INTRODUCTION

The green sea urchin *Hemicentrotus pulcherrimus* is widely distributed throughout Japan. It is an important coastal fishery product along the south-west coasts of Japan, but is hardly utilized along the north-east coasts (i.e. Fukushima Prefecture). The edible parts of a sea urchin are its gonads. At Iwaki, Fukushima Prefecture, green sea urchins with bitter-tasting gonads are often found and, thus, they usually have little commercial value. In the Iwaki area, grilled sea urchin gonads named *kaiyaki* are a special local product, and is obtained from the sea urchin *Strongylocentrotus nudus* and not the green sea urchin. In southern Hokkaido, the green sea urchin is also known to have bitter-tasting gonads. A previous investigation we conducted at Iwaki has suggested that the bitterness of the green sea urchin is specific to mature ovaries. From the mature ovaries of the green sea urchin, a novel sulfur-containing amino acid, pulcherrimine, was isolated and its structure was determined as 4S-(2'-carboxy-2'S-hydroxyethylthio)-2R-piperidine-carboxylic acid. This compound exhibits bitterness with a threshold value of 0.31 mM. Subsequently, an analytical method for pulcherrimine using dimethylaminobenzene sulfonyl chloride (Dabs-Cl) has been developed, and the pulcherrimine content in green sea urchin individuals sampled in November 1998 were determined. These analytical experiments, together with sensory testing, demonstrated that the bitterness of the green sea urchin and the pulcherrimine content in its gonads are significantly correlated. Previous studies we have done have suggested that the main bitter...
principle in the mature ovaries of the green sea urchin is pulcherrimine.\textsuperscript{2–5}

Many studies about the maturation and spawning of the sea urchin have been carried out.\textsuperscript{6–12} However, seasonal changes of maturity and the concurrent changes in the frequency of bitter-tasting gonads of the green sea urchin in the Iwaki area have not been investigated.

Therefore, the relationship between maturation and the bitterness of green sea urchin gonads has not been fully clarified.

In the present study, we carried out tri-monthly investigations of the frequency of bitter gonads of the green sea urchin and their pulcherrimine contents, which were sampled off Iwaki in Fukushima Prefecture, in order to clarify the relationships among maturation, bitterness, and pulcherrimine content.

MATERIALS AND METHODS

Green sea urchin

At each tri-monthly sampling, 100 green sea urchins were collected off Iwaki, Fukushima Prefecture, from November 1998 to November 1999. After collection, they were stored in a cool box kept below 5°C and transferred to the laboratory. Sea urchin individuals were measured, weighed, and their gonads removed.

The gonad index (GI) of each individual was calculated as follows:

\[ \text{GI(%)} = \left( \frac{\text{Gonad weight}}{\text{bodyweight}} \right) \times 100 \quad (1) \]

Determination of sex and maturity

The individuals examined were divided into mature and immature specimens. The present study defines mature individuals as those with gametes that ooze from the gonads; whereas immature individuals are those with gametes that do not ooze from the gonads. The sex of mature individuals was identified from the oozed gametes.\textsuperscript{13}

Four to seven immature individuals of those collected every 3 months were observed histologically in order to determine the stage of their gonadal development. This was done by fixing small pieces of gonad from each individual in Bouin’s solution, embedding them in paraffin, and sectioning at a thickness of 6 \( \mu \)m. The sections were stained with hematoxylin and eosin, and then observed under a microscope to determine the sex and gametogenic stage of the gonads. The gonadal maturity of each individual was assessed according to the stage classification of Fuji.\textsuperscript{13}

Analysis of pulcherrimine

For the analysis of pulcherrimine, 20 specimens were randomly selected from each of the mature ovaries and testes obtained every 3 months. Samples were prepared and analyzed as described elsewhere.\textsuperscript{5}

Sensory test

First, the gonads underwent a brief sensory test for the preliminary testing for bitter taste in the same manner as that described elsewhere.\textsuperscript{2} After a brief test, all gonad extracts were tested for bitterness by three subjects using a single sample test. In this test, each subject judged and evaluated the bitterness of a sample after tasting the prepared extracts. A sample was defined as being ‘bitter-tasting’ if judged bitter by more than two subjects.

Statistical analysis

To compare groups from tri-monthly samplings, we performed the \( F \)-test for the difference of variance in each group. When the variance was equal, Student’s \( t \)-test was used, but if the variance was different, the Cochran–Cox test was used.\textsuperscript{14}

RESULTS

Frequency of mature males, females, and immature individuals

Table 1 shows the frequency of mature males, females, and immature green sea urchins. In November 1998 and February 1999, all of the sea

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<tr>
<th>Table 1</th>
<th>Number of mature males and females, and immature individuals*</th>
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<tr>
<td></td>
<td>Mature Male</td>
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<tr>
<td>November 1998</td>
<td>56</td>
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<tr>
<td>February 1999</td>
<td>57</td>
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<tr>
<td>May 1999</td>
<td>50</td>
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<tr>
<td>August 1999</td>
<td>20</td>
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<tr>
<td>November 1999</td>
<td>56</td>
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* In each month, 100 individuals were examined.
urchins sampled were mature, of which males comprised 60%. In May and August 1999, the frequency of immature individuals was relatively high; that is 20% and 60%, respectively. Seven specimens from each sample out of the immature individuals collected in May and August 1999 were subjected to histological observations. The observations revealed that these individuals were at the recovering spent stage. The frequency of mature males was 50% and 20%, and that of mature females was 30% and 20% in May and August, respectively. In November 1999, 56% of individuals were identified as male and 40% as female. Four immature individuals (4%) were identified as females by microscopy observations; two of them were judged to be at the growing stage, and the other two at the premature stage.

**Test diameter, test height, bodyweight, and gonad index**

The mean test diameter, test height, and bodyweight of individuals were approximately the same throughout the 5 months' observation period; that is, 22 mm, 41 mm, and 23 g, respectively (Table 2).

Seasonal changes in the GI of each sex of the sea urchin are shown in Fig. 1. The GI values varied considerably among the mature specimens for each season. The mean GI values of mature male and female gonads decreased significantly from February to May and increased significantly from May to August, suggesting that spawning occurs from February to May. The GI of the immature specimens obtained in May, August, and November 1999 were 8.3 ± 0.8%, 12.7 ± 0.5%, and 7.5 ± 1.0%, whereby these values were relatively lower than those of mature specimens.

**Frequency of bitter-tasting gonads of the green sea urchin for each season**

Figure 2 shows the frequency of bitter gonads for each season. More than 95% of the ovaries of sea urchins collected in November 1998, February 1999, and November 1999 tasted bitter. Conversely, in May and August 1999, 60% of the mature ovaries were bitter. Immature gonads had no bitter taste in May and November, but 20% of immature gonads in August 1999 tasted bitter.

**Pulcherrimine content of the tri-monthly samples**

Twenty specimens were selected randomly from each of mature ovaries and testes and analyzed for pulcherrimine content for each month sampled. No pulcherrimine was detected in mature testes and they had no bitter taste.

Figure 3 shows histograms representing the distribution of pulcherrimine content among mature female individuals for each of the months sampled. Pulcherrimine content distributions had large variances for all months examined and were different among months. Many individuals with pulcherrimine levels greater than 0.5 mg/100 g were found in November 1998, February 1999, and November 1999. Histograms for February and

<table>
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<th>Table 2. Test height, test diameter and bodyweight of green sea urchin caught between November 1988 and November 1999 (mean±SD)</th>
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<tr>
<td><strong>Month</strong></td>
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<td>------------</td>
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<tr>
<td>Mature males</td>
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Fig. 1  Seasonal changes in the values of gonad indices (mean ± SE) of: (a) mature males; and (b) mature females of the green sea urchin inhabiting the sea area off Iwaki. (a–c) indicate that the mean values are significantly different from each other (P<0.05).

Fig. 2  Ratios of bitter-tasting (□) mature males, (●) mature females, and (▲) immature individuals in each month sampled.

Fig. 3  Frequency distribution of pulcherrimine contents among mature female individuals for each season. (□) Non-bitter ovaries and (■) bitter ovaries. ND, pulcherrimine was not detected.
November 1999 show that the mean pulcherrimine content (1.59 mg/100 g and 0.93 mg/100 g) was located within the mode column. Also in November 1998, the mean pulcherrimine content (1.37 mg/100 g) was located near the mode. In contrast, during May and August 1999, the mode of each pulcherrimine level was at the lowest column, and many mature ovaries with no pulcherrimine were found. Distributions were highly skewed for these two months. Some non-bitter ovaries have been found to have pulcherrimine, but at a content of less than 0.5 mg/100 g.

In August, we analyzed 11 bitter immature gonads for pulcherrimine content. Their pulcherrimine contents ranged from 0.6 mg/100 g to 2.3 mg/100 g.

**DISCUSSION**

In previous investigations we did in March 1996 and 1997, most of the green sea urchins examined were mature and 95% of female individuals had a bitter taste.2

In the present tri-monthly study, seasonal changes in the maturation, bitterness, and pulcherrimine content of the green sea urchin collected off Iwaki were examined. The fact that the GI values showed a large variation among the mature specimens for each season indicates that the maturation process of the sea urchins might vary among individuals. The mean GI values decreased significantly during the period between February and May 1999, were at their lowest values in May 1999, and increased thereafter. These seasonal changes in the GI values suggest that the major spawning season is during the period from February to May. In May and August 1999, many immature individuals were observed but, concurrently, many mature female and male individuals were also observed, which suggests that mature individuals occur during all seasons in the sea off Iwaki. After considering the variations in the individual GI values and the presence of mature individuals throughout all seasons, the reproductive cycle of the green sea urchin seems to be unclear, and the occurrence of the mature stage extends over a long-term, in this area. The maturation and subsequent spawning behavior are generally considered to be controlled by the seawater’s temperature.1 Ito et al. have described that, for the green sea urchin, a temperature depression after a high water temperature period accelerates the maturation of gonads.10 In the sea area off Saga Prefecture, it has been observed that spawning starts when the water temperature drops to 15°C.10 In the sea off Iwaki, the water temperature remains above 20°C from August to October, reaches its highest value in September and then declines, while from December to June, the water temperature is usually below 15°C.15 Therefore, the variation of the maturation process and the long-term maturation period among individual sea urchins in the sea off Iwaki may be due to the relatively low water temperature.

The frequency of bitter ovaries accounted for more than 95% of the total examined in November 1998, February 1999 and November 1999, and accounted for 60% of the total number of mature ovaries, even in May and August 1999. From these results, the seasonal change in the frequency of bitter ovaries is in agreement with that of the distribution of mature ovaries.

Pulcherrimine content distributions of mature ovaries had a large variance for every month sampled and were also different among months. Pulcherrimine levels of mature ovaries were highest in February, and those of mature ovaries in November 1998 were relatively high. In November 1999, most of the mature ovaries analyzed had more than 0.5 mg/100 g of pulcherrimine. Conversely, in May and August 1999, pulcherrimine levels of mature ovaries were relatively low and we found many ovaries without pulcherrimine. These results suggest that the amount of pulcherrimine is related to the seasonal change of the ovaries of the green sea urchin.

In August, we found 11 immature gonads to be bitter and their pulcherrimine content ranged from 0.6 mg/100 g to 2.3 mg/100 g. From these results, we propose that pulcherrimine is initiated during the early stages of gonadal development; however, further examination of the relationship between the presence of pulcherrimine and gonadal development of the green sea urchin is necessary to confirm this.

From the analysis of 20 mature testes every 3 months, it was demonstrated that pulcherrimine is not detected in mature testes. These results are consistent with a previous report.5

Gonads that did not contain pulcherrimine had no bitter taste. Alternatively, less than 0.5 mg/100 g of pulcherrimine was detected occasionally in non-bitter gonads. It is probable that the pulcherrimine content was lower than the taste threshold in these gonads.

One of the reasons why the green sea urchin has no commercial value in the Iwaki area is thought to be the presence of mature sea urchins whose gonads taste bitter during all seasons. Similarly, it has been suggested that the reason why the green sea urchin in south Hokkaido is unappetizing due to its bitter taste is because it matures over a long period, therefore resulting in long-term bitterness.
It is recommended that further investigations of the distribution of the green sea urchin and the seasonal occurrence of pulcherrimine be done in other geographical areas.

ACKNOWLEDGMENT

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REFERENCES


