INDUCTION SPAWNING FOR THE TROPICAL ABALONE (*Haliotis asinina*) IN THE LABORATORY

D.E. Djoko Setyono

ABSTRACT

The main purpose of this study was to test and understand the effectivity of previous successful treatment to induce spawning in temperate abalone, including the use of hydrogen peroxide, vigorous aeration, desiccation, thermal shock, and UV-irradiated seawater, to induce spawning in the tropical abalone (*Haliotis asinina*) from southern Lombok waters, NTB. Approximately 90% of mature animals conditioned in the laboratory could be spawned successfully. Wild freshly mature broodstock collected in the morning during spring low tide failed to spawn under any treatment tried, while in wild freshly mature broodstock collected in the afternoon during spring low tide, approximately 45% of the males and 37% of the females, spawned successfully under all treatments. Mature animals ready to spawn were usually found creeping up the tanks close to the water surface, and were very active but relaxed, and the foot was soft and flabby. In the laboratory, *H. asinina* from southern Lombok waters released gametes at night between 11:00 pm and 01:00 am. The conditioned broodstock had average batch fecundity ranged from 50,000 to 435,000 eggs and the freshly caught wild broodstock ranged from 50,000 to 105,000 eggs. Approximately 75% of spawned eggs were found to be ripe and the remaining 25% were unripe. In general, no artificial spawning induction is required to spawn tropical abalone (*H.* asinina) in laboratory (hatchery).

KEYWORDS: spawning induction, tropical abalone, *Haliotis asinina*

INTRODUCTION

Successful aquaculture is dependent on the ease with which cultured animals can be induced to reproduce in captivity. The first step in abalone culture is to develop reliable procedures for spawning, which is ultimately dependent on the success of the control of artificial induction of spawning (Hahn, 1989; Fleming & Hone, 1996).

Although many species of abalone can be induced to spawn under laboratory conditions, the induction methods used often have little relevance to natural stimuli. In temperate regions, the control of spawning is vital steps for successful seed production, as the abalones often only spawn during a short period of time, usually in spring and summer (Kikuchi & Uki, 1974; Shepherd & Laws, 1974; Grange, 1976; Ikenoue & Kafuku, 1992; Saout *et al.*, 1999). However, in tropical regions, spawning may not be subject to such constraints as the abalone breed continuously, can often spawn throughout the year (Jarayabhand & Paphavasit, 1996; Capinpin *et al.*, 1998; Setyono, 2003a).

Induction of spawning of abalone in nature is not completely understood, and several endogenous and exogenous factors have been proposed for its control (Hahn, 1989). With regards to exogenous factors, spawning might be caused by sudden changes in sea temperature, exposure to the air during low tide, changes in photoperiod, phase of the lunar cycle, release of gametes from other individuals in the same population, or by a combination of these factors or others (Webber, 1977; Tutschulte & Connell, 1981; Runham, 1988; Hahn, 1989).

A combination of desiccation (exposed to air) and sudden change in water temperature (thermal shock) is considered to play an important role in spawning of Taiwanese abalone (*H. diversicolor supertexta*) (Chen, 1984). In the laboratory, vigorous aeration was applied successfully to induce spawn-

Research Center for Oseanography, Jakarta

ing in *Trochus niloticus* (Dwiono *et al.*, 1995; 1997), *Turbo marmoratus* (Setyono & Dwiono, 1998) and *Turbo chrysostomus* (Setyono, 2003b).

Among the endogenous factors, prostaglandins (PGs) and some amines produced by nerve cells are considered to play an important role in mollusc spawning (Khotimchenko & Deridovich, 1991). There are two widely used methods to induce abalone to spawn which are putatively correlated with a precursor to prostaglandins (Moss *et al.*, 1995). The first involves exposing the abalone to ultraviolet (UV) - irradiated seawater for several hours, and the second involves the addition of hydrogen peroxide (H2O2) (Morse *et al.*, 1977; Morse, 1984). Both ultraviolet (UV)-irradiation and addition of hydrogen peroxide (H_2O_2) to seawater are believed to produce hydroperoxy free radicals, HOO-, or peroxy radicals, -OO- (Morse *et al.*, 1977; Uki & Kikuchi, 1984). These radicals act as a donor of electrically activated oxygen suitable for the cyclooxygenase-catalysed addition of oxygen to a precursor to form prostaglandin (Morse, 1984; Hahn, 1992).

Hydrogen peroxide (H_2O_2) has been used successfully to induce spawning in *H. coccinea canariensis* (Pena, 1986) and *H. iris* (Tong & Moss, 1992; Moss *et al.*, 1995). Desiccation/thermal shock and UV-irradiated seawater has been used successfully to induce spawning in *H. diversicolor* (Chen, 1984), *H. rufescens* (Ebert & Houk, 1984), *H. tuberculata* (Clavier, 1992), and *H. asinina* (Jarayabhand & Paphavasit, 1996).

In this study, successful previous procedures to induce spawning in temperate abalone, including the use of hydrogen peroxide, vigorous aeration, desiccation, thermal shock, and UV-irradiated seawater were tested to induce spawning in the tropical abalone (*H. asinina*) from southern Lombok waters, NTB.

MATERIALS AND METHODS

Source of Broodstock

Abalones used for this study were collected from coastal areas of southern Lombok waters, NTB. Collection was carried out during the low tides around the new moon and full moon, by picking up the abalone gently from their shelter under rocks, dead coral or within crevices. Animals having a size >50 mm shell length were collected for this part of the study. It is known that male and female *H. asinina* become sexually mature at a size of 45 mm and 50 mm, respectively (Setyono, 2003a). After field collection the abalone were brought to the 'Loka Lombok Marine Laboratory', where the experiments took place.

To understand the spawning behaviour of tropical abalone (*H. asinina*), the abalone were separated between conditioned broodstock which were held in indoor concrete tanks for more than a month; and freshly captured broodstock collected from the wild prior to induction of spawning.

The first groups, i.e., the conditioned broodstock, were held for use in a regular spawning programme (fortnightly) in 'Loka Lombok Marine Laboratory', Lombok, eastern Indonesia. One hundred males and 100 females abalone >50.0 mm were held in concrete rectangular tanks (2 m long, 0.75 m wide, 1.5 m deep). The tanks were filled with approximately 2,000 liters of fresh seawater, pumped directly from the sea. Animals were fed *ad libitum* on fresh algae, mainly *Gracilaria* spp., which was collected from nature. New fresh algae were added every 3—4 days. Fresh seawater was pumped into the tanks every 1—2 days for about 3—4 hours. The tank was shaded from direct sunlight during the day and was in darkness during the night, and the water was aerated. Water temperature ranged from 26°C in the morning to 28°C in the afternoon. In this group, animals were induced to spawn using thermal-shock only.

The second group, consisting of 150 males and 150 females of freshly mature captured wild abalone of sizes >50 mm shell length, collected from the wild during low tide at around four days before and after the new moon and full moon, on the day prior to treatment. Freshly captured broodstock were separated into two groups: animals collected during low tide occurring in the morning (6—9 am) and animals collected during low tide occurring in the afternoon (3—6 pm). All methods of spawning induction were treated in this second group.

A visual examination on gonad bulk (Setyono, 2004) was carried out in all broodstock prior to treatment to induce spawning. Mature animals with gonad bulk >49% were selected. Animals were sexed, the shell was brushed using a toothbrush, washed, and labeled with a numbered plastic tag glued onto the outside of the shell.

After selection and measurement, broodstock were put in black cylindrical plastic containers. The containers were then filled with about 20 liters of filtered seawater and aerated. When it was possible, equal numbers of 5 male and 5 female abalone were used in each spawning induction.

Spawning Induction

The experiment was set up the day before the intended trial. Five spawning tanks (black cylindrical plastic containers, 48 cm in diameter and 28 cm height) were cleaned and filled with 20 liters of filtered seawater. The same numbers of 5 mature males and 5 mature females (if possible) were placed in the same tank (Tables 1 and 3). Each individual was used as a pseudoreplicate.

In the afternoon, mature broodstock were subjected to various treatments, including hydrogen peroxide, vigorous aeration, desiccation/thermal shock, and UV-irradiated seawater. Untreated mature broodstock were held in a spawning tank under identical condition as a control. All spawning tanks were held in darkness and the animals given a very low level of aeration, and no food. The animals were left undisturbed and observed regularly every 15 minutes to see if there was any spawning. Spawning experiment started at 5 pm and stopped at 3:00 am.

Hydrogen peroxide

Mature broodstock were placed in a spawning tank filled with 20 litres of filtered seawater at ambient temperature (28°C). Hydrogen peroxide (H_2O_2; 30%) was then added to the water in the spawning tank to make a concentration of 0.015% v/v (modified from Buchal *et al.*, 1998). Mature broodstock were exposed to the H_2O_2 for one hour and then the spawning tank was rinsed and refilled with filtered seawater at ambient temperature (28°C).

Vigorous aeration

Mature broodstock were placed in a small plastic bucket filled with filtered seawater, just high enough to cover the animals. An open ended aeration line was placed in the bottom of the bucket. Vigorous aeration (speed was not noted) was then given for 3—6 hours. Following this treatment, the mature broodstock were transferred into a spawning tank filled with 20 liters of filtered seawater at ambient temperature (28°C).

Desiccation and thermal shock

Mature broodstock were first exposed to the air for about 1 hour (30°C—31°C). Following this, 20 liters of filtered seawater, which had been heated to 3°C—4°C above the ambient temperature (28°C), was poured into the tank to give the animals a temperature shock.

UV-irradiated seawater

Mature broodstock were first placed in spawning tanks. Filtered seawater, which had been passed through a UV-light device (Made in USA, Portable UV no: 4971687, 360U/50Hz, POR modular system) was poured directly onto the animals to a volume of approximately 20 liters.

Control

Mature broodstock were placed in spawning tanks that had been filled with filtered UV-irradiated seawater, prepared in the morning or one day prior to the experiment. It was assumed that the effect of UV-irradiated seawater on spawning had disappeared after 2—3 hours (Dr. Barrie Peake, Department of Chemistry, University of Otago, personal communication). Reason for using UV-irradiated seawater was to provide the control animals with suitable clean seawater. Control animals were placed in the spawning tank at 3—5 pm and left in the tank till the following day.

Batch Fecundity and Fertilization Rates

After spawning was completed, broodstock were lifted out of spawning tanks and placed in holding tanks. The water in the spawning tank was stirred gently to maximize fertilization, and then left undisturbed for about 30—45 minutes before the eggs were washed and transferred into hatching tanks. All post-spawning work used filtered and UV-irradiated seawater.

Eggs were washed by decantation (3—5 times) until the water was clean. Eggs were then siphoned gently from the spawning tank through a 100 µm Nitex screen placed on top of a plastic bucket filled with seawater. Following filtration, eggs were put into a beaker filled with one liter of seawater and stirred gently to thoroughly mix the eggs through the entire water column. At least three replicates of a one ml sample were taken from a one liter mixture of eggs and observed under a stereo-microscope and the number of eggs in the sample

counted. Average batch fecundity was determined by divided the calculated total eggs with the number of spawned female. Ten replicates of ten eggs were also examined to estimate fertilisation rates. Fertilisation rate was determined by counting the number of cleaved eggs and expressing as percentage of the total eggs counted.

Statistical Analysis

The 'Fisher exact test' was used to analyze the proportion of spawned animals in treated broodstock compared to the proportion of spawned animals in the control. Data of batch fecundity and fertilization rates were first tested for normality before analysis of variance was performed. The Scheffe Post Hoc test was used to identify significant differences between mean value of each variable. All tests were performed using software packages of Microsoft-Excel 98 (Microsoft Corporation) and DataDesk 4.1 (Data Description, Inc. Ithaca, N.Y.). The statistical significance level (P) was set at 0.05.

RESULTS

Conditioned Broodstock

The ability to induce spawning of conditioned broodstock was independent of lunar cycle (new moon and/or full moon). Mature animals which were ready to release gametes were generally creeping near the water surface. The results of spawning induction experiments are presented in Table 1 and the average percentage of spawned broodstock, average batch fecundity, and fertilization rates in relation to lunar period and treatment is shown in Table 2.

In conditioned broodstock, the proportion of spawned male and female in treated group is not greater than the proportion of spawned male ($P = 0.3091$) and female ($P = 0.1756$) in the control. Average batch fecundity per gram of body weight was not significantly different between period ($P = 0.9714$) and between the control and thermal schock ($P = 0.8464$). Fertilization rate was significantly higher ($P<0.0053$).in the control group than in the treated animal with thermal shock.

Freshly Caught Wild Broodstock

During collection, freshly caught wild broodstock were carried in a collecting bag and were therefore stressed for about 1—3 hours before arriving in the laboratory. In the laboratory, mature animals were induced to spawn using vigorous aeration, thermal-shock, desiccation, hydrogen peroxide, and UV-irradiated seawater as previously described. The results of these spawning induction experiments are presented in Table 3 and the average percentage of spawned broodstock, average batch fecundity, and fertilization rates in relation to treatment is shown in Table 4.

Freshly wild broodstock that were collected in the morning failed to spawn using any type of spawning induction, except for one of the females that released immature gametes in response to a treatment of the combination of desiccation and thermal-shock (Table 3). For freshly caught wild broodstock which were collected in the afternoon, about 45% of the males and 37% of the females spawned (Table 4).

In freshly caught wild broodstock, the proportion of spawned male and female in treated group is not greater than the proportion of spawned male ($P = 0.0521$) and female ($P = 0.1977$) in the control. Average batch fecundity per gram of body weight was not significantly different between treated and the control groups ($P = 0.0896$). Fertilization rate was significantly higher in the control group than in the treated animals ($P = 0.0273$).

The eggs of *H. asinina* are spherical. Mature eggs are composed of three layers. The first outer layer is a transparent coating, which surrounds the eggs, called the 'jelly coat' and has a thickness of 37 ± 2 µm. The second layer is the egg membrane, which measures 203 ± 3 µm in diameter. The third (inner) layer is the egg yolk (cytoplasm), which measures 155 ± 2 µm in diameter. Not all of the eggs that had been released during spawning were mature. The mature eggs (>200 µm in diameter) dominated (about 75%). The remaining (about 25 %) was immature.

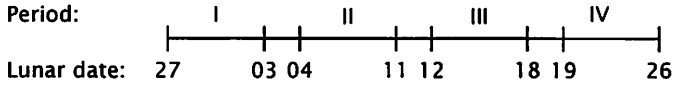
DISCUSSION

The control of spawning is very essential in the operation of a hatchery. A successful regular spawning out of the natural spawning season allows for the production of larvae and juveniles over a longer period. Although tropical abalone have a prolonged spawning season and can spawn at any time of the year, culturists can not rely solely on natural stock for spawning.

Table 1. Results of spawning induction experiments on conditioned broodstock using thermal-shock during March-October 2000 in 'Loka Lombok Marine Laboratory', Lombok, NTB. Lunar date: 1 is new moon and 15 is full moon

Period (lunar date)	Date	Treatment	Number M : F	Spawned M : F	Average batch fecundity (10^3)	Fertilization rate (%)
I (1)	4/4/2000	Thermal-shock	2:02	2:02	302.8	68
I (3)	6/5/2000	Thermal-shock	3:04	2:03	78.4	55
I (3)	5/7/2000	Thermal-shock	2:02	2:02	130.3	56
I (3)	6/5/2000	Control	3:02	2:01	142.7	75
I (3)	5/7/2000	Control	3:02	2:01	86.5	78
II (7)	11/3/2000	Thermal-shock	3:02	2:02	138.2	64
II (6)	9/5/2000	Thermal-shock	2:03	2:03	192.2	68
II (7)	11/3/2000	Control	2:02	2:02	183.8	75
II (6)	9/5/2000	Control	1:01	1:01	184.3	73
II (6)	9/5/2000	Control	3:02	2:02	50.7	74
III (14)	17/5/2000	Thermal-shock	3:02	2:02	116.3	63
III (18)	18/10/2000	Thermal-shock	1:02	1:02	87.2	56
III (14)	17/5/2000	Control	1:01	1:01	321	82
III (14)	17/5/2000	Control	2:01	2:01	126.3	75
III (18)	18/10/2000	Control	4:03	3:02	123.8	79
IV (25)	28/4/2000	Thermal-shock	1:01	1:01	434.7	29
IV (26)	28/7/2000	Thermal-shock	2:01	2:01	326.3	71
IV (26)	28/7/2000	Thermal-shock	1:03	1:03	137.2	66
IV (25)	28/4/2000	Control	1:02	1:02	85.8	69
IV (26)	28/7/2000	Control	2:02	2:02	66.2	87

Note:



Spawning the broodstock of tropical abalone (*H. asinina*) that have been conditioned in the laboratory environment was not difficult. Mature conditioned broodstock spawned successfully, with the average frequency of spawning about 89% for males and 92% for females (Table 2). This successful spawning was not really affected by the lunar cycle, as the results have shown that more than 80% of the mature conditioned male and female *H. asinina* could be spawned in the laboratory every week. The exception to this is during the new moon period, where only about 75% of the mature conditioned females spawned.

The results of spawning induction of thermal shock in conditioned broodstock are in contrast with the freshly caught wild broodstock that have been collected directly from nature prior to the spawning treatment. The reason for the differences in the success of spawning between broodstock collected in the morning and in the afternoon is not clearly understood. One possible explanation is that animals collected in the morning had not received sufficient stimuli to trigger their natural spawning behaviour. It is known that exposure to air and rapid temperature changes are natural spawning stimuli for a variety of abalone species (Hahn, 1989d). In pinto abalone, *H. kamtschatkana*, tidal warm sea temperature has been suggested as a spawning stimulus (Breen & Adkins, 1981), and spawning has been found to occur shortly after high tide in the

Table 2: The percentage of spawned broodstock, average batch fecundity, and fertilization rates in relation to period (refer to Table 1) and treatment to spawn for conditioned broodstock during March-October 2000 in 'Loka Lombok Marine Laboratory, Lombok, NTB

Group	Spawned males (%)	Spawned females (%)	Average batch fecundity (10^3)	Average batch fecundity per gram of body weight (10^3)	Fertilization rate (%)
I	80	75	148.2	2.72	66.6
II	86.7	90	137.2	2.68	71.8
III	86.7	100	154.9	2.42	69.5
IV	97.8	98.4	201	2.73	72.2
Thermal-shock	90	97.7	188.1	2.71	59.8
Control	87.5	86.7	137.1	2.56	76.6

evening (Stekoll & Shirley, 1993). Therefore, exposure of the abalone (*H. asinina*) to air and increased sea temperature during the afternoon low tides, may induce abalone to spawn or release their gametes in the night. In this study spawning occurred between 11.00 pm and 01.00 am which was shorter than in the Philippines that occurred between 11.00 pm and 3.00 am (Capinpin *et al.*, 1998).

The difficulty in spawning *H. asinina* which were collected directly from the wild prior to the spawning treatment is common among abalone. For example, spawning of temperate species (*H. fulgens*, *H. corrugata*, and *H. cracherodii*) in California was found to be difficult when the animals were collected directly from the wild (Hahn, 1989). Adult *H. australis* collected directly from the wild cannot usually be induced to spawn (Moss, 1998), as well as for mature South African abalone, *H. midae* (Sales & Britz, 2001).

The need to condition the broodstock to produce a successful spawning was mentioned by Sales & Britz (2001). Pena (1986) kept the broodstock of *H. coccinea canariensis* under natural laboratory conditions for three months before the animals were spawned successfully following treatment with hydrogen peroxide. Holding mature animals in conditioning tanks before spawning is also practiced for the New Zealand abalone (Tong & Moss, 1992). Kabir (2001) reported that to produce a fully mature and spawnable gonad, abalone need to be conditioned and attain an effective accumulative temperature (EAT) e" 1,400°C-days for *H. australis* and e" 2,700°C-days for *H. iris*.

The present studies show that to maintain a better successful spawning, mature *H. asinina* broodstock need to be conditioned in the laboratory for more than one month under ambient temperature and photoperiod but fed *ad libitum* with fresh algae (*Gracilaria* spp.). However, when freshly wild broodstock of *H. asinina* need to be spawned directly in the laboratory, they should be collected from the wild during afternoon low tides.

This study shows that *H. asinina* spawned in response to all the treatments tested, although the results were not significantly different from the control. In the control group of conditioned broodstock, about 87% of male and female *H. asinina* spawned. This result is in agreement with Castanos (1997); Capinpin *et al.* (1998) that *H. asinina* could spawn spontaneously without treatment several days before or during the new and full moon. Spontaneous spawning without treatment was practiced successfully for *H. asinina* from Heron Island, Australia (Norris & Preston, 2003). However, spawning induction by thermal-shock in this study increased the number of spawned broodstock, i.e., 90% for males and 97% for females (Table 2).

It was found that mature animals which were ready to spawn were usually found creeping up around the water surface. They were very active but relaxed. The animals did not retract their foot when touched, and would even creep freely on one's hand when gonad maturation was being checked. Their foot felt soft and flabby. This sign of spawning readiness was also observed by Breen & Adkins (1981) in *H. kamtschatkana*. They found that during

Table 3. Results of spawning induction experiments on freshly caught wild broodstock during May-July 2000 in 'Loka Lombok Marine Laboratory', Lombok, NTB. Collection times were between 6—9 am in the morning and 3—6 pm in the afternoon

Date	Collection times	Treatment	N M : F	Spawned M : F	Average batch fecundity (10^3)	Fertilization rate (%)
1/6/2000	Morning	VA + TS	5:05	2:00	-	-
		TS	5:05	0:00	-	-
1/7/2000	Morning	Des	5:05	0:00	-	-
		Des + TS	5:05	0:01	*	-
		UV	5:05	0:00	-	-
		Control	5:05	0:00	-	-
2/7/2000	Morning	VA	5:05	0:00	-	-
		VA + TS	5:05	0:00	-	-
		UV	5:05	0:00	-	-
		Control	5:05	0:00	-	-
5/5/2000	Afternoon	H_2O_2	5:05	3:02	50.4	64
		Control	5:05	3:01	80.3	87
5/6/2000	Afternoon	TS	5:05	1:00	-	-
		TS	5:05	1:01	79.3	71
		UV	4:04	1:01	101.3	87
		Control	3:03	3:3 **	20.3	90
6/6/2000	Afternoon	Des + TS	5:05	5:05	43.1	52
		TS	5:05	4:03	26.3	54
16/7/200	Afternoon	VA	5:05	2:02	93.7	69
		VA + TS	5:05	1:02	59.1	58
		VA	5:05	3:02	105.2	71
		UV	5:05	0:00	-	-
		H_2O_2	5:05	0:00	-	-

* = Animals released immature eggs, 50—140 µm in diameter
** = Animals spawned the following day. VA = vigorous aeration
TS = thermal-shock
Des = desiccation
UV= UV-irradiated seawater
H_2O_2 = hydrogen peroxide

spawning, *H. kamtschatkana* were very loosely attached to the substratum, remained relaxed but active, and it was easy to check the gonad. FAO (1990) noted that mature *H. discus hannai* with a fleshy foot are desirable as spawners.

Combination of desiccation and thermal-shock produced good spawning results in terms of percentage of spawned animals, i.e., 100% for both males and females. Similar good results using desiccation and thermal-shock were found for *H. diversicolor supertexta* (Chen, 1984), and *H. tuberculata* (Clavier, 1992).

Poor spawning results were obtained in *H. asinina* treated using UV-irradiated seawater and hydrogen peroxide (H_2O_2) alone (Table 4). A similar poor spawning result was also reported by Moss *et al.* (1995) using UV-irradi-

Table 4. The percentage of spawned broodstock, average batch fecundity, and fertilization rate under particular spawning induction for freshly wild broodstock during May—July 2000 in 'Loka Lombok Marine Laboratory', Lombok, NTB

Treatment	Spawned males (%)	Spawned females (%)*	Average batch fecundity (10^3)	Average batch fecundity per gram of body weight (10^3)	Fertilization rate (%)
Hydrogen Peroxide	30.0	20.0	50.4	0.88	31.9
Vigorous aeration	50.0	40.0	99.4	1.45	70.3
Thermal-shock	40.0	26.7	26.3	0.80	41.6
UV-irradiated seawater	12.5	12.5	101.3	1.70	43.7
Vigorous aeration and thermal-shock	20.0	40.0	69.2	1.01	57.8
Desiccation and thermal-shock	100.0	100.0	43.1	0.82	51.9
Control	80.0	60.0	50.3	0.68	88.7

ated seawater to induce spawning in *H. iris* (i.e. only about 4% of males and no females spawned). The poor results using UV-irradiated seawater and H_2O_2 treatments in the present study may due to inappropriate levels of UV-irradiation and concentration of H_2O_2. Successful spawning induction using UV-irradiated seawater and/or H_2O_2 has been found in *H. rufescens* in Chile (Owen *et al.*, 1984), *H. coccinea canariensis* in Spain (Pena, 1986), *H. iris* in New Zealand (Moss *et al.*, 1995), and *H. asinina* and *H. ovina* in Thailand (Jarayabhand & Paphavasit, 1996).

Higher batch fecundity in conditioned animals may be associated in some way to the fact that the animals have acclimatized to laboratory conditions. These broodstock may therefore release most of their mature eggs. On the other hand freshly caught wild broodstock may only release a portion of their mature eggs, as it is known that partial spawning occurs in the wild population. Also conditioned broodstock which have been well fed for a period and probably will not have had an opportunity to spawn during this period resulting in an accumulation of ripe oocytes in females.

On average, *H. asinina* in southern Lombok waters produces equivalent numbers of eggs per spawning (i.e., 66,000 eggs/individual for freshly caught wild broodstock and 163,000 eggs/individual for conditioned broodstock) compared to the same species in Thailand (Jarayabhand & Paphavasit, 1996) and in the Philippines (Capinpin *et al.*, 1998). However, this average batch fecundity was slightly higher than the small-sized species, *H. coccinea canariensis* (i.e., 11,000-56,000 eggs) (Pena, 1986).

The success of spawning depends also upon the fertilization rates of spawned gametes. Poor fertilization rates could be a serious problem for culturists (Hahn, 1989). In this study, average fertilization rate was about 68% in conditioned broodstock and 70% in freshly caught wild broodstock. The highest fertilization rate was achieved in the gametes that were released by animals that spawned spontaneously without artificial induction of spawning, and was about 77% in conditioned broodstock and 89% in freshly caught wild broodstock. The higher fertilization rate in freshly caught wild animals may be due to the variety and availability of good quality of natural food, while conditioned broodstock were fed only on *Gracilaria* spp. There is evidence that *H. australis* that has been fed on artificial diet produces eggs with higher fertilization rate compared to animals fed on seaweeds (*Gracilaria chilensis* and *Macrocytis pyrifera*) (Harrington, 2000). The fertilization rates of *H. asinina* in the present study were lower compared to temperate species, i.e. about 95% for *H. rufescens* (Ebert & Houk, 1984), 89% for *H. iris* (Moss *et al.*, 1995), and 90% for *H. australis* fed with artificial food (Harrington, 2000).

A possible explanation for the lower percentage of fertilization rate in those abalone induced to spawn by thermal shock compared to the control group is that the higher temperature may have damaged some eggs or sperm or reduced sperm motility. McCardle (1984) reported that gamete viability of *H. virginea virginea* was related to time of gamete mixing and temperature. He found that fertilization rates was 9.5% at 15.9°C and decreased to 0.3% at 18.0°C.

Artificial induction of spawning has been noted to cause animals to release some eggs that are not fully mature (Hahn, 1989d). Artificial induction of spawning in *H. asinina* in this study resulted in the release of eggs, about 75% of which were ripe. The remaining of 25% was unripe eggs and could not be fertilized.

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