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ORIGINAL ARTICLE

Biology

Extension of immature stage of *Hemicentrotus pulcherrimus* ovary for reduction of the bitter compound (pulcherrimine) by water temperature manipulation

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Abstract

Hemicentrotus pulcherrimus is usually consumed in summer when the ovaries and testes are immature and tasty, and is not utilized from autumn to spring as a bitter compound, pulcherrimine accumulates in the ovaries. However, in the northern distribution edge (Tohoku and Hokkaido), it is not consumed because the ovaries are bitter nearly all year. Our previous studies suggested that the short palatable season in northern *H. pulcherrimus* is caused by a lower seawater temperature. To test this hypothesis, we reared *H. pulcherrimus* collected in Hokkaido at manipulated temperatures. Higher temperatures than the natural temperature at the collection site from August to March delayed gametogenesis and pulcherrimine accumulation, which usually begins in autumn. Temperature elevation during the spawning season (April) advanced the transition of mature ovaries to immature ovaries and led to the disappearance of the remaining pulcherrimine. Seawater temperature estimated using an ocean forecast system revealed that the warmer season, when the temperature exceeds 20 °C, is shorter where *H. pulcherrimus* is not consumed. We believe that the short palatable season of *H. pulcherrimus* in the northern edge of the distribution is due to the short summer season. Such *H. pulcherrimus,* however, can be improved by cultivation for several months at elevated temperatures.

Keywords Bitterness · Egg · Gametogenesis · Gonad · *Hemicentrotus pulcherrimus* · Maturation · Pulcherrimine

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Introduction

Hemicentrotus pulcherrimus is a popular edible sea urchin found in Japan. It occurs in the intertidal and subtidal zones from Kyushu to Tohoku in the Pacific Ocean and from Kyushu to Hokkaido in the Sea of Japan (Agatsuma 2020). *Hemicentrotus pulcherrimus* is one of the most important fishery products from the central to the southwest coast, for example in Fukui and Yamaguchi Prefectures (Kawana 1938). Like other sea urchins, the ovaries and testes comprise the edible parts of this species (Unuma and Walker 2009). The species spawns from winter to spring, depending on the location (Agatsuma 2020; Ogasawara et al. 2011), and is mainly caught in summer (non-reproductive season) (Murata et al. 2021). They are usually used to produce salted gonads such as Echizen-Uni (produced in Fukui Prefecture) and Shimonoseki-Uni (produced in Yamaguchi Prefecture) (Kawana 1938; Miwa 1970; Unuma 2015). However, *H. pulcherrimus* with a distribution in the Hokkaido and Tohoku (such as Fukushima Prefecture) areas are not used because of the extremely bitter-tasting ovaries, which persist for most of the year (Murata et al. 2002).

We previously clarified that the bitter taste is caused by the ovarian accumulation of pulcherrimine (Pul), a sulfur-containing amino acid [4*S*-(2′-carboxy-2′*S*-hydroxyethylthio)-2*R*-piperidinecarboxylic acid] (Murata et al. 1998, 2000, 2002, 2021; Murata and Sata 2000; Sata et al. 2001). *Hemicentrotus pulcherrimus* ovaries containing more than 0.5 mg/100 g of Pul taste bitter (Murata et al. 2002). We recently examined the annual reproductive cycles and Pul accumulation in the ovaries of *H. pulcherrimus* collected from Fukui and Fukushima Prefectures (Murata et al. 2021). In sea urchins obtained from both regions, Pul levels increased in maturing ovaries as oogenesis proceeded (from autumn to winter) and accumulated primarily in the eggs, rather than in the nutritive phagocytes (nutrient storage cells in the gonad). Most of the Pul was released from the ovaries along with the eggs during spawning (in spring), but some Pul remained in the spent ovaries with the residual eggs (Murata et al. 2021). In Fukui Prefecture, the remaining Pul gradually degraded and was barely detectable in the ovaries over several months in summer. However, in Fukushima Prefecture, late spawning delayed the dissipation of Pul in summer, and the early initiation of oogenesis coincided with the early restart of Pul accumulation in autumn. Consequently, the period when the ovaries were palatable due to the absence of Pul was shortened or completely lost (depending on the year) in the Fukushima Prefecture.

The reproductive cycles of sea urchins are regulated by environmental factors such as water temperature and photoperiod (Kirchhoff et al. 2010; Kayaba et al. 2012; Unuma et al. 2015; Walker et al. 2020; Ishii et al. 2022). The gonadal maturation of Japanese sea urchins, such as *H. pulcherrimus*, *Heliocidaris crassispina*, and *Pseudocentrotus depressus*, is more strongly affected by water temperature than by photoperiod (Yamamoto et al. 1988; Sakairi and Yamamoto 1989). *Hemicentrotus pulcherrimus* gonads are induced to mature when the water temperature decreases from 25–26 to 15 °C (Ito et al. 1989; Sakairi and Yamamoto 1989). In our previous study (Murata et al. 2021), we observed that the seawater temperature at the study site in Fukushima Prefecture was 1–7 °C lower than that in Fukui Prefecture throughout the year. Considering these facts, we hypothesized that the cause of the short period for utilization of this species in the northern edge of the distribution (Tohoku and Hokkaido) (which is attributable to the delayed dissipation of Pul in summer and the advanced restart of Pul accumulation in autumn) could be the lower seawater temperature (Murata et al. 2021).

The present study had two main objectives: (1) to experimentally demonstrate our hypothesis that the short palatability of northern *H. pulcherrimus* is caused by low seawater temperature, and (2) to find a way to extend the palatable season of *H. pulcherrimus* in the regions. To this end, we reared *H. pulcherrimus* collected from Hokkaido, where it is not utilized because of the bitter-tasting ovaries, under different temperature conditions and compared the maturational status of the gonads and the Pul content in the ovaries.

Materials and methods

Animals

Wild individuals of *H. pulcherrimus* were collected by scuba diving at depths of 2–5 m in Funahama, Otaru city, Hokkaido (43°10′51.60″ N, 141°02′19.10″ E) on 18 May 2009, for Experiment 1 and at depths of 2–3 m in Shioya, Otaru city, Hokkaido (43°12′43.45″ N, 140°54′41.77″ E) on 12 April 2010, for Experiment 2. After collection, urchins were placed in cool boxes with wet paper and transported to the National Research Institute of Fisheries Science, Yokohama, Kanagawa Prefecture. They were acclimated in 0.1-tonne tanks that were aerated and supplied with sand-filtered seawater (flow-through system) at ambient temperature. Throughout the experiment, the photoperiod in the aquarium house was manipulated to simulate the natural photoperiod at Yokohama, using a timer switch and a fluorescent lamp. The urchins were starved until they were used in the rearing experiments.

Experiment 1

The first experiment examined the relationship between seawater temperature and gametogenesis progress. The

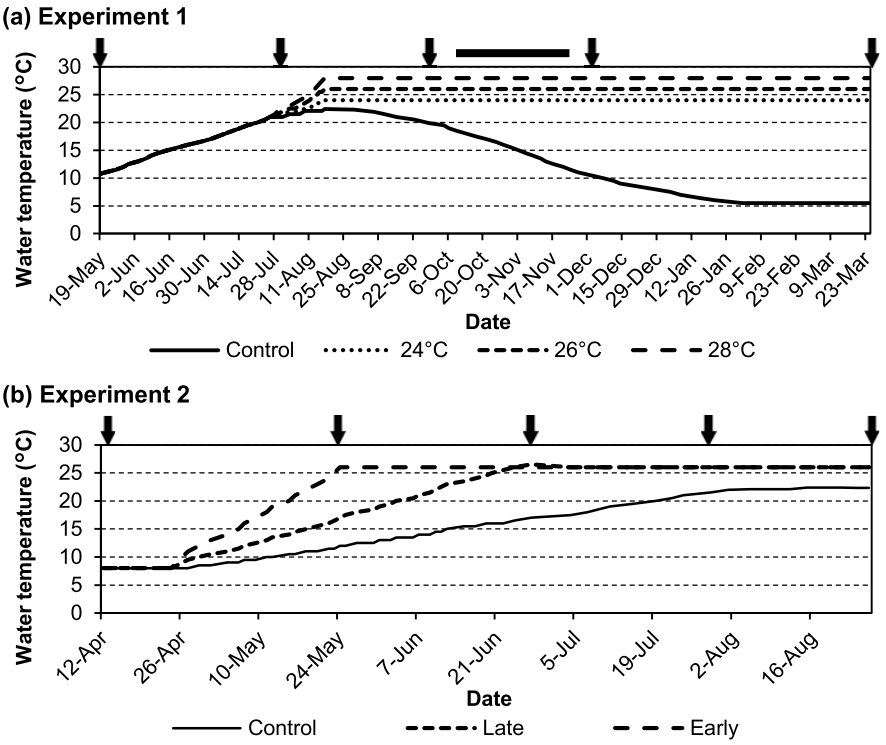

Fig. 1 The experimental design for rearing *Hemicentrotus pulcherrimus* under manipulated water temperatures. **a** Experiment 1. Sea urchins were cultivated at four different temperatures from 29 July 2009, to 24 March 2010. In the control group, the temperature was continuously adjusted to the 30-year mean seawater temperature in Yoichi. In the 24, 26, and 28 °C groups, the water temperature was gradually increased until it reached 24, 26, and 28 °C, respectively, on 17 August, and was kept constant until the end of the experiment. Data represent the preset temperature of the heating–cooling equipment. Sixteen to 24 urchins were sampled for dissection on 19 May, 29 July, 28 September, 2 December, and 24 March as indicated with *arrows*. Daily food intake was measured from 12 October to 24 November as indicated with a *bar*. **b** Experiment 2. Sea urchins were cultivated at three different temperatures from 26 April to 26 August 2010. In the control group, the temperature was adjusted to the 30-year mean seawater temperature in Yoichi. In the late and the early groups, the water temperature was gradually increased at a rate of 0.3 and 0.6 °C/day until it reached 26 °C. Data represent the preset temperature of the heating–cooling equipment. The difference between the preset and actual temperature was 0.1–0.6 ℃. Fifteen to 20 urchins were sampled for dissection on 13 April, 25 May, 29 June, 27 July, and 27 August as indicated with *arrows*

experimental design, including the water temperature and sampling schedule, is illustrated in Fig. 1a. Three hundred urchins (46 ± 2 mm test diameter) were reared in four circular tanks (inner dimensions, φ 56.5 × D 45 cm; water depth, 30 cm) that were aerated and supplied with sand-filtered seawater (2 l/min/tank, flow-through system) from 19 May to 29 July 2009. The seawater temperature was controlled using electric heating–cooling equipment (Model APS-1, Koito Manufacturing Co., Ltd., Tokyo, Japan). The preset temperature of the heating–cooling equipment was adjusted every 1–5 days to simulate the 30-year mean seawater temperature from 1971 to 2000 in Yoichi, Hokkaido (43°12′14″ N, 140°46′29″ E), which was measured daily at 09:00 on the sea surface and averaged for three 10-day periods in each month by the Central Fisheries Research Institute of the Hokkaido Research Organization (http://www.hro.or.jp/list/fisheries/research/central/section/kankyou/suion/index.html; accessed 23 Dec 2021). This temperature was adopted as the control temperature for Experiments 1 and 2. On 29 July 2009, 224 urchins were randomly divided into four groups for temperature treatments (56 individuals per group): control, 24 °C, 26 °C, and 28 °C. These temperatures reflect the average seawater temperatures in August at Otaru (22 ℃) and Mikuni (28 °C). Thirty-six urchins were not used for temperature treatment. The urchins in each group were housed in a circular tank (internal dimensions, φ 36.5 × D 31.5 cm; water depth, 20 cm) that was aerated and supplied with sand-filtered seawater (2 l/min/tank, flow-through system). In the control group, the water temperature was adjusted to the control temperature described above, until the end of the experiment (24 Mar 2010). In the 24, 26, and 28 °C groups, the water temperature was gradually increased until it reached 24, 26, and 28 °C on 17 August 2009, and was kept constant until the end of the experiment. As daily

temperature variations were not recorded during the rearing experiments, the difference between the temperature measured in the rearing tanks and the preset temperature of the heating–cooling equipment was determined after the experiments, and was observed to be within ± 0.5 °C.

The urchins were fed dried *Saccharina japonica* (from May to July and from December to March), or live *Eisenia bicyclis* and *Ecklonia cava* (from August to November), *ad libitum*. To examine the influence of rearing temperature on feeding, food intake was measured from 12 October to 24 November, when live macroalgae could be stably obtained because dried *S. japonica* was not suitable for this measurement due to its perishability in seawater. *Eisenia bicyclis* was used from 12 October to 26 October and *Ecklonia cava* from 27 October to 24 November. The wet weight of the provided food and uneaten food was measured every 1 to 3 days (13 time points for *Eisenia bicyclis* and 16 time points for *Ecklonia cava*). The daily food intake of the urchins in each group was calculated separately for *Eisenia bicyclis* and *Ecklonia cava* using the following equation:

Daily food intake (g/individual/day)
= (wet weight of provided food−wet weight of uneaten food)/
number of individuals/days for food intake measurement.

On 19 May and 29 July 2009, 20 individuals that were not included in the four experimental groups were dissected, following the sampling procedure described below, as the initial controls for acclimation (19 May) and temperature treatment (29 July). On 28 September and 2 December 2009, 16 individuals were randomly sampled from each experimental group for dissection. At the end of the experiment (24 Mar 2010), all remaining urchins were dissected.

Experiment 2

The second experiment was conducted to examine the effects of the time of initiating higher temperature treatment on the disappearance of gametes from the gonads. As 26 °C was shown to be effective at delaying the initiation of gametogenesis in autumn without a decrease in food intake in Experiment 1, we chose this temperature for the higher temperature treatments in Experiment 2. The experimental design is illustrated in Fig. 1b. On 13 April 240 urchins (46 ± 4 mm test diameter) were randomly divided into three groups for temperature treatments (80 individuals per group): control, late, and early. The urchins in each group were accommodated in a circular tank (inside dimensions, φ36.5 × D 31.5 cm; water depth, 20 cm) that was aerated and supplied with sand-filtered seawater (2 l/min/tank, flow-through system). The water temperature was maintained at 8 °C from 13 April to 26 April in all groups, and then altered as follows. In the control group, the water temperature was adjusted to the control temperature described above, until the end of the experiment (27 August 2010). In the late and early groups, the water temperature was gradually increased at a rate of 0.3 and 0.6 °C/day until it reached 26 °C on 29 June and 25 May, respectively, and the temperature was then kept at 26 °C until the end of the experiment. On 13 April, 20 individuals that were not included in the three experimental groups were sampled for dissection as an initial control group. On 25 May, 29 June, 27 July, and 27 August, 15 to 16 individuals were randomly sampled from each group for dissection. The other procedures performed were the same as those described in Experiment 1, except that food intake was not measured.

Sampling procedure

The test diameter and wet body weight of the sampled sea urchins were measured, after which they were cracked open for dissection. The gonads were completely removed, rinsed with ice-cold seawater, blotted with a paper towel, and weighed. The gonad index (GI) of each individual was calculated using the following equation:

GI (%) = (wet gonad weight/wet body weight) × 100.

A small piece (ca. 0.1 g) of gonad obtained from each sea urchin was fixed in Bouin's solution for histological observation. The remaining gonads were stored at − 80 °C for Pul analysis.

Histological observation

The fixed gonad was dehydrated, embedded in paraffin, sectioned (10-μm thick), dewaxed, stained with hematoxylin and eosin, and examined under a light microscope. The gonadal maturity of each sea urchin was classified into Fuji's (1960) five stages with some modifications (Unuma 2002), as follows: recovering (stage 1, before gametogenesis), growing (stage 2, early gametogenesis), pre-mature (stage 3, mid gametogenesis), mature (stage 4, late gametogenesis), and spent (stage 5, after spawning).

Pulcherrimine analysis

Pul analysis was performed using Dabs-Cl as the labeling reagent, according to Murata et al. (2001).

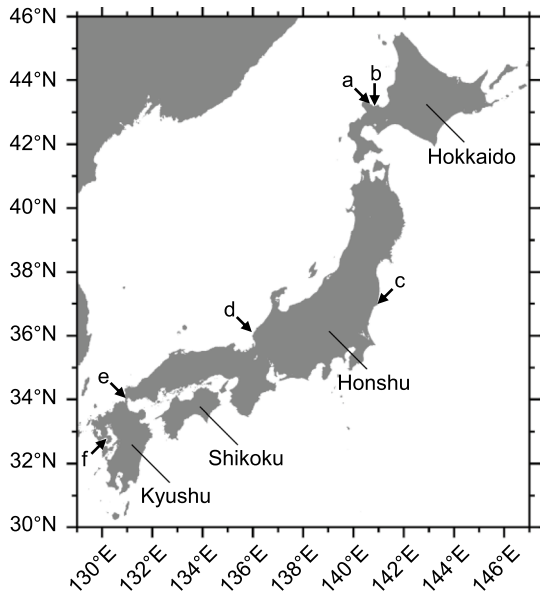

Fig. 2 Location of the sites for *Hemicentrotus pulcherrimus* collection and seawater temperature estimation. *H. pulcherrimus* used for rearing experiments were collected from Yoichi (**a**). Changes in the seawater temperatures were estimated from Otaru (**b**), Iwaki (**c**), Mikuni (**d**), Shimonoseki (**e**), and Unzen (**f**) using FRA-ROMS, an ocean forecasting system specialized for fisheries science (Kuroda et al. 2015). *H. pulcherrimus* are not utilized as food at **a**–**c**, and utilized as food at **d**–**f**

Seawater temperature estimation

Seawater temperatures at five sites where *H. pulcherrimus* is utilized or not utilized as a food product were compared using FRA-ROMS, an ocean forecasting system specialized for fisheries science (Kuroda et al. 2017). Daily changes in temperature from 1993 to 2018 were estimated off Otaru (Hokkaido), Iwaki (Fukushima Prefecture), Mikuni (Fukui Prefecture), Shimonoseki (Yamaguchi Prefecture), and Unzen (Nagasaki Prefecture), at a depth of 2 m. The locations of these sites are indicated in Fig. 2. The 26-year mean seawater temperature was calculated, and the number of days per year for which the water temperature exceeded a specific temperature (for temperatures ranging from 15 to 24 °C) was counted for each site.

Statistical analysis

Data are expressed as mean ± standard deviation (SD) unless otherwise specified. Statistical analyses were performed using Statistica v13.1 (Dell Software, Inc., Aliso Viejo, CA, USA) or BellCurve for Excel v3.21 (Social Survey Research Information Co. Ltd., Tokyo, Japan). The frequency of stage 1 gonads in the temperature-treated groups was compared with that in the control group for each sex using the Cochran–Mantel–Haenszel

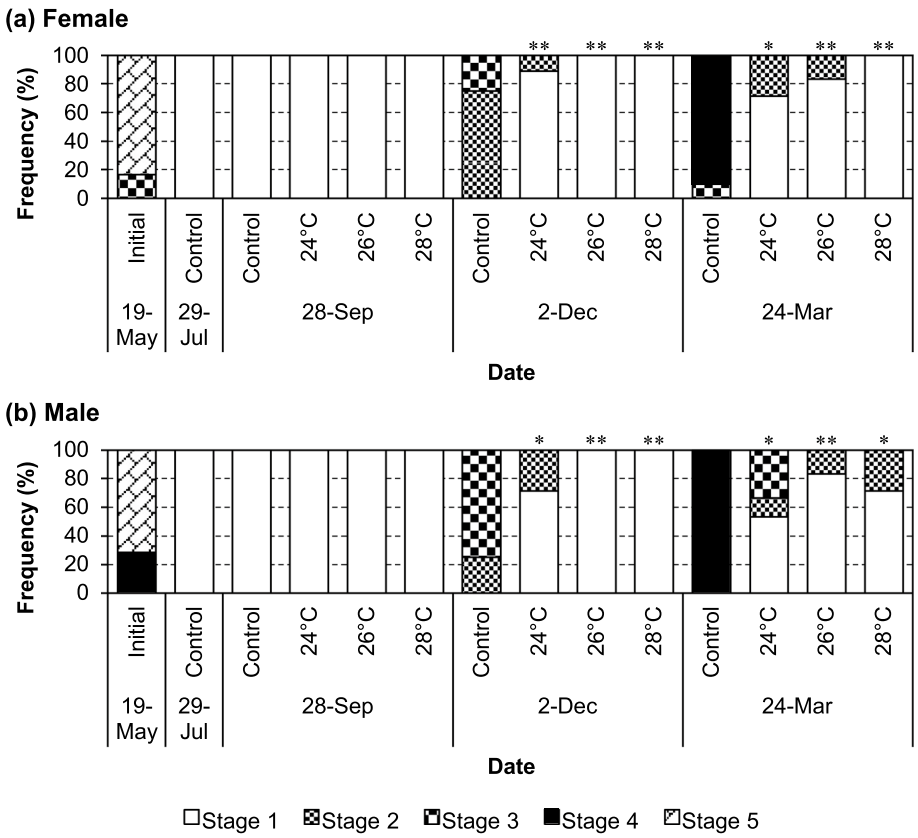

Fig. 3 Frequency distribution in maturation stages of *Hemicentrotus pulcherrimus* gonads reared under manipulated water temperatures in Experiment 1. **a** Females. **b** Males. Gonadal maturity of each animal (4–18 individuals) was classified as stages 1–5 according to Fuji (1960) with some modifications (Unuma 2002). *Asterisks* indicate the frequencies of stage 1 in the experimental groups that are significantly different from that in the control group on the same date (*, $P < 0.05$; **, $P < 0.01$)

test with Fisher's exact test. The significance level for multiple comparisons was adjusted using a Bonferroni correction. The GI and Pul content values in the temperature-treated groups were compared with those in the control group for each sex using Dunnett's multiple comparison test. The GI data were arcsine-transformed before analysis. Statistical significance was set at $p < 0.05$.

Results

Experiment 1

During the rearing period, 4, 2, 0, and 9 urchins died in the control, 24 °C, 26 °C, and 28 °C groups, respectively.

The frequency distribution of the maturation stages of the gonads is shown in Fig. 3. At the beginning of the experiment, all female and male urchins were in the pre-mature, mature, or spent stages (stages 3–5). On 29 July and 28 September, all female and male urchins were in the recovering stage (stage 1). On 2 December, all urchins in the control group had started gametogenesis, with some of them exhibiting gametes (stage 3). In contrast, a larger proportion of urchins in the 24 °C group and all urchins in the 26 °C and 28 °C groups remained immature, regardless of sex. By 24 March, most of the female and male urchins in the control group were fully mature. However, in the other groups, a large proportion of urchins remained immature in both sexes. The proportion of urchins in the recovering stage (stage 1) was significantly higher in the temperature-treated groups than in the control group from December to March in both sexes.

The variations in the mean GI values are presented in Fig. 4. At the beginning of the experiment, the mean GI was 15.3 ± 6.7% in females and 12.8 ± 3.3% in males. Similar values were maintained until 2 December in the control, 24 °C, and 26 °C groups, respectively. By 24 March, both the female and male GI values in the control group were

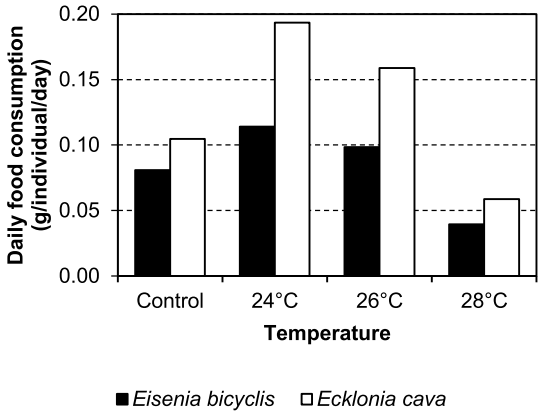

Fig. 5 Daily food intake of *Hemicentrotus pulcherrimus* reared under the manipulated water temperatures in Experiment 1. Values represent results obtained from single tanks ($n = 1$)

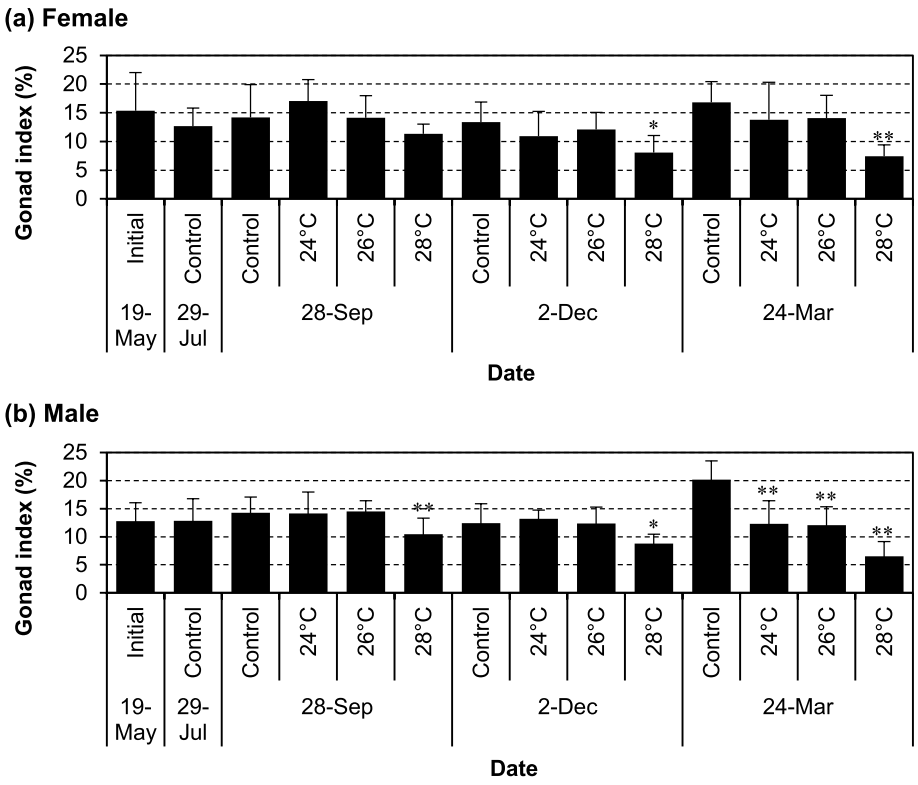

Fig. 4 Gonad indices of *Hemicentrotus pulcherrimus* reared under the manipulated water temperatures in Experiment 1. **a** Females. **b** Males. Values are the means ± SD of 4–18 individuals. *Asterisks* indicate values that are significantly different from that in the control group on the same date (*, $P < 0.05$; **, $P < 0.01$)

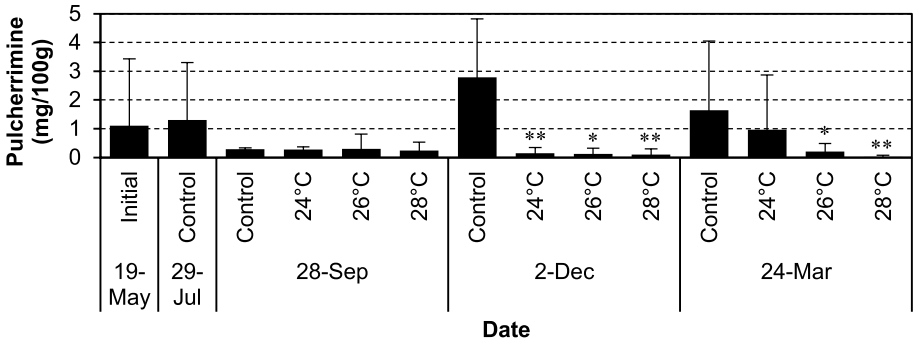

Fig. 6 Pulcherrimine contents of *Hemicentrotus pulcherrimus* ovaries reared under the manipulated water temperatures in Experiment 1. Values represent the mean ± SD of 4–18 individuals. *Asterisks* represent values that are significantly different from that in the control group on the same date (*, $P < 0.05$; **, $P < 0.01$)

raised. The GI value of males in the control group was significantly higher than those in the other groups. The GI values of the 28 °C group were always lower than those of the other groups for both sexes. The values in the 28 °C group were significantly lower than those in the control group from December to March for females and from September to March for males.

Figure 5 shows the daily food intake from 12 October to 24 November. For both *E. bicyclis* and *E. cava*, the urchins consumed more macroalgae in the 24 °C and 26 °C groups compared with the control during this period, while urchins in the 28 °C group exhibited reduced consumption compared with the control. The values were slightly higher for *E. cava* in each group.

Figure 6 shows the variations in mean Pul content in the ovaries over time. At the beginning of the experiment (19 May), the mean Pul content was 1.1 ± 2.3 mg/100 g, and this level was maintained up to 29 July. By 28 September, however, the mean values decreased to less than 0.3 mg/100 g in all of the groups. On 2 December, the mean Pul content increased to 2.8 ± 2.0 mg/100 g in the control group. In contrast, no increase was observed in the temperature-treated groups, which exhibited mean Pul content values that were significantly lower than that of the control group. On 24 March, the mean Pul content increased to 1.0 ± 1.9 mg/100 g in the 24 °C group, while they did not increase in the 26 °C and 28 °C groups. The values in these groups were significantly lower than that of the control group.

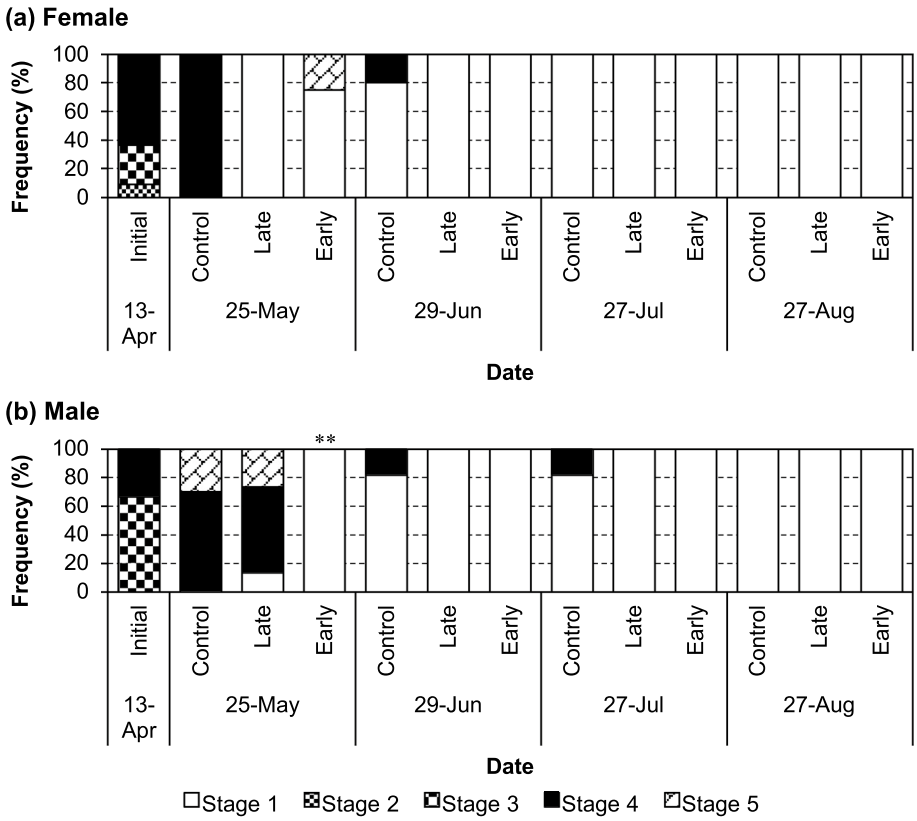

Fig. 7 Frequency distribution in maturation stages of *Hemicentrotus pulcherrimus* gonads reared under the manipulated water temperature in Experiment 2. **a** Females. **b** Males. Gonadal maturity of each animal (1–15 individuals) was classified into stages 1–5 according to Fuji (1960) with some modifications (Unuma 2002). Asterisks indicate frequencies of stage 1 in the experimental groups that are significantly different from that in the control group on the same date (**, $P < 0.01$)

Experiment 2

During the experimental period, 4, 1, and 5 urchins died in the control, late, and early groups, respectively.

The frequency distribution of the maturation stages of the gonads is shown in Fig. 7. At the beginning of the experiment, most female and male urchins exhibited pre-mature (stage 3) or mature (stage 4) gonads. On 25 May, all females in the control group possessed mature ovaries, while most females in the temperature-treated groups had returned to the recovering stage (stage 1). Most of the males in the control and late groups had mature testes. In contrast, all males in the early group had returned to the recovering stage at this time point. The proportion of urchins in the recovering stage (stage 1) was significantly higher in the early group than in the control group. By 29 June, all urchins had returned to the recovering stage in the temperature-treated groups. In the control group, however, mature ovaries and testes were found until 29 June and 27 July, respectively.

The variations in the mean GI values are shown in Fig. 8. At the beginning of the experiment, the mean GI was 12.3 ± 3.4% in females and 17.1 ± 3.7% in males. The GI values in both female and male urchins exhibited a decreasing tendency throughout the experiment across all groups. The female GI of the late group was significantly lower than that of the control group on 27 July, while the male GI of the early group was significantly lower than that of the control group on both 27 July and 27 August.

Figure 9 shows the variation in mean Pul content in the ovaries over time. At the beginning of the experiment, the mean Pul content was 1.6 ± 1.2 mg/100 g. In the control group, mean Pul content slowly decreased during

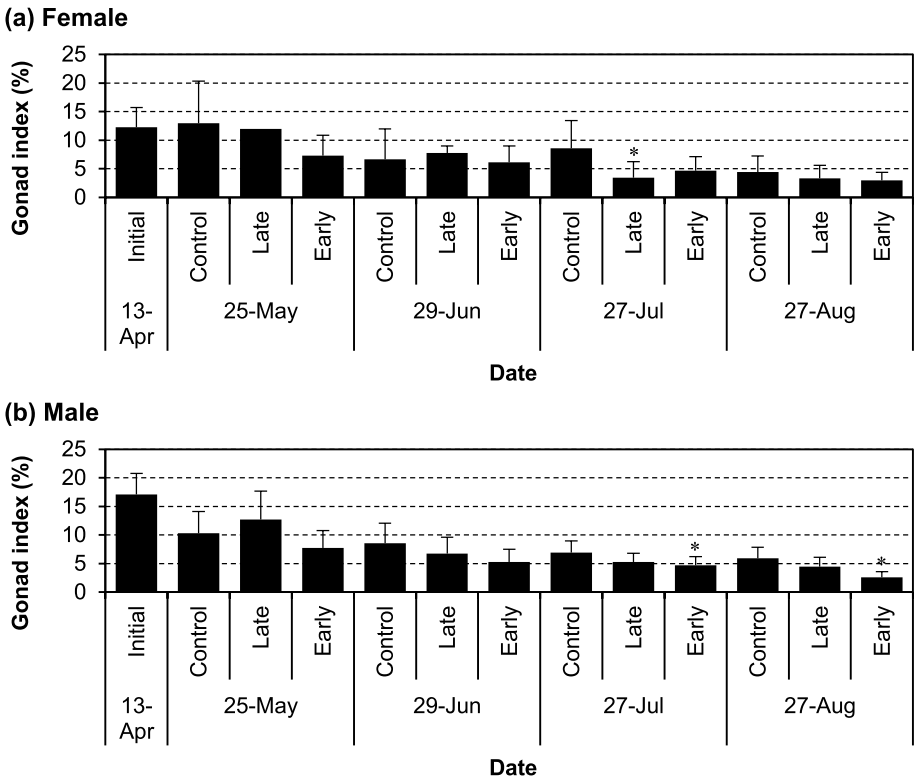

Fig. 8 Gonad indices of *Hemicentrotus pulcherrimus* reared under the manipulated water temperature in Experiment 2. **a** Females. **b** Males. Values represent the mean ± SD of 1–15 individuals. *Asterisks* represent values that are significantly different from that in the control group on the same date (*, $P < 0.05$)

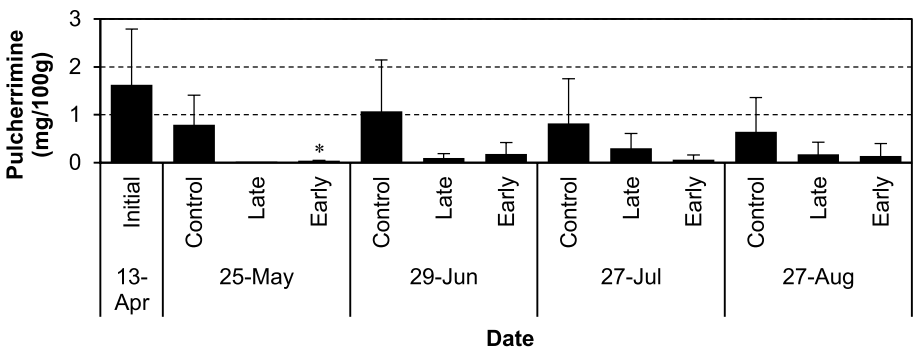

Fig. 9 Pulcherrimine contents of *Hemicentrotus pulcherrimus* ovaries reared under the manipulated water temperature in Experiment 2. Values represent the mean ± SD of 1–11 individuals. *Asterisks* represent values that are significantly different from that in the control group on the same date (*, $P < 0.05$)

the experimental period, but remained detectable in the immature ovaries at a level of 0.7 ± 0.7 mg/100 g on 27 August. In contrast, mean Pul content rapidly decreased in the temperature-treated groups during the experimental period. On 25 May, the mean value in the early group was 0.04 ± 0.01 mg/100 g, which was significantly lower than that in the control group. Only one female was found in the late group with low levels of Pul. Thereafter, similar low levels of Pul were maintained in these groups until the end of the experiment.

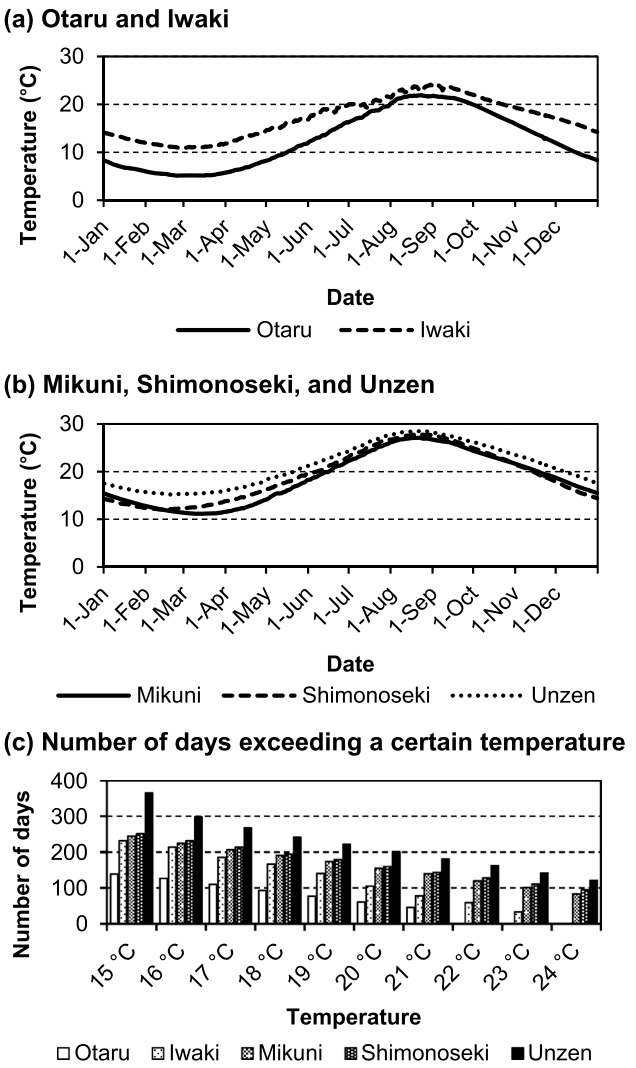

Fig. 10 The 30-year mean seawater temperature off Otaru, Iwaki, Mikuni, Shimonoseki, and Unzen. Daily changes in the temperature at 2-m depths were estimated from 1993–2022 using FRA-ROMS (an ocean forecast system; Kuroda et al., 2015), and averaged. **a** Otaru and Iwaki, where *Hemicentrotus pulcherrimus* is not utilized as food due to bitter ovaries. **b** Mikuni, Shimonoseki, and Unzen, where *H. pulcherrimus* is utilized as food. **c** Number of days exceeding a certain temperature from 15 to 24 °C at each site in a year

Comparison of seawater temperature among habitats

The 26-year mean seawater temperatures estimated using FRA-ROMS for five *H. pulcherrimus* habitats are shown in Fig. 10. The annual mean temperatures were 12.7 °C at Otaru, 17.1 °C at Iwaki, 18.6 °C at Mikuni, 19.2 °C at Shimonoseki, and 21.3 °C at Unzen. The mean temperatures were higher in habitats where *H. pulcherrimus* is utilized as food (Mikuni, Shimonoseki, and Unzen) than in habitats where *H. pulcherrimus* is not utilized as food (Otaru and Iwaki). The difference between Iwaki and Mikuni was 1.5 °C. The number of days exceeding a certain temperature, from 15 to 24 °C, per year was compared among the five habitats (Fig. 10c). The number of days exceeding 15 °C was similar in Iwaki (231 days), Mikuni (244 days), and Shimonoseki (251 days), but these values were different at Otaru (139 days) and Unzen (365 days). As the temperature increased, the difference between the number of days exceeding a certain temperature in Iwaki and those in Mikuni and Shimonoseki expanded. The days exceeding 20 °C numbered 105 in Iwaki, 155 in Mikuni, and 160 in Shimonoseki. Days exceeding 24 °C numbered 1 in Iwaki, 83 in Mikuni, and 95 in Shimonoseki.

Discussion

The present study clarified that seawater temperature affects the timing of initiation and completion of *H. pulcherrimus* gametogenesis. The first experiment showed that higher temperatures delayed the initiation of gametogenesis, which usually begins in autumn in wild *H. pulcherrimus* (Agatsuma 2020; Ogasawara et al. 2011; Murata et al. 2021) (Fig. 3). After the ovaries and testes were maintained in an immature state from July to September, both oogenesis and spermatogenesis in the control group began in December and proceeded rapidly thereafter. In contrast, gametogenesis began in only a few females and males in the 24 °C group and no animals in the 26 °C and 28 °C groups in December. A large increase in Pul in December was observed only in the control group (Fig. 6), which exceeded the acceptable Pul level for palatability (0.5 mg/100 g) (Murata et al. 2002). Thus, higher temperatures were able to delay Pul accumulation in ovaries by suppressing gametogenesis.

The second experiment showed that higher temperature treatment after the spawning season accelerated the transition of the mature gonads to the recovering stage (stage 1) (Fig. 7). For females, all animals in the temperature-treated groups proceeded to the spent or recovering stages (stages 5 or 1) in May. In males, all animals in the early group returned to the recovering stage in May. In contrast, a portion of females and males in the control group were still mature

in June and July. Pul content in the ovaries slowly decreased from April to August in the control group, whereas it was very low in the temperature-treated groups from as early as May (Fig. 9). Thus, higher temperature treatment promoted the disappearance of residual Pul after spawning by advancing the transition of mature gonads to immature ones.

The water temperature used for the control group was adjusted to the average temperature at Yoichi, at the northern edge of the distribution. The results from our experiments indicate that seawater kept at temperatures higher than the average temperature at Yoichi during summer extends the duration of the immature gonad stage (stage 1), which is associated with low Pul content, and thus makes the gonads suitable for consumption. This suggests that the lower seawater temperatures during summer in the northern edge of the distribution are the cause of the short palatable season for *H. pulcherrimus* ovaries in this region. The annual mean seawater temperatures at the northern edge of the distribution (Otaru and Iwaki) were lower than those at the sites where *H. pulcherrimus* is used as a food product (Mikuni, Shimonoseki, and Unzen) (Fig. 10). In particular, Iwaki and Mikuni exhibited a similar number of days when the seawater became warmer than 15 °C. However, the number of days when the seawater became warmer than 20 °C or more was much higher at Mikuni than at Iwaki, indicating that the duration of the warmer season is important for the palatability of *H. pulcherrimus*. We believe that the short palatability of *H. pulcherrimus* at the northern edge of the distribution is caused by the shorter summer season.

In wild *H. pulcherrimus*, gametogenesis is initiated in autumn when the temperature drops to approximately 20 °C regardless of the locality (Semura 1991; Agatsuma 1992; Agatsuma et al. 2006; Murata et al. 2021). When we tracked maturational status in relation to temperature, the results from the control group in Experiment 1 confirmed that the temperature drop in autumn is a factor that induces gametogenesis (Fig. 3). However, in some urchins in the temperature-treated groups, where constant higher temperatures were continued until the end of the experiment, gametogenesis was initiated in December or March. This suggests that the temperature drop is not the sole factor that induces gametogenesis in *H. pulcherrimus*. Sea urchin gametogenesis is regulated by both temperature and photoperiod (Kirchhoff et al. 2010; Kayaba et al. 2012; Unuma et al. 2015; Walker et al. 2020; Ishii et al. 2022), although temperature manipulation is more effective to promote gametogenesis in *H. pulcherrimus*, *Heliocidaris crassispina*, and *P. depressus* (Yamamoto et al. 1988; Sakairi and Yamamoto 1989). In the temperature-treated groups in Experiment 1, gametogenesis may have been induced by seasonal changes in photoperiod in the aquarium house, where the photoperiod was manipulated to simulate the natural photoperiod at the location.

The results obtained in this study suggest that the response patterns of sea urchin gametogenesis to temperature manipulation (temperature increase and decrease) can be divided into two groups. In hatcheries producing seeds of *H. pulcherrimus*, where gametogenesis proceeds from autumn to early spring, maturation can be advanced to allow collection of gametes in October by lowering the rearing water temperature to 15 °C from summer to autumn (Ito et al. 1989). Similarly, in *P. depressus* hatcheries, where gametogenesis proceeds from early to late autumn, maturation can be advanced to promote collection of gametes in September by lowering the rearing water temperature to 20 °C from summer to autumn (Noguchi et al. 1995). Therefore, similar to *H. pulcherrimus*, it may be possible to suppress *P. depressus* maturation by maintaining higher water temperatures from summer to autumn, when the natural seawater temperature decreases. In contrast to these two species, in *S. intermedius* hatcheries in eastern Hokkaido, where gametogenesis proceeds from early spring to early summer, maturation can be advanced to allow collection of gametes in March by raising the rearing water temperature from winter to spring (Sakai 2015). Conversely, in land-based *S. intermedius* aquaculture farms, maturation can be suppressed in order to prolong the harvest season and provide gonads of high food quality by rearing them in cold seawater drawn from the deep sea from spring to summer (Kayaba et al. 2012). In addition, maturation of *Mesocentrotus nudus*, where gametogenesis proceeds from early summer to autumn, can be suppressed by keeping them at temperatures that are lower than that of natural seawater (Unuma et al. 2015). In this way, temperature manipulation methods (temperature increase and decrease) can be employed to promote or suppress gonadal maturation in specific sea urchin species depending on whether gametogenesis proceeds during seasons of increasing or decreasing temperature.

The results of our experiments can be applied to short-term aquaculture to make use of the unexploited *H. pulcherrimus* in Tohoku and Hokkaido as a fishery product. These sea urchins can be collected from the fishery ground in spring and cultivated in tanks under elevated temperature conditions for several months to improve the gonad quality. Both advancing the start of the palatable period and delaying its end are possible. The results from Experiment 2 suggest that a temperature manipulation strategy can be used to promote the disappearance of Pul from the ovaries after the spawning season and advance the start of the palatable period. Similarly, the results from Experiment 1 indicate that temperature manipulation can be used to delay the initiation of gametogenesis in autumn and prolong the palatable period. However, temperatures above 26 °C may reduce food consumption and result in smaller gonad sizes (Figs. 4 and 5), while the GI values of the 24 and 26 °C groups were comparable to that of the wild *H. pulcherrimus* in Fukui

Prefecture during the fishery season (10–15%; Murata et al. 2021). Hatanaka et al. (2007b) also reported that the food consumption rate of *H. pulcherrimus* gradually decreased along with an increase in seawater temperature from 25–26 to 30 °C, which is consistent with our results. In addition, higher temperatures incur higher energy costs and may cause health issues in *H. pulcherrimus*. For example, mass mortalities of *H. pulcherrimus* have been observed off the coast of Fukui Prefecture in summer, which is probably attributable to decreased resistance against bacterial infection when the urchins are exposed to temperature conditions around 30 °C (Hatanaka et al. 2007a, b). Therefore, 24 °C may be sufficient to prolong the palatable season by several months. Future research needs to explore the optimal temperature to improve the size and quality of gonads, reduce energy costs, and avoid health issues during *H. pulcherrimus* cultivation.

In conclusion, higher seawater temperatures during summer extends the duration of immature gonads by promoting the transition from mature to recovering stages and suppressing the re-initiation of gametogenesis, thus providing several months of palatable harvesting during which Pul is absent from the ovaries. If *H. pulcherrimus* with bitter ovaries are collected in early summer and cultivated for several months under elevated water temperatures, it should be possible to reduce the bitter taste and use them as food products.

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References

Agatsuma Y (1992) Annual reproductive cycle of the sea urchin, *Hemicentrotus pulcherrimus* in southern Hokkaido. Suisanzoshoku 40:475–478 (**in Japanese with English abstract**)

Agatsuma Y (2020) *Hemicentrotus pulcherrimus, Pseudocentrotus depressus*, and *Heliocidaris crassispina*. In: Lawrence JM (ed) Sea urchins: biology and ecology, 4th edn. Elsevier, Amsterdam, pp 643–659

Agatsuma Y, Yamada H, Taniguchi K (2006) Distribution of the sea urchin *Hemicentrotus pulcherrimus* along a shallow bathymetric gradient in Onagawa Bay in northern Honshu, Japan. J Shellfish Res 25:1027–1036

Fuji A (1960) Studies on the biology of the sea urchin. I. Superficial and histological gonadal changes in gametogenic process of two sea urchins, *Strongylocentrotus nudus* and *S. intermedius*. Bull Fac Fish Hokkaido Univ 11:1–14

Hatanaka H, Takagaki M, Kawashiro M (2007a) Study of resources recovery measures of sea urchin *Hemicentrotus pulcherrimus*. Bull Fukui Pref Fish Exp Stn in 2005:122–129 (**in Japanese**)

Hatanaka H, Narita H, Takagaki M (2007b) Study of resources recovery measures of sea urchin *Hemicentrotus pulcherrimus*. Bull Fukui Pref Fish Exp Stn in 2006:96–105 (**in Japanese**)

Ishii M, Unuma T, Masadate A, Hoshikawa H, Takahashi K, Kosaka S, Masuda A, Murakami K (2022) Accelerated photoperiod promotes gonadal maturation in the sea urchin *Strongylocentrotus intermedius*. Fish Sci 88:299–310

Ito S, Shibayama M, Kobayakawa A, Tani Y (1989) Promotion of maturation and spawning of sea urchin *Hemicentrotus pulcherrimus* by regulating water temperature. Nippon Suisan Gakkaishi 55:757–763 (**in Japanese with English abstract**)

Kawana T (1938) On the propagation of *Strongylocentrotus pulcherrimus*. Suisan Kenkyu Shi 33:104–116 (**in Japanese**)

Kayaba T, Tsuji K, Hoshikawa H, Kikuchi Y, Kawabata K, Otaki I, Watanabe T (2012) Effect of low temperature rearing, using deep-sea water, on gonadal maturation of the short-spined sea urchin, *Strongylocentrotus intermedius*, in Rausu, Hokkaido. Fish Sci 78:1263–1272

Kirchhoff NT, Eddy S, Brown NP (2010) Out-of-season gamete production in *Strongylocentrotus droebachiensis*: photoperiod and temperature manipulation. Aquaculture 303:77–85

Kuroda H, Setou T, Kakehi S, Ito S, Taneda T, Azumaya T, Inagake D, Hiroe Y, Morinaga K, Okazaki M, Yokota T, Okunishi T, Aoki K, Shimizu Y, Hasegawa D, Watanabe T (2017) Recent advances in Japanese fisheries science in the Kuroshio-Oyashio region through development of the FRA-ROMS ocean forecast system: overview of the reproducibility of reanalysis products. Open J Mar Sci 7:62–90

Miwa K (1970) Studies on a freezing storage of sea urchins-I On pungent components in a frozen sea urchin gonad. Nippon Suisan Gakkaishi 36:617–622 (**in Japanese with English abstract**)

Murata Y, Sata NU (2000) Isolation and structure of pulcherrimine, a novel bitter-tasting amino acid, from the sea urchin (*Hemicentrotus pulcherrimus*) ovaries. J Agric Food Chem 48:5557–5560

Murata Y, Yamamoto T, Kaneniwa M, Kuwahara R, Yokoyama M (1998) Occurrence of bitter gonad in *Hemicentrotus pulcherrimus*. Nippon Suisan Gakkaishi 64:477–478 (**in Japanese with English abstract**)

Murata Y, Sakaguchi M, Sata NU, Kuwahara R, Kaneniwa M, Oohara I (2000) Bitterness of a novel sulfur-containing amino acid, pulcherrimine from the green sea urchin, *Hemicentrotus pulcherrimus*. Jpn J Taste Smell Res 7:615–618 (**in Japanese with English abstract**)

Murata Y, Sata NU, Yokoyama M, Kuwahara R, Kaneniwa M, Oohara I (2001) Determination of a novel bitter amino acid, pulcherrimine, in the gonad of the green sea urchin *Hemicentrotus pulcherrimus*. Fish Sci 67:341–345

Murata Y, Yokoyama M, Unuma T, Sata NU, Kuwahara R, Kaneniwa M (2002) Seasonal changes of bitterness and pulcherrimine content in gonads of green sea urchin *Hemicentrotus pulcherrimus* at Iwaki in Fukushima Prefecture. Fish Sci 68:184–189

Murata Y, Saito K, Sugimoto K, Kiyomoto M, Unuma T (2021) Accumulation of a bitter amino acid, pulcherrimine, in sea urchin ovaries and its relevance to annual reproductive cycle. Fish Sci 87:371–381

Noguchi H, Kawahara I, Goto M, Masaki K (1995) Promotion of gonadal maturation by regulating water temperature in the sea urchin *Pseudocentrotus depressus* –II. Sci Rep Saga Pref Mariculture Center 4:101–107 (**In Japanese**)

Ogasawara M, Matsui T, Agatsuma Y (2011) Growth and rapid gonad recovery of the sea urchin *Hemicentrotus pulcherrimus* after spawning in an *Undaria pinnatifida* and *Saccharina japonica* kelp bed. J Shellfish Res 30:159–166

Sakai Y (2015) Hatchery technology (production of seed). In: Brown NP, Eddy SD (eds) Echinoderm aquaculture, John Wiley & Sons, Inc, Hoboken, pp 85–93

Sakairi K, Yamamoto M, Ohtsu K, Yoshida M (1989) Environmental control of gonadal maturation in laboratory-reared sea urchins,

Anthocidaris crassispina and *Hemicentrotus pulcherrimus*. Zool Sci 6:721–730

Sata NU, Kuwahara R, Murata Y (2001) Synthesis of all isomers of pulcherrimine, a bitter principle in the sea urchin ovary. Tetrahedron Lett 42:115–118

Semura H (1991) Ecology of the sea urchin *Hemicentrotus pulcherrimus* off the central coast of Shimane Prefecture. Saibaigiken 19:67–74 (**in Japanese**)

Unuma T (2002) Gonadal growth and its relationship to aquaculture in sea urchins. In: Yokota Y et al (eds) The Sea Urchin: from basic biology to aquaculture. Swets & Zeitlinger, Lisse, pp 115–127

Unuma T (2015) Introduction: sea urchin fisheries in Japan. In: Brown NP, Eddy SD (eds) Echinoderm Aquaculture. John Wiley & Sons Inc, Hoboken, pp 77–85

Unuma T, Walker CW (2009) Relationship between gametogenesis and food quality in sea urchin gonads. Proceedings of the 36th U.S.-Japan Aquaculture Panel Symposium, pp.45–54

Unuma T, Murata Y, Hasegawa N, Sawaguchi S, Takahashi K (2015) Improving the food quality of sea urchins collected from barren grounds by short-term aquaculture under controlled temperature. Bull Fish Res Agen 40:145–153

Walker CW, Lesser MP, Unuma T (2020) Gametogenesis in regular sea urchins: structural, functional, and molecular/genomic biology. In: Lawrence JM (ed) Sea urchins: biology and ecology, 4th edn. Elsevier, Amsterdam, pp 29–50

Yamamoto M, Ishine M, Yoshida M (1988) Gonadal maturation independent of photic conditions in laboratory-reared sea urchins, *Pseudocentrotus depressus* and *Hemicentrotus pulcherrimus*. Zool Sci 5:979–988