabit land within 60 km of the world's coastlines, and they have placed considerable pressure on estuaries [7]. Anthropogenic impacts induce changes of estuary environments, such as habitat loss, deterioration of water quality, and degradation of resources [7–12].

Knowledge about the relationship between estuarine biota and habitat characteristics is important to establish appropriate conservation plans. A multidisciplinary approach was adopted to evaluate estuarine ecological integrity in the United States using the indicators of water quality, bed material, and habitats [13]. Ecological conditions were evaluated using the Benthic Quality Index, based on the recognition of regional benthic fauna [14]. In Australia, a conservation plan for each estuary has been designed and conservation actions have been progressing. In the Australian system, estuaries are classified by the strength of impact of the energy of their tide, waves, and rivers [15,16]. In this Australian system, estuaries are categorized into six main types (tide-dominated, tide-dominated estuary, tidal flat, wave-dominated delta, wave-dominated estuary, and strandplain), and potential habitat types associated with each estuary type are suggested [17]. Research on the relationships between fauna and estuary type and reference conditions for each estuary type was also conducted [18]. In European countries, a Water Framework Directive (WFD) is used to define a framework for the protection of European waters in order to reach "good status" objectives for water bodies throughout the EU [19]; many studies have also evaluated the environmental condition of estuaries to achieve their integrated management [20]. Phytoplankton or benthic fauna have been used as biological indicators [21,22] and physicochemical indicators have also been used to evaluate the condition of estuaries [23–25]. In addition, Galván (2010) classified Northeast Atlantic estuaries using hydrological and geological data and revealed that transitional waters with a complex morphology showed the highest values of species diversity, while those with a smaller tidal influence showed lower species diversity [26].

A conservation plan for estuaries should be based on knowledge of an estuary's classification results based on biological or physical/environmental factors. Many studies have thus established systems for classifying estuaries; their geography, physical environment, and biota are representative of the indicators that have been used. Pritchard (1952) focused on estuarine geography and established three classification types (coastal plain estuaries, fjords, and bar-built estuaries) [27]. In contrast, Davies (1964) classified estuaries into microtidal, mesotidal, macrotidal, and hypertidal, in accordance with the difference of tidal levels [28]. In addition, Fairbridge (1980) presented a comprehensive classification method using geomorphological history, river discharge of water and sediment, tidal current and waves, and coastal processes [29]. Darlymple et al. (1992) further developed the classification concept of Fairbridge and presented the estuarine habitat type based on river energy, wave energy, and tide energy [15]. On the other hand, fish fauna was used for classifying South African estuaries and the classification results were associated with water temperature or geological formation [30–34]. Colloty et al. (2002) categorized 92 estuaries of Eastern Cape Province using 54 plant species and suggested a relationship between the classification results and topographical factors (permanently open and temporarily open/closed) [35].

The examples of conservation efforts or research tools developed in the US, Europe, Australia, and South Africa mentioned above are mainly intended for estuaries of large rivers or coastal zones. However, few studies or conservation plans have been established for river estuaries of small and medium-sized rivers. In Japan and Southeast Asia, where the land is composed of many peninsulas or islands, there is an extended and complex coastline, geographically variable tides, and rivers of various sizes. Therefore, there are many types of estuaries according to the complex geography or physical condition of these areas, and the relationship between the ecological system and the physical environment has not been revealed. Furthermore, the anthropogenic impact on estuaries is extremely high in Japan because of the high population density in lowland areas which means that the reference condition is difficult to determine. Therefore, to establish conservation strategies for the great diversity of existing estuaries, it is important to comprehend the environmental condition by drawing comparisons with other estuaries. Furthermore, predicting the habitat or biota occurring in the river estuaries from physical factors enables the establishment of specific targets for environmental restoration.

Against this background, the aims of this study were to reveal the relationship between physical factors and the composition of the biotic community. To achieve this objective, we investigate the relationship between physical indicators and molluscan fauna and compare the classification results of physical indicators and molluscan fauna and consider the cause of non-correspondence between physical indicators and molluscan fauna.

We selected molluscan fauna to evaluate the integrity of the river estuaries. Molluscan fauna respond sensitively to water quality or bottom sediment and include species that inhabit only one particular environment or have a low capacity to thrive in different habitats. Molluscan species at individual locations directly reflect the environmental conditions at these sites [36]. Therefore, molluscan species are ideal for evaluating the environmental conditions or determining the impact of human activities [37–39].

2. Materials and Methods

2.1. Study Area

The present study focused on 19 river estuaries in Kyushu, Japan (Figure 1). River estuaries are characterized by large environmental gradients of water quality, riverbed material, and microtopography in the longitudinal and transverse directions [40]. We selected river estuaries for study here by including those with a variety of physical environments.

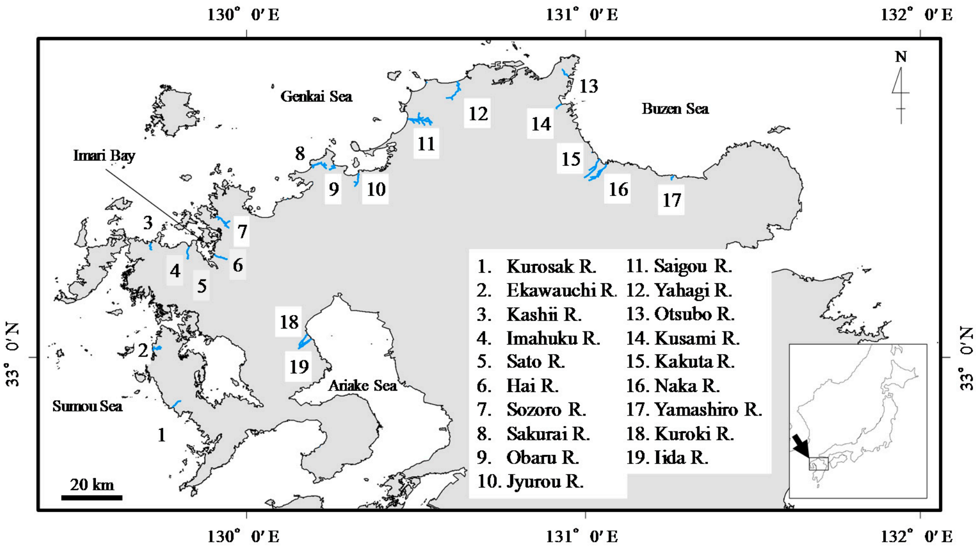

Figure 1. Location of the study sites.

2.2. Molluscan Fauna Data

Molluscan fauna collections were performed at low tide at the middle and spring tides from 28 April 2015 to 22 January 2016. The molluscan species were sampled at a dried out habitat one reach (about ten times of the river width) from the mouth. We defined the land of low flow channel as a habitat and set one to three sampling points at each habitat according to the habitat area. Eight kinds of habitats were set from the viewpoint of particle size of the sediment, vegetation, and artificial structure (silt, sand, gravel, boulder, bedrock, riprap, concrete construct, and vegetation). We collected molluscan (Bivalvia and Gastropoda) fauna in a region of a 50 cm square and 10 cm deep at each onshore habitat in a stream reach.

2.3. Physical Environment Data

We collected riverbed material at each molluscan sampling site. The grain size accumulation curve was obtained by a sieve analysis test, as specified in JIS Z 8815 [41], for the grain size range of 3.0–75 mm. For grain sizes greater than 75 mm, we measured the short diameter (d_s) and long diameter (d_l) of 50 pebbles and calculated the grain diameter as $(d_s \times d_l)^{0.5}$. When the sampled material did not contain particles larger than 3.0 mm, the grain size accumulation curve was calculated by laser diffraction particle size analysis (SALD, 2000). In addition, the morphology of each habitat and the cross section and longitudinal shape of the river were surveyed by Real Time Kinematic-Global Positioning System (RTK-GPS). The habitat planform and cross-sectional profiles of each river were created by the measurements.

2.4. Calculation of Physical Indicators

In this study, we focused on physical indicators at the watershed scale and the habitat scale. The indicators of river energy, tide energy, and wave energy were adopted for the watershed scale, and the indicators of river bed material and habitat type were adopted for the habitat scale. Specific indicators were as follows: indicator of tide energy: (1) tidal range; indicators of wave energy; (2) wave exposure; (3) direct fetch; indicators of river energy; (4) topographic gradient; (5) form ratio; (6) specific discharge of occurrence probability 1/5, and indicators of habitat scale; (7) silt; (8) sand; (9) gravel; (10) boulder; (11) bedrock; (12) riprap; (13) concrete construct; and (14) vegetation.

(1) Tidal range was calculated by the average of the difference of monthly maximum and minimum tide levels for the period from January–December 2014. The tidal data were obtained from the Japanese Coastguard. To represent the tide at the investigated site, the recorded value from the nearest Japanese Coastguard observation point was adopted; (2) Wave exposure was quantified using the Baardseth Index [42]. To calculate this index, the center of a transparent circular disc with a radius of 15 cm (representing 7.5 km) was placed at the investigation site on a 1:50,000 scale chart. The disc was divided into 40 sectors, with the angle of each sector being 9°. Sectors containing peninsulas, islands, or parts of the mainland shore were ignored [43]; (3) We calculated direct fetch by integrating the distance to the opposite shore from the investigation point, extending the radius in each direction with a width of ±22.5° from the center point [44]. A maximum distance of 200 km was set as the distance to the opposite shore, in accordance with the concept of wave height saturation [45]; (4) The topographic gradient was obtained by dividing the altitude of the headstream by the length of the main river; (5) The form ratio was calculated by dividing the basin area by the square of the length of the main river; (6) The specific discharge of occurrence probability 1/5 was calculated by a rational run-off formula using a rainfall intensity formula released by each river's administrator; For (7) silt; (8) sand; (9) gravel; and (10) boulder; based on the grain size accumulation curve of riverbed materials collected at each habitat, we defined silt as an average grain diameter of 0.005–0.075 mm, sand as an average grain diameter of 0.075–2.0 mm, gravel as an average grain diameter of 2.0–75 mm, and boulder as an average grain diameter greater than 75 mm; For (12) riprap and (13) concrete construct, when the function of a habitat was changed between a concrete construct or stone material, we defined large stones acting as coastal defenses as riprap and bed beaching as a concrete construct. We used presence-absence data of the indicators (7–14).

2.5. Statistical Analysis

To identify the possible groupings of similar molluscan fauna between river estuaries, we conducted non-metric multidimensional scaling analysis (nMDS) to summarize the composition of molluscan fauna using the Bray–Curtis similarity. nMDS was conducted for 14 physical indicators and species that appeared in over three rivers, to exclude the influence of species appearing at a low frequency. To approach a normal distribution, the number of individual molluscs was used after logarithmic conversion [log(e + 1)]. As a result of the permutation test, physical indicators ($p < 0.05$) were shown as vectors. Additionally, indicator species of each group were obtained using the indicator value method (IndVal) [46]. Secondly, molluscan fauna and physical indicators were classified by hierarchical cluster analysis (Ward method). Euclidean distance was used for calculating the distance between objectives. To investigate the difference of a physical indicator among groups, the average values of physical indicators of each group were analyzed with the Kruskal–Wallis test and Steel Dwass test. These analyses were conducted using the statistical analysis software R.

3. Results

3.1. Molluscan Fauna Survey

In total, 27 families, 55 species, and 6003 individuals were collected in 19 river estuaries (Table 1). The species for which the highest number of individuals were collected was *Batillaria multiformis*, for which a total of 1105 individuals were confirmed in 12 rivers. At the other end of the spectrum, species belonging to Bivalvia, such as *Nuttallia commode*, *Moerella iridescens*, and *Laternula boschasina*, were confirmed in a few rivers. Regarding the numbers of species and individuals in each river, the Hai River was associated with the highest numbers of species and individuals (17 species and 1249 individuals). In contrast, no molluscan species were found in the Obaru River.

Table 1. Molluscan fauna of 19 river estuaries.

Species	River No																		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Chitonidae																			
Ischnochiton comptus	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nacellidae																			
Cellana nigrolineata	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0
Lottiidae																			
Nipponacmea gloriosa	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nipponacmea radula	5	5	0	12	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Nipponacmea nigrans	0	0	8	9	1	43	10	0	0	0	0	0	0	0	0	0	0	0	0
Patelloida pygmaea	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Patelloida pygmaea form conulus	0	0	2	1	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0
Pattelloida pygmaea form heroldi	0	2	4	0	0	8	4	0	0	0	0	0	0	0	0	0	0	0	0
Trochidae																			
Chlorostoma xanthostigma	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monodonta labio form confusa	4	19	1	11	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0
Omphalius rusticus	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Turbinidae																			
Turbo coronoatus coronatus	0	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Turbo cornatus coreensis	0	5	1	3	0	2	13	0	0	19	0	0	0	0	0	0	0	0	0
Neritidae																			
Nerita japonica	42	79	19	0	0	23	40	0	0	0	0	0	0	0	0	0	0	0	0
Clithon retropicta	0	0	14	9	6	0	4	44	0	0	5	0	0	0	8	0	0	0	0
Neripteron cornucopia	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	12	0	0
Phenacolepadidae																			
Phenacolepas unguiformis	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Cinnalepeta pulchella	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cerithiidae																			
Ceritium coralium	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Batillariidae																			
Batillaria multiformis	0	28	138	111	2	216	76	101	0	16	0	0	0	205	1	208	0	0	3
Batillaria attramentaria	0	3	10	89	0	73	0	92	0	52	0	0	0	1	0	0	0	0	0
Potamididae																			
Cerithidea djadjariensis	0	0	0	0	0	396	136	0	0	0	0	0	0	0	0	0	0	0	0
Cerithidea largillierti	0	0	0	0	0	0	3	0	0	0	0	0	8	0	0	0	33	0	0
Cerithidea ornata	0	0	1	0	0	15	11	0	0	0	0	0	0	2	1	0	13	0	0
Cerithidea rhizophorarum	0	0	18	0	1	16	53	0	0	0	0	0	54	46	3	0	99	0	0
Cerithidea cingulata	0	0	0	0	0	319	85	0	0	0	0	0	0	4	0	0	675	0	0
Littorinidae																			
Cerithidea rhizophorarum	11	0	0	0	0	4	2	13	0	0	7	0	0	29	23	21	1	43	13
Littoraria intermedia	0	0	0	0	0	0	0	0	0	0	0	0	1	14	87	104	1	0	6
Littoraria articulata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0	73	0
Assimineidae																			
Assiminea sp.	0	0	38	0	0	0	0	0	0	0	0	0	18	274	2	3	280	0	442
Muricidae																			
Thais clavigera	2	0	0	4	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0
Reishia bronni	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nassariidae																			
Reticunassa festiva	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nassarius multigranosa	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0
Buccinidae																			
Japeuthria ferrea	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ellobiidae																			
Laemodonta exaratoides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

Table 1. *Cont.*

Species	River No																		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Arcidae																			
Barbatia virescens	0	2	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Mytilidae																			
Modiolus nipponicus	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	0	0	0
Hormomya mutabilis	2	15	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Mytilus galloprovincialis	4	0	6	0	5	90	7	2	0	0	0	11	0	1	1	5	0	32	10
Mactridae																			
Raetellops pulchellus	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Mesodesmatidae																			
Coecella chinensis	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tellinidae																			
Nitidotellina hokkaidoensis	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Moerella iridescens	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Psammobiidae																			
Nuttallia commoda	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Psammotaea virescens	0	0	0	0	0	0	0	0	0	0	8	10	0	0	0	0	0	0	0
Psammotaea minor	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
Trapezidae																			
Trapezium oblongum	0	0	0	0	1	2	11	0	0	0	0	0	0	0	0	0	0	9	0
Corbiculidae																			
Corbicula japonica	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Glauconomidae																			
Glauconome chinensis	0	0	0	0	0	0	0	0	0	0	0	0	7	50	0	0	5	0	1
Veneridae																			
Ruditapes philippinarum	0	0	0	2	0	0	0	1	0	83	1	0	0	0	0	0	0	0	0
Cyclina sinensis	0	0	1	0	0	25	0	0	0	1	0	0	0	0	0	0	0	0	0
Meretrix lusoria	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Laternulidae																			
Laternula boschasina	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Pomacea canaliculate	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0
number of species	8	14	16	15	7	17	17	12	0	10	5	3	7	13	8	7	10	4	6
number of individuals	72	168	268	264	34	1249	459	264	0	185	54	22	89	630	126	362	1125	157	475

3.2. Relationship between Molluscan Fauna and Physical Indicators

Figure 2 indicates the plotted molluscan species and physical indicators on nMDS dimensions. As a result of the permutation test, tidal range, direct fetch, silt, sand, boulder, concrete construct, and vegetation were selected. *Littoraria intermedia*, *Cerithidea rhizophorarum*, *Assiminea* sp., or *Cerithidea cingulate* were plotted in the first quadrant, characterized by a large tidal range and abundant silt and vegetation habitat. The second quadrant represented habitats with large direct fetch and an abundant concrete construct. *Mytilus galloprovincialis* and *Littorina brevicula* were plotted in this quadrant. *Ruditapes philippinarum*, *Monodonta labio form confusa*, or *Nipponacmea radula* belonged to the third quadrant, characterized by habitats with large direct fetch and sand habitat. *Pattelloida pygmaea form heroldi*, *Nipponacmea nigrans*, and *Turbo cornatus coreensis* were plotted in the fourth quadrant, characterized by abundant sand and boulder habitat.

In the nMDS dimensions, the *Cerithidea* genus, which inhabits muddy bottoms or reed fields in tidal wetlands, was plotted in the first quadrant. The first quadrant in this analysis represented a high tide and vegetation-rich environment. Additionally, *Mytilus galloprovincialis*, which inhabits wave-dominated rock reefs or concrete constructs via byssus attachment, was plotted into the second quadrant. The second quadrant in this analysis represented high direct fetch and a gravel-abundant environment. Therefore, the nMDS results appropriately reflect the habitat environment of these species.

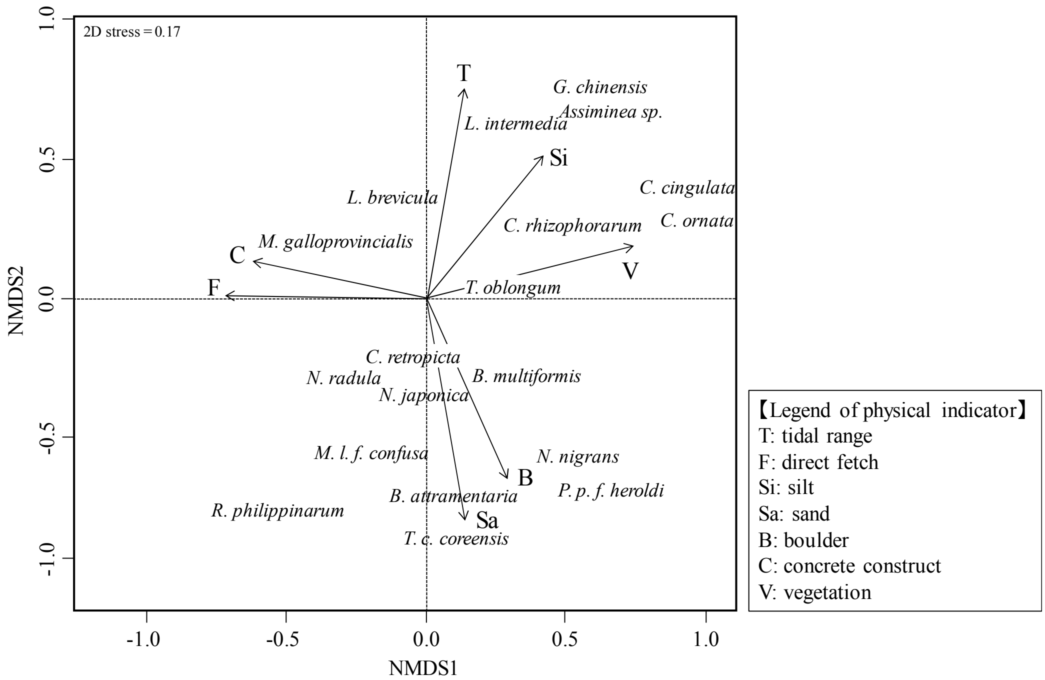

Figure 2. Plotted molluscan species and physical indicators on non-metric multidimensional scaling analysis (nMDS) dimensions.

3.3. River Estuary Classification Using Molluscan Fauna

From the results of the nMDS analysis, the molluscan fauna of the 19 rivers were divided into four groups (Figure 3). Group A comprised fauna from three rivers flowing into the Genkai Sea and the Kurosaki River flowing into the Sumou Sea. Group B comprised five rivers flowing into the Genkai Sea and Imari Bay. Group C comprised four rivers flowing into the Imari Sea and the Kusami River. Group D comprised six rivers flowing into the Imari Sea and the Buzen Sea. The Obaru River was not classified into any groups because no molluscan species were found.

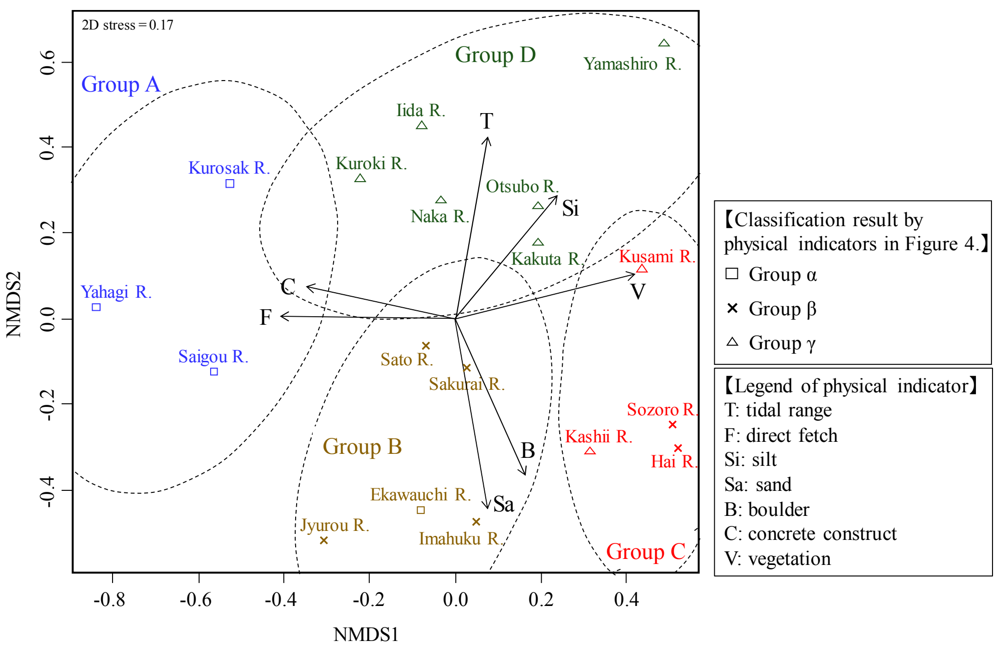

Figure 3. nMDS for similarity between the rivers based on molluscan fauna.

Table 2 shows the IndVal index of molluscan fauna in the four groups.

In group A, the IndVal score of all species was lower than 20. The IndVal indices of *Littorina brevicula* and *Mytilus galloprovincialis* were relatively higher than those of the other species. The IndVal indices of *Batillaria multiformis* and *Batillaria attramentaria* exceeded 40 in group B. In group C, the IndVal indices of eight species exceeded 40, and especially, the value of *Cerithidea ornata* was the highest. The IndVal indices of *Littoraria intermedia* and *Assiminea* sp. exceeded 50 in group D.

Table 2. IndVal index of molluscan species in each group.

Species	Indval			
	A	B	C	D
Nipponacmea radula	11	16	6	0
Nipponacmea nigrans	0	14	49	0
Pattelloida pygmaea form heroldi	0	4	59	0
Monodonta labio form confusa	8	25	17	0
Turbo cornatus coreensis	0	27	42	0
Nerita japonica	9	3	44	0
Clithon retropicta	7	23	16	2
Batillaria multiformis	0	40	40	10
Batillaria attramentaria	0	41	36	0
Cerithidea ornata	0	0	75	8
Cerithidea rhizophorarum	0	2	59	15
Cerithidea cingulata	0	0	61	3
Littorina brevicula	18	2	23	28
Littoraria intermedia	0	0	6	64
Assiminea sp.	0	0	19	52
Mytilus galloprovincialis	16	6	37	16
Trapezium oblongum	0	5	29	3
Glauconome chinensis	0	0	8	33
Ruditapes philippinarum	12	39	0	0

3.4. River Estuary Classification Using Physical Indicators

Figure 4 shows the results of the cluster analysis using physical indicators. In this case, the 19 rivers were divided into three groups (groups α–γ). Group α was composed of the rivers flowing into the Ariake Sea and the Genkai Sea. All of the rivers flowing into the Imari Sea belonged to group β. Group γ was mainly composed of the rivers flowing into the Buzen Sea and the Ariake Sea.

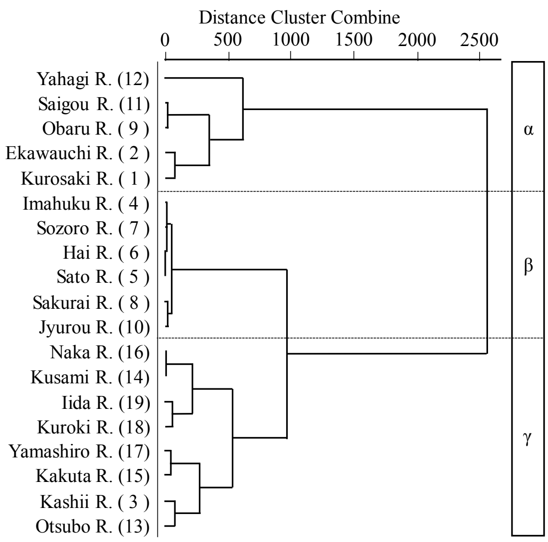

Figure 4. Cluster analysis dendrogram for the environmental variables.

The differences of six quantitative physical indicators (tidal range, wave exposure, direct fetch, topographic gradient, form ratio, specific discharge) among the three groups are indicated in a boxplot (Figure 5). Group γ exhibited the highest tidal range (388.4 cm), followed by group α (238.5 cm) and group β (220.0 cm). The results of the Kruskal-Wallis test indicated a significant difference in this regard among the three groups ($p < 0.01$). The Steel-Dwass multiple comparison test also showed a significant difference between group γ and the other two groups ($p < 0.05$). Direct fetch varied in the order of group α (583.0), group γ (110.7), and group β (11.7). The Kruskal-Wallis test showed a significant difference among the three groups ($p < 0.001$), as did the Steel-Dwass multiple comparison test. Wave exposure was the highest (9.4) in group α. The results of the Kruskal-Wallis test also indicated a significant difference among the three groups for this variable ($p < 0.001$), and the Steel-Dwass multiple comparison test indicated a significant difference between group α and the other two groups ($p < 0.05$). In contrast, topographic gradient, form ratio, and specific discharge were not confirmed to differ significantly among the three groups in one-way analysis of variance. The average values of eight qualitative physical indicators in each group are shown in Table 3. The value of sand was higher than those of the other bed materials in group α. The rivers belonging to group α were dominated by sand-based habitats. In addition, vegetation was not observed in group α. In group β, the values of silt, sand, gravel, boulder, and riprap were high. The rivers belonging to group β were characterized by various types of riverbed material. The rivers classified into group γ were characterized by various riverbeds and vegetation habitats, except for the Yamashiro River.

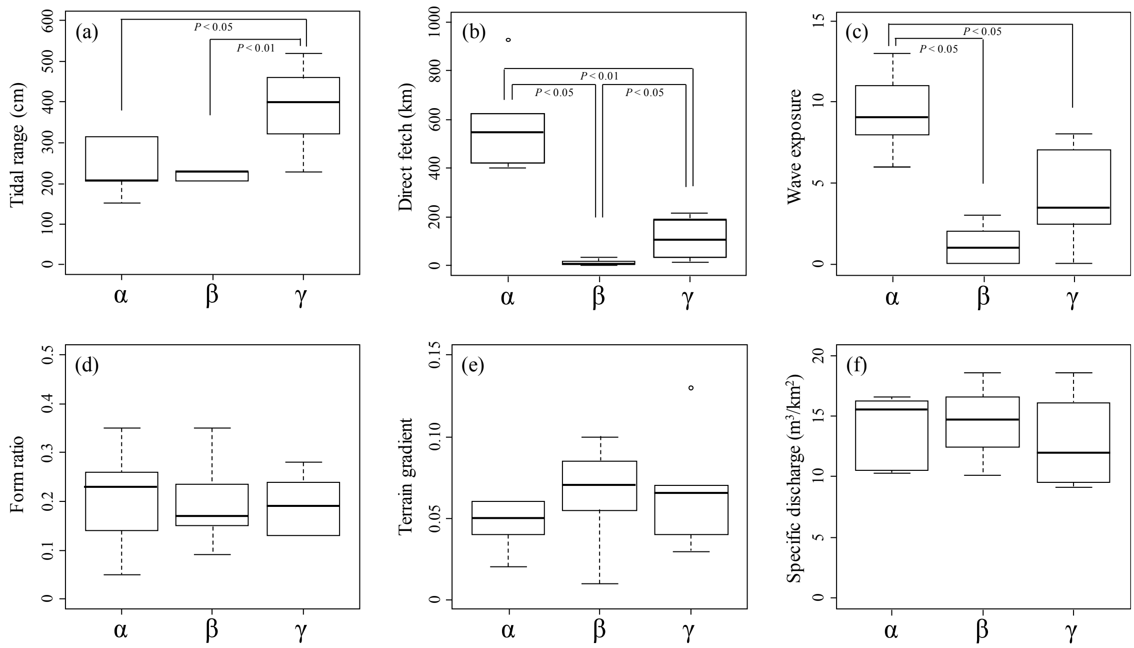

Figure 5. Comparison of tidal range (**a**); direct fetch (**b**); wave exposure (**c**); form ratio (**d**); terrain gradient (**e**) and specific discharge (**f**) among the three groups.

Table 3. Average value of qualitative physical indicators.

Group	River	Silt	Sand	Gravel	Boulder	Bed Rock	Riprap	Concrete Construct	Vegetation	Number of Habitats
α	Kurosak.R	0	0	1	0	1	0	1	0	3
	Ekawachi.R	0	1	1	1	0	0	0	0	3
	Ohara.R	0	1	0	0	0	0	0	0	1
	Saigou.R	0	1	0	1	0	0	1	0	3
	Yahagi.R	0	1	0	0	0	0	1	0	2
	Average	0	0.8	0.4	0.4	0.2	0	0.6	0	2.4

Table 3. *Cont.*

Group	River	Silt	Sand	Gravel	Boulder	Bed Rock	Riprap	Concrete Construct	Vegetation	Number of Habitats
β	Imahuku.R	0	1	1	1	0	1	0	0	4
	Sato.R	1	1	0	0	0	0	0	0	2
	Hai.R	1	1	1	1	1	1	0	1	6
	Sozoro.R	1	1	1	1	0	1	0	1	6
	Sakurai.R	1	1	1	1	0	1	0	0	5
	Jyurou.R	0	1	0	1	0	0	0	0	2
	Average	0.7	1	0.7	0.8	0.2	0.7	0	0.3	4.2
γ	Kashii.R	0	1	1	1	0	0	0	1	4
	Otsubo.R	1	1	1	1	0	0	0	1	5
	Kusami.R	0	1	0	1	0	1	0	1	4
	Kakuta.R	1	1	1	0	0	0	0	1	4
	Naka.R	1	0	1	1	0	1	0	0	4
	Yamashiro.R	1	0	0	0	0	0	0	1	2
	Kuroki.R	1	0	1	1	0	1	0	0	4
	Iida.R	1	0	1	0	0	0	1	0	3
	Average	0.75	0.5	0.75	0.63	0	0.38	0.13	0.63	3.75

4. Discussion

4.1. Physical Indicators Affecting Molluscan Fauna

As a result of the nMDS, seven physical indicators were selected as factors having a strong relationship with molluscan fauna. In Australia, estuarine morphology has been reported to be strongly influenced by the strength of impacts of energy from the tide, waves, and rivers [15,16]. Also in this study, the tidal range reflecting tide energy and direct fetch reflecting wave energy were selected as important variables to the inhabiting molluscan species. The reason why the indicator representing the energy of the river was not selected is that the river estuaries included in this study belong to the same climate zone and the difference in specific flow rate is not significant (Figure 5). The habitat scale indicators of silt, sand, boulder, concrete construct, and vegetation were also selected, in addition to watershed scale indicators. These findings indicate that the planning of estuary conservation schemes, including for molluscan fauna, requires information on the habitat at the micro-habitat scale, in addition to the watershed scale.

Regions of vegetation in estuaries, such as reed fields, have important functions as habitats for estuarine organisms [47–49]. However, reed fields have been modified to paddy fields and fishery ponds in recent years [50,51], which has led to discussions about the need for conservation in this context. On the other hand, the invasion of common reeds (*Phragmites australis*) has reduced ecosystem quality due to a change in the hydroperiod of salt marshes and its planform [52–54]. However, the results of this study suggest that the vegetation habitat is an important environmental element of river estuaries for rarer species, such as *Cerithidea rhizophorarum* and *Assiminea* sp.

The findings obtained here indicate that many molluscan species are closely related to physical indicators at the watershed scale and the habitat scale; therefore, molluscan species at individual locations directly reflect the environmental conditions of a particular site.

4.2. Comparison of the Classification Results of Molluscan Fauna and Physical Indicators

In this section, we discuss a comparison of the classification results of molluscan fauna and physical indicators. As a result of the classification using physical indicators, 19 rivers were classified into three groups.

Group α comprised all rivers belonging to group A (the Yahagi River, the Saigou River, and the Kurosaki River) in the classification of molluscan fauna and the Obaru River and the Ekawauchi River. Common species that inhabit all rivers were not confirmed in group α. In contrast, species closely related to direct fetch in nMDS, such as *Nipponacmea radula* and *Monodonta labio form confuse*, were confirmed in multiple rivers belonging to group α. The characteristic of the physical environment of rivers in group α was high wave energy (Figure 5); therefore, the results of a classification using physical indicators may reflect the characteristics of molluscan fauna.

The physical characteristic of group β was low wave energy (Figure 5). Compared with the classification results of molluscan fauna, this group comprises five rivers, each belonging to groups B (the Imahuku River, the Sato River, the Sakurai River, and the Jyurou River) and C (the Hai River and the Saozoro River). The species belonging to the *Cerithidea* genus were frequently confirmed in these rivers. These species were marked at the low direct fetch and high tide range areas in nMDS.

The tidal range of group γ was significantly larger than those of the other two groups (Figure 5), and rivers dominated by tide energy belonged to this group. Compared with the classification results of molluscan fauna, six rivers (the Naka River, the Iida river, the Yamashiro River, the Kakuta River, the Kuroki River, and the Otsubo River) belonging to this group were classified into group D. *Littoraria intermedia* and *Assiminea* sp. were frequently confirmed in these rivers. These species were marked at the high tide area in nMDS. Therefore, the classification results of physical indicators fit with the molluscan fauna.

Martins et al. (2014) investigated benthic molluscan communities on the Portuguese continental shelf; they evaluated the utility of using molluscan fauna as an environmental indicator because of the strong relationship between molluscan fauna and sediment particle size [55]. Moreover, they pointed out the application of other fauna such as polychaete assemblages in areas where the molluscan fauna is poor [56,57]. In this study, many rivers belonging to group C showed poor molluscan fauna; therefore, it is necessary to consider the introduction of other taxa to evaluate environmental integrity.

4.3. Physical Environment Degradation by Human Activities and Molluscan Fauna

As mentioned above, the classification results of molluscan fauna and those of physical indicators were generally in agreement. However, the results of the classification of molluscan fauna and physical indicators did differ in multiple rivers. This was thought to have been due to the molluscan fauna being modified by human activities, such as river channel improvement. In this section, we discuss the relationship between the impact of channel improvement and molluscan fauna by comparing the Sato River and the Hai River. These two rivers flow into Imari Bay (Figure 1) and have very similar physical indicators (Figure 4). Therefore, their molluscan fauna would also be expected to be similar. However, the classification results using molluscan fauna indicated a difference between the two rivers (Figure 3). In total, 17 molluscan species and 1249 individuals were confirmed in the Hai River, whereas 7 species and 34 individuals were confirmed in the Sato River. We consider that this difference can be explained by habitat characteristics.

Habitat distributions of these two rivers are indicated in Figure 6. Six types of habitat (silt, sand, gravel, bedrock, vegetation, and riprap) were confirmed in the Hai River. However, only two types (silt, gravel) were confirmed in the Sato River. Moreover, the proportion of total habitat area to river channel area was 59% in the Sato River, whereas it was 10% in the Hai River. Regarding the difference of molluscan fauna, *Batillaria attramentaria*, *Cerithidea djadjariensis*, and *Cerithidea cingulata*, which were abundant in the Hai River, were not confirmed in the Sato River. Since these species mainly inhabit muddy bottoms or reed fields in tidal flats, the difference of habitat diversity and habitat size may influence the molluscan fauna in the two rivers.

The cross-sectional profiles of the Hai River and the Sato River are shown in Figure 7. The riverbed of the Sato River has been flattened by channel improvement, and the area of dried habitat that appears at low tide is small. In contrast, the Hai River exhibits substantial variation in the elevation of its channel. The existence of various elevations along a river channel is thought to have a major influence on the habitat of shellfish fauna because it causes variations in the concentration of salt in the river and in the type of material on the riverbed.

As mentioned above, the molluscan fauna was expected to be similar between these rivers because of their closely analogous physical environments. However, river channel improvement had affected the molluscan fauna of the Sato River. These results of cluster analysis of molluscan fauna and physical indicators suggested that this approach could be used to identify rivers that have been degraded by human activities.

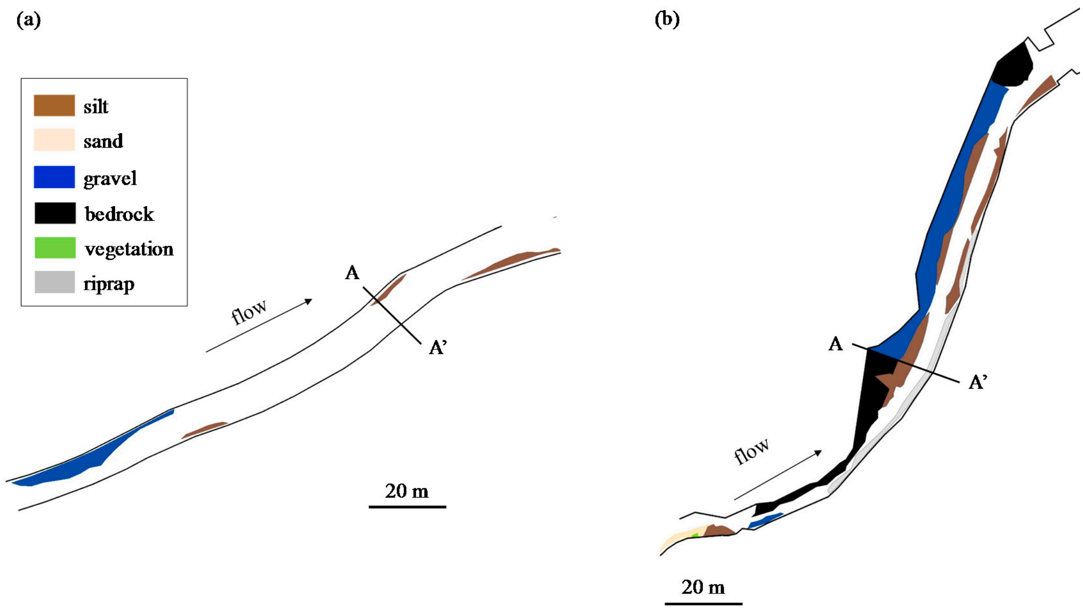

Figure 6. Habitat distribution of the Sato River (**a**) and the Hai River (**b**).

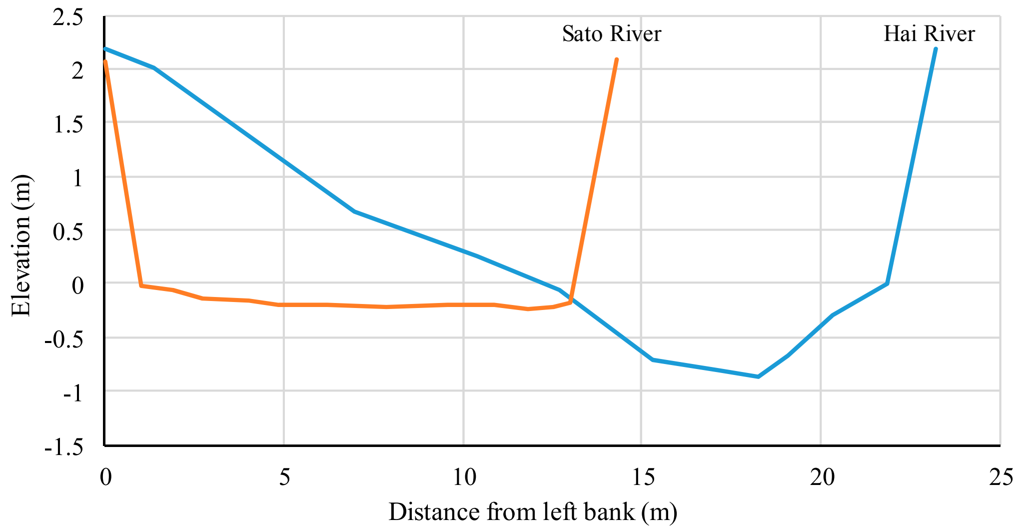

Figure 7. Comparison of the cross-sectional profile of the Sato River and the Hai River.

5. Conclusions

The objective of this study was to classify river estuaries by their molluscan fauna and physical indicators, in order to reveal the relationship between molluscan fauna and physical environments. The major conclusions and recommendations of this study include the following:

1. As a result of nMDS, seven physical indicators (tidal range, direct fetch, specific discharge, silt, gravel, concrete construct, and vegetation) were selected with a strong relationship with molluscan fauna. At the watershed scale, the energy levels of the tide and waves were found to influence the molluscan fauna of a river estuary, while at the habitat scale, the factors of silt, gravel, concrete construct, and vegetation exerted this same influence.

2. The classification results using physical indicators indicated three types of river estuaries (wave energy-dominated group, tide energy-dominated group, and low tide and wave energy group). This classification result was similar to the classification of molluscan fauna. Therefore, it was suggested that molluscan fauna is extremely useful as a feature representing the river estuary environment.
3. From the comparison between molluscan fauna and the physical environment, some rivers were not classified into the same group as in the classification of molluscan fauna, despite them having similar physical environments. Some of these rivers with molluscan fauna that diverged from expectations had undergone channel modification, which is expected to have caused a shift in the fauna group. Comparing the classification results of the biota and the physical indicators suggested that it was possible to extract rivers with degraded biota by artificial influence.

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

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