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Original Article

Ecological responses of pen shell, *Atrina lischkeana*, and ark shell, *Scapharca kagoshimensis*, to sediment characteristics in a temperate tidal flat

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Abstract: To investigate the ecological responses of pen shells and ark shells to sediment characteristics in a temperate tidal flat in Japan, field surveys were conducted during spring low tides. Bivalve distributions were recorded, and sediment samples were collected from 32 sites. Regression analyses were performed to examine the relationships between bivalve density and sediment parameters, including mean grain size (Mdφ), mud layer thickness, moisture content, mud content, and loss on ignition (LOI) at 600°C and 800°C. Pen shells showed significant negative correlations with Mdφ, mud layer thickness, moisture content, mud content, and LOI at 600°C, but a positive correlation with LOI at 800°C. In contrast, ark shells were positively correlated only with Mdφ and mud content, with no significant associations with other parameters. These patterns were further supported by correlation matrix analysis and principal component analysis. Overall, the results suggest that pen shells exhibit selective habitat preferences, favoring sandy sediments with low mud content and high coarse shell material. In contrast, ark shells demonstrate broader ecological tolerance to sediment variation, resulting in a wider distribution.

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Introduction

Pen shells of the genus *Atrina* are widely distributed along the coasts of East and Southeast Asia and represent a significant fishery resource in countries such as China, Korea, and Japan (Akimoto et al., 1994; Liu et al., 2009; Liu et al., 2011; An et al., 2012; Xue et al., 2014; Yang et al., 2015; Kim et al., 2017). Due to their large adductor muscles and rapid shell growth, these bivalves have emerged as promising candidates for aquaculture. In recent years, suspended aquaculture techniques have been explored for their commercial cultivation (Ohashi et al., 2008; Maeno et al., 2009). A critical challenge in the development of pen shell aquaculture is understanding the factors that influence the mass settlement of natural spat, which is essential for collecting wild seed. During the transition from the planktonic to the benthic stage, pen shells attach to substrates using fibrous byssal threads (Tsukada et al., 1991), suggesting that specific, yet unidentified, sediment characteristics may play an important role in their settlement.

Another economically important bivalve species in East Asian aquaculture is the ark shell, *Scapharca kagoshimensis* (Masaki and Onohara, 2003; Yurimoto et al., 2007; Yurimoto et al., 2008b; Kim et al., 2018). This species is notable for the presence of hemoglobin in its body fluid (Bao et al., 2013). A well-established method for collecting natural spat involves the use of artificial collectors made of bamboo and/or palm fibers, after which the juveniles are transplanted and cultured in aquaculture grounds (Masaki and Onohara, 2003). The ark shell spat initially attaches to substrates such as shells, gravel, or collectors using byssal threads. Upon reaching a shell length of approximately 15 mm, they detach and transition to the benthic stage (Masaki and Onohara, 2009). The ecology of this species has been extensively studied, and methods for the natural collection of seedlings are nearly fully established.

The tidal flats off the Tsukushi Plain in western

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Kyushu Island are characterized by a maximum tidal range of approximately 6 meters during spring tides, resulting in the formation of extensive intertidal zones (Sato et al., 2003). These tidal flats support diverse bivalve communities, including pen shells, ark shells, and Manila clams (*Ruditapes philippinarum*), whose distributions are influenced by variations in bottom sediment conditions (Aoyama, 1977; Sekiguchi and Ishii, 2003). Pen shells are typically found on sandy-mud substrates at depths of 5-20 meters in coastal and offshore areas. A field survey by Akimoto et al. (1994) reported that this species occurs at densities of 0.1-0.5 ind./m^2 in sediments with a median particle diameter (Mdφ) of 2-4 on the phi scale and a loss on ignition (LOI) of ≤ 10% at 550°C. In contrast, ark shells are primarily distributed in muddy substrates up to approximately 7 meters deep, including intertidal mudflats (Aoyama, 1977; Ikematsu, 1980; Koga, 1991; Masaki and Onohara, 2003; Sekiguchi and Ishii, 2003). In Kasaoka Bay, Okayama Prefecture, their distribution has been associated with sediments containing 81-84% mud fraction (particle size < 0.04 mm) and LOI values of 9.2-9.9% at 700°C (Ting et al., 1972).

Although pen shells and ark shells are known to inhabit specific sediment types, the relationship between the density distribution of their natural populations and sediment characteristics in the tidal flats off the Chikushi Plain remains insufficiently understood. In recent years, especially, the pen shell population in this area has experienced a significant decline due to environmental changes, including topographical changes, slowing of ocean currents, red tides and hypoxia (Maeno et al., 2006; Tsutsumi, 2006, 2021; Yurimoto et al., 2008a), making it increasingly difficult to investigate the environmental factors that facilitate mass settlement. To address this issue, the present study analyzed the population density and sediment quality associated with pen shells and ark shells using data collected during a period when pen shell resources were still present in appreciable numbers. The author then examined the sediment parameters correlated with high-density occurrences of both species. Understanding these relationships may help improve resource management and enhance natural spat settlement by optimizing sediment conditions.

Materials and Methods

Survey design and site description: A total of 32 survey points were established in a grid pattern (approximately 5 m intervals, covering ~ 450 m²) on a tidal flat located in the lower intertidal zone off the Tsukushi Plain, western Kyushu Island. The area included both muddy and sandy-mud substrates, as identified through visual observation. Field surveys were conducted during the ebb tide of the spring tide period in March 2003, when the tidal range was near its annual maximum (Fig. 1).

Bivalve density measurements: At each sampling point, the number of pen shells (*Atrina lischkeana*, shell length: 77±15 mm, mean±standard deviation [SD]) and ark shells (*Scapharca kagoshimensis*, shell length: 22±5 mm, mean±SD) were recorded using direct visual counting. These individuals primarily comprised young clams younger than 1 year (Ting et al., 1972; Yurimoto et al., 2005). Bivalve densities per square meter were calculated from these counts.

Sediment sampling and preservation: Sediment samples were collected using a core sampler (2.7 cm in diameter, 10 cm in depth) at each site, after measuring the thickness of the surface mud layer with a ruler. Samples were chilled immediately and transported to the laboratory, where they were stored at –30°C until analysis. Prior to analysis, the sediment samples were thoroughly mixed and used to determine the following parameters: mean grain size (Mdφ), moisture content, mud content, and loss on ignition (LOI) at 600°C and 800°C.

Grain size composition and mud content calculation: Grain size composition was analyzed from approximately 10 g of sediment, which was washed under running water and sieved through mesh sizes of 4, 2, 1, 0.5, 0.25, 0.125, and 0.063 mm. Each fraction was strained using Whatman No. 5 filter paper and dried overnight at 105°C to determine dry weight. The fraction of particles ≤ 0.063 mm was calculated by subtracting the total weight of larger

Table 1. Sediment quality parameters measured on a temperate tidal flat in Japan. Variables include median particle diameter (Mdφ), surface mud layer thickness, moisture content, mud content, and loss on ignition (LOI) at 600°C and 800°C.

	Mdφ	Mud layer	Moisture content	Mud content	LOI at 600°C	LOI at 800°C
		cm	%	%	%	%
n	32	32	32	32	32	32
Maximum	4.0	9.0	66	51	15	14
Median	1.7	2.3	43	28	9	5
Minimum	0.6	0.0	25	11	4	1
Mean ± SD	2.0±1.0	2.5±2.0	43±12	27±12	9±2	5±3

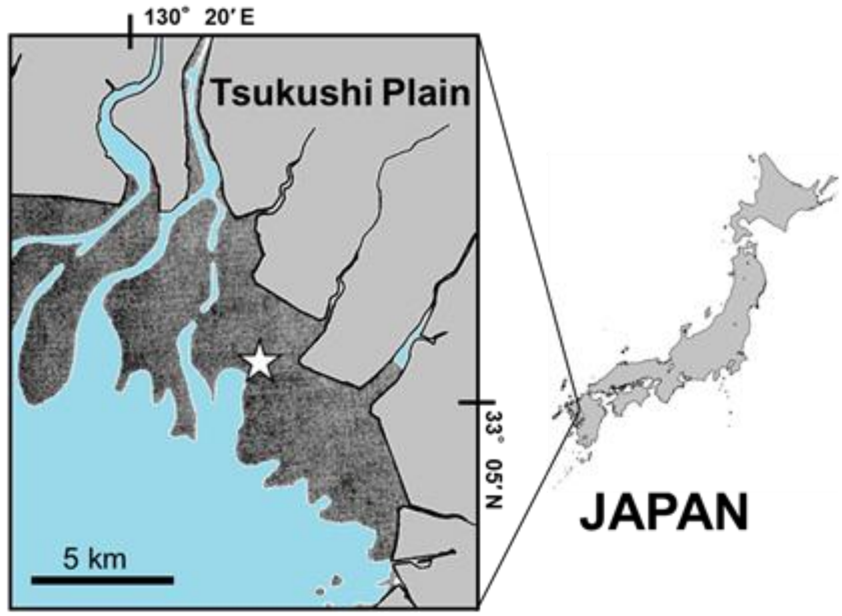

Figure 1. Map of the bivalve density survey and sediment sampling sites (★) on a temperate tidal flat (lower intertidal zone) off the Tsukushi Plain, western Kyushu, Japan. A total of 32 sampling points were arranged in a grid pattern at approximately 5-meter intervals, covering an area of about 450 m². The survey was conducted during the ebb tide of the spring tide period.

fractions from the total dry sediment weight. Mud content was expressed as the percentage of particles ≤ 0.063 mm. Mdφ was calculated from the cumulative frequency distribution of particle sizes.

Moisture content and loss on ignition (LOI): Moisture content was determined as the ratio of wet to dry weight (drying at 105°C overnight) using approximately 10 g of sediment. LOI values were measured using the same dried sediment samples, which were heated at 600°C for 4 hours, weighed, and then reheated at 800°C for another 4 hours and weighed again. The weight loss between 105°C and 600°C, and between 600°C and 800°C, was used to calculate LOI at each temperature (Saito et al., 1977).

Statistical analysis of environmental and biological data: To evaluate the relationships between bivalve densities and sediment parameters, linear regression analyses were performed using Microsoft Excel 2010 (Microsoft Corp., USA). Coefficients of determination (R^2) and *P*-values were calculated using Excel's regression analysis functions. Additionally, correlation matrix analysis and principal component analysis (PCA) were conducted using Microsoft 365 and Copilot to explore multivariate relationships among sediment parameters and their influence on bivalve distribution patterns.

Results

Sediment quality: The sediment quality parameters were summarized as follows (mean±SD, n = 32): Mdφ 2.0±1.0, mud thickness 2.5±2.0 cm, moisture content 43±12%, mud content 27±12%, loss on ignition (LOI) at 600°C 9±2%, and LOI at 800°C 5±3% (Table 1). The minimum and maximum values were Mdφ 0.6-

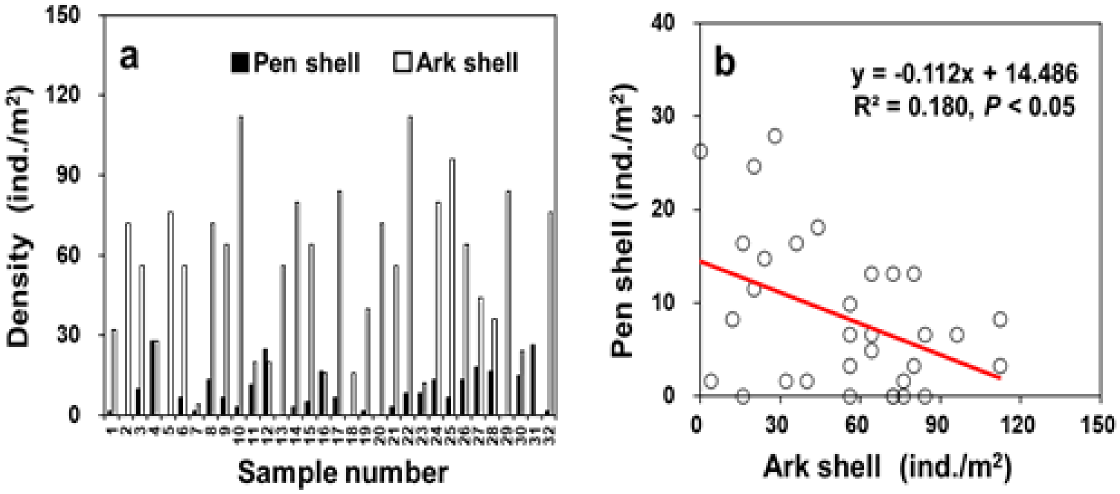

Figure 2. Distribution densities of pen shell (*Atrina lischkeana*) and ark shell (*Scapharca kagoshimensis*) at each sampling site on a temperate tidal flat in Japan (a). Relationship between the densities of pen shell and ark shell across all sites (n = 32) (b). Regression analysis was used to assess the significance of the correlation between species densities (P<0.01 or P<0.05).

4.0, mud thickness 0.0-9.0 cm, moisture content 25-66%, mud content 11-51%, LOI at 600°C 4-15%, and LOI at 800°C 1-14% (Table 1).

Relationship between pen shell and ark shell: The range in densities of pen shell was 0-28 ind./m^2 with mean±SD of 8±8 ind./m^2, and for ark shell was 0-112 ind./m^2 with 54±31 ind./m^2 on the 32 sampling points (Fig. 2a). Moreover, the two distributions exhibited a significant negative relationship with each other (R^2 = 0.180, P<0.05, Fig. 2b).

Relationship between pen shell and sediment: The distribution densities of pen shell were significantly negatively related (P<0.01 or P<0.05) with Mdφ, mud layer thickness, moisture content, mud content, and LOI at 600°C. On the other hand, the densities were significantly and positively related (P<0.01) to LOI at 800°C (Fig. 3).

Relationship between ark shell and sediment: The density of ark shell was significantly positively related (P<0.05) to Mdφ and mud content. However, density was not significantly related to the other sediment parameters: mud layer thickness, moisture content, and LOIs at 600°C and 800°C (Fig. 4).

Interrelationships of various parameters: A heat map of Pearson correlation coefficients among eight variables—median particle diameter (Mdφ), mud thickness, moisture content, mud content, LOI at 600°C and 800°C, and the densities of pen shells and ark shells—is presented in Figure 5a. In the heat map, red indicates positive correlations, blue indicates negative correlations, and color intensity reflects the strength of the correlation (range: -0.81 to 1.00). A particularly strong positive correlation was observed between Mdφ and mud content (r = 0.94), followed by moisture content and mud thickness (r = 0.81), and moisture content and mud content (r = 0.71). A moderate positive correlation was also found between LOI at 800°C and pen shell density (r = 0.74). In contrast, strong negative correlations were observed between moisture content and LOI at 800°C (r = –0.81), and between mud content and LOI at 800°C (r = –0.75). For ark shells, weak positive correlations were observed with Mdφ (r = 0.40) and mud content (r = 0.35).

Two-dimensional biplot: A two-dimensional biplot based on principal component analysis (PCA) is shown in Figure 5b. The biplot was constructed from the first two principal components, PC1 (62.17%) and PC2 (11.57%). Blue dots represent individual observations, while red arrows indicate the contribution of each variable to the principal components. Variables such as moisture content and mud content showed strong positive loadings on PC1, indicating that samples located in this direction tend to have higher values for these variables. The vector for ark shell density was primarily aligned with PC2,

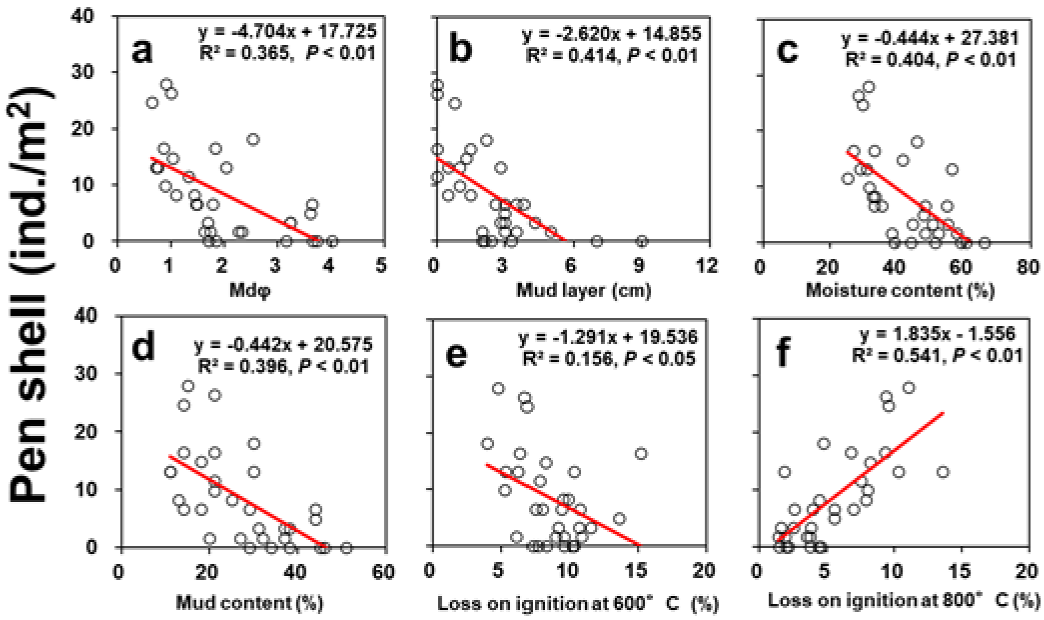

Figure 3. Relationships between the density of pen shell (*Atrina lischkeana*) and various sediment quality parameters in a temperate tidal flat, Japan: (a) Mdφ of sediment particle size, (b) mud layer thickness on surface bottom, (c) moisture content in bottom sediment, (d) mud content in bottom sediment, and loss on ignition of bottom sediment at (e) 600°C and (f) 800°C. Regression analysis was used to determine the significance of the relationship between the density and sediment quality parameters (P<0.01 or P<0.05, n = 32).

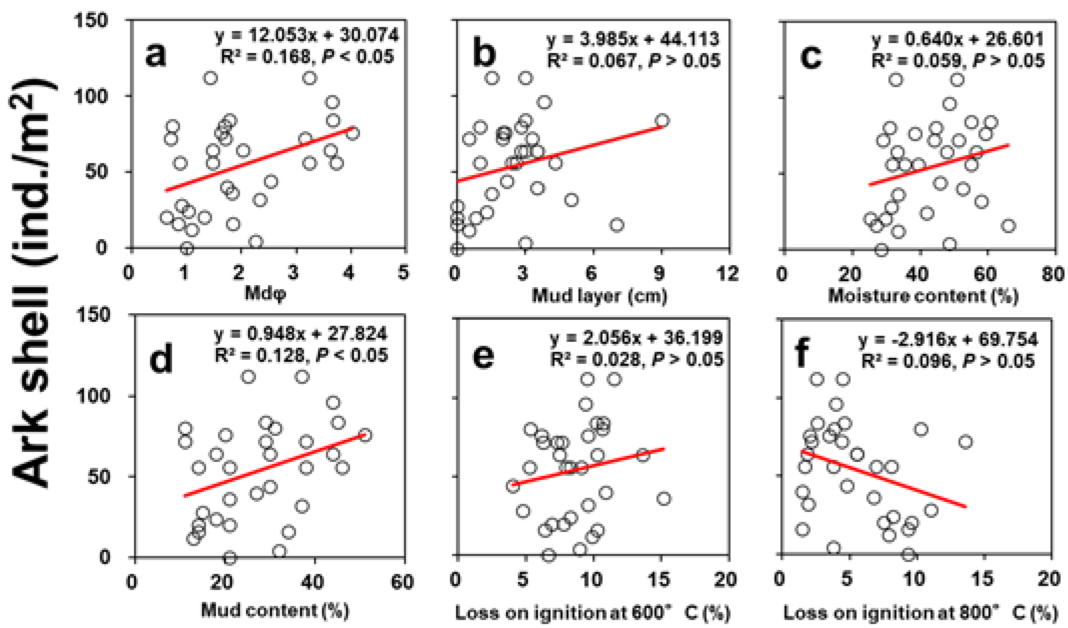

Figure 4. Relationships between the density of ark shells (*Scapharca kagoshimensis*) and various sediment quality parameters in a temperate tidal flat, Japan: (a) Mdφ of sediment particle size, (b) mud layer thickness on surface bottom, (c) moisture content in bottom sediment, (d) mud content in bottom sediment, and loss on ignition of bottom sediment at (e) 600°C and (f) 800°C. Regression analysis was used to determine the significance of relationships between the density and sediment quality parameters (P<0.01 or P<0.05, n = 32).

indicating its influence on that component. Additionally, mud thickness, moisture content, and LOI at 600°C were oriented in similar directions, as were Mdφ and mud content. The LOI at 800°C showed a directional similarity to pen shell density and showed a potential negative association between organic content and pen shell occurrence.

Discussions

Previous studies on pen shell distribution: Akimoto et al. (1994) surveyed 17 subtidal sites in the central and inner regions of Ariake Bay, reporting sediment

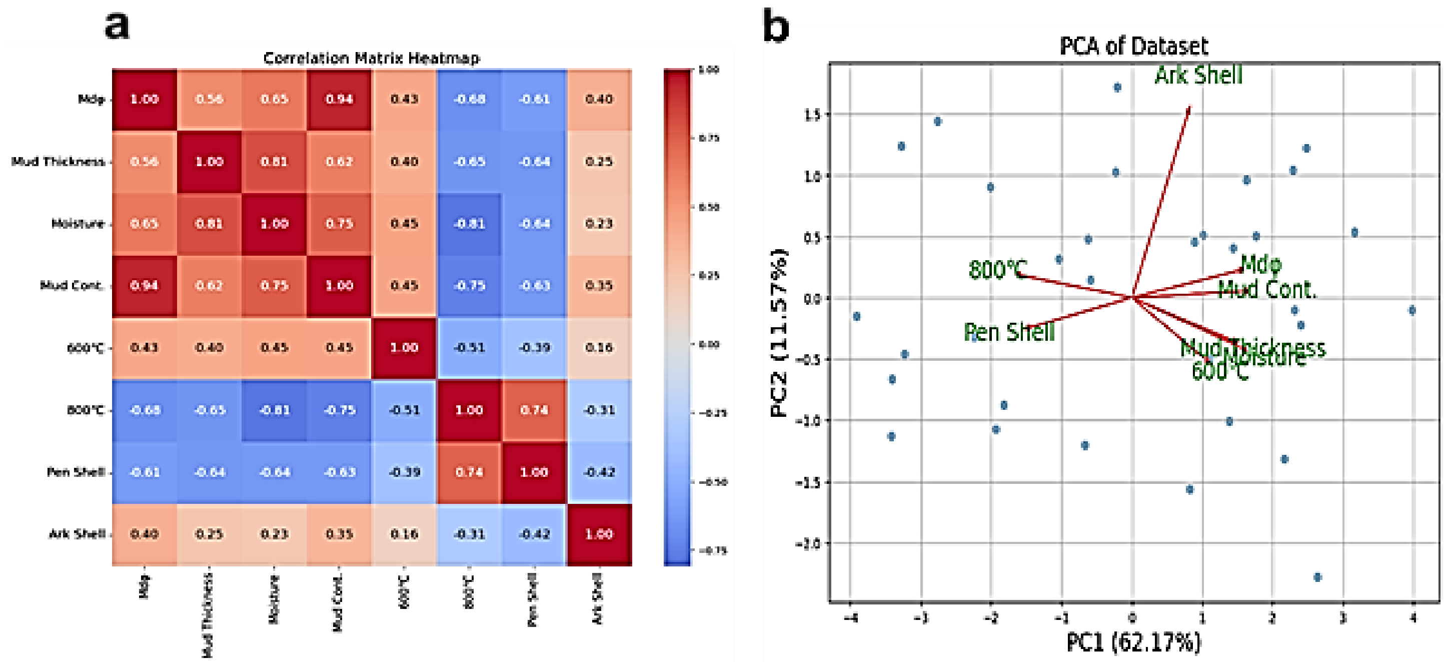

Figure 5. Pearson Correlation Coefficient Heatmap (a) and 2D Biplot from Principal Component Analysis (PCA) (b). Figure a show a heatmap of Pearson correlation coefficients among eight variables: Mdφ, Mud Thickness, Moisture, Mud Cont., LOI at 600°C, LOI at 800°C, Density of Pen Shell, and Density of Ark Shell. Red indicates positive correlations, blue indicates negative correlations, and the color intensity reflects the strength of the correlation (range: -0.81 to 1.00). Figure b presents a biplot based on the first principal component (PC1: 62.17%) and the second principal component (PC2: 11.57%) obtained through PCA. Blue dots represent the projected observations, while red arrows indicate the contribution of each variable to the principal components.

conditions with Mdφ values ranging from 2 to 8 and LOI values up to 35% at 550°C. Their study found no clear relationship between pen shell density and surface sediment chlorophyll-a or phaeopigment content—indicators of phytoplankton abundance as a food source. However, pen shells were typically found at densities of 0.1-0.5 ind./m² in sediments with Mdφ values of 2-4 and LOI ≤ 10% at 550°C. Suzuki et al. (2009) expanded on this by surveying 46 subtidal sites in the same region, identifying a clearer association between pen shell density (≥ 2.5 cm shell length, ~10 ind./m²) and sediment parameters: Mdφ of 1.5-4.5, mud content of 0-55%, and LOI of 2-7% at 550°C.

Findings from the present study: In the present study, pen shells were observed in the tidal flat region of Ariake Bay at densities exceeding 10 ind./m² under sediment conditions of Mdφ 0.6-2.5, mud layer thickness 0-2.8 cm, moisture content 25-56%, mud content 11-30%, and LOI values of 4-15% at 600°C and 2-14% at 800°C. These sediment characteristics—ranging from coarse to medium sand with variable mud content and organic matter—closely align with the findings of Akimoto et al. (1994) and Suzuki et al. (2009), suggesting that pen shells inhabit similar sediment types across both tidal flat and subtidal zones.

Optimal conditions for high-density pen shell populations: Furthermore, when focusing on sites with pen shell densities ≥20 ind./m², the most favorable sediment conditions were: Mdφ 0.6-1.0, mud layer thickness 0-0.8 cm, moisture content 28-31%, mud content 14-21%, LOI at 600°C of 5-7%, and LOI at 800°C of 9-11%. These results indicate a strong relationship between pen shell distribution and sediment parameters, particularly Mdφ, mud layer thickness, moisture content, mud content, and LOI values.

Role of shell material in pen shell settlement: Murata (1964) demonstrated that calcium carbonate, the primary component of bivalve shells, does not decompose at 600°C but rapidly degrades above 700°C. Thus, LOI at 800°C reflects the presence of shell material in the sediment (Ueta and Sumitomo, 2003). Previous studies have shown that adding shell sand or oyster shell debris to muddy bottoms in Ariake Bay promotes stable settlement of pen shell spat (Koga and Yamashita, 1986; Koga and Nakatake, 1991). This suggests that shell gravel in the sediment plays a key role in pen shell settlement and survival, likely due to its physical and chemical properties.

Contrasting distribution patterns of ark shells: In contrast, ark shell distribution was significantly positively correlated (P<0.05) only with Mdφ and mud content. No significant relationships were found with mud layer thickness, moisture content, or LOI values at either temperature. While ark shells in Kasaoka Bay typically inhabit substrates with >80% mud content (Ting et al., 1972), the present study found high densities (~50 ind./m²) in sandy mud sediments with ~37% mud content. This suggests that ark shells can tolerate a broader range of sediment conditions. Unlike pen shells, which remain fixed to the substrate via byssal threads during their benthic life stage, ark shells detach after settlement and do not remain anchored. This mobility likely contributes to their broader distribution across tidal flats, enabling them to adapt to environmental disturbances, such as tidal fluctuations and wave action.

Statistical analysis of environmental and biological relationships: In this study, to comprehensively assess the relationships between environmental factors and biological indicators, correlation matrix analysis and principal component analysis (PCA) were employed. This multifaceted statistical approach enabled both visual and quantitative exploration of variable associations and the underlying structure of the dataset. The PCA results revealed a clear separation of environmental gradients along the first PC1 and second PC2 principal components. PC1 was primarily associated with muddy sediment conditions, including mud layer thickness, moisture content, and mud composition. In contrast, PC2 was strongly influenced by biological and organic factors, such as LOI at 800°C and the abundance of *A. lischkeana*. This two-dimensional structuring provided a clearer understanding of how environmental conditions correspond to species distribution patterns.

Implications for environmental monitoring and conservation: By integrating the findings from both correlation and PCA analyses, the influence of sediment characteristics on bivalve distribution was evaluated from multiple perspectives. This integrative approach offers robust, evidence-based insights that are valuable for environmental monitoring, ecosystem assessment, and the development of conservation strategies. The analysis demonstrated distinct structural relationships between environmental variables and biological indicators, which may serve as reference indices in future environmental evaluations. Moving forward, incorporating time-series data and comparative studies across different regions will be essential for developing more comprehensive and generalizable assessment methodologies.

Conclusion

This study revealed that the distribution of pen shells was significantly negatively correlated with several sediment parameters, including median particle diameter (Mdφ), mud layer thickness, moisture content, mud content, and LOI at 600°C. Conversely, a significant positive correlation was observed with LOI at 800°C. These findings showed that pen shells preferentially inhabit coarse sandy sediments enriched with shell fragments and low in organic and fine particulate matter. In contrast, the distribution of ark shell showed a significant positive correlation with Mdφ and mud content, indicating greater tolerance of sediment conditions. Additionally, unlike pen shells, which exhibit localized settlement patterns and remain anchored via byssal threads, ark shells were found across a wider area of the tidal flat, reflecting their greater mobility and reduced dependence on specific sediment types. Although pen shell populations have declined markedly in recent years within the study area, the results suggest that shell sand may play a critical role in promoting spat settlement. This insight has potential for habitat restoration and aquaculture seed-collection strategies to conserve and enhance pen shell populations.

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