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Research Article

Seasonal Changes in Glycogen Contents in Various Tissues of the Edible Bivalves, Pen Shell *Atrina lischkeana*, Ark Shell *Scapharca kagoshimensis*, and Manila Clam *Ruditapes philippinarum* in West Japan

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The types of tissues accumulating glycogen and seasonal changes in glycogen content were investigated in the following shell species: pen shell *Atrina lischkeana*, ark shell *Scapharca kagoshimensis*, and Manila clam *Ruditapes philippinarum*. Comparison of the results showed that the adductor muscle or foot was the main glycogen reservoir and the levels varied seasonally. The adductor muscle in the pen shell showed higher glycogen content during spring and lower content during autumn. The ark shell, on the other hand, showed higher content during winter and spring and lower content during summer and autumn, while the Manila clam showed higher glycogen content during spring and summer and lower content during autumn and winter. These results revealed that the adductor muscle in pen shells and the foot in ark shells and Manila clams act as the main storage tissues for glycogen in the three species studied and that these tissues are suitable to analyze glycogen prevalence to estimate individual physiological condition.

1. Introduction

Glycogen is a water-soluble polysaccharide, composed by polymerized glucose molecules joined through glycosidic bonds, forming a network-like structure [1]. It represents the main form of energy storage in the animal bodies along with body fat. In particular, bivalves present higher percentage of glycogen compared to fat, and its contents are influenced by internal factors, such as growth and sexual maturation, and external factors, such as food availability and other environmental factors [2–6]. Thus, glycogen content varies depending on the physiological state of the organism, and it can be used to evaluate physiological condition in different bivalve species, including Pacific oysters (*Crassostrea gigas*), pearl oysters (*Pinctada fucata martensii*), Manila clams (*Ruditapes philippinarum*), and ark shells (*Scapharca kagoshimensis*) [7–11].

Ariake Bay is a one of the most famous fishery grounds for bivalves such as pen shells (*Atrina lischkeana*), ark shells, and Manila clams in West Japan. However, landings of these species have remained at low levels for recent years, due to environmental changes that coastal development is considering to contribute the frequently occurrence of red tide and hypoxic water. Therefore population stabilization of these in the bay has become a pressing issue for local fisheries [12, 13]. Set against this background, identifying a maker able to provide information on bivalve physiological condition *in situ* would facilitate reaching stable fishing production for these species. So far, glycogen content and its variation have been assessed in pen shells as an important taste component of the adductor muscle [14]. In addition, previous studies have assessed the relationship between sexual maturation and annual variation in adductor muscle, mantle, and digestive gland glycogen content in pen shells [6], and the organism nutritional status based on the variations in glycogen in the foot of ark shells [10, 11]. In the Manila clam, few studies have also assessed the effect of annual and seasonal variations in soft tissues on its taste [15, 16] and the glycogen variability associated with growth and sexual maturation [17]. However, whether glycogen content varies among different tissues

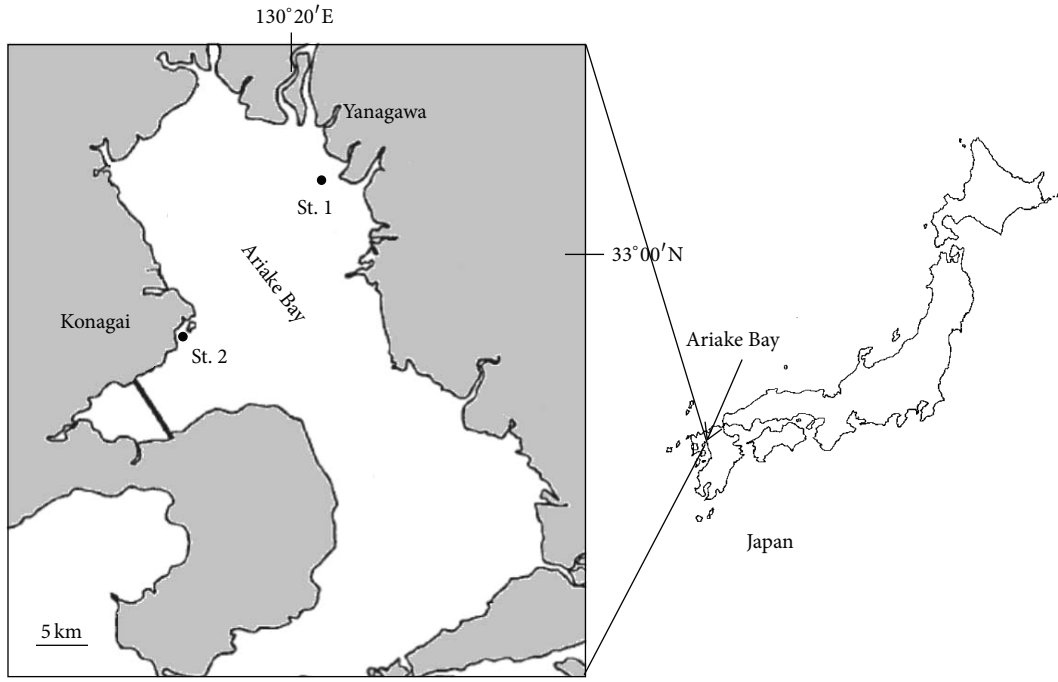

Figure 1: Sampling locations in Ariake Bay, West Japan.

and the seasonal changes in these bivalves has never been assessed. Then we have restricted the detailed estimation using the glycogen contents on physiological conditions of these species. Thus, in order to understand accumulation and seasonality of glycogen at the tissue level, seasonal variation in the amount of glycogen stored in different tissues was assessed in the three most commercially important bivalve species in the Ariake Bay: the pen shell, the ark shell, and the Manila clam.

2. Materials and Methods

Pen shells (shell length 120–155 mm) and ark shells (shell length 22–33 mm) were collected from station 1 (St 1 in Figure 1), a tidal flat offshore from Yanagawa City, Fukuoka Prefecture, at four independent time points: in January (winter), April (spring), July (summer), and October (autumn), 2003. Manila clams (shell length 25–35 mm) were collected from station 2 (St. 2 in Figure 1), a tidal flat offshore from Konagai Town, Isahaya City, Nagasaki Prefecture, in January, April, July, and October, 2007. After collection, the specimens were transported back to the laboratory, where all the shells were open after shell length was measured, and the different tissues were excised and stored frozen at −40°C until further analysis. The glycogen contents of the mantle, gill, adductor muscle, digestive gland, foot, kidney, and gonad were measured in pen shells. In ark shells, the glycogen contents of the mantle, gill, adductor muscle, digestive gland, foot, and gonad were measured, whereas in Manila clams, the glycogen contents in the mantle, gill, adductor muscle, digestive gland, foot, siphon, and gonad were measured.

Glycogen analysis was performed following the anthrone method as previously described [18]. Through this method, 0.1–0.5 g of wet weight from various bivalve tissues is isolated and boiled in 1.5 mL solution of 30% potassium hydroxide for 20 minutes until all the tissue is dissolved. Subsequently, the samples were centrifuged, and 2.0 mL 95% ethanol solution and 0.25 mL of saturated sodium sulfate were added to each sample after cooling. The precipitate resulting from centrifuging the sample was resuspended in distilled water and boiled in anthrone reagent, and the absorbance at 620 nm was measured after cooling. Commercially available glycogen (Wako Pure Chemical Industries, Ltd., Japan) was used for the glycogen standard curve. The glycogen content was expressed per 1 g wet weight of bivalve tissue. Multiple comparison of the glycogen content in each tissue on each month was analyzed to make clear main storage tissues on each month and the seasonality on each tissue for glycogen by the Tukey-Kramer test with α set at $P < 0.05$.

3. Results and Discussion

3.1. Pen Shells. Glycogen was detected in all the tissues (the mantle, gill, adductor muscle, digestive gland, foot, kidney, and gonad), and the glycogen contents measured throughout the study period were 2–13, 4–8, 8–33, 3–5, 9–15, 7–14, and 2–9 mg/g, respectively (Figure 2). In addition, glycogen was clearly present in the adductor muscle throughout the year. In this tissue, glycogen content increased in January and April and decreased in July and October. Similar variation was also observed in the other tissues, but it was not as pronounced due to the low amounts of glycogen detected

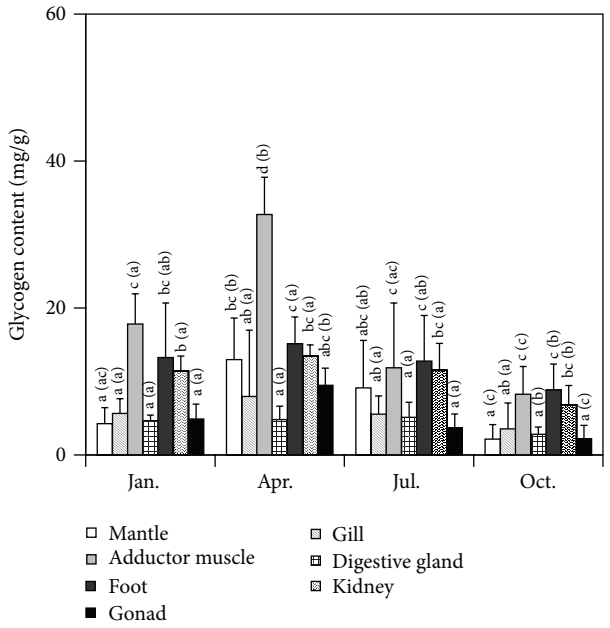

Figure 2: Seasonal changes in glycogen content in different tissues collected from the pen shell, *Atrina lischkeana*. Bars represent means and whiskers represent standard deviation. 3–6 samples (replicates) were used for the analysis of each organ. No common letters out of parentheses indicate significant differences of glycogen contents in different tissues on each month and no common letters in parentheses indicate significant differences of seasonal changes on each tissue ($P < 0.05$; Tukey-Kramer test).

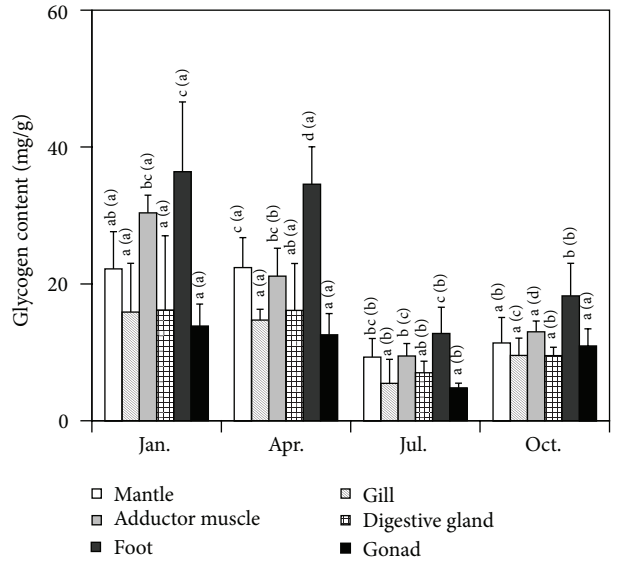

Figure 3: Seasonal changes in glycogen content in different tissues from ark shells, *Scapharca kagoshimensis*. Bars represent means and whiskers represent standard deviation. 4–6 samples (replicates) were used for the analysis of each organ. No common letters out of parentheses indicate significant differences of glycogen contents in different tissues on each month and no common letters in parentheses indicate significant differences of seasonal changes on each tissue ($P < 0.05$; Tukey-Kramer test).

compared to the adductor muscle. On the other hand, glycogen content in April, when it showed highest level in the adductor muscle, varied among tissues, decreasing from the adductor muscle, followed by the foot, kidney, mantle, gonad, gill, and digestive gland in this order. Yurimoto et al. [6] monitored annual changes in glycogen content in adductor muscle, digestive gland, and mantle in pen shells from the Ariake Bay and showed an almost lack of seasonal variation in the digestive gland glycogen content, which remains low, below 10 mg/g throughout the year. The content in the adductor muscle and mantle, on the other hand, showed a clear variation from a few to 78 mg/g and from a few to 23 mg/g, respectively. In both of these organs, glycogen content peaked in spring (April) and remained low, around only few mg/g, in the autumn season (October to December) after spawning. In the present study, only a small seasonal variation was observed in gill, digestive gland, foot, and kidney, where the glycogen content remained nearly constant throughout the year. On the other hand, glycogen contents in the mantle, adductor muscle, and gonad showed large variations, increasing in spring (April) and decreasing in autumn (October). In addition, glycogen content in the adductor muscle in April was significantly higher, by twofold, than that of the other tissues during the same period. These results also revealed that the adductor muscle in pen shells was the main storage tissue for glycogen and it was peaked during spring.

3.2. Ark Shells. Glycogen was also detected in all the tissues analyzed in ark shells (mantle, gill, adductor muscle, digestive gland, foot, and gonad), and the amount measured throughout the study period in each of these tissues was 9–22, 6–16, 10–30, 7–16, 13–36, and 5–14 mg/g, respectively (Figure 3). In addition, seasonal variation in glycogen content followed a similar pattern in all the tissues, with the glycogen increasing markedly in January and April, and decreasing in July and slightly in October. On the other hand, glycogen content varied among tissues, being larger in the foot in January, to decrease in the adductor muscle, mantle, digestive gland, gill, and gonad in this order. Yurimoto et al. [10] assessed the nutritional status of ark shells reared in captivity and its association with foot glycogen content. They showed that glycogen content in between spring and autumn (April to October) varied between 17 mg/g and 61 mg/g, showing the highest and lowest values in April and July, respectively. In addition, Yurimoto et al. [11] revealed that glycogen was present in different organs, including mantle, gill, adductor muscle, digestive gland, and foot in ark shells from the Ariake Bay, measured in April. In the same study, the shell foot showed a significantly higher amount of glycogen (60 mg/g over) compared to the other tissues, varying between about 20 mg/g in the summer and 40 mg/g or higher in the spring. However, Yurimoto et al. [10, 11] did not estimate seasonal variation in glycogen content in any of the other tissues. In this study, seasonal variation in the glycogen content was measured in six different organs, including gill, adductor muscle, digestive gland, foot, and gonad, in ark shells from a tidal flat in Ariake Bay. Glycogen was detected in all

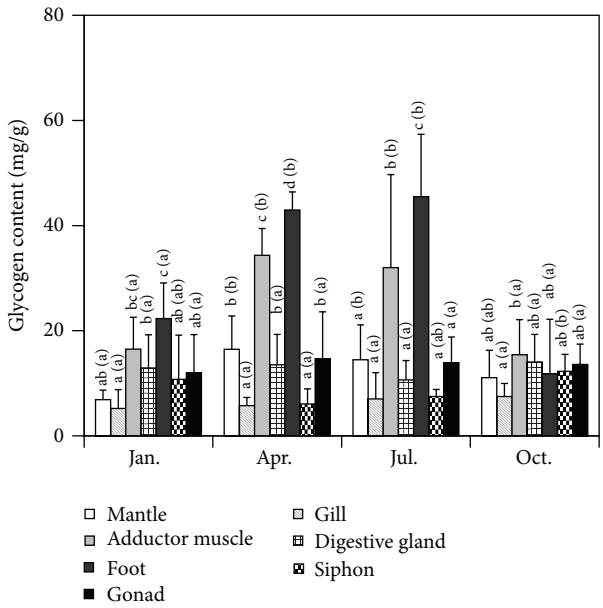

FIGURE 4: Seasonal changes in glycogen content in different tissues removed from Manila clams, *Ruditapes philippinarum*. Bars represent mean and whiskers represent standard deviation. 3–6 samples (replicates) were used for the analysis of each organ. No common letters out of parentheses indicate significant differences of glycogen contents in different tissues on each month and no common letters in parentheses indicate significant differences of seasonal changes on each tissue ($P < 0.05$; Tukey-Kramer test).

the tissues measured, presenting its higher concentration in the foot, following in the adductor muscle and mantle. In addition, the amount of glycogen accumulated in gill, digestive gland, and gonad was lower than the amount detected from the other three organs, although, quantitatively, the differences among these tissues remained unclear. Following the results by Yurimoto et al. [10, 11], the glycogen content measured from all the tissues, including the foot, decreased in summer (July). In this study, the glycogen content remained constant in the different tissues measured in ark shells between winter and spring, and decreased in summer; however, the extent of the decrease varied among tissues. The foot appeared as the main storage location for glycogen in this species.

3.3. Manila Clams. Glycogen was also detected in all Manila clam tissues (mantle, gill, adductor muscle, digestive gland, foot, siphon, and gonad), and the amounts measured in these tissues throughout the study period were 7–17, 5–8, 15–34, 11–14, 12–46, 6–12, and 12–15 mg/g, respectively (Figure 4). In addition, glycogen was clearly present in the adductor muscle and foot throughout the year, increasing in both tissues in April and July, and decreasing in January and October. Glycogen prevalence was not observed in other tissues, where the content remained nearly constant through the year. Glycogen content also varied among tissues in this species, being higher in the foot in July, to decrease in the adductor muscle, mantle, gonad, digestive gland, siphon, and gill, in this order. Takagi and Simidu [15] analyzed the amount of glycogen in soft tissues of Manila clams from Maizuru Bay, in West Japan in January, March, June, September, and November. The proportion of glycogen, in wet weight of soft tissue, varied between 1.0% and 2.5%, increasing in June and decreasing in November. In a similar study, Shiraishi et al. [16] sampled Manila clams throughout a full year in the Fukuoka coast, Fukuoka Prefecture, West Japan. The proportion of glycogen, in wet weight of the soft tissue, ranged between 0.5% and 5.2%, showing its highest values in August and minimum values in December. Robert et al. [17] collected Manila clams in the inner part and the mouth of Arcachon Bay, a section of the Bay of Biscay in France. They monitored monthly variation in glycogen content in soft tissues and showed that glycogen content significantly decreased with sexual maturity. However, they did not assess seasonal variation in glycogen in different tissues. In this study, data on seasonal variation in glycogen content were collected from seven organs, including mantle, gill, adductor muscle, digestive gland, foot, siphon, and gonad, from Manila clams collected in Ariake Bay. Glycogen was detected from all the tissues analyzed. In addition, high levels of glycogen and a marked seasonal variation were observed in foot, adductor muscle, and mantle. On the other hand, the gill, digestive gland, siphon, and gonad showed lower glycogen contents and less marked seasonal variation than the foot, adductor muscle, and mantle. In addition, the seasonal variation in glycogen contents among tissues was high in April and July and low in January and October. These results agree with the seasonal variation in glycogen content from Manila clam soft tissues reported by Takagi and Simidu [15] and Shiraishi et al. [16]. In addition, the foot was shown as the main glycogen reservoir in this species.

4. Conclusion

Glycogen accumulation and seasonal variation in different tissues in pen shells, ark shells, and Manila clams collected in Ariake Bay were examined throughout a year. The adductor muscle appeared as the main glycogen reservoir in pen shells, while foot appears as the main reservoir organ in ark shells and Manila clams. The periods of higher and lower accumulation of glycogen in the reservoirs also varied among species. Pen shells showed a glycogen peak in spring and the lowest values in autumn, ark shells showed a peak in winter-spring and the lowest values in summer-autumn, while Manila clams showed a peak in spring-summer and the lowest values in autumn-winter. These results revealed as useful organs to assess individual physiological condition based on glycogen content variation on the three species.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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