Synchronized spawning of abalone *Haliotis diversicolor* triggered by typhoon events in Sagami Bay, Japan

Toshihiro Onitsuka^{1,*}, Tomohiko Kawamura¹, Toyomitsu Horii²,
Naoyuki Takiguchi³, Hideki Takami⁴, Yoshiro Watanabe¹

¹Ocean Research Institute, The University of Tokyo, 1-15-1 Minamidai, Nakano-ku, Tokyo 164–8639, Japan

²National Research Institute of Fisheries Science, Fisheries Research Agency, 6-31-1 Nagai, Yokosuka 238–0316, Kanagawa, Japan

³Fisheries Division, Environment and Agriculture Department, Kanagawa Prefectural Government, 1 Nihon-odori, Naka-ku, Yokohama, Kanagawa 231–8588, Japan

⁴Tohoku National Fisheries Research Institute, Fisheries Research Agency, 3-27-5 Shinhamacho, Shiogama, Miyagi 958, Japan

ABSTRACT: To elucidate the external cues triggering spawning of *Haliotis diversicolor*, changes in the gonads of adults and the occurrence of newly settled post-larvae were monitored from 2001 to 2004 in Sagami Bay, Japan. The gonad index (GI) and gonad somatic index (GSI) of both sexes began to increase between April and June 2002, peaked in September, and then decreased significantly by early October. In other years, GI and GSI were significantly lower in autumn. Between June and September in each year, almost all females had a mature ovary and most were capable of spawning, but the occurrence of newly settled post-larvae (<500 µm in shell length) was strictly limited to the periods just after typhoons passed through the study area. The GI and GSI of both sexes also decreased significantly only after typhoon events. Although mature adults experienced various changes in seawater temperature, tidal height and lunar phase during the survey periods, evidence of spawning was observed only after typhoon events. There were no specific trends in seawater temperature and lunar phase before newly settled post-larvae were collected. Our results suggest that spawning of *H. diversicolor* is strongly associated with typhoon events. Most individuals had an immature ovary just after the passage of a typhoon. However, 1 mo after spawning, most individuals had a mature ovary with a high GI, suggesting that *H. diversicolor* requires an interval of about 1 mo between successive spawning events.

KEY WORDS: Synchronous epidemic spawning · Spawning cue · Typhoon · Abalone · *Haliotis diversicolor* · Post-larvae · Maturation

—Resale or republication not permitted without written consent of the publisher—

INTRODUCTION

Fertilization is critical for successful reproduction, and many marine organisms have evolved traits to maximize the rate of fertilization. For most broadcast spawners, fertilization occurs in the water column after the gametes are released. Viability of the gametes is not maintained for long (e.g. Kikuchi & Uki 1974b, Benzie & Dixon 1994); therefore, the fertilization rate is enhanced by synchronous spawning that is commonly observed in marine

invertebrates, e.g. echinoderms, sponges, mollusks (Babcock et al. 1992), polychaetes (Babcock et al. 1992, Watson et al. 2000), scallops (Williams & Babcock 2004) and corals (Babcock et al. 1986). Environmental factors such as lunar and tidal cycles (Babcock et al. 1986, Watson et al. 2000, Counihan et al. 2001), storm events (Creese & Ballantine 1983, Sasaki 1985, Sasaki & Shepherd 1995) and temperature changes (Tanaka et al. 1986, Minchin 1992) are potential cues that induce spawning of shallow-water animals.

*Email: oni2z@ori.u-tokyo.ac.jp

The spawning and recruitment of abalone (*Haliotidae*, *Mollusca*) is of particular interest due to the value of abalone fisheries and the role of abalone as a dominant grazer in coastal ecosystems. Abalone are dioecious broadcast spawners. Eggs must encounter a high density of sperm within a short period of time after release to be fertilized (e.g. Kikuchi & Uki 1974a,b, Babcock & Keesing 1999). Consequently, synchronous spawning of neighboring males and females induced by distinctive external cues is important for successful fertilization. The limited data available for abalone provide conflicting evidence regarding spawning cues. *Haliotis discus hannai* larvae were detected primarily after typhoons or minor storms (Sasaki 1985, Sasaki & Shepherd 1995), while *H. kamtschatkana* and *H. rubra* were suggested to spawn during calm periods (Breen & Adkins 1980, Prince et al. 1987). Tanaka et al. (1986) concluded that sudden temperature changes induced spawning of *H. discus discus*, *H. madaka* and *H. gigantea*, while spawning of *H. asinina* appears to be triggered by the lunar cycles and/or tidal amplitude (Counihan et al. 2001).

Most authors have inferred abalone spawnings from either the occurrence of larvae or changes in gonad index. Both methods are subject to uncertainty, such as that caused by patchy larval distribution or partial spawnings. A combination of larval sampling and observation of gonad changes can provide more reliable evidence of synchronous spawning.

This study addresses spawning cues in a species not previously studied, *Haliotis diversicolor*. Data on the occurrence of newly settled post-larvae and changes in gonad indices of adult *H. diversicolor* were correlated with a range of potential spawning cues to provide strong evidence of the environmental factors triggering synchronous epidemic spawning of *H. diversicolor* at Nagai in Sagami Bay, Japan. *Haliotis diversicolor* is a relatively small abalone species about 10 cm in maximum shell length (SL) and inhabits shallow subtidal areas in subtropical and temperate zones of the western North Pacific Ocean (southern Japan, southern Korea, Taiwan and southern China; Geiger 1999). Newly settled post-larvae and early juveniles of *H. diversicolor* were widely observed on cobblestones covered with crustose coralline algae

(CCA) in shallow rocky reefs around Nagai in 2001 and 2002 (T. Onitsuka unpubl. data). In laboratory experiments CCA induced strong and rapid larval attachment and metamorphosis of *H. diversicolor* (Onitsuka 2006) and all other abalone species tested (Roberts 2001).

Histological observations of gonads have been carried out on many abalone species (Tomita 1967, Young & Demartini 1970, Okuno et al. 1978, Takashima et al. 1978, Jebreen et al. 2000, Fukazawa et al. 2007). These observations indicate that the maturation processes of the ovary are very similar among the species (Fukazawa et al. 2007). In previous studies, changes in the gonad index (GI) and oocyte development in the ovary suggested that the spawning season of *Haliotis diversicolor* was between June and November at

Fig. 1. Locations of sampling stations and the National Research Institute of Fisheries Science at Nagai in Sagami Bay, the meteorological observatory in Yokohama and the tide station in Aburatsubo

Tateyama and Kominato on the Boso Peninsula (Fig. 1; Oba 1964, Okuno et al. 1978).

MATERIALS AND METHODS

Study sites. This study was carried out on the rocky shore subtidal area at Nagai on the coast of Sagami Bay in central Japan (35° 11' N, 139° 36' E; Fig. 1). Sampling stations 1 and 2 (Fig. 1) were established at 0.5 to 3.0 m depth and consisted of rocky reefs with many cobblestones covered with CCA. Few large macroalgae were observed at either station.

Gonad development and changes in the condition factor. Changes in the GI, the gonad somatic index (GSI) and condition the factor (CF) of adult *Haliotis diversicolor* were monitored from August 2001 to November 2002 (only the GI of males was measured from August to November in 2001). From July to October 2003 and June to September 2004, the GI of both sexes was measured. Individuals larger than 35 mm SL, which were expected to have a mature gonad (Oba 1964), were defined as adults following the definition criteria established for *H. discus hannai* (Takami 2002). Between 2 and 24 males (in 2001 to 2004) and 2 and 21 females (in 2002 to 2004), caught by skin-diving fishers around the study area, were examined every 2 to 4 wk (Table 1). In both sexes, mean SL did not differ greatly among sampling dates (Table 1), although significant differences were detected among samples in 2002 owing to variability in SL of the abalone caught.

The gonad of abalone species surrounds the digestive organ and it is impossible to separate the 2 organs. Therefore, for all collected abalone, the gonad portion included both the digestive organ and gonad, and was removed from the shell and muscle after measuring SL. The wet weight of the whole body (not including the shell), gonad portion and muscle portion were measured separately, and the gonad portion was preserved in Davidson's solution. The GI and GSI were calculated to display volumetric and gravimetric changes, respectively, in the ovary. The GI was calculated as the ratio of the diameter of the gonad

(excluding the digestive organ) to the diameter of the cross section at 1 cm from the tip of the preserved gonad portion (Ino & Harada 1961). The GSI was calculated as the ratio of the weight of the gonad portion to the whole body weight using Eq. (1), following the method of Webber & Giese (1969). The CF was calculated as the ratio of the weight of the muscle portion to the SL using Eq. (2) (Ishida & Tanaka 1983).

$$\text{GSI} = W_g \times W_b^{-1} \times 10^2 \quad (1)$$

$$\text{CF} = W_m \times \text{SL}^{-3} \times 10^5 \quad (2)$$

Table 1. Shell length and number of males and females used for the gonad index measurement. The differences in the range of shell length between the sampling dates of each year were tested by ANOVA with Tukey-Kramer multiple comparison tests. Different letters (a,b,c) indicate significant differences detected among sampling dates in 2002 ($p < 0.05$). In other years, there was no significant difference. * Samples excluded from statistical treatments

Sampling date	Male		Female	
	No. examined abalone	Range of shell length (mm)	No. examined abalone	Range of shell length (mm)
2001				
06 Aug	24	48.1–88.9	0	–
28 Aug	20	39.5–80.9	0	–
18 Sep	11	41.6–84.7	0	–
12 Oct	9	43.9–84.3	0	–
17 Dec	4	42.7–69.5	9	38.7–72.0
2002				
25 Jan	3	59.5–62.1 abc	4	52.8–62.7 ab
20 Mar	3	53.9–65.0 abc	3	50.2–70.1 ab
15 Apr	2	54.9, 56.7*	4	58.5–68.0 ab
03 Jun	6	41.0–48.8 c	6	38.2–57.1 b
02 Jul	4	38.6–71.9 bc	8	51.0–75.2 ab
01 Aug	10	52.5–76.9 ab	10	55.7–73.8 a
12 Aug	15	50.7–80.2 ab	18	48.4–81.7 ab
11 Sep	20	42.0–80.3 ab	18	41.5–84.4 ab
25 Sep	12	46.0–73.3 ab	13	46.3–82.6 a
11 Oct	14	53.8–84.2 a	14	44.7–83.1 a
01 Nov	3	52.3–61.0 abc	2	45.7, 51.2*
2003				
18 Jul	15	43.6–71.9	11	47.3–82.5
04 Aug	15	51.0–80.1	15	45.1–74.8
11 Aug	16	47.3–81.6	15	50.0–85.9
04 Sep	15	53.4–79.6	15	51.78–88.15
17 Sep	15	45.7–78.6	15	45.1–73.0
25 Sep	14	46.0–73.5	15	43.5–78.9
23 Oct	15	44.5–80.9	14	49.5–67.2
2004				
04 Jun	17	38.4–78.5	17	42.9–71.9
24 Jun	15	46.1–85.9	15	42.3–80.1
08 Jul	16	45.6–77.1	16	41.3–79.5
23 Jul	18	45.7–77.0	18	43.7–76.0
05 Aug	18	44.0–75.6	18	45.7–83.5
23 Aug	16	47.8–76.1	16	49.9–78.0
02 Sep	16	50.8–80.7	21	50.1–78.2
14 Sep	17	38.7–80.0	17	45.4–75.3
29 Sep	16	46.2–83.4	14	41.6–76.6

where W_g and W_b are the weight of the gonad portion and whole body, respectively; W_m is the weight of the muscle portion.

Approximately 5 mm thick tissue pieces were taken 1 cm from the tip of the preserved gonad portion of females caught in 2001 to 2002 and in 2003. The pieces were soaked in 70% ethanol for a few days, dehydrated through a graded ethanol series, cleared in xylene and embedded in paraffin wax. Transverse sections 4 to 6 μ m thick were cut from the tissue pieces using a microtome. The sections were subjected to standard histological procedures and stained with Mayer's haematoxylin and eosin. Oocytes were observed under a dissecting optical microscope and classified into 5 developmental stages following the definitions established for *Haliotis discus hannai* (Fukazawa et al. 2007): Stage 1, chromatin nucleolus stage; Stage 2, early/pre-vitellogenic primary stage; Stage 3, oil droplet stage; Stage 4, primary yolk globule stage; Stage 5, mature stage (Fig. 2). The maturation stage of the ovary from each individual was defined by the developmental stage that dominated in the ovary.

Sampling of newly settled post-larvae. To monitor the occurrence of newly settled post-larvae, 3 cobblestones (10 to 40 cm in the longest dimension) covered with CCA were collected haphazardly from each station, weekly in principle, from August to November 2001 and 2002, from July to October 2003 and from

June to September 2004. Individuals <500 μ m SL, which had probably settled within a week prior, were defined as newly settled post-larvae. Each stone was put into a Ziplock plastic bag underwater at the station, immediately returned to the laboratory (National Research Institute of Fisheries Science at Nagai; Fig. 1) and immersed in 10% ethanol in seawater for approximately 10 min to anesthetize the abalone. Post-larvae were then detached from the cobblestone in 10% ethanol in seawater using a brush and collected in a plankton net of 110 μ m mesh. Post-larval abalone were counted and their SL was measured under a dissecting microscope. Then, post-larval *Haliotis diversicolor* were identified based on larval SL (Onitsuka 2006).

Environmental factors. Changes in wave height, atmospheric pressure, tidal height, lunar stage and seawater temperature were monitored during the periods when newly settled post-larvae were collected in 2001 to 2004. Wave height was categorized into 5 grades: 0, calm sea; 1, little ground swell; 2, ground swell; 3, a few white-crested waves with a south wind; 4, many white-crested waves with a strong south wind. The wave height grade was determined daily by the National Research Institute of Fisheries Science at Nagai (Fig. 1). Atmospheric pressure in Yokohama, about 30 km north of the study area, was provided by the Japan Meteorological Agency (Fig. 1). Tidal height was measured by the Geographical Survey Institute 5 km south of the study area (Fig. 1). Tidal height could not be measured from 10 to 24 September 2001 owing to equipment failure. The seawater temperature was measured daily at the coast adjacent to the National Research Institute of Fisheries Science at Nagai. Lunar stage data were provided by the National Astronomical Observatory of Japan.

Statistical analyses. Differences among survey periods in the GI, GSI and CF were tested using analysis of variance (ANOVA) with Tukey-Kramer multiple comparison tests. Data were arcsine transformed before ANOVA to homogenize the variances. Homoscedasticity was assessed using Levene tests. Samples taken on 15 April and 1 November 2002 were excluded from the analyses because the sample size was too small (Table 1). All statistical analyses were carried out using JMP v. 6, SAS).

Fig. 2. *Haliotis diversicolor*. Classification of the 5 developmental stages of oocytes. (A) Chromatin nucleolus stage. (B) Early/pre-vitellogenic primary stage. Active rRNA gene transcription is occurring in the germinal vesicle (gv), as indicated by the presence of nucleoli (n) in (A) and (B). (C) Oil droplet stage. (D) Primary yolk globule stage. (E) Mature stage. Abbreviations: o = Oil droplet; jc = jelly coat; ve = vitelline envelope. Scale bars: A–C: 10 μ m; D,E: 50 μ m

RESULTS

Seasonal changes in GI, GSI, CF and mature stage

Seasonal changes in the GI, GSI and CF of adult *Haliotis diversicolor* collected in 2001 to 2002 were similar for males and females (Fig. 3). Relatively low GI and GSI were observed from January to March 2002. The GI and GSI began to increase in April and June, respectively, and peaked in September 2002, although the GI of males showed another peak in June. The gonad indices (i.e. GI and GSI) of both sexes decreased significantly in early October (ANOVA with Tukey-Kramer multiple comparison test: $p < 0.01$). The CF began to increase in January 2002 and reached its highest value in April. The CF decreased in June and remained at a low level between June and November.

The proportion of females in each maturation stage was evaluated (Fig. 4). No individuals had a Stage 4

or 5 ovary from January to March. The proportion of individuals with a Stage 3 ovary was higher in March than in January. In April, many individuals had a Stage 4 ovary and some had a Stage 5 ovary. Almost all individuals had a Stage 5 ovary between early June and late September. However, no individuals had a Stage 4 or 5 ovary in mid-October.

In 2003, the GI of both sexes peaked in early August and decreased significantly by mid-August (ANOVA with Tukey-Kramer multiple comparison test: $p < 0.01$; Fig. 5). The GI increased again in September and decreased slightly in October. From mid-July to early August, the ovaries of all individuals were at Stage 5 (Fig. 5). In mid-August, however, the proportion of individuals with a Stage 5 ovary was lower, and the proportion of individuals with a Stage 3 or 4 ovary increased. The proportion of individuals with a Stage 5 ovary increased again in early September and reached 100% in late September.

Fig. 3. *Haliotis diversicolor*. Changes in the mean gonad index (GI), gonad somatic index (GSI) and condition factor (CF) of adults from December 2001 to November 2002. Males and females are indicated by ● and ◇, respectively. Error bars indicate SD

Occurrence of newly settled post-larvae and changes in GI

Newly settled post-larvae (NSP) appeared in samples twice in 2001: on 29 August (first cohort) and 25 September (second cohort) at Stn 1, and on 27 and 30 August (first cohort) and 18 September (second cohort) at Stn 2 (Fig. 6a). The GI of adult males decreased significantly between 28 August and 18 September 2001 (ANOVA with Tukey-Kramer multiple comparison test: $p < 0.01$; Fig. 6b). A large-scale storm caused by a typhoon passed through the study area on 22 August, 5 d before the first cohort appeared, as indicated by the low atmospheric pressure and wave heights of Grade 4 (Fig. 6c). On 11 September, 7 d before the second cohort occurred, another typhoon passed through the study area as indicated by the low atmospheric pressure and caused a storm with Grade 4 wave heights (Fig. 6c). The first cohort occurred after the first quarter moon and during a neap tide (Fig. 6d). No distinctive change in seawater temperature was detected before the occurrence of the first cohort (Fig. 6e). The second cohort occurred during the new

Fig. 4. *Haliotis diversicolor*. Changes in the proportion of individuals in each ovary maturation stage at each sampling date from December 2001 to October 2002

Fig. 5. *Haliotis diversicolor*. Changes in the mean gonad index (GI) and proportion of individuals in each ovary maturation stage at each sampling date from July to October in 2003. The GI of males and females are indicated by white and black bars, respectively. Error bars indicate SD

moon and a spring tide (Fig. 6d). Again, no clear change in seawater temperature was observed (Fig. 6e).

In 2002, NSP were found only once, on 11 October, at both stations (Fig. 7). The GI of males and females decreased significantly between 25 September and 11 October (ANOVA with Tukey-Kramer multiple comparison test: $p < 0.01$). A typhoon, as indicated by the low atmospheric pressure and wave heights of Grade 4 (Fig. 7), passed through the study area on 1 October, 10 d before NSP were found.

The occurrence of NSP in 2003 was observed on 13 and 18 August at both stations (Fig. 7). The GI of males and females decreased significantly between 4 and 11 August (ANOVA with Tukey-Kramer multiple comparison test: $p < 0.01$). A typhoon passed through the study area on 9 August, as indicated by the low atmospheric pressure, 4 d before the occurrence of NSP (Fig. 7). The typhoon caused a large-scale storm in the study area, but the wave height grade could not be recorded that day.

In 2004, NSP appeared in our samples on 6 September at Stn 1 and on 14 September at Stn 2 (Fig. 7). The GI of both sexes decreased significantly between 23 August and 2 September (ANOVA with Tukey-Kramer multiple comparison test: $p < 0.01$). A typhoon, as indicated by the low atmospheric pressure and wave height of Grade 4 (Fig. 7), passed near the study area on 31 August, 6 d before the occurrence of NSP at Stn 1. During this year, 2 other typhoons passed the study area on 22 June and 1 July, causing wave heights of Grade 3 (Fig. 7). NSP $< 500 \mu\text{m}$ SL were not found, but several larger post-larvae were collected at Stn 1 on 7 July (640 and 720 μm SL) and 14 July (560, 600, 940 and 1200 μm SL). Slight decreases in the GI of males and females were recorded between 24 June and 8 July, but they were not significant (ANOVA: $p > 0.05$; Fig. 7).

Changes in both the biological characteristics of abalone and the environmental variables in the 10 d before collection of NSP are listed in Table 2. The cohorts that appeared in July 2004 were excluded from this analysis because no NSP were collected. All NSP in each cohort were collected after the passage of typhoons,

Fig. 6. *Haliotis diversicolor*. Occurrence of newly settled post-larvae (NSP), changes in the gonad index (GI) of adults and environmental factors at Nagai from August to November 2001. (a) Total numbers of NSP collected from 3 cobblestones at Stn 1 (▲) and Stn 2 (●). (b) Mean GI of adult male abalone. Error bars indicate SD. (c) Wave height grade (white bars) and atmospheric pressure (solid line). Wave height grades: 0, calm sea; 1, little ground swell; 2, ground swell; 3, a few white-crested waves with a south wind; 4, many white-crested waves with a strong south wind. (d) Tide height and moon stage. Tide heights were not measured from 10 to 24 September. Full moon and new moon are indicated by ○ and ●, respectively. (e) Seawater temperature

as indicated by the low atmospheric pressure (<1000 hPa), all of which caused large storms with wave heights of Grade 4. No trends in the lunar stage were observed. The seawater temperature was within 2°C at all collections, which was not a distinctive change (Table 2).

DISCUSSION

The GI data revealed that gonad maturation was initiated in April 2002. The CF then changed in inverse proportion to GI, suggesting that energy reserves were used for gonad maturation. The seasonal timing of gonad maturation in *Haliotis diversicolor* in the study area in 2002 was similar to that at Kominato, Boso Peninsula, Japan, in 1976 to 1977 (Okuno et al. 1978).

Two cohorts of oocytes were observed within an ovary before spawning events; the primary cohort consisted of Stage 5 oocytes and the secondary cohort consisted of Stage 1–3 oocytes. The oocyte composition of the ovary in *Haliotis diversicolor* was similar to that in *H. asinina* (Jebreen et al. 2000) and *H. discus hannai* (Tomita 1967, Fukazawa et al. 2007), in which the secondary cohort begins to mature immediately after the primary cohort is released. The secondary cohort is ready to be spawned after at least 2 wk at about 26°C in *H. asinina* (Jebreen et al. 2000) and after about 1 mo at 20°C in *H. discus hannai* (Fukazawa et al. 2007). In *H. diversicolor* changes in the proportion of individuals in various stages of ovary maturation in 2003 suggested that a new cohort of oocytes began to develop shortly after the previous oocyte cohort was spawned, and the secondary cohort was ready to be spawned after approximately 1 mo. Therefore, it is inferred that *H. diversicolor* requires an interval of at least 1 mo between spawning events.

The spawning season of *Haliotis diversicolor* in Japan was previously reported to be from June to November (Oba 1964, Okuno et al. 1978). Takashima et al. (1978) suggested that *H. diversicolor* becomes capable of releasing gametes at a GI of 70 in

males and 90 in females. In *H. discus hannai*, females with Stage 5 oocytes are able to spawn (Fukazawa et al. 2007). In August 2001, August and September 2002, August 2003 and June and August 2004, the mean GI in both sexes exceeded the cutoff values and most females had a Stage 5 ovary. Consequently, most

Fig. 7. *Haliotis diversicolor*. Occurrence of newly settled post-larvae (NSP), changes in the gonad index (GI) of adults and environmental factors at Nagai from August to November 2002, July to October 2003 and June to September 2004. (a) Total no. of NSP collected from 3 cobblestones at Stn 1 (▲) and Stn 2 (●). (b) Mean GI of adult abalone. Males and females are indicated by ● and ◇, respectively. Error bars indicate SD. (c) Wave height grade (white bars) and atmospheric pressure (solid line). Wave height grades: 0, calm sea; 1, little ground swell; 2, ground swell; 3, a few white crested waves with a south wind; 4, many white crested waves with a strong south wind

Table 2. Changes in abalone and environmental parameters in the 10 d before the first newly settled post-larvae (NSP) of each cohort were collected in 2001 to 2004. The first cohort in 2004 was not included because no NSP were collected. nd: no data, nsd: no significant decrease, sd: significant decrease

Cohorts	GI and mature stage of abalone			Environmental parameters				
	Gonad index Male	Gonad index Female	Dominant mature stage in cohort	Typhoon passed?	Atmospheric pressure (hPa)	Highest wave height grade	Moon phase ^a	Seawater temperature (°C)
First in 2001	nsd	nd	nd	○	989–1016	4	☾ → ☽	25.3–26.5
Second in 2001	sd	sd	nd	○	993–1015	4	☾ → ●	25.5–26.0
2002	sd	sd	Stage 5 → Stage 3	○	1000–1023	4	☾ → ☽	21.6–23.2
2003	sd	sd	Stage 5 → Stage 3	○	994–1012	nd	☽ → ☾	25.0–26.2
2004	sd	sd	nd	○	1000–1016	4	☾ → ☾	24.0–25.3

^aFull moon (○); new moon (●)

adults inhabiting the study area appeared ready to release their gametes in these periods. However, the occurrence of NSP of *H. diversicolor* was strictly limited to the periods just after the typhoons passed near the study area (a total of 6 times). The GI of both sexes also decreased significantly only after typhoon events, and few or no females with a Stage 5 ovary were observed following the typhoons. These results suggest that spawning of *H. diversicolor* is strongly associated with typhoon events. For other abalone species, sudden changes in seawater temperature (Tanaka et al. 1986) and tidal amplitude and/or lunar cycle (Counihan et al. 2001) have been suggested as spawning cues. During our survey, mature adult *H. diversicolor* experienced various changes in seawater temperature, tidal height and lunar phase, but evidence of spawning corresponding with those environmental changes was not found.

Larvae of *Haliotis discus hannai* were often collected following minor storms as well as typhoons (Sasaki & Shepherd 1995). Although a few minor storms passed our study area and caused a wave height of Grade 3, NSP of *H. diversicolor* did not occur after these minor storms. Unlike other abalone species studied to date, the spawning of *H. diversicolor* appears to be triggered only by typhoon-scale storms. Given this conclusion, it is interesting to consider the regularity of this spawning cue and the implications that the timing and frequency of typhoons may have for recruitment in *H. diversicolor*.

In the past 50 yr, an average of 1.5 typhoons yr⁻¹ passed Honshu Island between June and October, and there were only 3 yr in which no typhoon came to Honshu Island. Most typhoons passed the western and middle portions of Honshu Island, whereas fewer typhoons passed the northeastern region of Honshu.

Therefore, the typhoon and/or its associated events are favorable cues for *Haliotis diversicolor* in Sagami Bay to synchronize its spawning and may be more reliable for *H. diversicolor* than for *H. discus hannai*, which is found in the northeastern region of Honshu.

Storms caused by typhoons are often advantageous to abalone. For example, the strong current caused by the storms may enhance larval dispersion (Sasaki & Shepherd 1995) and provide a favorable substratum for the settlement of larval abalone by removing sediments from the surfaces of CCA, as has been demonstrated for *Haliotis diversicolor* in a laboratory experiment (Onitsuka 2006). The photosynthetic activity of CCA also decreases as surface sediments increase (Harrington et al. 2005). Overturning of stones caused by a very strong water current can remove surface sediments and may dislodge epiphytes on CCA surfaces, which negatively affect the growth of CCA. Storms may also have negative effects on abalone. The occurrence of deformed larvae (Tanaka 2000) and high mortality of post-larvae and early juveniles (Sasaki 1985) may be caused by water turbulence during storms. The passage of a typhoon and/or its attendant storm seems to be a necessary cue for triggering the synchronous spawning of *H. diversicolor* and could be important in creating favorable conditions for larval settlement, but may negatively affect the survival of post-larvae and juveniles.

The timing of typhoons at the spawning ground may also be important for successful recruitment of *Haliotis diversicolor*. The hatch rate of abalone eggs and subsequent larval development are greatly influenced by changes in seawater temperature (Lu et al. 2004). The hatch rate of *H. diversicolor* eggs was about 95% at 27°C, but decreased to about 40% at 21°C (Lu et al. 2004). If spawning triggered by a typhoon occurs late in the spawning season, the hatch rate of eggs and survival rate of larvae might decrease due to the lower seawater temperature. Individuals born late in the spawning season have a limited time before winter, and thus might not be able to grow large enough to survive in low seawater temperature during winter, as discussed for *H. discus hannai* (Saito 1981, Shibui 1984, Takami 2002).

The timing of typhoon-triggered spawning in *Haliotis diversicolor* may affect its egg quality and influence subsequent post-larval survival. The egg quality of *H. discus hannai*, which influences larval and post-larval survival and growth (Fukazawa et al. 2005), can change within a spawning season (Fukazawa et al. 2007). In *H. discus hannai*, 2 oocyte cohorts can develop during a single spawning season. The eggs developed in the latter oocyte cohort are of higher quality and have higher post-larval survival and growth rates (Fukazawa et al. 2005, 2007). Also, the

quality of eggs developed from the same oocyte cohort become higher later in the spawning season (Fukazawa et al. 2005). A similar phenomenon may occur in *H. diversicolor*, although studies of egg quality have not been conducted in this species.

In this study, we clearly revealed that the spawning of *Haliotis diversicolor* was triggered only by the passage of a typhoon and its associated events by comparing the timing of occurrence of NSP and the changes in gonads of adult abalone. As the next step, direct cues associated with typhoons should be determined and physiological mechanisms underlying resultant spawning examined. The timing of typhoon passage may be a potential factor controlling recruitment success of *H. diversicolor*. Further work is needed to examine the relationship between the timing of spawning and the subsequent post-larval survival and growth.

Acknowledgements. We thank Mr. Y. Kajigaya for providing data on wave height grade and seawater temperature. Dr. C. B. Clarke and Dr. H. Fukazawa helped with surveys, and Mr. H. Kuroki and Mr. H. Aono provided helpful comments. The critical reading of this manuscript by Dr. R. D. Roberts is gratefully acknowledged. This study was partly supported by a Grant-in-aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan (No. 16380126). T.O. was supported financially by a research fellowship from the Japan Society for the Promotion of Science for Young Scientists, Japan (JSPS).

LITERATURE CITED

- Babcock R, Keesing J (1999) Fertilization biology of the abalone *Haliotis laevis*: laboratory and field studies. *Can J Fish Aquat Sci* 56:1668–1678
- Babcock RC, Bull GD, Harrison PL, Heyward AJ, Oliver JK, Wallace CC, Willis BL (1986) Synchronous spawning of 105 scleractinian coral species on the Great Barrier Reef. *Mar Biol* 90:379–394
- Babcock RC, Mundy C, Keesing J, Oliver J (1992) Predictable and unpredictable spawning events: in situ behavioural data from free-spawning coral reef invertebrates. *Invertebr Reprod Dev* 22:213–228
- Benzie JAH, Dixon P (1994) The effects of sperm concentration, sperm-egg ratio, and gamete age on fertilization success in crown-of-thorns starfish (*Acanthaster planci*) in the laboratory. *Biol Bull (Woods Hole)* 186:139–152
- Breen PA, Adkins BE (1980) Spawning in a British Columbia population of northern abalone, *Haliotis kamtschatkana*. *Veliger* 23:177–179
- Counihan RT, McNamara DC, Souter DC, Jebreen EJ, Preston NP, Johnson CR, Degnan BM (2001) Pattern, synchrony and predictability of spawning of the tropical abalone *Haliotis asinina* from Heron Reef, Australia. *Mar Ecol Prog Ser* 213:193–202
- Creese RG, Ballantine WJ (1983) An assessment of breeding in the intertidal limpet, *Cellana radians* (Gmelin). *J Exp Mar Biol Ecol* 67:43–59
- Fukazawa H, Takami H, Kawamura T, Watanabe Y (2005) The effect of egg quality on larval period and post-larval

- survival of an abalone *Haliotis discus hannai*. J Shellfish Res 24:1141–1147
- Fukazawa H, Kawamura T, Takami H, Watanabe Y (2007) Oogenesis and relevant changes in egg quality of abalone *Haliotis discus hannai* during a single spawning season. Aquaculture 270:265–275
- Geiger DL (1999) A total evidence cladistic analysis of the Haliotidae (Gastropoda: Vetigastropoda). PhD thesis, University of Southern California, Los Angeles
- Harrington L, Fabricius K, Eaglesham G, Negri A (2005) Synergistic effects of diuron and sedimentation on photosynthesis and survival of crustose coralline algae. Mar Pollut Bull 51:415–427
- Ino T, Harada K (1961) Spawning season of abalone in Ibaraki prefecture. Bull Tokai Reg Fish Res Lab 31:275–281
- Ishida O, Tanaka S (1983) Spawning seasons of *Haliotis discus discus* and *H. gigantea* in the coast of Amatsuka, Futomi, Chiba prefecture. Bull Chiba Pref Fish Exp Stn 41: 11–22
- Jebreen EJ, Counihan RT, Fielder DR, Degnan BM (2000) Synchronous oogenesis during the semilunar spawning cycle of the tropical abalone *Haliotis asinina*. J Shellfish Res 19:845–851
- Kikuchi S, Uki N (1974a) Technical study on artificial spawning of abalone, genus *Haliotis* III. Reasonable sperm density for fertilization. Bull Tohoku Reg Fish Res Lab 34: 67–71
- Kikuchi S, Uki N (1974b) Technical study on artificial spawning of abalone, genus *Haliotis* IV. Duration of fertility related to temperature. Bull Tohoku Reg Fish Res Lab 34:73–75
- Lu J, Lin Q, Sun Y, Sheng J, Chen Q (2004) Effect of temperature on the early development of *Haliotis diversicolor* Reeve. J Shellfish Res 23:963–966
- Minchin D (1992) Induced spawning of the scallop, *Pecten maximus*, in the sea. Aquaculture 101:187–190
- Oba T (1964) Studies on the propagation of an abalone, *Haliotis diversicolor supertexta* Lischke – I. On the spawning habits. Bull Jpn Soc Sci Fish 30:742–748
- Okuno M, Takashima F, Yamakawa H (1978) Reproductive cycle of *Haliotis diversicolor aquatilis* Reeve. J Tokyo Univ Fish 65:9–13
- Onitsuka T (2006) Studies on the reproduction and early life ecology of *Haliotis diversicolor* at Nagai in Sagami Bay. PhD thesis, The University of Tokyo
- Prince JD, Sellers TL, Ford WB, Talbot SR (1987) Experimental-evidence for limited dispersal of haliotid larvae (genus *Haliotis*; Mollusca: Gastropoda). J Exp Mar Biol Ecol 106: 243–263
- Roberts RD (2001) A review of settlement cues for larval abalone (*Haliotis* spp.). J Shellfish Res 20:571–586
- Saito K (1981) The appearance and growth of 0-year-old Ezo abalone. Bull Jpn Soc Sci Fish 47:1393–1400
- Sasaki R (1985) Larval identification and occurrence of Ezo abalone, *Haliotis discus hannai*, in the adjacent waters of Kesennuma Bay, Miyagi Prefecture. Suisanzoshoku 32: 199–206
- Sasaki R, Shepherd SA (1995) Larval dispersal and recruitment of *Haliotis discus hannai* and *Tegula* spp. on Miyagi Coasts, Japan. Mar Freshw Res 46:519–529
- Shibui T (1984) The relationship between fluctuation in abalone catch and environmental factors in Iwate. Saibai-giken 13:1–20
- Takami H (2002) Studies on the feeding, growth and survival in early life stages of an abalone *Haliotis discus hannai*. PhD thesis, The University of Tokyo
- Takashima F, Okuno M, Nishimura K, Nomura M (1978) Gametogenesis and reproductive cycle in *Haliotis diversicolor diversicolor* Reeve. J Tokyo Univ Fish 65:1–8
- Tanaka K, Tanaka T, Ishida O, Ohba T (1986) On the distribution of swimming and deposited larvae of nursery ground of abalone at the southern coast of Chiba prefecture. Bull Jpn Soc Sci Fish 52:1525–1532
- Tanaka T (2000) On the abnormal development of abalone egg large collected after the low pressure passage. Bull Chiba Pref Fish Exp Stn 56:25–29
- Tomita K (1967) The maturation of the ovaries of the abalone, *Haliotis discus hannai* Ino, in Rebun Island, Hokkaido, Japan. Sci Rep Hokkaido Fish Exp Stn 7:1–7
- Watson GJ, Williams ME, Bentley MG (2000) Can synchronous spawning be predicted from environmental parameters? a case study of the lugworm *Arenicola marina*. Mar Biol 136:1003–1017
- Webber HH, Giese AC (1969) Reproductive cycle and gametogenesis in the black abalone *Haliotis cracheroidii* (Gastropoda: Prosobranchiata). Mar Biol 4:152–159
- Williams JR, Babcock RC (2004) Comparison of multiple techniques to evaluate reproductive variability in a marine bivalve: application to the scallop *Pecten novaezelandiae*. Mar Freshw Res 55:457–468
- Young JS, Demartini JD (1970) The reproductive cycle, gonadal histology, and gametogenesis of the red abalone, *Haliotis rufescens* (Swainson). Calif Fish Game 56:298–309

Editorial responsibility: Otto Kinne (Editor-in-Chief), Oldendorf/Luhe, Germany

Submitted: December 22, 2006; Accepted: July 10, 2007
Proofs received from author(s): November 14, 2007