Original Article

Long-term Assessment of the Environmental Conditions of Lake Nakaumi (Japan) Using Multivariate AZTI Marine Biotic Index (M-AMBI)

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ABSTRACT

This study aimed to identify and understand the temporal changes in the benthic ecosystem of Lake Nakaumi, which is an enclosed brackish water body, using the multivariate AZTI marine biotic index (M-AMBI) as biotic indicators and to evaluate the benthos response to the reclamation project for the lake. The monitoring data collected during 1994–2012 were used. Paraprionospio patiens, classified as second-order opportunistic species, appeared with high frequency in all the survey sites throughout the investigation period and was mostly dominant. Therefore, the variation of AMBI was small during the survey period. However, the species richness and Shannon diversity significantly increased from 1994 at the center of the lake. The benthic community of Lake Nakaumi was characterized by a coexistence of marine and brackish benthos and controlled by the Cl− concentration through the change in their proportions.

Keywords: macrobenthic community, brackish lake, organic enrichment, biodiversity

INTRODUCTION

Sensitive environmental characteristics of water bodies and sediments have been significantly impacted by human activities. In addition, benthic communities are also influenced by both natural and artificial disturbances and, thus, their constituent organisms can be used to evaluate the environmental conditions [1–4]. Certain regulations, such as the Clean Water Act in the United States and the Water Framework Directive and the Marine Strategy Framework Directive in the European Union, include the monitoring of such communities in estuaries and coastal regions; the European ones have also required the development of new biological indices to evaluate the ecological quality of European water environments [5]. For these reasons, Borja et al. have developed the AZTI marine biotic index (AMBI) [6], which is based on an already existing bio-indexing system and focused on soft-bottom benthic organisms [7]. In the AMBI analysis, the macrobenthos taxa are categorized into five ecological groups (EG) based on the sensitivity to organic enrichment: EG I, consisting of species very sensitive to organic enrichment and present in normal conditions; EG II, consisting of species indifferent to organic enrichment and always present in low densities with no significant temporal variation; EG III, consisting of species tolerant to excess organic matter enrichment and that may occur in normal conditions, although their populations are stimulated by it; EG IV, consisting of second-order opportunistic and small species that have a short life cycle, adapted for reduced sediments where they can proliferate, and are subsurface deposit feeders essentially related to the Cirratulidae; EG V, consisting of first-order opportunistic species that are surface
deposit feeders able to proliferate in reduced sediments [8].

The AMBI software has allowed the EG classification of approximately 2,000 of the most important representative benthic organisms inhabiting estuaries and coastal areas and more than 6,300 benthic species have been registered in 2012 [9,10]. The AMBI could be useful for the analysis of temporal and spatial impacts by the change of a benthic environment. Although AMBI provides information about the relative abundance of sensitive species facing increasing levels of organic enrichment in the sediments where they live, it can sometimes be misleading due to the presence of stress-tolerant species that can lead to a natural increase in the opportunistic ones and, subsequently, in the AMBI values [11]. To minimize these problems, the introduction of a multi-index approach has been reported in a previous research; the recently developed multivariate AMBI (M-AMBI) [12], which is calculated via a principal component analysis (PCA) involving the AMBI, species richness, and Shannon diversity indices, could be a proper solution. AMBI and M-AMBI have been applied not only in Europe [9,13–15] but also in South America [16], North America [17], and for few cases in Asia [18,19] although, this type of research has been limited to the estuaries and coastal regions.

Lake Nakaumi, our study area, is connected to Lake Shinji and the Sea of Japan and is the second largest brackish lake in Japan (Fig. 1). Since the water in this lake is stratified due to a halocline and thermocline development, the dissolved oxygen (DO) below the pycnocline is consumed via the decomposition of organic matters and its concentration in the bottom layer decreases to hypoxic levels in many areas during summer [20]. In 1963, a large-scale environmental modification plan, called the Lake Nakaumi Reclamation Project,
to obtain farmland and paddy fields and convert the brackish water into freshwater for their irrigation has been approved (Table S1) [21]. A water gate, called Nakaura Watergate, has been constructed in 1974 at the connection site between Lake Nakaumi and the Sea of Japan to prevent seawater intrusion and the Honjo area, located in the northwest part of Lake Nakaumi, has been separated from the main body of the lake by the Omisaki Dike and the Moriyama Dike, respectively built in 1978 and 1981 (Fig. 1). Although the Nakaura Watergate has not been used, the water quality of the lake has declined after the construction of the two dikes and, therefore, in 2000, the Japanese Ministry of Agriculture, Forestry and Fisheries decided to return it to its previous healthy ecosystem. The removal of the Nakaura Watergate started in 2005 and was completed in 2009; in addition, Western Drainage Channel Dike was also removed by 2010. The various reclamation project activities have had a large impact on the environment of Lake Nakaumi [22]. Despite these artificial modification were implemented, few studies have revealed long-term changes in the benthic environment during these period in Lake Nakaumi [23,24]. In addition, it has been reported that changes in water quality have occurred in the period 1975 to 1984 in Lake Nakaumi [25], but analysis including recent data has not been conducted.

In Lake Nakaumi, quantitative observation of benthos has been continuously performed since 1994, but no research has been conducted on the analysis of long-term environmental changes using these data. In this manuscript, by applying M-AMBI to observation results, we have shown for the first time and clearly that the environment surrounding benthos in Lake Nakaumi is gradually improved.

MATERIALS AND METHODS

Study area

Lake Nakaumi is connected to Lake Shinji via Ohashi River, which is about 7 km in length, and the Sea of Japan via the Sakai Channel, which is 200–600 m in width (Fig. 1); due to its area of 86.2 km² and mean water depth of 5.4 m. Its major affluents are the Hii and Inashi Rivers and its salinity is approximately half that of seawater, resulting in a unique ecosystem that includes both freshwater and marine species. The long water residence time (0.4 year) is due to a small amount of water discharge. The DO concentration in the bottom water layers often decreases below 3 mg/L (40% of the saturation level) during summer and many benthic animals die during summer and autumn [26,27].

The survey sites were set up at four locations: Nagami (Na), Kamiubeo (Ka), Center of Nakaumi (CN), and Yonago Bay (YB) (Fig. 1). YB is an enclosed area characterized by a low flow rate because of the discharge from small rivers. The Ka and Na sites were located in the water area surrounded by the Omisaki and Moriyama Dikes. The (minimum, mean, maximum) water depths at the Na, Ka, CN, and YB sites were (5.6, 6.1, 6.5 m), (6.0, 6.5, 7.1 m), (6.3, 6.7, 7.1 m), (4.0, 4.4, 4.7 m), respectively.

Sample collection and analysis

In this study, the monitoring data of the Japanese Ministry of Land, Infrastructure and Transport (MLIT) were used. The survey data about water quality and macrobenthos were obtained from the hydrological and water quality database [28] regarding the 2006–2016, 2006–2016, 1972–2016, and 2004–2016 periods for the Na, Ka, CN, and YB sites, respectively, within Ohashigawa River Improvement Project and Environmental Monitoring Program at Na collected in February [29], April, June, August, October, November, and December during 2006–2012, Ka collected in February, April, May, June, August, October, November, and December during 2006–2012, CN during 1994–2012, and YB collected in February, May, June, August, and November during 2004–2012. The sampling depths for the upper and lower layers of each site were 0.1 m below the water surface and 1.0 m above the lake bottom, respectively.

The benthic survey was based on the census of river environment flora and fauna [30]. The sampling was conducted four times at each site using an Ekman–Birge grab sampler (20 cm × 20 cm, Rigo, Tokyo, Japan); the residue was shaken with a D-frame net (mesh size 0.493 mm, NGG 38) and added to formalin, with a final concentration of 5–10%. Then, the formalin-fixed samples were sorted and stored in 60–70% ethanol for identification. The macrobenthos density was expressed as the number of individuals per 0.16 m².

Continuous DO measurements were conducted using an oxygen membrane electrode (Tsurumi Seiki KW-2, Kanagawa, Japan). The various parameters were determined according to Japanese Industrial Standard: the chemical oxygen demand was obtained with the potassium permanganate (CODMn) method; the turbidity was measured via the integrating-sphere photoelectric photometry. Total nitrogen (TN) was determined by the potassium peroxodisulfate decomposition method, combined with the cadmium–copper reduction method during 2001–2011, and the ultraviolet absorption spectrophotometry in 2012. Total phosphorus (TP) was determined by the potassium peroxodisulfate decomposition method, combined with the molybdenum blue spectroscopy.
Data analysis

A PCA, a one-way analysis of variance (ANOVA) and Fisher’s least significant difference (LSD) test were performed using the BellCurve for Excel (version 3.2) (BellCurve, Tokyo, Japan) software. A PCA was performed using normalized data to identify any relationship among the environmental variables observed. ANOVA and LSD test were performed to evaluate the differences in the species richness, Shannon diversity, and M-AMBI indices among the four survey sites. For the analysis of the benthic organisms, both AMBI [6] and M-AMBI [12,31] were used. The M-AMBI is a statistical multivariate tool using factor analysis and incorporating species richness, Shannon diversity, and AMBI values [12]. At ‘high’ status of the benthic habitat quality, the reference condition may be regarded as an optimum where the M-AMBI approaches 1. At ‘bad’ status, the M-AMBI approaches 0. The threshold values for the M-AMBI classification are based upon the European intercalibration (Table 1) [32].

For some species not included in the software, the taxonomic level was reduced to the genus or family one if their higher levels were assigned in the list or they were assigned to the EG consisting of similar species [33,34]; when none of these two methods could be applied, the species was ignored. These species represented mean abundance of 5%, for the total species of samples. The following formula (1) was used for the AMBI calculation.

\[
AMBI = \frac{[(0 \times \%EGI) + (1.5 \times \%EGII) + (3 \times \%EGIII) + (4.5 \times \%EGIV) + (6 \times \%EGV)]}{100}
\]  

The AMBI was determined based on the percentage of abundance of each EG in each sample. Several thresholds have been established over the scale of the AMBI, based upon proportions amongst the various ecological groups (Table 1) [19,32].

RESULTS AND DISCUSSION

Differences among the survey sites

Figure 2 shows the mean values of species richness,
Shannon diversity, and M-AMBI at each sampling site for the 2008–2012 periods. M-AMBI, species richness, and Shannon diversity exhibited the lowest values at the YB site, which was located in the innermost part of Lake Nakaumi. A statistically significant difference was observed between the YB site and the Na and CN sites, in terms of M-AMBI and species richness ($p < 0.05$); M-AMBI were $0.32 \pm 0.14$, $0.52 \pm 0.09$ and $0.51 \pm 0.02$ at the YB, Na and CN sites, respectively, while the species richness were $3.40 \pm 0.14$, $8.63 \pm 1.57$ and $8.50 \pm 0.55$ at the YB, Na and CN sites, respectively. A statistically significant difference was observed between the YB site and the others in terms of Shannon diversity ($p < 0.05$); Shannon diversity was $1.13 \pm 0.44$ at the YB site and ranged between $1.58 \pm 0.24$ and $1.87 \pm 0.35$ at the others.

In terms of benthic habitat quality and according to the M-AMBI classification (Table 1), the YB site was identified as poor status, the other sites as moderate status.

**Temporal changes in the biological indicators**

Figure 3 shows the temporal changes in species richness, Shannon diversity, and M-AMBI at each site during the 1994–2012 periods. Long-term data were available only for the CN site; although the values of all the biological indicators temporarily declined in the early 2000s at this site, they increased throughout the monitoring period from 1994. As regards the period for which the data from all sites were available (2006–2012), no increasing nor decreasing trends were observed, but the fluctuation range was large at the YB site and small at the CN one (Fig. 3). The M-AMBI values were affected by the Shannon diversity and species richness at each site. On the other hand, no significant correlation was observed between the AMBI and M-AMBI for the CN and YB sites (Fig. 4).

To identify the dominant species (the species with the highest proportion of number of appearances to the total...
number) of each microbenthic community, the appearance frequencies (proportion of number of appearances to the survey number) and dominance ratios (proportion of number of appearances to the total number) of the three most common macrobenthos observed at each site are shown in Table 2, which were estimated by, respectively, the ratio of occurrences in the survey count and the ratio of the number of individuals in the total number of individuals at each site.

The number of species collected between 2008 and 2012 at the Na, Ka, CN, and YB sites were 39, 37, 47, and 25, respectively, and 18 of them were found in all the sites. To further clarify the temporal change from 1994, the appearance frequencies and dominance ratios of the three most common macrobenthos observed at the CN site between 1994 and 1998 are shown in Table 3. At the CN site, the number of species collected in the 2008–2012 period (47) was approximately two times higher than that for the 1994–1998 period (20). The values of M-AMBI, species richness, Shannon diversity, and AMBI were, respectively, 0.33, 3.43, 0.93, and 3.29 during 1994–1998 and 0.51, 8.50, 1.85, and 3.27 during 2008–2012. Therefore, M-AMBI, species richness, and Shannon diversity increased between these two periods, while AMBI slightly decreased. During 1994–1998, the most dominant species was *Paraprionospio patiens*, with appearance frequency and dominance ratio of 85% and 65%, respectively; during 2008–2012, however, the appearance frequency and dominance ratios were 97% and 42%, respectively. This change caused the AMBI reduction and the highest dominance ratio decreased from 65% to 42%. Since a decrease in the proportion of the most dominant species lead to an increase in the Shannon diversity, M-AMBI also increased at the CN site. Figure S1 shows the total

Fig. 4 Relationships between the multivariate AZTI marine biotic index (M-AMBI) and the other biological indicators (species richness, Shannon diversity, and AZTI marine biotic index (AMBI)) at the four survey sites: Nagami (Na) (n = 7), Kamiubeo (Ka) (n = 7), Center of Nakaumi (CN) (n = 19), and Yonago Bay (YB) (n = 9).
number of benthic individuals and the dominant proportion of *P. patiens* at the CN site, which was above 50% from the 1990s to the early 2000s and then decreased.

### Relationship between water quality and M-AMBI

The PCA was conducted on the data for the CN site because its larger data set allowed a better evaluation of the factors affecting the M-AMBI variation (Fig. 5); the annual mean values of COD$_{Mn}$, DO, TN, TP and Cl$^-$ concentration, and water temperature in the bottom layer during 1994–2012 were used. Since the contribution ratios of the first (PC1) and second principal components (PC2) were 48.6% and 16.1%, respectively, the cumulative contribution of the first two axes accounted for 64.7%.

The PC1 was positively loaded with the COD$_{Mn}$ and DO, and negatively loaded with the Cl$^-$ concentration, M-AMBI, Shannon diversity and species richness. In a brackish water body with both marine and freshwater benthos such as Lake Nakaumi, not only organic pollution but also salinity would control the benthic community via species changes.

### Environmental factors controlling M-AMBI

We divided the benthic organisms inhabiting Lake Nakaumi into two groups, one for those present also in Lake...
Shinji (salinity range: 1–10 practical salinity unit (PSU), mean value: 3.5 PSU), hereafter referred to as freshwater benthos [35–37], and the other for those found only in Lake Nakaumi, hereafter referred to as brackish and marine benthos, respectively. Although this benthos classification is not strictly based on salinity tolerance or preference, it allows us to predict the trend of species shift from the change in the salinity. **Figure 6** shows the proportion of brackish and marine benthos to the total species number, which decreased together with the Cl$^-$ concentration from 1994 to 2002 and then increased onwards. M-AMBI exhibited a similar trend; the proportion of brackish and marine benthos and M-AMBI were both correlated with the Cl$^-$ concentration during the period under study, indicating that the salinity controlled the benthos population dynamics. Hence, the improvement of the ecological state of benthos represented by the M-AMBI change, as observed at the CN site since 2002, mainly resulted from an increase in salinity. By contrast, the Cl$^-$ concentration increased from 9.5 and 12.6 g/L during 2006–2007 to 12.2 and 14.1 g/L during 2008–2012 at the

**Fig. 5** Principle component analysis performed using the water quality parameters and biotic indices at the Center of Nakaumi (CN) site (n = 19).

**Fig. 6** Temporal changes in the proportion of brackish and marine benthos to the total species number, Cl$^-$ concentrations, and multivariate AZTI marine biotic index (M-AMBI) at the Center of Nakaumi (CN) site.
Na and Ka sites, respectively, that is, it increased after the opening of the western drainage channel and seawater input in 2007. Since this event, the proportion of brackish and marine benthos to the total species number at these sites also increased in parallel with the increase in the Cl\(^{-}\) concentration, from 0.29 and 0.23 during 2006–2007 to 0.38 and 0.43 during 2008–2012 at the Na and Ka sites, respectively. On the other hand, the YB site, which is located in the innermost part of Lake Nakaumi, exhibited a relatively low and steady Cl\(^{-}\) concentration (10.3–12.7 g/L) compared to the other sites and the proportion of brackish and marine benthos to the total species number was also stable (19.8% ± 15.7%); no significant correlation was observed between the appearance ratio of brackish and marine benthos and M-AMBI for this site. The large increase in M-AMBI from 2009 to 2010 was probably due to the increase in the number of Sigambra hanaokai (EG II), P. patiens (EG IV), and Pectinariidae sp. as well as the increase in the total number of species. These benthic organisms were also found in Lake Shinji and, therefore, the M-AMBI variation at the YB site was likely caused not by a species shift from the change in salinity as observed at the other sites but by a species shift among the freshwater benthos.

**Figure 7** shows the relationship between the proportion of the first dominant benthos species to the total species number and the M-AMBI at the four sampling sites. The proportion of the first dominant species to the total species number was high enough (0.62 ± 0.22 (mean ± SD) and as a result, it significantly influenced the M-AMBI. Therefore, the long-term improvement in sediment quality could be explained by the appearance and increase of non-dominant species at Lake Nakaumi and whose importance would be remarkable in such polluted sediment. On the other hand, in slightly polluted sediment like Huanghe Estuary, where the benthic environment has been evaluated as moderate and good status in most sites according to the M-AMBI, the correlations between M-AMBI and the species richness and Shannon diversity are relatively low because of the low dependence on specific species [19].

**Influence of Lake Nakaumi Reclamation Project on the salinity change**

Salinity was one of the important factors controlling the benthic community in Lake Nakaumi. Hence, the PCA was performed to clarify the factors affecting its change at the bottom layer at the CN site (Fig. 8). The Cl\(^{-}\) concentration at the bottom layer; tidal level in the Sakai; wind speed at Yonago; flow rate of Hii River; water temperature difference between the upper and lower layer at the CN; and sea level pressure in Sakai 1970–2016 were used in the analysis. The contribution ratios of PC1 and PC2 were 35.0% and 21.8%, respectively, and the cumulative contribution ratio up to PC2 was 56.8%. The sea level pressure and tidal level in Sakai exhibited strong negative and positive correlations, respectively, suggesting that the seawater input into Lake Nakaumi was enhanced by the increased water level in the Sea of Japan and, as a result, the salinity in the bottom water layer increased [38,39]. Moreover, the wind speed at Yonago was negatively correlated with Cl\(^{-}\) at the bottom layer at the CN site, suggesting that the vertical mixing of surface water with low salinity was enhanced by the strong wind lowered salinity in the bottom layer. The water temperature difference between the upper and lower layers in the CN site was positively correlated with Cl\(^{-}\) in PC2; its large value indicated a thermocline formation preventing the vertical mixing of the water column.

The relationship between the mean tidal level in Sakai and the Cl\(^{-}\) concentration in the bottom layer at the CN site during 1972–2016, except for the missing data from 1974 and 1975, is shown in Fig. 9 to clarify the effects of the activities conducted within Lake Nakaumi Reclamation Project; a difference in this relationship can be observed before and after 1979, except for the 1997–2002 period. Among the works carried out from 1963 to 1981, the construction of Omisaki...
Fig. 8 Principle component analysis performed using the weather parameters and flow rate of Hii River (n = 362).

Fig. 9 Relationship between the mean tidal level in Sakai and the Cl\textsuperscript{−} concentration at the bottom layer of the Center of Nakaumi (CN) site in the 1972–2016 period, except for 1974 and 1975.
Dike in 1978 influenced the most the seawater intrusion into the CN site. Despite its two small openings, the dike largely changed the water flow in Lake Nakaumi, i.e., it completely cut off the freshwater flow from Ohashi River into the Honjo area and lowered the seawater flow from Sakai Channel into the same area. The digging of Sakai (1969–1972) and Nakaura Channels (1968–1974) was expected to enhance the intrusion of seawater into the CN site, but this implied cutting off the seawater flow into the Honjo area. However, this digging contributed to increasing the Cl$^-$ concentration, which was higher after 1979 at the same tidal level in Sakai. Although part of Moriyama Dike was cut off, the situation did not change because no cut off was conducted for Omisaki Dike. Although the cause for the difference in the Cl$^-$ concentration observed between the 1997–2002 period and after 1979 was not clear, the higher wind speed in Yonago during 1997–2002 (3.35 ± 0.56 m/s) than in other periods (3.12 ± 0.65 m/s) ($p < 0.01$) could be a good candidate.

CONCLUSIONS

We investigated the temporal changes in the benthic ecosystem in Lake Nakaumi, an enclosed brackish lake, using M-AMBI as biotic indicators and evaluated the effects of Lake Nakaumi Reclamation Project. *P. patiens*, classified as second-order opportunistic species, appeared with high frequency in all the survey sites throughout the investigation period and was mostly dominant. Therefore, the variation of AMBI was small during the survey period. However, the species richness and Shannon diversity significantly increased from 1994 at the center of the lake; therefore, M-AMBI, which is a statistical tool derived by combining AMBI, species richness, and Shannon diversity, also increased. The benthic community of Lake Nakaumi was characterized by a coexistence of marine and brackish benthos and controlled by the Cl$^-$ concentration through the change in their proportions. The Lake Nakaumi Reclamation Project, especially the construction of Omisaki Dike in 1978, changed the water flow and seawater intrusion into the lake, affecting the benthic ecosystem at the center through the change in the Cl$^-$ concentration in the bottom water layer.

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SUPPLEMENTARY MATERIALS

Supplementary Materials for this article are available at the journal website as separate file.

REFERENCES


