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Pattern, synchrony and predictability of spawning of the tropical abalone *Haliotis asinina* from Heron Reef, Australia

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ABSTRACT: The spawning biology of the tropical abalone *Haliotis asinina* on Heron Reef, Australia, was investigated to identify putative environmental and endogenous factors controlling spawning. Spawnings by *H. asinina* were highly regular and, in comparison to most other haliotids and marine invertebrates, frequent and extremely synchronous. These events appeared to be regulated by more than 1 environmental cue. The spawning season of *H. asinina* extends from October to April and is associated with an increase in water temperature. During the spawning season, recently captured abalone, housed in flow-through aquaria, released gametes for 2 nights every 2 wk during the new and full moons. However, the exact date of spawning did not correlate precisely with the lunar cycle. Occasionally the spawning events between 2 populations of *H. asinina* on Heron Reef that were 1.5 km apart differed by 1 d, suggesting that differential tidal regimes might influence the date of spawning. The population that was exposed to slightly longer spring low tides occasionally spawned 1 d earlier. In the aquaria, the onset of male spawnings was earlier than the onset of female spawnings by an average of 31 min. The time of spawning of either sex was highly correlated with the evening high tide; males spawned an average of 19 min prior to the high tide, and females spawned 11 min after the high tide. Spawnings were highly synchronous amongst individuals, with 90% commencing spawning within 89 min of the first individual that spawned. A greater percentage of individuals spawned when in the presence of the opposite sex and the frequency of male ejaculation was greater when in the presence of females. Synchronous spawning patterns persisted for 6 wk in *H. asinina* maintained in aquaria; after this period, spawnings continued but were irregular and asynchronous. We propose that low tide exposure and time of high tide indirectly regulate the date and time of spawning respectively, and that these tidal elements influence the spawning biology of *H. asinina* by maintaining endogenous rhythms that persist in non-tidal environments for at least 6 wk.

KEY WORDS: Abalone · Mollusc · Spawning · Synchronous · Tidal cycle · Lunar cycle · Endogenous

INTRODUCTION

Many marine invertebrates, whose life histories include external fertilization, synchronise gamete release among individuals within a population to increase fertilization success (e.g. echinoderms, sponges, molluscs, polychaetes, Babcock et al. 1992; scleractinian corals, Fan & Dai 1995; ascidians, Bingham 1997). While synchronous spawning is a common phenomenon amongst marine invertebrates, the spawning events themselves are often rare (e.g. occurring once per year) or unpredictable. Amongst the haliotids (Mollusca: Vetigastropoda) there is enormous variation in the length of the spawning season and the number of spawning events

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per season (e.g. Webber & Giese 1969, Shepherd & Laws 1974, Tutschulte & Connell 1981, Wells & Keesing 1989). Natural spawnings for most abalone are unpredictable and patterns of spawning are most often determined by indirect measures such as gonad indices (e.g. Webber & Giese 1969, Shepherd & Laws 1974). As such, the mechanisms regulating haliotid spawning remain largely unknown. A number of detailed examinations of abalone spawning biology have identified water temperature (Ino 1952, Webber & Giese 1969, Young & DeMartini 1970), photoperiod (Uki & Kikuchi 1984) and food availability (Shepherd & Laws 1974) as key environmental factors. Factors that directly *synchronize* natural gamete release in wild abalone are not known, probably because of the unpredictable nature of spawning events for most haliotids. Several artificial exogenous factors are known to induce spawning of abalone, e.g. UV irradiated seawater, temperature shock, desiccation, hydrogen peroxide (reviewed in Hahn 1989) but these are only effective on fecund abalone.

In this study we investigated the spawning biology of the tropical abalone *Haliotis asinina*. This abalone is widely distributed throughout the Indo-Pacific, primarily inhabiting intertidal reef flats. Recent studies of the life history of this species indicate that it may be an ideal species for aquaculture and biological research (Singhagraiwan & Doi 1992, McNamara & Johnson 1995, Capinpin & Corre 1996, Counihan et al. 1998). *H. asinina* grows faster than other abalone (e.g. McNamara & Johnson 1995) and has frequent and regular spawning events, negating the need for induced spawnings (Singhagraiwan & Doi 1992, Capinpin 1995, Capinpin et al. 1998). In Thailand, the spawning season of *H. asinina* is year round except April and May, and peaks in October and November when the water temperature is lowest (Singhagraiwan & Doi 1992). In The Philippines, *H. asinina* are serial spawners, spawning asynchronously year round except during May and June (Capinpin et al. 1998). In both these studies (Singhagraiwan & Doi 1992, Capinpin et al. 1998) observations were made on captive abalone housed in aquaria for months, or abalone that had been bred in captivity.

Here we monitored gonad development and the spawning pattern of recently caught *Haliotis asinina* from Heron Reef, Great Barrier Reef, with the goal of detailing long-term spawning patterns and identifying putative environmental and endogenous regulatory factors. An assumption of this study is that the spawning patterns of recently captured abalone (i.e. 1 to 7 d) are the same as for natural populations. As yet there have been no observations of wild spawnings of this species. However, Capinpin et al. (1998) noted that recently captured (less than 6 wk) *H. asinina* exhibited spawning patterns that were more synchronous than longer-term captive abalone. This implies that recently captured abalone may more accurately reveal the spawning patterns of natural populations, and that spawnings by wild abalone will be at least as synchronous as recently captured abalone.

During the spawning season, we monitored the spawning patterns of individuals from 2 populations of *Haliotis asinina* from Heron Reef to determine inter- and intra-population spawning patterns. Since the presence of haliotid gametes is known to induce spawning of the opposite sex (e.g. Murayama 1935, Carlisle 1945, Shibui 1972, Morse et al. 1977) we also investigated the influence that the presence of the opposite sex has on the spawning behaviours of individuals.

METHODS

At Heron Reef, Great Barrier Reef, Australia (23° 27′ S, 151° 55′ E), *Haliotis asinina* inhabits coral bommies in the outer and mid-reef flat zones (Mather & Bennet 1993). This species appears to be nocturnal and feeds primarily on red algae (e.g. *Gracilaria* spp., *Laurencia* spp., *Hypnea pannosa*, McNamara & Johnson 1995). The reef flat at Heron Reef usually experiences 4 tides each day with a maximum tidal range of 3.3 m (Gourlay & Hacker 1997). The reef crest is 0.5 m above the lowest astronomical tide (Sunmap 1983). This results in ponding of reefal waters that change little in depth while the oceanic waters are below the reef crest (i.e. at low tides). This period is greatest at spring low tides. Heron Reef has an artificial harbour (Fig. 1) that influences water movement about the reef flat, particularly the timing and height of tidal changes (Hacker 1995). The wind is predominantly from the south-southeast (150°) and the average wind speed for October to April is

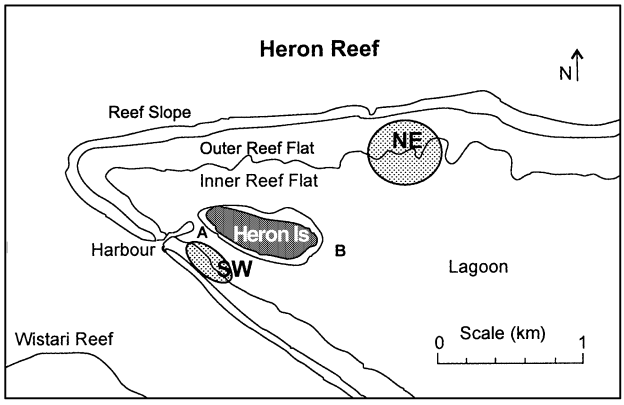

Fig. 1. Heron Reef, Great Barrier Reef, Australia (23° 27′ S, 151° 55′ E). Locations of the 2 populations of *Haliotis asinina* that were sampled in this study, northeast (NE) and southwest (SW) are shown. The artificial harbour is marked. The letters A and B represent sites monitored for tide movements by Hacker (1995), referred to in Fig. 7

6.3 knots (HIRS 1998). The water temperature range for the same period is 25.2 to 27.2°C and average salinity is 35.7 ppt (HIRS 1998). Precise lunar information was obtained from Auslig (Australian Surveying and Land Information Group, Dept of Industry Science and Resources, Canberra, Australia; available at www.auslig.gov.au/geodesy/astro/astro.html).

Gonad indices and spawning observations. The spawning season was determined by monthly examination of gonad development, as described in Shepherd & Laws (1974). Monthly gonad index values were obtained by measuring the cross-sectional area of the gonad and comparing it to the area of the hepatic gland, around which the gonad develops. Twenty adult abalone were collected approximately monthly from the southwest (SW) population (Fig. 1) of *Haliotis asinina* on Heron Reef between 1993 and 1996. The hepatic gland and surrounding gonad were removed and fixed in 5% buffered formalin for 4 d. Sections (5 mm thick) were taken one-third the distance from the tip of the hepatic gland to the tip of the body whorl. The section was photographed using a binocular microscope mounted with a camera, the image then digitized, and the area of the gonad and hepatic gland estimated using the AutoCAD® software (Autodesk, Inc., San Rafael, CA, USA). The gonad index was calculated as the proportion of the gonad area of the total area. During this period, other abalone were maintained for up to 1 mo in open circulation seawater aquaria, at Heron Island Research Station, The University of Queensland. Abalone were sexed according to the colour of the gonad (Singhagraiwan & Sasaki 1991) and maintained in either male-only or female-only aquaria. The abalone were fed daily to saturation with *Laurencia* spp., *Gracilaria* spp. and *Hypnea pannosa*. During regular maintenance, the abalone were observed several times per day. A number of spawnings were fortuitously observed during this period (1993 to 1996), the dates of which were recorded.

Observations of populations. Based on the results of the gonad index analyses and the observations of spawnings described above, collection and observation of *Haliotis asinina* were concentrated during October to April of the following 2 yr (1996/97, 1997/98). Adults of *H. asinina* were collected from Heron Reef approximately every 2 wk (between 2 and 7 d prior to full and new moons) during October 1996 to April 1997 and November 1997 to April 1998 (a few exceptions to the collections existed when access to the Heron Island populations was not possible). At least 30 individuals were collected from each of 2 sites on the reef flat, approximately 1000 m northeast (NE population) and 500 m southwest (SW population) of Heron Island (Fig. 1). Abalone were maintained as described above. PVC-pipe shelters (15 cm diameter, cut lengthwise, approximately 25 cm long) were provided and lights were on during daylight hours only. These animals were not directly exposed to lunar or tidal influences. The abalone were saturation fed daily with freshly collected *Laurencia* spp. Individuals were sexed as described above and maintained in male-only or female-only tanks (350 mm × 350 mm × 500 mm; 60 l). Tanks were checked several times each day for evidence of released gametes. On each potential spawning night, at least 30 individuals from the NE population (1996/97 season) and from each of the NE and SW populations (1997/98 season) were observed. The time that spawning began by the first individual of each sex was recorded as 'onset[all]'. These times were compared with local tide cycles: correlation analyses were used to compare the time onset[all] with the evening high tide.

To determine how long the observed spawning patterns would persist in captive abalone, individuals were maintained in the aquaria until their spawning patterns were no longer synchronous. Due to the logistical constraints of accurately monitoring many individuals concurrently, only casual observations were made of these longer-term captive individuals; spawnings that were asynchronous with recently captured abalone were noted.

Observations of individuals. For 1 set of spawning events (new moon, November 1998) 64 individuals were tagged: 16 of each sex from each population (NE and SW, Fig. 1). To examine what influence the presence of the opposite sex has on the spawning behaviour of individual abalone, abalone were maintained in 1 of 2 tank types: mixed-sex (male and female together), or same-sex (male or female only). For each population, the abalone were separated as follows: 2 tanks of 4 females, 2 tanks of 4 males, and 2 tanks of 4 females with 4 males. During the spawning period, the times of spawning of every individual throughout each spawning night was recorded. From these data, the total number of ejaculates (an independent release of gametes), the frequency of ejaculation and time period of spawning (for total ejaculations) for individual abalone were calculated. For each individual, the time period onset[ind.] was calculated as the time difference between the first ejaculation of each individual and the first ejaculation of the first individual to spawn from the same population, sex and tank type. Onset[ind.] values gave a measure of the spread of individual spawn times, i.e. the synchronicity of spawning.

The design of this experiment was such that the density of individuals in tanks differed between treatments, as a consequence of placing the same number of response-individuals in each tank (i.e. 4 males in tanks without females, and 4 males in tanks with 4 females). All statistical analyses were conducted using the software JMP IN version 3.2.1 (JMP IN Statistical Discovery Software, SAS Institute Inc., Cary, NC, USA).

RESULTS

Spawning season

The gonad indices were determined from monthly inspections of sectioned gonad with hepatic gland samples from *Haliotis asinina* (Fig. 2). An analysis of variance on the means pooled over 4 yr (1993 to 1996) separates the months that were sampled into 2 significantly different groups: October to April (excluding November and February which were not sampled) and May to September (Tukey Kramer's HSD, $p < 0.0001$, $n = 23$, Fig. 2). Nineteen spawnings by abalone were observed in aquaria during this period. The date of these spawnings was always closely associated with either a new or full moon. Of the 19 spawnings observed, 16 occurred within 2 d of a new or full moon, the remaining 3 occurred either 3 or 4 d following a new or full moon.

Spawning patterns of populations of *Haliotis asinina* from Heron Reef

Spawning of *Haliotis asinina* in aquaria occurred over 1 to 3 nights approximately every 12 to 15 d during the 1996/97 and 1997/98 spawning seasons (Fig. 3 shows the 1997/98 season). Spawning events always occurred around either the new or full moon, irrespective of how many days (2 to 7 d) prior to the new or full moon the abalone were collected. Occasionally a small number of male abalone (1 or 2 ind.) spawned the night before a major spawning of the rest of the same population. Only spawning events in which both males and females spawned were used for the analyses of population spawning patterns. Early in the season (e.g. October) spawning events began 1 d prior to, or on the same day as, the new or full moon. As the spawning season progressed the date of spawning shifted later relative to the lunar cycle, so that by April spawning began 1 to 2 nights after the new or full moon (Fig. 3).

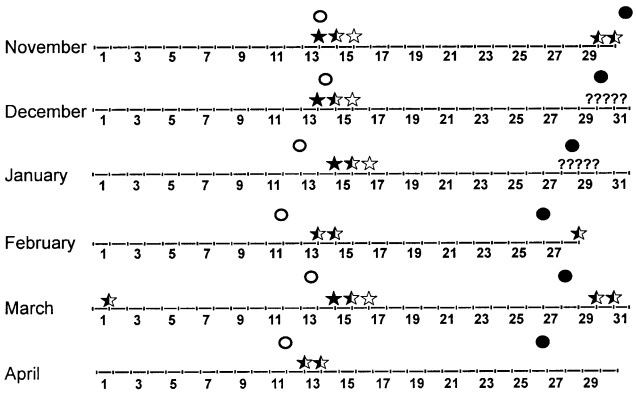

Fig. 3. Seasonal spawning pattern of *Haliotis asinina* on Heron Reef for 1997/98. (○) Full moon, (●) new moon, (★) spawning of the NE population only, (☆) spawning of the SW population only, (⯪) spawning of both NE and SW populations, (?) spawnings that could not be observed

Analysis of the spawning dates with respect to the collection site of the abalone (i.e. NE or SW reef flat populations) indicated that individuals from each population spawned for a maximum of 2 nights every 2 wk and that the 2 populations spawned on either the same nights or differed by 1 night (Fig. 3). This occasional population-specific pattern occurred in 4 of the 9 spawning events observed during the 1997/98 spawning season (only the NE population was monitored during the 1996/97 season) and resulted in only 1 night of overlap on which abalone from both populations spawned. Differences between the dates of spawning of the 2 populations were observed to occur during full-moon spawnings only, and when they occurred the NE population always spawned 1 d earlier than the SW population (Fig. 3).

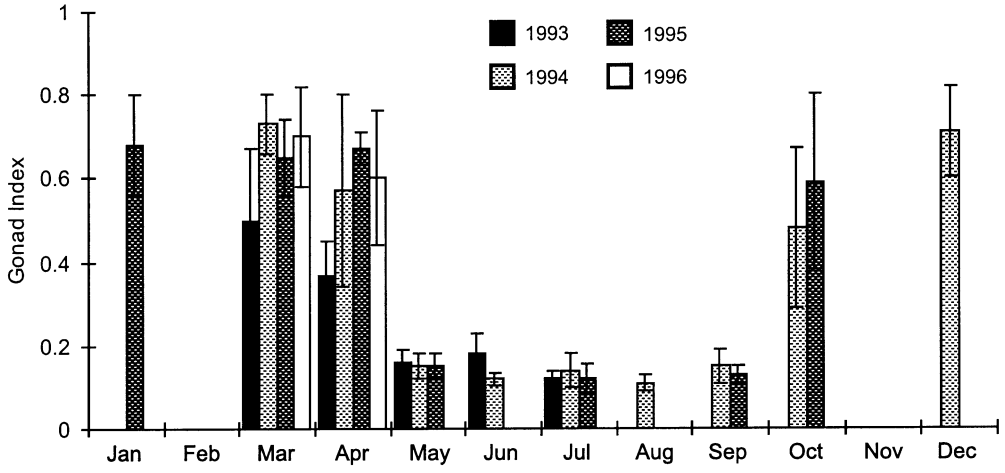

Fig. 2. Monthly means for gonad indices of *Haliotis asinina* (male and female) calculated from 1993 to 1996. For each month sampled, gonad indices were calculated from 20 individuals of each sex. Error bars are the standard deviations. The months sampled can be divided into 2 statistically significant different groups: May to September, and October to April (excluding November and February, which were not sampled) (Tukey Kramer's HSD, $p < 0.0001$, $n = 23$)

The time of the first spawning by individuals from both sexes (from either population) for each of the spawning events (n = 36 during 1996 to 1998) is represented as 'onset[all]'. The time of spawning of the first male (i.e. male onset[all]) differed from the female onset[all] (paired *t*-test, $p < 0.0001$, n = 36 spawning events). The average time difference of onset[all] between male and female spawnings was 30.7 min (SD = 37.4, n = 36, Fig. 4), with the females commencing spawning after the males. The onset of spawning of the population (onset[all]) on each day was strongly correlated with the tidal cycle. A pairwise correlation analysis between the times of onset[all] of male spawnings, and the times of the evening high tide (spawn times for the 2 populations were considered together) demonstrated that this relationship was significant (Pearson's coefficient = 0.68, $p < 0.0001$, n = 36 spawning events, Fig. 5), with males spawning on average 19.0 min (SD = 49.8, n = 36) before the time of high tide. The range of time differences between male onset[all] and the evening high tide time was −105 to 64 min. A correlation of female onset[all] time with the time of the evening high tide was also significant (Pearson's coefficient = 0.62, $p = 0.0001$, n = 36, Fig. 5). Females began spawning on average 11.6 min (SD = 58.6, n = 36) after the high tide (range = −100 to 136 min).

Observations of individuals maintained in flow-through aquaria (i.e. with no moonlight or tidal influences) for an extended period time indicated that the spawning patterns described above persist for 3 spawning cycles (i.e. 5 to 6 wk). During this time, captured individuals spawned in synchrony with each other and with newly collected individuals of the same population. After 6 wk, some individuals continued to spawn approximately every 2 wk, but were no longer completely synchronous with the lunar cycle, tidal cycle, or other individuals.

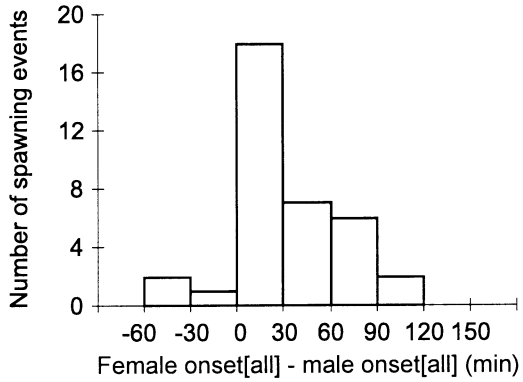

Fig. 4. Distribution of time differences (min) between the male onset[all] and female onset[all] for each spawning night. Each value is calculated as the time of the first ejaculation (male and female) from either population on a given spawning night (n = 36 spawning nights between 1996 and 1998)

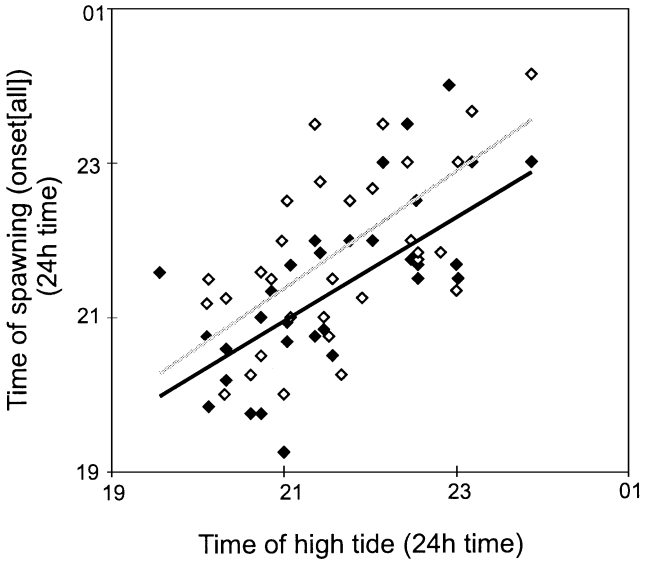

Fig. 5. Correlation analyses showing the relationship between the times of male onset[all] (♦) or female onset[all] (◇) and the time of the evening high tide. Each symbol represents the first individual (male and female) to spawn from either population for each spawning night (n = 36 spawning events between 1996 and 1998). The line of best fit for the male data is represented by the solid line, and for female data, by the gray line

Spawning patterns of individual *Haliotis asinina*

A detailed investigation was conducted of 1 complete spawning event (new moon, November 1998) in which 64 individuals were tagged, separated into same- and mixed-sex tanks, and monitored closely. Although the number of independent replicates was low, strong patterns were observed. Each individual spawned 1 or 2 nights, or not at all. Of all individuals, 78.1% spawned on at least 1 night during the spawning event.

No statistically significant differences were found between the number of nights that each individual spawned between either population or tank type (3 factor ordinal logistic regression, population $p = 0.34$, tank type $p = 0.31$). However, sex had a significant effect upon the number of nights any individual spawned ($p = 0.009$); a greater percentage of males spawned for at least 1 night than did females (89.7 and 65.7% respectively), and more males spawned for 2 nights than did females (21.9 and 6.3% respectively; Fig. 6). Although not statistically significant, the influence of the opposite sex (i.e. tank type) on the number of spawning nights is noteworthy. A greater percentage of both males and females spawned for 2 nights when in mixed-sex tanks (25 and 12% respectively) compared to males or females in same-sex tanks (19 and 0% respectively; Fig. 6).

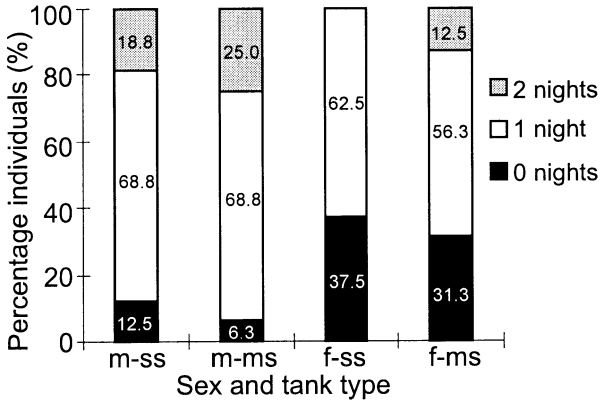

Fig. 6. Number of nights any individual spawned in a single spawning event (2 consecutive spawning nights), for male (m) and female (f) *Haliotis asinina* in mixed-sex (ms) or same-sex (ss) tanks. Numbers in the graph bars represent the percentage of individuals that spawned 0, 1 or 2 nights

The mean values of the onset[ind.] and ejaculation statistics for each individual were pooled within each tank, since individuals within the same tank could not be considered independent replicates. In general, spawnings were highly synchronous among all individuals. Fifty percent of the individuals that spawned began to release their gametes within 29.5 min of the first individual (across sexes, populations and tank types); 90% began to spawn within 89.1 min of the first individual. An analysis of the onset[ind.] times calculated as the time difference between the first ejaculation of each individual and the first ejaculation of the first individual to spawn (irrespective of sex, tank type, or population) suggested that both sex and population significantly influenced onset[ind.] (3 factor ANOVA, sex $p = 0.0331$ population $p = 0.0001$, tank type $p = 0.2263$, $n = 24$). However, when onset[ind.] was calculated according to the sex, population and tank type of each individual (i.e. the time difference between the first ejaculation of each individual and the first ejaculation of the first individual from the same sex, population and tank type), sex was no longer significant (3 factor ANOVA, sex $p = 0.8289$, population $p = 0.0079$, tank type $p = 0.5174$, $n = 24$). This suggests that the mean onset time for males and females differed (in comparison to each other), but the spread of the individual onset times within each sex, i.e. the synchrony of individuals, was the same for both sexes. The population of origin did influence the individual onset times; abalone from the NE population showed a higher degree of synchrony compared to SW abalone (mean onset[ind.]: NE population = 16.4 min, SD = 14.5 min; SW population = 40.7 min, SD = 27.1 min). All abalone (male or female) that spawned on any 1 night did so within 66 min of the first individual for the NE population, and within 102 min for the SW population.

In most cases the type of tank (i.e. mixed sex or same sex) did not influence the spawning behaviour of individuals, although the population of origin affected some ejaculation parameters (Table 1). However, the significance of the population effect cannot be commented upon without replication of observations of other spawning events. In some cases, the presence of the opposite sex did have an influence; males in tanks with females had a significantly higher ejaculation frequency than males without females (2-factor ANOVA, tank effect $p = 0.009$, population effect $p = 0.16$). Although not significant, the total number of ejaculations by male abalone was greater when in the presence of females, and the time to complete all ejaculations was shorter, compared to males without females (for both populations, Table 1). Males had no influence on female ejaculation number, frequency, or total spawn time period (Table 1).

Table 1. Summary of ejaculation statistics for *Haliotis asinina* individuals. Means (and standard deviations) are shown for each sex, from either the northeast (NE) or southwest (SW) populations maintained in either mixed-sex (ms) or same-sex (ss) tanks. Mean time for individuals to complete all their ejaculations (ejac.), the total number of ejaculations, and ejaculation frequency are all calculated from pooled means of each replicate tank. The column Onset[ind.] represents the mean of the time difference between the first ejaculation of each individual and the first ejaculation of the first individual from the same population, sex and tank type, and was also calculated using pooled means

Sex	Tank type	Population	Time for all ejac. (min)	No. of ejac.	Ejac. frequency (min^{-1})	Onset[ind.] (min)
Female	ms	NE	3.83 (4.91)	1.17 (0.29)	0.85 (0.26)	4.33 (5.13)
		SW	9.33 (7.54)	1.46 (0.29)	0.61 (0.26)	30.58 (18.27)
	ss	NE	1.75 (1.50)	1.13 (0.25)	0.91 (0.18)	13.00 (13.39)
		SW	15.83 (12.96)	1.67 (0.47)	0.39 (0.44)	18.33 (0.94)
Male	ms	NE	28.38 (9.03)	11.46 (9.72)	0.54 (0.20)	10.21 (7.89)
		SW	46.25 (30.30)	34.08 (20.92)	0.68 (0.20)	14.58 (14.38)
	ss	NE	50.56 (25.70)	8.67 (7.37)	0.22 (0.18)	6.33 (9.56)
		SW	52.50 (28.83)	18.83 (13.61)	0.37 (0.14)	30.00 (14.47)
Female	ms + ss	NE + SW	6.26 (7.62)	1.29 (0.34)	0.75 (0.30)	14.80 (13.38)
Male	ms + ss	NE + SW	43.20 (23.08)	17.74 (15.41)	0.46 (0.24)	14.89 (13.52)

DISCUSSION

The season, date and time that *Haliotis asinina* spawns appear to be regulated by different factors. These factors influence the length and time of the spawning season, the precise timing of the spawning event, and the level of spawning synchronicity within and between populations and individuals.

The spawning season of *Haliotis asinina* on Heron Reef is from October to April, coinciding with the warmest water temperatures (25.2 to 27.2°C, HIRS 1998). In contrast, *H. asinina* in the waters surrounding Thailand and The Philippines spawn all year round, except the summer months when the water temperature is at its warmest (Singhagraiwan & Doi 1992, Capinpin et al. 1998). Combined, these data indicate that *H. asinina* spawns within a defined temperature range, rather than above a minimum or below a maximum temperature value. Water temperature has been identified as an important influence of spawning season in other haliotids (e.g. *H. cracheroidii*, Webber & Giese 1969; *H. discus hannai*, Uki & Kikuchi 1984).

The highly regular spawnings observed for the 2 Heron Reef populations of *Haliotis asinina* suggest that the factors controlling these events are cyclic in nature. Although it has often been suggested the lunar cycle may be a primary environmental cue regulating the synchronous spawning of marine invertebrates (e.g. Olive & Garwood 1983, Parsons et al. 1992), only in a very few cases does the lunar cycle exactly correlate with spawnings (e.g. the limpet *Acanthozostera gemmata*, Korringa 1947). Two observations indicated that the lunar cycle alone does not control the pattern and synchrony of spawning of *H. asinina* on Heron Reef. First, there is variation in the date of spawning about the new or full moon during the spawning seasons (e.g. 1997/98, Fig. 3). Early in the season abalone tend to spawn prior to, or on the same days as, new and full moons, while later in the season abalone spawn after new and full moons. Second, the spawning dates of the NE and SW populations occasionally differ by 1 d. Since these 2 populations are approximately 1.5 km apart on contiguous reef it is unlikely that exposure to moonlight (duration or intensity) or gravity differs between these 2 sites.

When the 2 populations of *Haliotis asinina* spawned on different dates, the NE population always spawned before the SW population, suggesting consistency in the variation of regulating factors. Although a number of abiotic and biotic factors may vary between the NE and SW habitats, variation in local tidal regimes appear to be the best candidate. Differences in semi-diurnal tidal regimes (timing and height) between sites on the Heron Reef flat have been previously detected (Fig. 7, modified from Hacker 1995; refer to Fig. 1 for site locations), with some sites exposed at low tide for longer periods of time or to lower water levels than others (Figs. 1 & 7). Theses differences are particularly apparent during spring tides and are likely to be caused by the artificial harbour and/or variation of the reef's geomorphology (Hacker pers. comm.). Based on Hacker's (1995) observations (Fig. 7), it is likely the NE and SW populations of *H. asinina* on Heron Reef experience slightly different exposure times to ponding low water and/or to low water levels. This suggests that *H. asinina* on Heron Reef can detect subtle changes in tidal profile, and that there is a threshold tidal height or exposure time required to induce the physiological changes and behaviour associated with spawning. These small differences may result in the NE population being exposed to a threshold tide 1 d before the SW population. Evidence that a spawning cue is detected earlier on the day of a spawning is presented by the analyses of *H. asinina* gonad development over a 2 wk spawning period on Heron Reef. This analysis indicates that the final stage of oocyte maturation does not begin until about 12 to 18 h, or 2 to 3 tides, prior to spawning (i.e. after being exposed to an early morning spring low tide) (Jebreen et al. 2000).

Abalone in this study were collected at least 2 d prior to spawning and as such were not exposed directly to

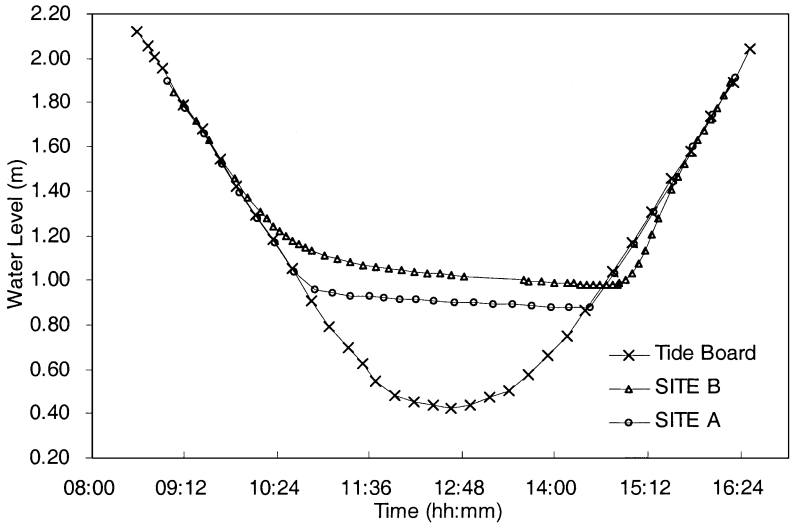

Fig. 7. Tide height (m) with time of day (h:min) for 2 sites on Heron Reef flat (A and B, refer to Fig. 1) during a spring low tide (1 November 1994). The tide board refers to oceanic water height outside the reef flat. Site B is exposed for approximately 10 min longer at low tide than Site A; however Site A experiences a lower water level during low tide than Site B

the tidal influences that appear to be inducing spawning. Furthermore, synchronous spawning patterns persisted for up to 6 wk in *Haliotis asinina* maintained in aquaria. We propose that an environmental factor associated with local tidal cycles regulates an endogenous clock that in turn controls the physiological state of the gravid abalone. The loss of spawning periodicity and synchrony in long-term captive abalone (Capinpin et al. 1998, this study) suggests that these natural environmental signals are required to maintain these endogenous rhythms. Variations in the timing of spawning have been reported between populations of *H. cracherodii* that are only 10 km apart (Webber & Giese 1969). In addition, Shepherd & Laws (1974) also described variability in the timing and duration of spawning of *H. ruber* at different locations, concluding that local environmental variables are of great importance in regulating reproduction. However, it is yet unknown if other haliotids share spawning regulatory mechanisms similar to *H. asinina*.

Other marine invertebrates can detect and respond to small differences in tide. For example, the spawning behaviour of the scallop *Placopecten magellanicus* within 1 bay is tidally cued and sometimes differs between populations (Parsons et al. 1992). In addition, foraging and spawning activities of siphonarian limpets are cued to low tides, but vary between habitats (Iwasaki 1995). The spawning of many free-spawning marine invertebrates is regulated by a combination of exogenous and endogenous factors (e.g. the gastropod *Gibbula umbilicalis*, Clare 1986; the polychaete *Typosyllis prolifera*, Franke 1986). Rarely are exogenous factors alone responsible for synchronizing spawning (Naylor 1976, Olive & Garwood 1983).

The precise timing of gamete release by *Haliotis asinina* is highly synchronous between all individuals of the 2 populations and is closely associated with nighttime spring high tides (Fig. 5). Nighttime with spring tides is particularly favoured for spawning by broadcast spawners, especially gastropods, probably because they provide protection from predation and increased dispersal (Berry 1986). Spawning by *H. asinina* during the relatively slack water of high tide may actually minimize gamete dispersal, thereby increasing fertilization success. Fertilization success in *H. asinina* may also be increased by the release of gametes by males prior to females, such that sperm are likely to be present when the eggs are released. The timing of spawning remains highly correlated with evening tides in abalone maintained in aquaria for up to 6 wk, suggesting that a second component of the endogenous rhythm, regulated by an environmental factor related to the high tide, determines the time of gamete release.

In other haliotids, the presence of gametes induces spawning of the opposite sex (e.g. Murayama 1935, Carlisle 1945, Shibui 1972, Morse et al. 1977). The percentage of both female and male *Haliotis asinina* that spawns on any given night increases in the presence of the opposite sex (although not statistically significant in this study). This suggests that in the absence of the opposite sex, some individuals of *H. asinina* may delay releasing gametes, perhaps until another date when the opposite sex is in the close vicinity and the chances of fertilization are therefore greater. Neither the frequency nor synchronicity of *H. asinina* female ejaculations is affected by the presence of males. However, the presence of *H. asinina* females appears to affect male spawning behaviour by inducing more frequent sperm release. Since the females generally release their gametes *after* the males, it seems the females may release a factor *prior* to egg release that influences male spawning behaviour. The sex pheromones of most marine invertebrates are released at the same time as the gametes (e.g. Hardege et al. 1996, Zeeck et al. 1998), and other haliotid studies suggest it is the presence of the eggs themselves that induces sperm release (e.g. Morse et al. 1977). It appears here that both individuals may release factors that affect the spawning behaviour of the opposite sex. Whether in fact these factors are sex-specific cannot be determined from these experiments. In this study, the effects of the presence of the opposite sex cannot be separated from the effects of the presence of more individuals, irrespective of sex. However, if the effects observed are the result of a higher density of abalone rather than the opposite sex specifically, the implications are largely unchanged: abalone may delay releasing their gametes until more abalone surrounds them, and males may ejaculate more frequently when more abalone are present. Both scenarios result in spawning behaviours that will potentially encourage successful fertilizations. We do not know of any other study with marine invertebrates that shows that either the density of animals or the density of the opposite sex specifically influences the ejaculation behaviour of individual animals. There is, however, evidence to show that fertilization success is significantly influenced by population density because of the resultant density of gametes (e.g. Levitan 1991, Young et al. 1992, Claereboudt 1999). Experiments designed specifically to investigate the presence of spawning-related factors are necessary to confirm their presence, elucidate their nature and detail their effects.

No other abalone has spawning patterns that are as predictable, synchronous and regular as *Haliotis asinina*. *H. discus hannai*, a cold water Japanese abalone, spawns in a very predictable manner (1 h after sunset on the day the water temperature drops below 20°C), but spawning is not frequent (Ino 1952). *H. gigantea* and *H. sieboldii* also release gametes when the water temperature drops below 20°C, but the exact timing

varies (Ino 1952). Many species of haliotids spawn naturally only once or twice per year, e.g. *H. fulgens*, *H. sorenseni*, *H. cracherodii*, *H. iris* and *H. australis* (Webber & Giese 1969, Poore 1973, Leighton 1974, Tutschulte & Connell 1981). Other species spawn more frequently but with less synchrony, e.g. *H. corrugata* and *H. roei* (Shepherd & Laws 1974, Tutschulte & Connell 1981). There is also often considerable variation in spawning seasons and spawning patterns between allopatric populations, making prediction of spawning events difficult, e.g. *H. ruber* and *H. rufescens* (Shepherd & Laws 1974, see Hahn 1989). In all cases, factors that directly synchronize natural gamete release are not known. This is probably because the unpredictability of spawning events for most haliotids has impeded detailed studies of natural spawning behaviours. However, there are other marine invertebrates whose spawning events are predictable and frequent, and controlled by a combination of endogenous and exogenous factors. For example, the spawning of the polychaete *Perinereis nuntia* var. *brevicirrus* is regulated by an increase in water temperature which induces maturation, the lunar cycle which regulates swarming, and species-specific signals which regulate swarm times and induce gamete release (Hardege et al. 1994).

The highly rhythmic and remarkably synchronous and predictable spawning patterns of *Haliotis asinina* from Heron Reef allow for detailed analyses of the environmental and endogenous factors controlling its reproduction. We have shown that reproduction is regulated by water temperature, by local tidal regimes that maintain endogenous rhythms, and by conspecific factors. This study, when combined with other abalone studies, suggests that a variety of species-specific mechanisms underlie the regulation of haliotid spawning.

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