

Relationship Between Environmental Food and Glycogen Contents in Pen Shells

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Abstract

We investigated several factors influencing changes in the glycogen content in pen shells *Atrina pectinata* and made a standard health index for glycogen content of pen shells living in Ariake Bay. Seasonal variation of glycogen content and sexual maturation was determined as followed: (February, 2000 ~ February 2001) the glycogen content increased in adductor muscle from February-April (70~80mg/g), but decreased gradually following the development of reproductive glands, showing 15mg/g in spawning season (July ~ September). After spawning season (October ~ December) low-levels (3mg/g) of glycogen content were observed, and rose to 13mg/g following high availability of phytoplankton (January). The fluctuating glycogen content could be related to reproductive gland development, as glycogen is an energy source needed for life and reproductive gland development. Results were achieved the following measures for the influence of feed quantity on environment. For experiment 1, "Tolerance to food deprivation", specimens were fed *Chaetoceros* sp. for 4 days after sampling and divided into fed group and unfed group. Changes of glycogen content in the unfed group decreased continuously (12mg/g to 2mg/g over 1 week) with specimens dying after day 7 until all had died by day 15. However, the glycogen content of the fed group remained at 10~12mg/g with a survival rate of 80%. For experiment 2, "recuperation from unfed conditions", pen shells were used in this study 2 months after spawning. This experiment was divided into two groups: one group was starved for 1 week and the other for 1 month. Recuperation of glycogen intake for 40 days was observed thereafter. Both groups differed in tolerance for food deprivation. The 1 week group accumulated glycogen rapidly (2 to 20mg/g over 1 week), but the 1-month group did not (2 to 12mg/g over 1 month). The 1 month group survival rate was lower (70 %) than in the one-week group (100%). These results suggest that the pen shell survival rate is influenced by short-term lack of food in spawning season. Also, pen shells after spawning season can accumulate glycogen rapidly in the presence of high food availability after spawning season. Therefore, it is suggested that pen shells had low glycogen content (3mg/g) in Ariake Bay during autumn 2000 due to low phytoplankton levels over several weeks.

Introduction

The pen shell *Atrina pectinata* grows up to a shell length of 20-25cm (**Fig. 1**) and lives in a sandy mud bottom under tidal areas in bays at a depth of 5-20m. The pen shell is an expensive food in western Japan. However, pen shell fishing production has been

decreased significantly in recent years in Ariake Bay, Kyushu Island (**Fig. 2**). Pen shell fishing production has been less than 1,000 tons in Ariake Bay since 1999 and comprised about 90% of domestic fishing production the until early 1980 (Okutani 1997). It was reported that pen shell stocks have decreased due to mass mortality in the growth stage in recent years (Matsui 2002, Kawahara and Ito 2003).

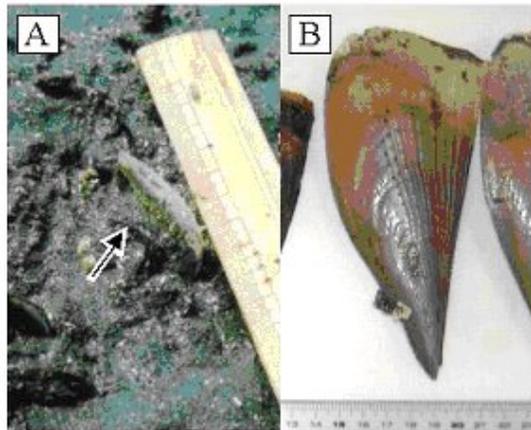


Fig.1 Environmental conditions and pen shell morphology
 A: Pen shell living in tidal land B: Pen shell caught in Ariake Bay

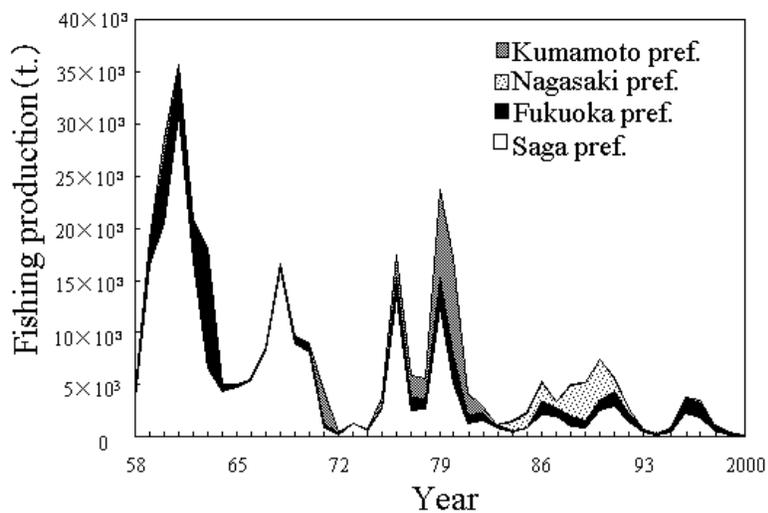


Fig.2 Annual changes of pen shell fishing production in Ariake Bay
 (Data from annual prefectural fisheries production statistical reports)

In this study, we investigated changes in factors affecting glycogen content in pen shells and made a standard health index for glycogen content of pen shells living in Ariake Bay. Glycogen content has been commonly used as a health index for bivalves such as scallops *Patinopecten yessoensis* (Miyazono and Nakano 2000, Yamanaka 2002), oysters *Crassostrea gigas* (Akashige and Fushimi 1992, Mori *et.al.* 1965, Yamamura and Watanabe 1964), pearl oysters *Pinctada martensii* (Shinomiya *et.al.* 1997, Uchimura 1999) and Manila clams *Ruditapes philippinarum* (Takagi and Shimizu 1963).

Materials and Methods

Seasonal Variation of Glycogen Contents and Sexual Maturation

Two types of pen shell *Atrina pectinata* were found in Japan in a recent study. Yokogawa (1996) distinguished them according to shell type (non-scaly vs. scaly form) and isozymic patterns. In this study, we used scaly form pen shells, because pen shells of this type are the main objects of fishing in Ariake Bay.

Pen shells that were of the same age group cohort occurring in 1999 were collected every month (February, 2000-February, 2001) by helmet-type diving in the fishing grounds of Ariake Bay (**Fig. 3**). Adductor muscle was analyzed for glycogen content by the Anthron method (Kamada and Hamada 1985) and the organization block was isolated from reproductive glands, fixed in 10% formalin solution, embedded in paraffin, cut into thin sections, stained with HE (Hematoxylin and Eosin) and observed with an optical microscope.

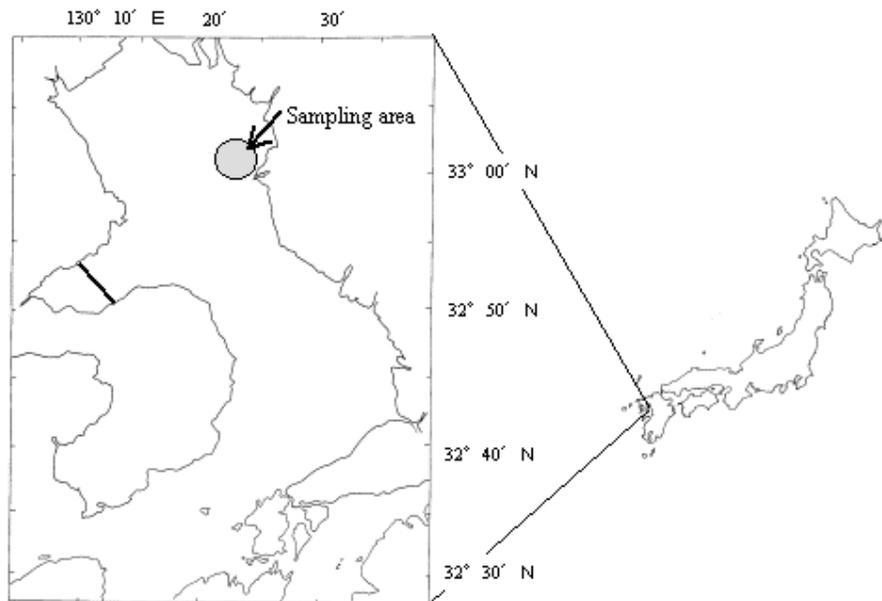


Fig.3 Location of sampling area in Ariake Bay

The classification of sexual maturation stages in shellfish has already been reported in Scallops *Patinopecten yessoensis* (Mori *et al.* 1977), Umitake *Barnea dilatata* (Yamasaki 1993) and Manila clams *Ruditapes philippinarum* (Toba *et al.* 1993), thus we compared our results of pen shells with these previous studies.

Influence of Changes in Glycogen Contents According to Feed Quantity Environment

Experiment 1: Tolerance for Food Deprivation

Pen shells collected in tidal lands of Ariake Bay during spawning season were used for the experiment. Specimens fed phytoplankton (*Chaetoceros* sp.) for 4 days after sampling were divided into two groups, fed and starved. The experiment was conducted in 50L containers containing sandy mud collected in Ariake Bay, water temperature 24.5 ~ 26.5°C, salinity 31.0 ~ 32.5PSU, and specimens were fed *Chaetoceros* sp. 18×10^6 cells/day (for fed group). Both groups were observed for changes in the survival rates and glycogen content over two weeks. During the experiment, three specimens of each group were analyzed for adductor muscle glycogen content on days 0, 2, 5, 9, and 13.

Experiment 2: Recuperation From Unfed Conditions

Specimens were collected in Ariake Bay from September to October. Before taking the samples, we took some specimens to check their sexual stage. During this time, pen shells were already spent or the spawning season was already finished. The animals were brought to the laboratory and kept in a 100L aquarium. The animals were divided into two groups. One group was starved for 1 week and another for 1 month respectively. Survival was monitored every 2 days. The recuperation experiment was conducted in the seaside of Nagasaki Bay with high plankton density. Experimental animals were put inside net cage and hung near the seaside. On days 0, 4, 10, 17, 24 and 31, survival rates were checked and three specimens were collected from each group and the glycogen content in their adductor muscles was analyzed.

Results

Seasonal Variation of Glycogen Contents and Sexual Maturation

The glycogen content was increased in adductor muscle from February to April with a peak of 70~80mg/g in April (**Fig. 4**). The glycogen contents decreased gradually following the development of reproductive glands, showing 15mg/g in August spawning season with continued low-levels (3mg/g) for three months from October to December and accumulating again to 13mg/g following the availability of phytoplankton food in January of the next year. The fluctuating glycogen content could be related to the stage of reproductive gland development during sexual maturation. Reproductive glands of male and female pen shells were observed to have developed into full sexual maturation in May. Both phenomena were well correlated.

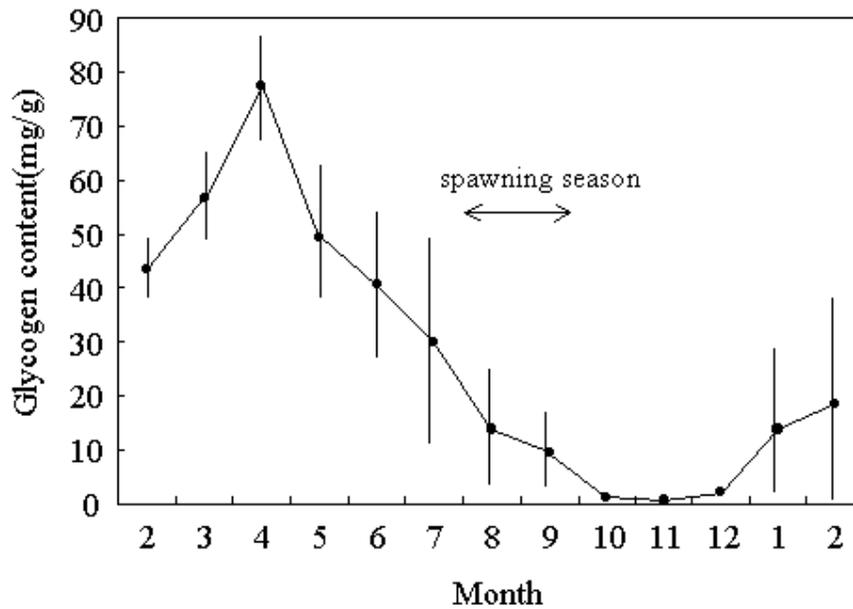


Fig.4 Seasonal changes of glycogen content in pen shells.

Influence of Changes in Glycogen Content by Feed Quantity Environment

(Experiment 1)

The glycogen content of the unfed group decreased continuously from about 12mg/g to 2mg/g over one week (**Fig. 5**) and the specimens started to die after day 7. All specimens had died by day 15. On the other hand, the glycogen content of the fed group remained at the level of 10~12mg/g (**Fig. 6**) and the survival rate was 80% during the course of the experiment.

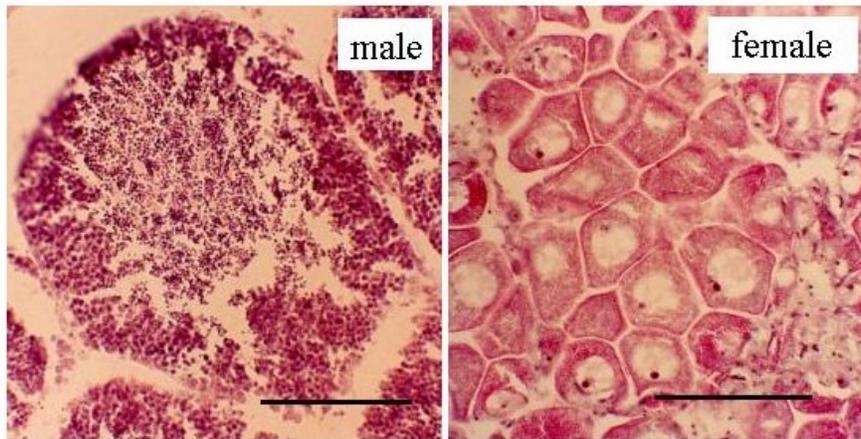


Fig.5 Histological observation of gonads of pen shells in May stained with HE (Hematoxylin and Eosin) . bars 50 μ m.

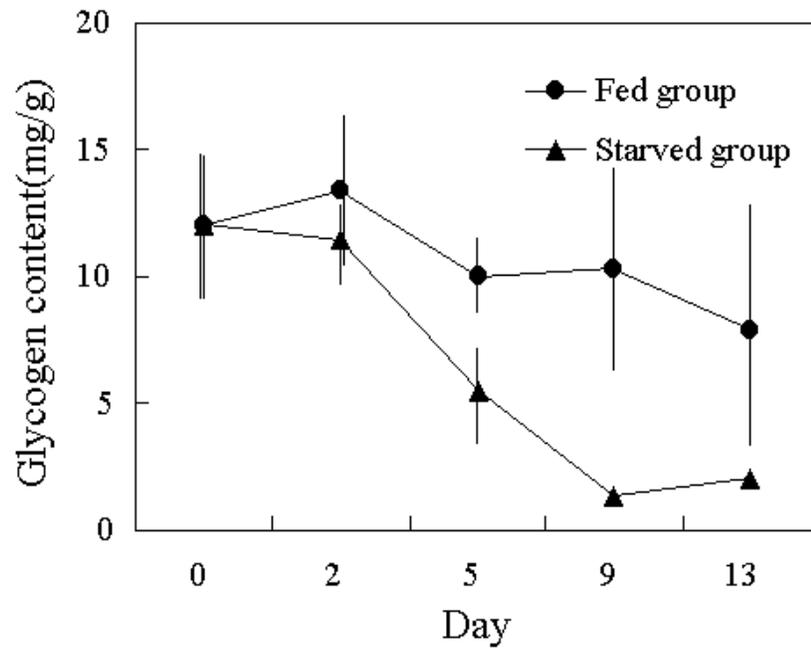


Fig.6 Comparison of glycogen content between fed and starved group

No mortality was observed in the group starved for 1 week, while in the group starved for 1 month, mortality was 15% during the period before the experiment commenced. During the experiment, the survival rate of the one-month group (70%) was lower than the one-week group (100%). Both groups showed differing recuperation processes. The one-week group accumulated glycogen rapidly from 2mg/g to 20mg/g over a one week period while the one-month group couldn't accumulate glycogen rapidly, accumulating glycogen from 2mg/g to 12mg/g over a one month period (Fig. 7).

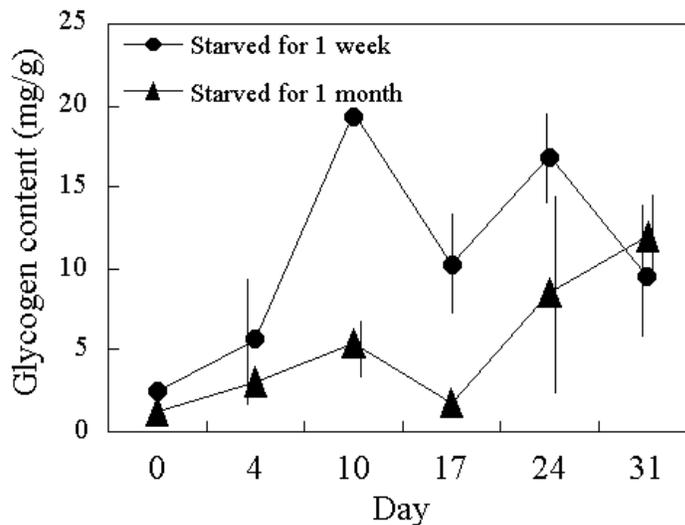


Fig.7 Glycogen content of pen shells during recuperation

Discussion

Seasonal changes of glycogen content in pen shells are deeply influenced by the development of reproductive glands. Glycogen contents were decreased gradually leading up to the spawning season. It was thought that this phenomenon is similar to the seasonality of glycogen content of scallops (Yamanaka 2000), oysters (Yamamura and Watanabe 1964) and pearl oysters (Uchimura 1999). In this study, the glycogen contents in pen shells started to decrease when the reproductive glands entered sexual maturation in May. It is suggested that pen shells rapidly consume glycogen during sexual maturation stage because glycogen is well known as an energy source needed for both life and reproductive gland development.

Numaguchi (1995) investigated tolerance for lack of food for pearl oysters. He reported the relationship between survival rate and glycogen contents in pearl oysters,

observing that the survival rate became low when the glycogen contents were about 3mg/g. In this study, we investigated the tolerance for food deprivation in pen shells and found that survival rate lowered when the glycogen contents in adductor muscle indicated about 2mg/g.

Matsui (2002) surveyed the relationship between survival rate and environmental conditions in the fishing grounds of pen shells in Ariake Bay, 2000. He found the survival rate became gradually decreased during the summer to autumn, 2000. We analyzed the glycogen contents in adductor muscle of pen shells that were caught in the same place during the same period and found the glycogen contents were about 3mg/g during autumn. These results suggest that the survival rate decreased when the glycogen contents approximated only 2~3mg/g in adductor muscle of pen shells. On the other hand, it is considered that pen shell survival rate and glycogen content in spawning season is influenced by a 2 week period of food deprivation.

In the examination of recuperation occurring in beneficial feeding conditions, it was found that post-spawning season pen shells could accumulate glycogen rapidly if good feeding conditions were available. However, the group that had starved for 1 month was slower in accumulating glycogen content than 1 week starved group. This phenomenon suggested that if pen shells were placed in low-level environmental food conditions for a long time such as over 1 month, they would be unable to intake nutrition rapidly.

Bivalves such as scallops, oysters and pearl oysters had been already reported to accumulate glycogen contents rapidly if they had finished the spawning season (Yamanaka 2002, Yamamura 1964, Uchimura 1999). Matsui (2002) reported the environment of pen shell fishing grounds in Ariake Bay in 2000 and found that the occurrence of plankton was continued at low-levels for about 2 months in autumn, 2000. Therefore, it is suggested that pen shells had a low glycogen contents (3mg/g) in the fishing grounds of Ariake Bay for 3 months in autumn 2000 due to low phytoplankton levels in the environment for several weeks.

Literature Cited

- Akashige, S. and Fushimi, T.** 1992. Growth, survival, and glycogen content of triploid pacific oyster *Crassostrea gigas* in the waters of Hiroshima, Japan. *Nippon Suisan Gakkaishi* 58 (6): 1063-1071 (in Japanese).
- Kamada, H. and Hamada, T.** 1985. Comparison of anthrone and enzymatic methods for hepatic glycogen analyses. *N.I.A.I.451* 43: 85-91 (in Japanese).
- Kawahara, I. and Ito, S.** 2003. Mortality of pen shells, *Atrina pectinata* in northeast part fishery of Ariake Sound in summer, 2000 and 2001-I. *Bull. Saga Fish. Exp. Sta.* 21: 7-13 (in Japanese).

- Matsui, S.** 2002. Resources change of the pen shell *Atrina pectinata* in northeast part fishery of Ariake Sea. Bull. Fukuoka Fish. Mar. Technol. Res. Cent. 12: 29-35 (in Japanese).
- Miyazono, A. and Nakano, H.** 2000. Seasonal fluctuations in the protein and glycogen contents of the adductor muscle of scallops, *Patinopecten yessoensis* (Jay), in snowing culture grounds in the Okhotsk Sea, Hokkaido. Sci. Rep. Hokkaido Fish. Exp. Sta. 58: 23-32 (in Japanese).
- Mori, K. et. al.** 1965. Studies on the mass mortality of the oyster in Matsushima Bay IV. Changes in the oyster during the stages of sexual maturation and spawning. Bull. Touhoku. Nat. Fish. Res. Inst. 25: 49-63. (in Japanese).
- Mori, K. et. al.** 1977. Seasonal gonad changes in scallops under culture in Toni Bay, Iwate Prefecture. Nippon Suisan Gakkaishi 43 (1): 1-8 (in Japanese).
- Numaguchi, K.** 1995. Influences of unfed condition on the mortality of pearl oyster *Pinctada fucata martensii*. Fisheries Science 61 (5): 739-742.
- Okutani, T.** 1997. Basic Data of endangered marine species in Japan (IV). 11. Pen shell. JFRCA (Tokyo): 43-47 (in Japanese).
- Shinomiya, Y. et. al.** 1997. Relationship between autumn mortality, glycogen content, and carbohydrate metabolism enzyme activity in Japan Pearl Oyster *Pinctada martensii*. SUISANZOSHOKU 45 (1): 47-53 (in Japanese).
- Takagi, I and Shimizu, W** 1963. Studies on muscle of aquatic animals-XXXV. Seasonal variation of chemical constituents and extractive nitrogens in some species of shellfish. Nippon Suisan Gakkaishi 29(1): 66-70 (in Japanese).
- Toba, M. et. al.** 1993. Reproductive cycles of Manila Clam collected from Funabashi waters, Tokyo Bay. Nippon Suisan Gakkaishi 59 (1): 15-22 (in Japanese).
- Uchimura, U.** 1999. The pearl formation on physiological aspects in triploid Japanese pearl oyster, *Pinctada fucata martensii*. Bull. Ehime Fish. Exp. Sta. 7: pp.1-68 (in Japanese).
- Yamamura, Y. and Watanabe, T.** 1964. Seasonal changes of glycogen content in oysters

in Matsushima Bay, Japan. Nippon Suisan Gakkai Touhoku Shibu Kaihou 16: 22-26 (in Japanese).

Yamanaka, H. 2002. Relation between post mortem biochemical changes and quality in the muscle of fish and shellfish. Nippon Suisan Gakkaishi 68 (1): 5-14 (in Japanese).

Yamasaki, M. 1993. Reproductive cycle of the bivalve *Barnea dilatata* in Ariake Bay. Bull. Seikai Nati. Fish. Res. Inst. 71: 17-31.

Yokogawa, K. 1996. Genetic divergence in two forms of pen shell *Atrina pectinata*. VENUS (Jap. Jour. Malac.) 55 (1): 25—39 (in Japanese).