Original article

Long periods with closed shells observed from winter to spring in Akoya oysters (*Pinctada fucata martensii*)

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Introduction

Akoya oysters Pinctada fucata martensii show unusual shell movements, such as more frequent closing and opening during red tides caused by the dinoflagellates Heterocapsa circularisquama and Karenia mikimotoi, and under conditions of low dissolved oxygen [1-3]. We measured shell movements of Akoya oysters, and unusual shell movements were used as indicators to mitigate damage to oysters used for pearl culture. We have also noticed, from year-round monitoring, that unusual, continuous shell closings lasting over 1 h are rarely observed in summer but happen frequently in winter. Therefore, to develop a safe, spring pearl culture management technique that reduces damage to and death of cultured Akoya oysters, we investigated the relationship between long shell closings and water temperature, from winter to spring.

Materials and methods

We measured the shell movements of two-year-old Akoya oysters. Following an established method [4], a Hall element sensor and small magnet were connected to the left and right valves of an oyster shell, respectively. Shell opening distance was determined continuously from the output voltage recorded by Shell-Lingual (SM-108A Tokyo Sokki Kenkyujo Co., Japan). For these measurements, four oysters, each equipped with sensor and magnet, were suspended at a depth of 3 m at a pearl farm. We used winter data (December-April) from 2013 to 2017 to observe the relationship between continuous closed-shell time per day and daily average water temperature. Water temperature was measured by the Shell-Lingual temperature sensor at 3-m depth. The shell opening distance was averaged for each hour, and was considered "closed" if this average was less than 10% of the maximum shell opening distance of the previous day. These 1-h closed periods were summed daily for



each oyster. Shells that did not close to less than 10% of maximum opening were judged as inadequate shell closings and were not used to calculate continuous shell closing time.

Results and discussion

We determined the continuous closed shell times (hours per day) and daily average water temperatures for each year (Fig. 1). In each year, continuous shell closing times over 1 h were observed at water temperatures above about 10 °C, and many consecutive closed-shell periods adding up to 4-8 h were observed at temperatures from 10 to 13°C. However, at water temperatures over 13°C there was a noticeable difference between years in the appearance of extended shell closings.



Fig. 1. Relationship between continuous closed shell time and daily average water temperature. (A) Dec 2013-Apr 2014 (Minimum water temperature [MWT] 8.5°C, 22 Feb 2014), (B) Dec 2014-Apr 2015 (MWT 10.4°C, 24 Dec 2014), (C) Dec 2015-Apr 2016 (MWT 10.2°C, 7 Feb 2016), (D) Dec 2016-Apr 2017 (MWT 10.7°C, 19 Jan 2017). ○, Data from before MWT (water temperature decreasing); ●, data from after MWT (water temperature increasing).

From December 2016 to April 2017, daily shell closings over 4 h were rare at temperatures over 14°C,

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and at temperatures over 13° C cumulative shell closing time became shorter as water temperatures rose (Fig. 1D). From December 2014 to April 2015 and from December 2015 to April 2016, the period of continuous shell closing became shorter when the water temperature exceeded 14° C (Fig. 1B, C). From December 2013 to April 2014, there were some continuous shell closings over 4 h at water temperatures over 15° C, and despite the increasing water temperatures, closed-shell time was longer (Fig. 1A).

Table 1 shows water temperature and their frequencies (days) during the period from December to April of each year. There were three days with temperatures below 11°C during December 2016-April 2017 when the continuous closed-shell time was shortened at water temperatures over 13°C, whereas from December 2014 to April 2015, there were 18 days below 11°C and the continuous closed-shell time was shortened at water temperatures over 14°C, and from December 2015 to April 2016, there were 17 days below 11°C. In contrast, from December 2013 to April 2014 when the closed-shell time was long despite rising water temperatures, there were 25 days below 11°C. These results reveal a trend: the longer the period with water temperatures below 11°C in winter, the greater the delay before the decrease in continuous closed-shell time when water temperatures rise.

 Table 1. Water temperature and the number of days in each range from December to April of each year (e.g. Dec 2013-Apr 2014)

Water temperature (°C)	2013 - 2014	2014 - 2015	2015 - 2016	2016 - 2017
7	0	0	0	0
8	6	0	0	0
9	6	0	0	0
10	13	18	17	3
11	32	31	25	18
12	23	35	22	38
13	14	25	27	33
14	24	10	20	24
15	6	8	25	10
16	2	0	7	17
17	0	0	2	1
18	0	0	0	0
Total	126	127	145	144

Figure 2 shows typical shell movement patterns at different water temperatures from December 2013 to April 2014, including some periods with temperatures below 10°C. At temperatures below 10°C, the shell did not close completely; the pattern of incomplete shell closing appeared repeatedly and there was an increase in the frequency of shell closings and openings (Fig. 2A). The same Akoya oyster with incomplete shell closure at water temperatures below 10°C closed its shell completely when water temperatures exceeded 10°C (Fig. 2B). Because the incomplete shell closing at water temperatures below 10°C was temporary, we

suggest, although the details are not clear, that low water temperature causes a kind of functional impairment. As water temperatures rose, the frequency of continuous shell closings decreased (Fig. 2C).

As a general trend, the period of water temperature rise in spring coincides with the period of increasing activity of Akoya oysters. Therefore, we suggest that the shortening of closed-shell time during the period of water temperature rise indicates increasing activity of Akoya oysters, whereas any delay in the shortening of continuous closed-shell time indicates a delay in the activity of Akoya oysters because of stress from low water temperatures in winter.



Fig. 2. Typical waveform patterns of shell movements at different water temperatures. (A) 20 February 2014 (daily average water temperature, 8.7° C), (B) 11 March 2014 (daily average water temperature, 12.5° C), (C) 4 April 2014 (daily average water temperature, 15.0° C). All data are from the same individual oyster.

In conclusion, we suggest that the shortening of periods of continuously closed shells at the time of water temperature rise can indicate the recovery of cultured oysters stressed by low water temperatures. In the culture of pearls, working with pearl oysters during periods of low temperatures can cause damage and sometimes death; however, it should be possible to use the time of continuously closed shells as an indicator of the condition of oysters recovering from low water temperatures without stressing them. We anticipate that the monitoring of shell closing time during water temperature rise will provide useful information for spring management in cultured pearl farming and help to keep cultured oysters in good condition.

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