

The effect of temperature and conditioning interval on the spawning success of wild-caught blacklip (*Haliotis rubra*, Leach 1814) and greenlip (*H. laevis*, Donovan 1808) abalone

Mark A Grubert^{1,2} & Arthur J Ritar¹

¹Marine Research Laboratories, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Taroona, Tas., Australia

²School of Aquaculture, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Launceston, Tas., Australia

Correspondence: M A Grubert, RMIT Marine Research Centre, PO Box 156, Lakes Entrance, Vic. 3909, Australia. E-mail: mark.grubert@rmit.edu.au

Abstract

Wild-caught blacklip (*Haliotis rubra*, Leach 1814) and greenlip (*H. laevis*, Donovan 1808) abalone fed a formulated feed were held at 16 or 18 °C for different conditioning intervals ranging from 114 to 235 days and induced to spawn using ultraviolet-irradiated seawater. They were conditioned again for a second identical period before another induction. For *H. rubra*, mean spawning rate of both sexes was higher in groups held at 18 °C than at 16 °C, as was the repeat spawning rate. Conversely, animals held at 16 °C produced significantly more gametes than those at 18 °C. Egg production peaked in groups held at 16 °C for ≥ 165 days. While both mean and total sperm production of *H. rubra* varied significantly, both figures were always high. Unlike *H. rubra*, the spawning rate, repeat spawning rate and gamete production of both sexes of *H. laevis* were higher when cultured at 16 °C than at 18 °C. Egg production peaked in groups conditioned at 16 °C for ≥ 212 days. Both mean and total sperm production by *H. laevis* were much lower than for *H. rubra*. This study demonstrates that year-round hatchery production of seedstock of both species is possible providing broodstock are held under favourable environmental conditions, preferably 16 °C.

Keywords: *Haliotis rubra*, *Haliotis laevis*, effective accumulative temperature, broodstock conditioning, induced spawning

Introduction

Blacklip (*Haliotis rubra*, Leach 1814) and greenlip (*H. laevis*, Donovan 1808) abalone form the basis of a lucrative wild fishery and a rapidly expanding aquaculture industry in southern Australia. Culture of these species has generally relied upon the capture and induced spawning of wild broodstock but this process is often compromised by spatial and temporal variations in the availability of gravid broodstock and/or the stresses of capture and transport. Hence, a reliable means of ensuring continuity of larval supply is vital for consistent production of seedstock for farming.

Abalone broodstock can be conditioned and spawned predictably in the hatchery through the provision of a favourable physico-chemical environment. This includes a stable temperature that optimizes gonad growth, high levels of dissolved oxygen, low levels of nitrites and ammonia and a pH of 7.5–8.5. Broodstock must also be fed a high-quality diet in amounts slightly in excess of their needs (Uki & Kikuchi 1982a).

Temperature is the main factor influencing the rate of gonad development in most species of abalone. Its effect is cumulative above a certain threshold temperature that varies between species. Kikuchi and Uki (1974a, b) were the first to record this phenomenon, and named the threshold temperature the 'biological zero point' (BZP). By subtracting the BZP from the daily water temperature and summing this figure

over the culture time (in days) they were able to describe the effective accumulative temperature in degree days (EAT °C-days) for gonad conditioning of two Japanese abalone species. At present, there is only one account of the EAT for conditioning of southern hemisphere abalones, that of Kabir (2001) on *H. australis* and *H. iris*.

Previous reports on broodstock conditioning of blacklip and greenlip abalone have generally concentrated on a single species held at one temperature. Lleonart (1992) described changes in gonad histology and volume during conditioning of greenlip abalone held at 16 °C but did not examine spawning success. Plant (2002) conditioned *H. laevigata* at 17 °C and observed spawning response relative to a range of temperature treatments applied 1–4 days prior to induction. Those groups exposed to an increase in 5 °C over this interval showed the best results. Savva, Heasman and Brand (2000) examined the effect of broodstock diet on fecundity and egg quality in *H. rubra* conditioned at 16 °C. Broodstock on a formulated feed appeared to spawn more eggs and produce better quality larvae than those fed the brown alga *Phyllospora comosa* or a mix of both diets. Plant, Mozquiera, Day and Huchette (2002) documented the spawning success of groups of blacklip abalone held at 18 °C for periods of 30–150 days. The spawning response of each sex was highest in the group cultured for 120 days. Surprisingly, the proportion of female spawners was always higher than that of males, while the reverse is usually the case for this species.

The aim of this study was to determine the optimal conditioning regime for repeat spawnings of wild-caught blacklip and greenlip abalone broodstock conditioned on a formulated feed. This was achieved by holding both sexes of each species at temperatures of either 16 or 18 °C for one of five different conditioning intervals (calculated as EAT °C-days) over two cycles. The optimal regime was defined as the combination of time and temperature that maximized spawning response, in terms of the proportion of spawners (and repeat spawners) and gamete production.

Materials and methods

Broodstock collection

Greenlip abalone broodstock (100–120-mm shell length) were collected on 26 November 2001 near Flinders Island (between Vansittart and Puncheon Islands) and held in flow-through tanks at ambient temperature for 3 days. During this time it was no-

ticed that some animals spawned. Information from the fisher indicated that spawning commenced in the wild during the previous 4 weeks. Blacklip abalone broodstock (100–130-mm shell length) were collected on 3 December 2001 near Swan Island from *FRV Challenger* and some animals spawned in the boat's holding tanks. Both species are known to be sexually mature at these sizes and locations (Tarbath & Officer 2003; C. Mason, pers. comm.).

Animals were transferred to the Tasmanian Aquaculture and Fisheries Institute, Marine Research Laboratories and held in 600-L tanks supplied with ambient temperature seawater until examination and allocation to experimental treatments. They were measured, weighed, tagged and gonad condition assessed by visual score according to the following criteria: 0 = sex indistinguishable; 1 = sex distinguishable, thin gonad with pointed tip; 2 = gonad partially enlarged with pointed tip; 3 = gonad swollen with rounded tip. All animals were scored as 0 or 1 and allocated a putative spawning date of 15 November 2001.

Experimental design

At the start of the experiment, 10–15 abalone of each sex and species were randomly assigned to either 16 or 18 °C (the minimum and maximum temperatures typically used to condition these species; Fleming 2000) and one of five conditioning intervals (1200, 1400, 1600, 1800 or 2000 EAT °C-days, referred to in the text as 1200 group, 1400 group, etc.). Animals of each sex and species were held separately, with three tanks for each temperature treatment (i.e. $2 \times 2 \times 3 \times 2 = 24$ tanks in total). The EAT was calculated on the basis of the estimated BZP of 7.5 °C for both species. The BZP was subtracted from the actual holding temperature (e.g. $16 - 7.5 = 8.5$ °C or $18 - 7.5 = 10.5$ °C) and summed over the number of days that animals were held before spawning. At 16 °C, EAT intervals of 1200, 1400, 1600, 1800 and 2000 equated to 141, 165, 188, 212 and 235 days respectively. At 18 °C, they corresponded to 114, 133, 152, 171 and 190 days respectively. The true BZP values were calculated at the end of a concurrent study that examined gonad development at temperatures from 12 to 18 °C (Grubert & Ritar 2004).

Husbandry and monitoring

Broodstock were conditioned in 150-L round fibreglass tanks (about 25 animals tank⁻¹) receiving

flow-through seawater at a rate of 1.5 L min^{-1} tank $^{-1}$. Animals were fed daily to satiation on a broodstock conditioning feed (Adam and Amos Abalone Foods, Mount Barker, SA, Australia). Mortalities and spontaneous spawnings were recorded during feeding. Wastes were siphoned from each tank every second day. Photoperiod was maintained at 12L:12D starting at 06:00 hours and light intensity at the bottom of the tanks (when filled) was 90–100 lx. Water temperature was recorded using StowAway TidbiT temperature loggers (Onset Computer Corporation, Bourne, MA, USA).

Induction of spawning

On each induction day, abalone were removed from their holding tanks at 09:00 hours. The VGI of each animal was recorded prior to being placed into a 14-L polyethylene spawning tray containing 10-L seawater. Water temperature in the trays was the same as the conditioning temperature (i.e. 16 or 18 °C). The trays were then covered in black plastic panels to exclude light. The induction procedure involved the simultaneous application of ultraviolet (UV)-irradiated seawater and temperature change. One micron filtered seawater was irradiated using $2 \times 150\text{-W}$ UV sterilization units (Wedeco, Girraween, NSW, Australia), the quartz sleeves of which were cleaned after every 50 h of use. Water temperature was raised by $1 \text{ }^\circ\text{C h}^{-1}$ for 4 h then allowed to return to the original temperature at the same rate (i.e. $16 \rightarrow 20 \rightarrow 16 \text{ }^\circ\text{C}$ or $18 \rightarrow 22 \rightarrow 18 \text{ }^\circ\text{C}$). Flow rate to each tray was 300 mL min^{-1} . Animals were monitored from 14:00 to 22:00 hours and the time that they began spawning recorded. Once an animal began spawning, the supply of UV-irradiated seawater was stopped facilitating the retention of gametes.

When males spawned prior to 22:00 hours, sperm water was poured off, made up to a known volume, mixed and samples taken for density estimates (using absorbance at 340 nm, Ritar & Grubert 2002). The male trays were then refilled with fresh seawater. Both sexes were held overnight in their spawning trays with no water exchange. Each tray was aerated and a stable temperature maintained using a waterproof, thermostatically controlled heat mat (Thermofilm Australia, Springvale, VIC, Australia). The following morning, samples of sperm water (for sperm density estimates) were taken from those males that had continued, or started, spawning after 22:00 hours. At the same time, eggs released from each female were suspended in 10 L of seawater and

subsampled (1 mL) in triplicate to estimate fecundity. When sperm were collected from the same male on two occasions (at night and the following morning), sperm production estimates were summed. No mortalities of either sex or species occurred during the induction process.

Statistical analyses

For both sexes of each species, analyses of gamete production and spawning response time data were conducted using one-way and two-way (without replication) analysis of variance (ANOVA). Tukey–Kramer HSD tests were used for *post hoc* comparison. Bartlett's tests were used to check for homogeneity of variance and where necessary (i.e. P -value for the F -ratio < 0.05) the data were $\sqrt{\text{transformed}}$ (Sokal & Rohlf 1995) and re-analysed. Probabilities of < 0.05 (for the ANOVAs) were considered significantly different. Significant interactions between temperature and EAT conditioning interval are denoted by $T \times \text{EAT}$; non-significant differences are expressed as NS; data are presented as mean \pm SEM. The ANOVA tests on gamete production by each sex and species were structured in a number of ways. One analysis examined the effect of temperature and conditioning interval on mean gamete production of each EAT group at the first induction (i_1) then at the second induction (i_2), using data from only those animals that had spawned. A second analysis examined how total gamete production at both inductions (i.e. $x_i = i_1 + i_2$, where x_i = each individual that spawned one or more times) varied relative to temperature and EAT conditioning interval. These values are referred to as IndTotal in the text. Because of lack of independence in data from successive inductions (as gamete production at the first induction may affect that at the second), no direct comparisons of the first and second inductions were made (i.e. it was not appropriate to use two-way ANOVA with replication). However, the difference in gamete production between the first and second inductions was calculated for each individual that spawned at least once ($x_i = i_2 - i_1$) and standardized by adding the absolute value of the largest negative value (x_{\min}) plus one (i.e. $x_i = x_i + |x_{\min}| + 1$). These data are referred to as IndDiff in the text. One-way ANOVA was employed to test for differences in mean spawning, repeat spawning and mortality rates between each temperature treatment (percentage data was arcsine $\sqrt{\text{transformed}}$ prior to analysis). G-tests were used to check for independence between the frequencies of animals that

spawned zero, one or two times and EAT conditioning interval. If the frequencies of each spawning group were independent of EAT for the 16 and 18 °C groups then these values were pooled over all EAT categories and another G-test used to examine temperature effects on spawning patterns. Regression analyses were used to test for possible relationships between shell length and gamete production. Statistics were executed using Excel 2000 (Microsoft, Redmond, WA, USA) and JMP version 5.0 (SAS Institute, Cary, NC, USA).

Results

Spawning response of female blacklip abalone (*H. rubra*)

At the first induction, the mean spawning rate of female blacklip groups held at 16 and 18 °C was 49 ± 4% and 63 ± 3% respectively (NS, Table 1). Mean

spawning response times were 6 h 32 ± 17 min at 16 °C and 6 h 05 ± 13 min at 18 °C (NS). The mean egg production for spawning females at 16 and 18 °C were similar (1.39 ± 0.65 and 0.71 ± 0.18 × 10⁶ eggs female⁻¹ respectively) and did not differ with conditioning interval.

At the second induction, spawning rate in the 18 °C treatment was significantly higher than the 16 °C treatment (80 ± 9% and 43 ± 2% respectively), as was the number of repeat spawners (i.e. animals spawning at both inductions; 46 ± 9% and 32 ± 2% respectively). The proportion of females that spawned either repeatedly, only once, or not at all did not differ significantly between conditioning intervals when females were induced a second time. Hence, it was possible to compare the spawning patterns of all animals at both temperatures. The number of repeat spawners was significantly higher at 18 °C than at 16 °C, while the number of non-

Table 1 Spawning rate, gamete production (× 10⁶ for females and × 10¹¹ for males) and repeat spawning rate at successive inductions of blacklip abalone relative to sex, temperature (*T*, °C) and conditioning interval (EAT)

Sex	<i>T</i> (°C)	EAT	<i>n</i>	Induction 1			Induction 2			
				Percentage spawning	Gamete production, mean (± SE)	Mort	Percentage spawning	Gamete production, mean (± SE)	% repeat spawners	
Female	16	1200	12	17	1.98 ± 1.84 ^a	2	50	0.80 ± 0.20 ^a	20	
		1400	12	58	1.05 ± 0.29 ^a	1	46	2.11 ± 0.69 ^a	36	
		1600	12	67	1.26 ± 0.41 ^a	0	42	0.20 ± 0.06 ^b	33	
		1800	11	64	0.92 ± 0.28 ^a	0	36	0.77 ± 0.29 ^a	27	
		2000	12	50	1.76 ± 0.45 ^a	0	42	1.70 ± 0.46 ^a	42	
		Mean ± SE		49 ± 4 ^A	1.39 ± 0.65 ^A		43 ± 2 ^B	1.12 ± 0.34 ^{T × EAT}	32 ± 2 ^B	
	18	1200	15	67	0.67 ± 0.09 ^b	5	70	1.59 ± 0.58 ^a	40	
		1400	13	46	0.82 ± 0.20 ^b	3	70	0.31 ± 0.14 ^b	30	
		1600	10	60	1.49 ± 0.39 ^a	0	100	0.20 ± 0.05 ^b	60	
		1800	12	83	0.46 ± 0.19 ^b	2	100	0.65 ± 0.25 ^b	80	
		2000	12	58	0.10 ± 0.05 ^b	0	58	0.60 ± 0.29 ^b	42	
		Mean ± SE		63 ± 3 ^A	0.71 ± 0.18 ^A		80 ± 9 ^A	0.67 ± 0.26 ^{T × EAT}	46 ± 9 ^A	
	Male	16	1200	12	100	1.13 ± 0.37 ^c	0	92	1.46 ± 0.41 ^b	90
			1400	12	75	1.83 ± 0.57 ^{bc}	0	75	3.48 ± 0.73 ^{ab}	50
1600			13	100	4.65 ± 0.77 ^{ab}	0	92	5.25 ± 0.69 ^a	92	
1800			12	100	8.88 ± 1.31 ^a	2	90	3.70 ± 1.00 ^{ab}	90	
2000			13	92	4.58 ± 1.19 ^{ab}	1	67	3.99 ± 1.08 ^{ab}	58	
Mean ± SE				93 ± 5 ^A	4.21 ± 0.84 ^A		83 ± 5 ^A	3.58 ± 0.78 ^{T × EAT}	76 ± 9 ^B	
18		1200	11	100	1.21 ± 0.30 ^a	1	100	1.42 ± 0.33 ^b	100	
		1400	12	100	1.69 ± 0.52 ^a	0	100	0.99 ± 0.42 ^b	100	
		1600	12	100	2.35 ± 0.50 ^a	0	100	0.93 ± 0.23 ^b	100	
		1800	11	100	4.77 ± 1.17 ^a	1	80	1.21 ± 0.94 ^b	90	
		2000	12	83	3.73 ± 1.26 ^a	0	92	5.93 ± 1.04 ^a	83	
		Mean ± SE		97 ± 3 ^A	2.75 ± 0.75 ^B		94 ± 4 ^A	2.10 ± 0.59 ^{T × EAT}	93 ± 3 ^A	

n, sample size; mort, mortalities between inductions. Comparisons made within sex and within column. EAT groups (at each temperature) with the same lower case letter are not significantly different. Likewise, means for each temperature treatment with the same upper case letter are not significantly different. *T* × EAT superscript indicates an interaction effect (see text for details of each case). EAT, effective accumulative temperature in degree days.

spawners was highest in the 16 °C group. Mean response times were similar; 7 h 02 ± 20 min at 16 °C and 6 h 25 ± 10 min at 18 °C. Mortalities were 5% at 16 °C and 16% at 18 °C.

There was a significant $T \times \text{EAT}$ interaction on the group mean egg production of animals at the second induction. Of the females held at 16 °C, those in the 1600 group produced significantly fewer eggs ($0.20 \times 10^6 \text{ female}^{-1}$) than the other groups ($0.80\text{--}2.11 \times 10^6 \text{ female}^{-1}$). In the case of the animals held at 18 °C, the 1200 group produced significantly more eggs ($1.59 \times 10^6 \text{ female}^{-1}$) than all other groups ($0.20\text{--}0.65 \times 10^6 \text{ female}^{-1}$).

There were no significant temperature or conditioning interval effects on the difference in egg production between the first and second inductions (IndDiff), whereas total gamete production across both inductions (IndTotal) was significantly higher for groups at 16 °C than at 18 °C. There was no significant relationship between shell length and egg production ($r^2 = 0.001$).

Spawning response of male blacklip abalone (*H. rubra*)

At the first induction, spawning rate of male blacklip abalone in both temperature treatments was high ($93 \pm 5\%$ and $97 \pm 3\%$ at 16 and 18 °C respectively; NS, Table 1). Mean spawning response times were 6 h 15 ± 19 min at 16 °C and 5 h 45 ± 8 min at 18 °C. While these means were not significantly different, there was a $T \times \text{EAT}$ interaction in spawning response times, but there was no obvious trend in the data. Mean sperm production was significantly higher in the 16 °C treatment ($4.12 \pm 0.84 \times 10^{11} \text{ male}^{-1}$) than the 18 °C treatment ($2.75 \pm 0.75 \times 10^{11} \text{ male}^{-1}$) and in both cases the 1800 group produced the greatest mean sperm number ($8.88 \pm 1.31 \times 10^{11}$ and $4.77 \pm 1.17 \times 10^{11} \text{ male}^{-1}$ at 16 and 18 °C respectively).

Spawning rate at the second induction was also high in both temperature treatments ($83 \pm 5\%$ at 16 °C and $94 \pm 4\%$ at 18 °C, NS). The proportion of repeat spawners was $76 \pm 9\%$ and $93 \pm 3\%$ at 16 and 18 °C respectively. Since the proportion of males that spawned either repeatedly, only once, or not at all did not differ significantly between conditioning intervals at each temperature, this allowed a comparison of spawning patterns between animals held at 16 and 18 °C. Most males spawned twice, with the proportion of repeat spawners highest in the 18 °C groups and the proportion of animals that spawned

only once highest in the 16 °C group. Spawning commenced after 6 h 41 ± 18 min and 6 h 24 ± 11 min at 16 and 18 °C respectively. Again, there was a significant interaction between temperature and conditioning interval on spawning response times but no obvious trend in the data was discernible. Mortalities were low at both temperatures; 5% at 16 °C and 3% at 18 °C.

There was a significant $T \times \text{EAT}$ interaction on mean sperm production of animals at the second induction. At 16 °C, males in the 1600 and 1200 groups had the highest ($5.25 \pm 0.69 \times 10^{11} \text{ male}^{-1}$) and lowest ($1.46 \pm 0.41 \times 10^{11} \text{ male}^{-1}$) sperm production, respectively, whereas at 18 °C, males in the 2000 group produced significantly more sperm ($5.93 \pm 1.04 \times 10^{11} \text{ male}^{-1}$) than all other groups ($0.93\text{--}1.42 \times 10^{11} \text{ male}^{-1}$).

There was a significant interaction between temperature and conditioning interval on the means of the standardized difference in sperm production between the first and second inductions (IndDiff). However, the interaction was influenced by the 2000 group at 18 °C which showed a significantly greater increase in sperm production between inductions than all others. A $T \times \text{EAT}$ interaction was also detected for total sperm production over both inductions (IndTotal). IndTotal increased proportionally with EAT, although this difference was largely driven by animals in the 16 °C groups, which typically produced more sperm than the 18 °C groups. There was no significant relationship between shell length and sperm production ($r^2 = 0.001$).

Spawning response of female greenlip abalone (*H. laevigata*)

At the first induction, the mean spawning rate of female greenlip groups was $56 \pm 11\%$ at 16 °C and $37 \pm 5\%$ at 18 °C (NS, Table 2). Mean spawning response times were similar at both temperatures (9 h 06 ± 18 min at 16 °C and 9 h 15 ± 36 min at 18 °C) and there was no EAT effect on this variable. Significant temperature and EAT conditioning interval effects on mean egg production were detected. Females held at 16 °C produced more eggs ($1.17 \pm 0.32 \times 10^6 \text{ female}^{-1}$) than those held at 18 °C ($0.38 \pm 0.18 \times 10^6 \text{ female}^{-1}$) and the 1400 group at 16 °C produced fewer eggs ($0.36 \times 10^6 \text{ female}^{-1}$) than the remaining groups at that temperature ($0.93\text{--}1.69 \times 10^6 \text{ female}^{-1}$).

When induced a second time, the mean spawning rate of groups at 16 °C was $68 \pm 14\%$ and at 18 °C was

Table 2 Spawning rate, gamete production ($\times 10^6$ for females and $\times 10^{10}$ for males) and repeat spawning rate at successive inductions of greenlip abalone relative to sex, temperature (*T*, °C) and conditioning interval (EAT)

Sex	<i>T</i> (°C)	EAT	<i>n</i>	Induction 1		Mort	Induction 2		% repeat spawners
				Percentage spawning	Gamete production mean (\pm SE)		Percentage spawning	Gamete production, mean (\pm SE)	
Female	16	1200	13	31	1.20 \pm 0.47 ^a	1	17	1.57 \pm 1.02 ^a	8
		1400	11	83	0.36 \pm 0.08 ^b	0	73	1.53 \pm 0.29 ^a	63
		1600	12	33	1.69 \pm 0.40 ^a	2	100	1.27 \pm 0.32 ^a	30
		1800	10	60	0.93 \pm 0.25 ^a	0	75	1.93 \pm 0.62 ^a	60
		2000	12	75	1.69 \pm 0.42 ^a	0	75	1.66 \pm 0.47 ^a	75
		Mean \pm SE		56 \pm 11 ^A	1.17 \pm 0.32 ^A		68 \pm 14 ^A	1.59 \pm 0.54 ^A	47 \pm 2 ^A
	18	1200	12	25	0.71 \pm 0.50 ^a	1	36	0.69 \pm 0.01 ^a	9
		1400	12	50	0.33 \pm 0.07 ^a	0	33	0.98 \pm 0.42 ^a	25
		1600	12	25	0.10 \pm 0.04 ^a	2	70	0.95 \pm 0.33 ^a	10
		1800	11	45	0.25 \pm 0.07 ^a	0	100	0.75 \pm 0.22 ^a	45
		2000	12	42	0.53 \pm 0.21 ^a	2	80	1.56 \pm 0.24 ^a	50
		Mean \pm SE		37 \pm 5 ^A	0.38 \pm 0.18 ^B		64 \pm 13 ^A	0.99 \pm 0.24 ^B	28 \pm 9 ^B
Male	16	1200	12	55	0.07 \pm 0.02 ^{cd}	3	89	6.03 \pm 3.08 ^a	33
		1400	11	91	0.06 \pm 0.02 ^d	1	80	13.72 \pm 5.85 ^a	70
		1600	11	100	12.38 \pm 4.86 ^a	2	78	26.80 \pm 9.50 ^a	66
		1800	12	58	5.81 \pm 2.01 ^{abc}	2	80	20.21 \pm 5.57 ^a	55
		2000	12	83	7.99 \pm 2.33 ^{ab}	0	92	25.86 \pm 5.03 ^a	75
		Mean \pm SE		77 \pm 9 ^A	5.26 \pm 1.85 ^{T \times EAT}		84 \pm 3 ^A	18.50 \pm 5.81 ^A	60 \pm 7 ^A
	18	1200	12	25	0.67 \pm 0.17 ^a	2	80	5.85 \pm 1.49 ^a	20
		1400	12	25	0.12 \pm 0.04 ^a	1	100	5.07 \pm 1.95 ^a	18
		1600	12	75	0.08 \pm 0.02 ^a	0	100	7.06 \pm 2.74 ^a	75
		1800	11	27	0.27 \pm 0.11 ^a	2	78	4.00 \pm 2.08 ^a	11
		2000	12	58	0.80 \pm 0.43 ^a	2	80	4.21 \pm 2.25 ^a	40
		Mean \pm SE		42 \pm 10 ^B	0.39 \pm 0.15 ^{T \times EAT}		88 \pm 5 ^A	5.24 \pm 2.10 ^B	31 \pm 12 ^B

n, sample size; mort, mortalities between inductions. Comparisons made within sex and within column. EAT groups (at each temperature) with the same lower case letter are not significantly different. Likewise, means for each temperature treatment with the same upper case letter are not significantly different. *T* \times EAT superscript indicates an interaction effect (see text for details of each case). EAT, effective accumulative temperature in degree days.

64 \pm 13% (NS). Mean repeat spawning rate was 47 \pm 2% and 28 \pm 9% at 16 and 18 °C respectively. For females held at 16 °C, there was a significant EAT effect on the proportion of females that spawned either repeatedly, only once, or not at all. The 1200 group contained significantly more non-spawners and fewer repeat spawners, than the other groups. There was no EAT effect on the frequency of non-, single or repeat spawners in the 18 °C treatment. Spawning commenced after 9 h 12 \pm 23 min at 16 °C and 8 h 48 \pm 37 min at 18 °C and there was no temperature or EAT effect on this variable. Mortality rates were 5% and 9% at 16 and 18 °C respectively (NS). Females held at 16 °C produced significantly more eggs (1.59 \pm 0.54 $\times 10^6$ female⁻¹) than those held at 18 °C (0.99 \pm 0.24 $\times 10^6$ female⁻¹) but conditioning interval did not affect egg production at either temperature.

There were no significant temperature or EAT effects on the mean of IndDiff across the range of treat-

ments, implying no change in egg production between the first and second inductions. There was, however, a significant temperature effect on IndTotal in which egg totals for groups at 16 °C were higher than at 18 °C. There was no significant relationship between shell length and egg production (*r*² = 0.003).

Spawning response of male greenlip abalone (*H. laevis*)

At the first induction, mean spawning rate of greenlip males at 16 °C (77 \pm 9%) was significantly higher than that of males at 18 °C (42 \pm 10%; Table 2). Spawning commenced after 9 h 17 \pm 31 min at 16 °C and 8 h 48 \pm 26 min at 18 °C, with no temperature or EAT effects on this factor. A significant *T* \times EAT interaction on mean sperm production was detected, being greater for animals held at \geq 1600 EAT °C-days at 16 °C (5.81–12.38 $\times 10^{10}$ male⁻¹) than

remaining groups at 16 °C ($0.06\text{--}0.07 \times 10^{10}$ male⁻¹) and all groups at 18 °C ($0.08\text{--}0.67 \times 10^{10}$ male⁻¹).

When induced a second time, the spawning rate of all male groups was high: $84 \pm 3\%$ at 16 °C and $88 \pm 5\%$ at 18 °C. The mean repeat spawning rate was $60 \pm 7\%$ and $31 \pm 12\%$ at 16 and 18 °C respectively (NS). At 16 °C, the proportion of males that spawned either repeatedly, only once, or not at all did not differ significantly between conditioning intervals. However, at 18 °C, most males in the 1200, 1400 and 1800 groups spawned only once, whereas most males in the 1600 group spawned twice. In the 2000 group, the ratio of repeat spawners to single spawners was 2:3. The average spawning response time was 8 h 09 ± 26 min at 16 °C and 8 h 50 ± 14 min at 18 °C. There was a significant $T \times \text{EAT}$ interaction on spawning response times but no obvious trend in the data was discernible. Mortality rates were 16% at 16 °C and 13% at 18 °C. Males held at 16 °C produced significantly more sperm ($18.50 \pm 5.81 \times 10^{10}$ male⁻¹) than those held at 18 °C ($5.24 \pm 2.10 \times 10^{10}$ male⁻¹) but conditioning interval did not affect sperm production at either temperature.

Values of IndDiff (the standardized difference in sperm production between the first and second inductions) for animals held at 16 °C were significantly higher than those at 18 °C, indicating that the increase in sperm production between the two inductions was greatest for 16 °C males. There was a significant $T \times \text{EAT}$ interaction on the means of the individual totals over both inductions (IndTotal). IndTotal increased proportionally with EAT in the 16 °C groups, with a threefold difference between the longest and shortest conditioning intervals. No significant difference in this factor was detected at 18 °C. There was no significant relationship between shell length and sperm production ($r^2 = 0.032$).

Discussion

Spawning rate and gamete production

Conditioning of blacklip and greenlip abalone on a formulated feed for as little as 114 days at 18 °C (1200 EAT °C-days) or as long as 235 days at 16 °C (2000 EAT °C-days) before induction generally yielded gamete production and spawning rates that can be considered commercially acceptable for hatchery production. Likewise, inductions after a second, identical conditioning interval also resulted in moderate-to-high spawning success in most cases.

Table 3 Estimated EAT, based on a BZP of 7.5 °C, and true EAT for blacklip and greenlip abalone, based on BZP values of 7.8 and 6.9 °C respectively (Grubert & Ritar 2004)

Estimated EAT	True EAT	
	Blacklip	Greenlip
1200	1160	1280
1400	1350	1500
1600	1540	1700
1800	1740	1930
2000	1930	2140

True EAT is calculated using a water temperature of 16 °C. EAT, effective accumulative temperature in degree days; BZP, biological zero point.

Although this study used an estimated BZP of 7.5 °C for both species, a concurrent work showed that the actual BZP for gonad development was 7.8 °C for blacklips and 6.9 °C for greenlips (Grubert & Ritar 2004). Therefore, at any given temperature, it takes less time for greenlips, and longer for blacklips, to reach the designated conditioning interval than when the estimated BZP value was used in the original calculations. The recalculated EAT values (using the actual BZP) for each species are given in Table 3.

The mean (and total) gamete production of both sexes of each species was higher when broodstock were held at 16 °C than at 18 °C. The optimal conditioning interval to ensure a high spawning rate and gamete production for blacklip abalone at 16 °C is ≥ 188 days (≥ 1540 EAT °C-days) for males and ≥ 165 days (≥ 1350 EAT °C-days) for females. Corresponding figures for *H. laevisgata* are ≥ 188 days (≥ 1700 EAT °C-days) for males and ≥ 212 days (≥ 1930 EAT °C-days) for females.

The fact that reproductive conditioning of adult *H. rubra* and *H. laevisgata* was more successful at 16 °C than at 18 °C was unexpected, particularly given that the optimal temperature for growth of juveniles is 17.0 and 18.3 °C respectively (Gilroy & Edwards 1998). Thus, it appears that the optimal temperature for growth (be it somatic or gonadal) of *H. rubra* and *H. laevisgata* declines with size, a phenomenon previously documented in *H. rufescens* by Steinarsson and Imsland (2003).

Given that fecundity of abalone can be determined in a number of ways, it is important to distinguish between estimates of instantaneous fecundity (derived from counts of spawned eggs), as in this study, and potential fecundity (number of oocytes in the ovary).

Table 4 Instantaneous fecundity from induced spawnings of selected female Haliotidae relative to shell length, origin and diet

Species	Instantaneous fecundity ($\times 10^3$, mean \pm SD or range)	Shell length (mm, mean \pm SD or range)	Origin	Diet	Source
<i>Haliotis asinina</i>	102 \pm 1 137 \pm 1 126 \pm 2	49 \pm 1	Cult.	G.b. G.b. and formulated Formulated	Bautista-Teruel, Millamena and Fermin (2001)
<i>H. australis</i>	0.2–900	75 \pm 11	CWC	Various macroalgae	Moss (1998)
<i>H. coccinea canariensis</i>	11–75	28–48	CWC	–	Pena (1986)
<i>H. iris</i>	3–1750	–	WC	–	Moss, Illingworth and Tong (1995)
	1000–7000	125–140	CWC	–	G. Moss (pers. comm.)
<i>H. laevigata</i>	100–1000 162–508 340–8200 100–1400 100–1500† 15–5900	120–175 60–88 117–196 107–142 88–142 100–120	CWC Cult. WC CWC CWC CWC	Various macroalgae Various macroalgae – Formulated* Formulated* Formulated*	Lleonart (1992) Babcock and Keesing (1999) Plant (2002) K. Freeman (pers. comm.) This study
<i>H. rubra</i>	1910 \pm 290 1710 \pm 570 1110 \pm 300 54–5900 20–2600 15–4800	100–125 120–142 109 \pm 1 100–130	CWC WC CWC CWC	Formulated* P.c. and formulated* P.c. – Formulated* Formulated*	Savva and colleagues (2000) Litaay and De Silva (2001) Plant and colleagues (2002) This study
<i>H. rufescens</i>	112–5300 85–11 085 242 \pm 263 181 \pm 133	111–194 65–182 92 \pm 6 89 \pm 5	WC CWC Cult. Cult.	– N.I. N.I. P.m.	Ault (1985) Buchal, Levin and Langdon (1998)
<i>H. tuberculata</i>	20–1600	–	WC	–	Clavier (1992)

*Adam and Amos Abalone Feeds broodstock feed.

†Mean of all animals induced.

–, data not available; cult., cultured broodstock; CWC, conditioned wild-caught broodstock; WC, wild-caught broodstock; G.b., *Gracilaria-opsis bailinae*; P.c., *Phyllospora comosa*; N.I., *Nereocystis luetkeana*; P.m., *Palmaria mollis*.

This distinction is made as the latter tends to overestimate the former, particularly when the ovary contains multiple cohorts of oocytes (e.g. in *H. asinina*; Jebreen, Counihan, Fielder & Degnan 2000) or in the case of partial spawning. As we subsampled spawned eggs, the following discussion refers only to estimates of instantaneous fecundity.

The spawning response and egg production of *H. rubra* reported here was similar or slightly higher (for equivalent-sized animals) to previous research on this species (see Table 4). Plant and colleagues (2002) found that spawning success of female *H. rubra* peaked after 120 days conditioning at 18 °C (92% spawned; 2.0×10^6 eggs female⁻¹), which equates to approximately 1220 EAT °C-days, while females conditioned for longer (150 days or approximately 1600 EAT °C-days) were less responsive (65% spawned) and produced fewer eggs (1.1×10^6 eggs female⁻¹). By contrast, no reduction in egg produc-

tion by female *H. rubra* held for ≥ 1200 EAT °C-days was observed during this study.

Savva and colleagues (2000) found that for *H. rubra* held at 16 °C and repeatedly induced at intervals of 12 weeks, an average of only 8% of females spawned at each induction, producing 1.6×10^6 eggs female⁻¹ (Table 4). Only 4% of females spawned more than once, suggesting that a conditioning cycle of 12 weeks (approximating 690 EAT °C-days) was insufficient to complete oogenesis. However, these animals were collected from the northern limit of the species range where surface water temperatures can reach 25 °C in summer. Since high temperatures are stressful to abalone (Gilroy & Edwards 1998), the reproductive performance of the broodstock used by Savva and colleagues (2000) may have been compromised by their thermal history.

Maximum egg production estimates for *H. laevigata* (of a given size) reported here are greater than most

estimates in previous works, with the exception of Babcock and Keesing (1999). These authors used larger animals (see Table 4) and recorded a maximum fecundity of 8.2×10^6 eggs, approximately 2 million more than our highest estimate. In his trial on wild-caught *H. laevigata*, Leonart (1992) conditioned animals at 16 °C for 112 days (1020 EAT °C-days) prior to induction, at which point 38% of females and 17% of males spawned, resulting in the production of $0.1\text{--}1.0 \times 10^6$ eggs female⁻¹ while no sperm production was given.

When Plant (2002) induced wild-caught *H. laevigata* that were previously conditioned (but at different stages of maturity) at 17 °C, the initial spawning rate was 10–40% for females and 13–50% for males. At the second induction 4 months (approximately 1030 EAT °C-days) later, spawning rate was somewhat higher, 14–60% for females and 13–100% for males. This compares with spawning rates of 17–100% for females and 25–100% for males during this study. Egg production, which was recorded by Plant (2002) only at the second induction, ranged from 0.1 to 1.4×10^6 eggs female⁻¹, while there was a wider range ($0.03\text{--}5.9 \times 10^6$ eggs female⁻¹) of estimates in our study.

Groups of wild-caught *H. laevigata* conditioned at 17 °C and induced repeatedly at intervals of 6–12 weeks (equivalent to 420–840 EAT °C-days respectively) produced $0.1\text{--}1.5 \times 10^6$ eggs female⁻¹ (K. Freeman, pers. comm.) which is comparable with the values derived from our data ($0.1\text{--}1.3 \times 10^6$ eggs female⁻¹; calculated as total egg production/number of animals induced, not number of animals that spawned). However, animal weights in the former study were approximately 25% heavier than here (see Table 4 for comparison of SL). In that study, total egg production in the two shortest cycles (420 and 560 EAT °C-days) was low at the first induction and high at the second (equivalent to 840 and 1120 EAT °C-days respectively), whereas the reverse was the case for longer cycles (700 and 840 EAT °C-days). Beyond the second induction, total egg production progressively declined in all groups. This suggests that female *H. laevigata* can be conditioned within 700–1120 EAT °C-days at 17 °C, which is shorter than the optimal figure suggested in our study (≥ 1930 EAT °C-days at 16 °C). However, the reduction in egg production over a series of frequent inductions indicates that short conditioning periods (i.e. < 1200 EAT °C-days) are not conducive to consistent spawnings of this species.

Kabir (2001) found that the intervals required to condition the cool temperate abalone *H. australis* and *H. iris* to oocyte maturation (determined histologically) were moderate to long. *H. australis* (BZP = 5.0 °C) required ≥ 1400 EAT °C-days, similar to that for female *H. rubra* and *H. laevigata* (this study) and *H. discus hannai* (Kikuchi & Uki 1974a), whereas *H. iris* (BZP = 6.2 °C) required ≥ 2700 EAT °C-days, comparable with the figure for *H. discus* (Kikuchi & Uki 1974b).

The interval required for oogenesis of the tropical abalone, *H. asinina*, is short, ranging from 28–40 days during the spawning season (i.e. October–April; Jebreen *et al.* 2000). This equates to 310–440 EAT °C-days when calculated using the BZP for larval development (i.e. 15 °C; Sawatpeera, Upatham, Kruatrachue, Chitramvong, Songchaeng, Pumthong & Nugranad 2001) as a proxy for gonad development and a temperature of 26 °C (the mean seawater temperature during the spawning season; Counihan, McNamara, Souter, Jebreen, Preston, Johnson & Degan 2001). This interval range is less than one-third that required for oogenesis of temperate abalone.

The physiological reasons behind the large inter-specific differences in the EAT conditioning interval required for gametogenesis of abalone may relate to species differences in the activity and/or complexity of the biochemical pathways that control this process. Further studies on the hormonal control of gametogenesis in Haliotids relative to the EAT conditioning interval need to be undertaken to determine the factors responsible for these differences.

Fecundity and body size

Despite the large number of females induced to spawn during this study (> 100 for both species), we found no relationship between egg production and body size (as shell length) in either *H. rubra* or *H. laevigata*. Similar observations were reported by Babcock and Keesing (1999) for *H. laevigata* and Clavier (1992) for *H. tuberculata*, although we note that the latter author plotted instantaneous fecundity against body weight.

By contrast, Ault (1985) and Litaay and De Silva (2001) reported power and quadratic relationships between egg production and shell length in *H. rufescens* and *H. rubra* respectively. However, we are unsure of the reliability of the latter model given both the small sample size ($n = 19$) and the fact that it predicts a negative fecundity for *H. rubra* less than 120 mm.

There are several possible reasons for the apparent lack of a relationship between fecundity and shell length reported here. These include: spontaneous spawnings during the conditioning period (thereby reducing the number of eggs that could be released when induced to spawn), partial spawning at an induction (whereby only the most mature eggs are released) or the narrow size range (100–130 mm) of the broodstock used. In the case of partial spawners, residual eggs may have either been absorbed or released during subsequent (planned or unplanned) spawning events.

Spawning response time

Mean spawning response times were similar for both sexes of *H. rubra* (6 h 16 min for males and 6 h 29 min for females). These times are consistent with the report by Hone, Madigan and Fleming (1997), but less than for Plant and colleagues (2002). However, Plant and colleagues (2002) recorded spawning until 02:00 hours the next morning. Had we continued our observations until that time (rather than 22:00 hours) the mean spawning response times may have been greater. Spawning response times for *H. laevigata* were approximately 2.5 h longer than for *H. rubra*. Female *H. laevigata* took longer to respond than males, as seen previously by Plant (2002).

Kikuchi and Uki (1974c) found that the mean spawning response time of *H. discus hannai* was inversely related to the intensity of the UV induction stimulus. Males spawned after 5 h 39 min and females after 6 h 45 min when the stimulus was set at 96 mWh L⁻¹ but these figures declined to 2 h 42 min and 3 h 18 min, respectively, when the stimulus was increased to 803 mWh L⁻¹. Assuming there were no significant changes in the output of our UV lamps, the intensity of the stimulus (although unknown) was kept constant during our study by maintaining the flow (at 300 mL min⁻¹) to each tray at each induction. Despite this, variations in the spawning response times for both species exceeded those recorded by Kikuchi and Uki (1974c).

Altering the time when induction commences, relative to the photoperiod, can also affect spawning response times in abalone. Uki and Kikuchi (1982b) showed that spawning response times of *H. discus hannai* can be reduced to 1 h 20 min for females and 1 h 45 min for males by supplying UV-irradiated seawater to the broodstock 1 h before the onset of the dark phase. Despite the obvious advantages of photoperiod manipulation, which ensures spawning dur-

ing conventional work hours, few (if any) Australian abalone hatcheries employ this method to alter the timing of spawning. This may be because of the variability in spawning response times of local species, or perceived disruptions to husbandry practices. A systematic evaluation of spawning response times, relative to the application time of the UV stimulus and onset of the dark phase, should be undertaken on *H. rubra* and *H. laevigata* to determine the effectiveness of phase shifted spawning inductions.

Conclusions

Both the mean and total gamete production were higher for male and female *H. rubra* and *H. laevigata* cultured at 16 °C than at 18 °C. The optimal conditioning interval for blacklip abalone at this temperature is ≥ 1540 EAT °C-days (≥ 188 days) for males and ≥ 1350 EAT °C-days (≥ 165 days) for females. Corresponding figures for *H. laevigata* are ≥ 1700 EAT °C-days (≥ 188 days) for males and ≥ 1930 EAT °C-days (≥ 212 days) for females. There appears to be considerable flexibility in the duration that blacklip and greenlip abalone may be conditioned on formulated feeds to yield acceptable spawning performance for hatchery production. In most cases, males and females of both species produced large numbers of gametes when induced to spawn following both the first and second conditioning intervals. Conditioning either species at 16 °C and entraining them to a spawning cycle based on the recommended EAT conditioning intervals would allow hatcheries to consistently produce large numbers of gametes for use in seed production (or discarded when the spawning cycle fell between production periods). The groups of animals may be held in rotation while the size and number of groups, and how they are staggered for induction, will be a matter for hatchery management. This study indicates that seedstock can be produced from the hatchery at any time of the year when broodstock are held under favourable culture conditions and provided a formulated feed. Additional work on broodstock nutrition, preferably over two or more conditioning intervals, should be undertaken to determine if spawning performance of these species can be further improved.

Acknowledgments

This project was funded by the FRDC project number 2000/204 (managed by the Abalone Aquaculture Subprogram) awarded to A. J. R. We thank Charles

Mason of Furneaux Aquaculture and the TAFI Abalone Assessment Section for collection of abalone broodstock. Joel Scanlon of Adam and Amos Abalone Feeds kindly donated broodstock feed for the experiment. Assistance from Alan Beech and Bob Hodgson during the construction of the experimental system was greatly appreciated. We also thank Craig Mundy for statistical advice and Chris Burke, Leigh Gurney and Ann Fleming for reviewing the manuscript.

References

- Ault J.S. (1985) Some quantitative aspects of reproduction and growth of the red abalone, *Haliotis rufescens* Swainson. *Journal of the World Aquaculture Society* **16**, 398–425.
- Babcock R. & Keesing J. (1999) Fertilization biology of the abalone *Haliotis laevigata*: laboratory and field studies. *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 1668–1678.
- Bautista-Teruel M.N., Millamena O.M. & Fermin A.C. (2001) Reproductive performance of hatchery-bred donkey's ear abalone, *Haliotis asinina*, Linné, fed natural and artificial diets. *Aquaculture Research* **32**, 249–254.
- Buchal M., Levin J.-E. & Langdon C. (1998) Dulse *Palmaria mollis* as a settlement substrate and food for the red abalone *Haliotis rufescens*. *Aquaculture* **165**, 243–260.
- Clavier J. (1992) Fecundity and optimal sperm density for fertilization in the ormer (*Haliotis tuberculata* L.). In: *Abalone of the World: Biology, Fisheries and Culture* (ed. by S.A. Shepherd, M.J. Tegner & S.A. Guzman del Proo), pp. 86–92. Fishing News Books, Oxford, UK.
- Counihan R.T., McNamara D.C., Souter D.C., Jebreen E.J., Preston N.P., Johnson C.R. & Degnan B.M. (2001) Pattern, synchrony and predictability of spawning in the tropical abalone *Haliotis asinina* from Heron Reef, Australia. *Marine Ecology Progress Series* **213**, 193–202.
- Fleming A.E. (2000) *Conditioning Australian Abalone Broodstock: Best Practice Manual*. Marine and Freshwater Research Institute, Queenscliff, Australia, 39pp.
- Gilroy A. & Edwards S.J. (1998) Optimum temperature for growth of Australian abalone: preferred temperature and critical thermal maximum for blacklip abalone, *Haliotis rubra* (Leach), and greenlip abalone, *Haliotis laevigata* (Leach). *Aquaculture Research* **29**, 481–485.
- Grubert M.A. & Ritar A.J. (2004) Temperature effects on the dynamics of gonad and oocyte development in captive wild-caught blacklip (*Haliotis rubra*) and greenlip (*H. laevigata*) abalone. *Invertebrate Reproduction and Development* **45**, 185–196.
- Hone P.W., Madigan S.M. & Fleming A.E. (1997) *Abalone Hatchery Manual for Australia*. South Australian Research and Development Institute, Adelaide, Australia, 34pp.
- Jebreen E.J., Counihan R.T., Fielder D.R. & Degnan B.M. (2000) Synchronous oogenesis during the semilunar spawning cycle of the tropical abalone *Haliotis asinina*. *Journal of Shellfish Research* **19**, 845–851.
- Kabir N.M.J. (2001) *Environmental, chemical and hormonal regulation of reproduction in two commercially important New Zealand abalone, Haliotis iris and H. australis*. PhD dissertation, University of Otago, Dunedin, Australia, 236pp.
- Kikuchi S. & Uki N. (1974a) Technical study of artificial spawning of abalone, genus *Haliotis* I. Relationship between water temperature and advancing sexual maturity of *Haliotis discus hannai* Ino. *Bulletin of the Tohoku Region Fisheries Research Laboratories* **33**, 69–78.
- Kikuchi S. & Uki N. (1974b) Technical study of artificial spawning of abalone, genus *Haliotis* V. Relationship between water temperature and advancing sexual maturity of *Haliotis discus* Reeve. *Bulletin of the Tohoku Region Fisheries Research Laboratories* **34**, 77–85.
- Kikuchi S. & Uki N. (1974c) Technical study of artificial spawning of abalone, genus *Haliotis* II. Effect of irradiated seawater with ultraviolet rays on inducing to spawn. *Bulletin of the Tohoku Region Fisheries Research Laboratories* **33**, 79–86.
- Litaay M. & De Silva S.S. (2001) Reproductive performance indices based on physical characteristics of female blacklip abalone *Haliotis rubra* L. *Journal of Shellfish Research* **20**, 673–677.
- Leonart M. (1992) *A gonad conditioning study of the greenlip abalone Haliotis laevigata*. MS thesis, University of Tasmania, Launceston, Australia, 162pp.
- Moss G.A. (1998) Effect of temperature on the breeding cycle and spawning success of the New Zealand abalone, *Haliotis australis*. *New Zealand Journal of Marine and Freshwater Research* **32**, 139–146.
- Moss G.A., Illingworth J. & Tong L.J. (1995) Comparing two simple methods to induce spawning in the New Zealand abalone (paua), *Haliotis iris*. *New Zealand Journal of Marine and Freshwater Research* **29**, 329–333.
- Pena J.B. (1986) Preliminary study on the induction of artificial spawning in *Haliotis coccinea canariensis* Nordsieck (1975). *Aquaculture* **52**, 35–41.
- Plant R.J. (2002) *Conditioning and spawning the greenlip abalone (Haliotis laevigata) in an aquaculture facility*. Hons thesis, University of Melbourne, Melbourne, Australia, 46pp.
- Plant R.J., Mozquiera A., Day R.W. & Huchette S.M.H. (2002) Conditioning and spawning blacklip abalone. In: *Proceedings of the 9th Annual Abalone Aquaculture Workshop*, Queenscliff, Australia, 29–31 July (ed. by A.E. Fleming), pp. 136–144. Fisheries Research and Development Corporation's Abalone Aquaculture Subprogram, FRDC, Canberra, Australia.
- Ritar A.J. & Grubert M.A. (2002) Conditioning of wild-caught blacklip and greenlip abalone broodstock. In: *Proceedings of the 9th Annual Abalone Aquaculture Workshop*, Queenscliff, Australia, 29–31 July (ed. by A.E. Fleming), pp. 76–83. Fisheries Research and Development Corporation's Abalone Aquaculture Subprogram, FRDC, Canberra, Australia.
- Savva N.M., Heasman M.P. & Brand C.P. (2000) A recirculating temperature controlled system for the reproductive

- conditioning of blacklip abalone *Haliotis rubra*. In: *Proceedings of the 7th Annual Abalone Aquaculture Workshop*, Dunedin, Australia, 27–30 August (ed. by A.E. Fleming), pp. 61–76. Fisheries Research and Development Corporation's Abalone Aquaculture Subprogram, FRDC, Canberra, Australia.
- Sawatpeera S., Upatham E.S., Kruatrachue M., Chitramvong Y.P., Songchaeng P., Pumthong T. & Nugranad J. (2001) Larval development in *Haliotis asinina* Linnaeus. *Journal of Shellfish Research* **20**, 593–601.
- Sokal R.R. & Rohlf J.F. (1995) *Biometry: The Principles and Practice of Statistics in Biological Research*. Freeman, New York, USA, 486pp.
- Steinarsson A. & Imsland A.K. (2003) Size dependent variation in optimum growth temperature of red abalone (*Haliotis rufescens*). *Aquaculture* **224**, 353–362.
- Tarbath D.T. & Officer R.A. (2003) *Size limits and yield for blacklip abalone in Northern Tasmania*. Tasmanian Aquaculture and Fisheries Institute, Technical Report 17, Hobart, Australia, 37pp.
- Uki N. & Kikuchi S. (1982a) Technical study on artificial spawning of abalone, genus *Haliotis* IX. Influence of food levels on maturation and spawning of the abalone, *Haliotis discus hannai* related to effective accumulative temperature. *Bulletin of the Tohoku Region Fisheries Research Laboratories* **45**, 45–53.
- Uki N. & Kikuchi S. (1982b) Technical study on artificial spawning of abalone, genus *Haliotis* VIII. Characteristics of spawning behaviour of *H. discus hannai* induced by ultraviolet irradiation stimulus. *Bulletin of the Tohoku Region Fisheries Research Laboratories* **44**, 83–90.