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Spawning season, fecundity and proximate composition of the gonads of wild-caught blacklip abalone (*Haliotis rubra*) from Port Fairy waters, south eastern Australia

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Abstract

This study was based on wild-caught blacklip abalone *Haliotis rubra* L., from Port Fairy waters, south eastern Australia (142°15'E; 38°21'S), from July 1998 to November 1999, and was initiated to evaluate the spawning season and other aspects of its reproductive biology. The shell length and body weight of female and male abalone sampled ranged from 12.0 to 18.6 and 12 to 15 cm, and 137 to 529 and 148 to 585 g, respectively. The sex ratio did not vary significantly from 1:1 through the year. The gonadosomatic index (GSI) ranged from 3.0% to 8.4% in males, and 2.5% to 14.1% for females, and the highest GSI as well as the highest proportion of mature animals were recorded from September to October. During these months the hepatosomatic index (HSI) was low, and an inverse correlation between GSI and HSI ($P < 0.05$) was evident. Fecundity of blacklip abalone ranged from 1.09 to 7.5 million eggs for females of 12–14.5 cm in length, and 115–487 g in total body weight, respectively. The lipid content of the female gonad increased significantly from about July to November, and an opposite trend was observed for lipid content of the digestive gland. Seasonal changes in the protein and ash contents of the gonad and/or the digestive gland were not always significant.

Keywords: Spawning season; Gonadosomatic index; Fecundity; Digestive gland; Proximate composition; Abalone

1. Introduction

For effective management of a fishery, as well as for an aquaculture industry that is mostly dependent on wild broodstock, an understanding of the maturation cycle, and changes thereof, of the exploited populations is needed. The reproductive seasonality of many abalone species from various regions in the world has been studied previously (Booloottian et al., 1962; Tomita, 1967, 1968; Newman, 1967; Webber and Giese, 1969; Poore, 1970; Young and de Martini, 1970; Tutschulte and Connell, 1981; Capinpin et al., 1998; Counihan et al., 2001). Harrison and Grant (1971). and Shepherd and Law (1974) studied the reproductive biology of the blacklip abalone (*Haliotis rubra*) particularly from the South Australian region and other parts of Victoria. Most of the above studies have also contributed to the development and improvement of the artificial propagation techniques for a number of cultured abalone species, and to the growth of abalone aquaculture (Hahn, 1989; Hone et al., 1997).

In spite of the developments in artificial propagation techniques, abalone aquaculture is by and large dependent on wild broodstock. Apart from the seasonality factors, ascertaining the quality of gametes produced through the reproductive season and the energy resources utilized in maturation will enable improvements to broodstock and hatchery management to be effected. Hayashi (1983) observed that in *H. tuberculata* the glycogen content of the foot tissue decreased from 40% to 0% (by dry weight) as gonad maturation proceeded. Also in *H. cracherodii*, the total dry mass of polysaccharide (glycogen) in the foot decreased from 23% to 6%, the lowest value coinciding with late-summer spawning (Webber and Giese, 1969). Significant changes in the fat content in the viscera of *H. fulgens* have been linked to gonadal maturation (Hahn, 1989). The chemical composition of viscera of *H. fulgens*, *H. corrugata*, and *H. cracherodii* during the landing season was investigated by Viana et al.

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(1996) with a view to preparing a silage based feed for abalone. Various studies on other abalone species have mainly focused on seasonal variations in foot muscle in terms of the C:N ratio (Hayashi, 1983), extractive components (Watanabe et al., 1992), and chemical composition and textural properties (Hatae et al., 1995).

The present study is an extension of the previous work on the blacklip abalone, *H. rubra* L. from south eastern Australian waters. It was demonstrated that fertilizability in blacklip abalone was positively correlated to hatchability and larval survival, and that the physical characteristics and weight of the shell can be used as predictors of reproductive performance (Litaay and De Silva, 2001). In addition, Litaay et al. (2001) documented the changes in the amino acid profiles during embryonic development of blacklip abalone, and concluded that the energy requirements during early ontogeny are mostly met with from lipid reserves in the eggs, and that there was a tendency to conserve amino acids until pre-settlement. In general, there is a paucity of information on the biochemical changes associated with maturation in abalone species. In the present paper results on spawning seasonality, fecundity and biochemical changes associated with maturation in female blacklip abalone are dealt with.

2. Materials and methods

2.1. Source of wild-caught abalone

The study was based on wild-caught abalone collected (between July 1998 and November 1999) by divers from a particular stretch of waters in Port Fairy (142°15'E; 38°21'S), south eastern Victoria, Australia, and brought to the processing factory, Southwest Food Abalone Processors Ltd., Port Fairy. During this 18 month period of sampling the fishery was operational for only 12 months.

When samples were available, a minimum of 60 animals per month was examined for shell length, body weight and state of gonadal maturation. A sub-sample of at least 20 gonads from each monthly sample were preserved in 10% formalin for fecundity and histological studies, and another ten were kept at –30 °C for chemical analyses.

2.2. Parameters

2.2.1. Sex ratio

Sexes were determined macroscopically; mature males being identified from the milky white testis, and mature females from the green colored ovaries. The sex ratio of males and females of newly landed blacklip abalone were recorded monthly.

2.2.2. Gonadosomatic and hepatosomatic indices

Gonad index (GI) was determined on wet ovaries, and is expressed as a percent of ovary area against the total area of the conical appendage. At least 20 males and females were randomly selected, monthly for the estimation of gonadosomatic (GSI) and hepatosomatic (HSI) indices, which represent the gonad and digestive gland weight expressed as a percentage of the wet body weight, respectively.

Samples of ovaries were prepared for histology in order to evaluate the relationship between the macroscopic appearance and detail structural changes associated with maturation stages. Conical appendages of at least 20 females were preserved in a 10% formal-saline for histological studies. The middle point part of the conical appendage was carefully removed, and a thin slice (0.1 mm) was observed under a compound microscope to determine the stage of maturation (Young and de Martini, 1970). In the present study, criteria used to assess gonad development (five maturity stages) in *H. rubra* are a modification of criteria used commonly, elsewhere.

At least 2000 oocytes from 20 randomly selected, ripe females, were measured monthly in order to study oocyte size distribution throughout the reproductive cycle. Oocytes were loosened from trabeculae and their sizes were estimated using a compound microscope.

Fecundity, considered as the total number of oocytes present in a mature gonad (ripe) was estimated for at least 25 females per sampling month. Briefly, after fixing in formalin, gonads were carefully dissected, drained on filter paper and weighed to the nearest 0.1 g. Sub-samples were taken from three different locations of each ovary; near the base, mid and apical part and weighed to the nearest 0.0001 g (after Clavier, 1992), oocytes loosened from trabeculae and counted, and the total number of oocytes in the ovary was calculated, and is reported here as fecundity.

2.2.3. Chemical analysis

The proximate composition of ovaries and the digestive gland of females were analyzed using standard analytical procedures (AOAC, 1990) in order to observe seasonal changes in moisture, protein (through the estimation of Kjeldahl nitrogen using an automated Kjeltech 2300, Tecator, Sweden), total lipid (chloroform: methanol extraction, according to Folch et al., 1957), and ash (by burning for 12 h in a muffle furnace at 550 °C) contents as maturation progressed. Analyses were performed on five random samples of gonads and digestive glands from each sampling, and were done in duplicate.

2.3. Statistical analysis

Scatter plots were used to observe the distribution of data. Frequency distributions were used to evaluate oocyte distribution throughout maturation. Comparison of means of samples were done using SPSS version 9.0, while regression analyses were done using the software package Minitab version 12.1, respectively.

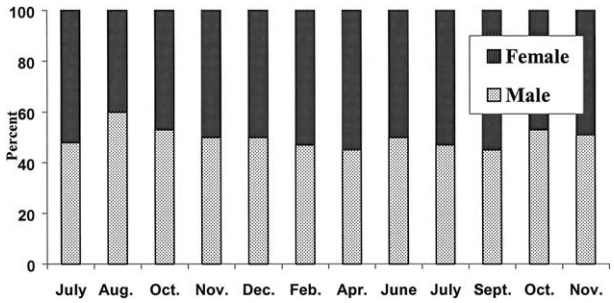

Fig. 1. Monthly distribution of sexes of *H. rubra*, landed from July 1998 to November 1999. Each month, a minimum of 60 animals, randomly selected from the commercial catches, were used for sex determination.

Table 1
The summary of morphological measurements of blacklip abalone sampled between July 1998 and November 1999

Parameters	Range	
	Males	Females
Shell length (cm)	12.0–18.6	12.0–15.1
Total weight (g)	137–529	148–585
Soft body (g)	58–386	50–500
Foot muscle (g)	84–307	84–302

3. Results

3.1. Sex ratio

A total of 1144 wild-caught abalone were examined during the study. There were 561 males and 573 females, respectively. Deviation from a 1:1 sex ratio of landed animals throughout the fishing seasons was not significant (χ^2-test, $P > 0.05$), and there was no recognizable trend in the monthly variations in sex ratio in blacklip abalone (Fig. 1).

3.2. Seasonal changes in morphometry

Size of sampled blacklip abalone ranged from 12 (minimum legal catch size) to 18.6 cm in length, and 137 to 592 g in weight. The biggest animal sampled was a male, 18.6 cm in length and 592 g in weight (Table 1). The overall mean length and weight of male and female abalone sampled were 12.7 ± 0.1 and 12.7 ± 0.1 cm, and 330 ± 8 and 326 ± 8 g, respectively. Shell length, total body weight, soft body weight, and foot muscle weight varied seasonally (Table 2). Bigger males (13.4 ± 0.1 cm) and females (13.3 ± 0.1 cm) were caught in July 1998, and slightly smaller sized animals

Table 2
Seasonal changes (means ± S.E.) in shell length, total body weight, soft body weight (viscera + foot muscle), and foot muscle weight of female and male blacklip abalone, caught between July 1998 and November 1999. Values in each column with the same superscript are not significantly different ($P > 0.05$)

Month	Female				Male			
	Length (cm)	Total weight (g)	Soft body (g)	Foot muscle (g)	Length (cm)	Total weight (g)	Soft body (g)	Foot muscle (g)
July	13.3 ± 0.1^{d}	395.3 ± 12.0^{c}	262.4 ± 8.0^{g}	171.9 ± 6.0^{g}	13.4 ± 0.0^{d}	394.8 ± 12.1^{e}	261.8 ± 7.5^{d}	176.8 ± 5.6^{e}
August	$12.5 \pm 0.1^{a,b}$	342.5 ± 12.4^{b}	$229.8 \pm 9.2^{e,f}$	$158.3 \pm 7.3^{e,f}$	12.9 ± 0.1^{c}	$339.8 \pm 13.5^{c,d}$	$222.7 \pm 9.1^{b,c}$	159.5 ± 6.7^{d}
October	$12.6 \pm 0.1^{a,b}$	334.9 ± 6.3^{b}	$212.7 \pm 4.8^{c,d,e}$	$145.6 \pm 3.7^{c,d,e}$	$12.5 \pm 0.1^{a,b}$	$324.2 \pm 9.9^{a,b,d}$	$209.9 \pm 7.8^{b,c}$	156.6 ± 4.4^{d}
November	$12.9 + 0.1^{b,c,d}$	$321.3 \pm 8.5^{a,b}$	$201.1 \pm 5.5^{b,d}$	$141.9 \pm 4.1^{b,d}$	$12.8 \pm 0.1^{b,c}$	$330.3 \pm 6.4^{b,c,d}$	$206.2 \pm 4.4^{b,c}$	$146.3 \pm 3.5^{c,d}$
December	12.4 ± 0.1^{a}	298.7 ± 6.3^{a}	188.3 ± 5.7^{b}	124.5 ± 5.1^{b}	12.4 ± 0.1^{a}	$301.1 \pm 5.7^{a,b}$	$189.0 \pm 3.4^{a,b}$	122.1 ± 2.5^{a}
February	$12.6 + 0.2^{a,b}$	298.2 ± 11.9^{a}	189.3 ± 12.7^{b}	129.2 ± 4.2^{b}	12.9 ± 0.1^{c}	$322.6 \pm 6.6^{a,b,c}$	$193.2 \pm 5.6^{a,b}$	$139.0 \pm 3.7^{b,c}$
April	$13.1 \pm 0.1^{b,c,d}$	337.6±7. 9^{b}	$201.3 \pm 4.5^{b,d}$	$146.7 \pm 4.2^{b,c,d}$	12.9 ± 0.1^{c}	348.8 ± 9.8^{d}	$210.9 \pm 5.7^{b,c}$	$152.4 \pm 4.6^{c,d}$
June	$12.3 + 0.1^{a,b}$	301.4 ± 4.0^{a}	$182.4 \pm 2.5^{a,b}$	$123.4 \pm 3.4^{a,b}$	12.3 ± 0.1^{a}	$300.6 \pm 3.6^{a,b}$	$189.2 \pm 3.3^{a,b}$	$129.2 \pm 1.8^{a,b}$
July	$12.9 \pm 0.1^{b,c,d}$	339.3 ± 7.7^{b}	$219.7 \pm 5.0^{d,e,f}$	$147.1 \pm 1.7^{d,e,f}$	12.9 ± 0.1^{c}	344.8 ± 8.2^{d}	223.7 ± 5.3^{c}	$150.9 \pm 3.9^{c,d}$
September	$12.4 \pm 0.1^{a,b}$	$311.6 \pm 4.8^{a,b}$	$192.4 + 3.3^{b,c}$	$128.3 \pm 3.5^{b,c}$	12.4 ± 0.7^{a}	$310.2 \pm 5.5^{a,b,c}$	$193.2 \pm 3.9^{a,b}$	$129.8 \pm 2.7^{a,b}$
October	$12.8 \pm 0.1^{b,c}$	338.3 ± 9.4^{b}	238.1 ± 7.2^{f}	165.5 ± 2.3^{f}	12.9 ± 0.1^{c}	342.7 ± 8.0^{d}	267.6 ± 6.9^{d}	192.8 ± 5.0^{f}
November	$12.4 \pm 0.1^{a,b}$	298.2 ± 4.9^{a}	167.0 ± 2.7^{a}	124.9 ± 5.4^{a}	$12.5 \pm 0.1^{a,b}$	297.8 ± 5.0^{a}	172.5 ± 3.4^{a}	$129.7 \pm 2.5^{a,b}$

Table 3
Seasonal changes in the GSI, HSI, and the GI (gonad index; percentage of a gonad area against the conical appendage area) of female (means ± S.E.), and mean (± S.E.) GSI and HSI of male blacklip abalone. Values in each column with the same superscript are not significantly different ($P > 0.05$)

Month	Female			Male	
	GSI	HSI	GI	GSI	HSI
July	$3.9 \pm 0.4^{a,b}$	8.5 ± 0.4^{d}	$23.7 \pm 2.4^{b,c}$	4.3 ± 0.3^{b}	7.0 ± 0.1^{d}
August	$4.0 \pm 0.3^{a,b}$	$3.0 \pm 0.3^{b,c}$	$26.9 \pm 1.6^{b,c}$	5.2 ± 0.2^{c}	5.5 ± 0.3^{b}
October	$6.8 \pm 0.5^{c,d}$	4.2 ± 0.3^{a}	32.7 ± 1.9^{c}	7.1 ± 0.4^{d}	$5.1 \pm 0.2^{a,b}$
November	$5.0 \pm 0.8^{a,b,c}$	4.2 ± 0.1^{a}	$21.9 \pm 2.8^{a,b}$	6.0 ± 0.4^{c}	4.3 ± 0.2^{a}
December	8.5 ± 1.1^{d}	$4.9 \pm 0.8^{a,b}$	$25.9 \pm 2.3^{b,c}$	8.0 ± 0.4^{e}	4.0 ± 0.1^{a}
February	3.6 ± 0.3^{a}	$6.1 \pm 0.4^{b,c}$	14.5 ± 1.5^{a}	$3.5 \pm 0.3^{a,b}$	5.4 ± 0.2^{b}
April	2.9 ± 0.4^{a}	6.5 ± 1.0^{c}	13.3 ± 1.9^{a}	3.2 ± 0.3^{a}	$6.6 \pm 0.2^{c,d}$
June	$4.2 \pm 0.6^{a,b}$	8.0 ± 0.5^{d}	$20.2 \pm 2.3^{a,b}$	$3.6 \pm 0.2^{a,b}$	$6.5 \pm 0.1^{c,d}$
July	$4.6 \pm 0.7^{a,b,c}$	$7.0 \pm 0.6^{c,d}$	$21.4 \pm 1.7^{a,b}$	$7.3 \pm 0.3^{c,d}$	6.3 ± 0.1^{c}
September	$7.1 \pm 0.6^{c,d}$	$5.8 \pm 0.7^{b,c}$	$27.4 \pm 2.5^{b,c}$	$7.2 \pm 0.3^{c,d}$	6.2 ± 0.2^{c}
October	$6.9 \pm 0.8^{c,d}$	$5.2 \pm 0.3^{a,b}$	$32.6 \pm 2.0^{b,c}$	$7.4 \pm 0.2^{d,e}$	$5.3 \pm 0.1^{a,b}$
November	$6.4 \pm 0.7^{b,c,d}$	4.4 ± 0.6^{a}	$25.6 \pm 2.6^{b,c}$	6.5 ± 0.2^{d}	$4.9 \pm 0.1^{a,b}$

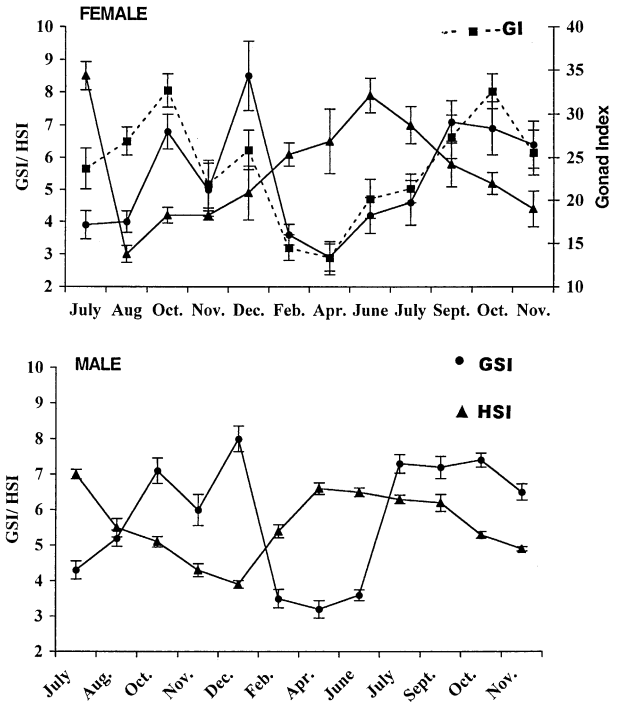

Fig. 2. Seasonal changes in GSI, HSI and GI of female, and GSI and HSI of male (means ± S.E.) blacklip abalone sampled from July 1998 to November 1999.

in July 1999. Also there was a decline in total body weight, soft body weight, and foot muscle weight from July to December 1998.

3.3. Seasonal changes in GSI and HSI

GSI of males ranged from 3.0 to 8.4, while that of females ranged from 2.5 to 10.4, and HSI of males ranged from 4.1 to 7.1, and for females from 4.0 to 9.0. Throughout the study, there were no significant differences between GSI of males and females caught in the same month (Table 3). However, there were significant increases in GSI of males from July to December (towards the spawning season) but declined thereafter (Fig. 2). The highest average GSI in males was noticed in December 1998 (8.0 ± 0.4), followed by 7.4 ± 0.2 in October 1999. Similarly, for females, the highest GSI occurred in December 1999 (8.5 ± 1.1), followed by 7.1 ± 0.6 in September 1999 (Table 3). In males, HSI declined from July 1998 towards December 1998, and increased from December 1998 to April 1999, followed by a decrease towards November. HSI of females showed a slightly different trend to that of males. Furthermore, it is evident from Table 3 that the HSI of females rapidly decreased from July to August 1998, and then gradually increased until July 1999 after which it gradually decreased. Furthermore, GSI in female *H. rubra* was inversely correlated to HSI (Pearson moment correlation $r = -0.577$, $P < 0.05$; Table 4).

GI, defined as the proportion of the gonad area to the area of the conical appendage expressed as a percentage, in females ranged from a minimum of 13.3 ± 1.9 in August to a maximum of 35.3 ± 2.0 in October (Table 3). GI in females increased from July 1998 to October 1998, followed by a decline from October 1998 to November 1998 ($P < 0.05$). An increase occurred again from November to December 1998 and sharply declined by February 1999 after which it increased gradually from April towards the second spawning season and reached a peak in October 1999. Months in which GI was high tended to coincide with those months with high GSI, and low HSI and these interrelationships are shown in Fig. 2.

3.4. Histological studies

Active gametogenesis appeared to occur during autumn and winter (June to August), when 46.2–79% of female gonads were in an active-maturing stage (Table 5). The highest percentage of animals in the spent stage occurred in November 1998, when 55% of females had completed spawning, followed by 30% in February 1999. Furthermore, ripe animals were found to be present throughout the study period. However, the proportion of ripe animals was high during the spawning seasons (October 1998 and 1999, and December 1998). In general, the months in which a proportion of stage 3 females were observed were the ones in which the GSI was high and HSI low.

3.5. Oocyte size distribution

A total of 24,000 oocyte diameters were measured for *H. rubra*. Oocyte diameter ranged from 40 μm in immature ovaries to 290 μm in mature ovaries. Seasonal changes in mean oocyte size (Fig. 3) indicates that oocyte size increased as maturation progressed and decreased after the spawning season.

Table 4
Correlation matrix of the relationship between GSI, HSI and GI, and protein and total lipid content of the gonad of female blacklip abalone. Value in the upper row is Pearson moment correlation, values in bottom row is *P* value

	GSI	HSI	GI	Protein	Total lipid
GSI	1	–0.579	0.8	ns	0.728
		0.049 *	0.002 **		0.008 **
HSI		1			
GI			1		
Protein				1	
Total lipid			0.852	0.64	1
			0.000 *	0.025 *	

ns, not significant. * $P < 0.05$; ** $P < 0.01$.

Table 5
Seasonal changes in gonad maturation stages of female *H. rubra*, expressed as a percent of the total number sampled each month throughout the study period

Month	Maturity stage				
	1	2	3	4	5
July	38.1	47.6	14.3	–	–
August	26.9	46.1	26.9	–	–
October	6.1	27.1	48.5	3.0	18.2
November	–	10.0	30.0	5.0	55.0
December	–	5.0	60.0	25.0	10.0
February	20.0	–	20.0	30.0	30.0
April	44.0	–	11.1	22.2	22.2
June	20.0	79.0	1.0	–	–
July	15.0	75.0	10.0	–	–
September	–	50.0	40.0	10.0	–
October	–	20.0	60.0	10.0	10.0
November	–	–	30.0	50.0	20.0

stage 1, Immature-Proliferative; stage 2, Active-Maturing; stage 3, Ripe; stage 4, Partly spawning; stage 5, Spent.

The mean oocyte size (Fig. 3) increased from July (120 ± 20 μm) to October 1998 (272 ± 8 μm), followed by a decrease in November 1998 (250 ± 25 μm). An increase in mean oocyte size again occurred from November to December 1998, reaching 280 ± 10 μm in December 1998 which was the maximum mean size observed in the study. There was a decrease in mean oocyte size recorded from December 1998 to February 1999 reaching a minimum value of 35 ± 5 μm (Fig. 3).

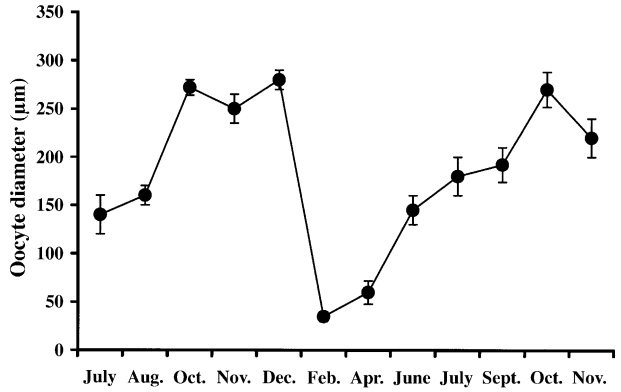

Fig. 3. Mean seasonal variations of oocytes diameter (means + S.D.). The mean for each month is based on approximately 2000 oocyte measurements from a minimum of ten randomly selected females (the stage of maturity of the individuals sampled was not taken into consideration).

3.6. Fecundity

Fecundity (*F*) of blacklip abalone ranged from 1.09 to 7.50 million eggs per female, for animals of 12 to 14.5 cm in shell length, and 147 to 487 g in total body weight, respectively. Fecundity was linearly correlated to length (*L*) ($P < 0.001$) and weight (*W*) ($P < 0.005$). In addition, GSI bore a linear relationship to fecundity ($P < 0.001$), and the respective relationships are best described by the equations:

$$F = 2.90\,L - 34.24 \quad (R^2 = 0.72)$$
$$F = 0.0325\,W - 6.39 \quad (R^2 = 0.50)$$
$$F = 0.5\,GSI - 2.68 \quad (R^2 = 0.63)$$

It is evident that shell length is a relatively more reliable parameter than weight and GSI for estimating fecundity in blacklip abalone.

Table 6
Seasonal changes (Mean ± S.E.) in proximate composition of the gonad and the digestive gland of female blacklip abalone, caught between, July 1998 and November 1999. Protein, crude fat, and ash are given on a dry weight basis. Values in each column with the same superscripts are not significantly different ($P > 0.05$)

Month	Gonad				Digestive gland			
	Moisture	Protein	Total lipid	Ash	Moisture	Protein	Total lipid	Ash
July	65.5 ± 1.5^{c}	$45.1 \pm 1.3^{b,c}$	$38.9 \pm 2.0^{c,d}$	$6.7 \pm 0.5^{b,c}$	$72.2 \pm 1.1^{b,c}$	47.9 ± 3.2^{a}	24.0 ± 2.8^{c}	9.3 ± 0.3^{a}
August	$62.1 \pm 1.0^{a,b}$	$44.8 \pm 1.4^{b,c}$	36.9 ± 0.5^{c}	5.9 ± 0.3^{b}	68.2 ± 0.9^{a}	$53.9 \pm 3.6^{b,c}$	$20.9 \pm 0.8^{b,c}$	$10.8 \pm 0.4^{a,b}$
October	63.7 ± 0.5^{b}	46.0 ± 2.0^{c}	$38.4 \pm 0.5^{c,d}$	5.5 ± 0.7^{b}	73.6 ± 0.5^{c}	$51.9 \pm 0.7^{a,b}$	17.5 ± 2.6^{b}	$10.1 \pm 0.6^{a,b}$
November	$64.9 \pm 0.8^{b,c}$	$44.9 \pm 1.6^{b,c}$	40.4 ± 0.8^{d}	5.4 ± 0.4^{b}	$71.2 \pm 0.8^{b,c}$	54.2 ± 2.1^{c}	16.1 ± 1.9^{a}	$10.8 \pm 0.5^{a,b}$
December	65.4 ± 0.9^{c}	$43.7 \pm 1.8^{b,c}$	37.9 ± 1.3^{c}	6.0 ± 0.3^{b}	$69.5 \pm 1.3^{a,b}$	$56.6 \pm 1.5^{b,c}$	18.7 ± 1.5^{b}	12.1 ± 1.0^{a}
February	65.8 ± 1.5^{c}	40.8 ± 1.0^{a}	27.5 ± 2.3^{a}	$6.9 \pm 0.5^{b,c}$	$70.2 \pm 0.4^{b,c}$	49.0 ± 0.6^{a}	$19.0 \pm 3.4^{b,c}$	$11.2 \pm 0.5^{a,b}$
April	63.2 ± 2.9^{b}	46.4 ± 1.3^{c}	30.5 ± 3.9^{b}	$5.3 \pm 0.4^{a,b}$	$68.7 \pm 0.8^{a,b}$	$51.8 \pm 0.1^{a,b}$	17.1 ± 1.6^{b}	$10.3 \pm 0.0^{a,b}$
June	56.9 ± 1.2^{a}	$42.0 \pm 1.3^{a,b}$	$29.9 \pm 1.3^{a,b}$	$4.7 \pm 0.3^{a,b}$	$69.7 \pm 0.4^{a,b}$	$48.7 \pm 1.6^{a,b}$	24.9 ± 1.8^{c}	$10.1 \pm 0.1^{a,b}$
July	58.7 ± 1.5^{a}	$40.0 \pm 3.2^{a,b}$	31.3 ± 1.3^{b}	$5.3 \pm 0.4^{a,b}$	$71.6 \pm 0.7^{b,c}$	$51.6 \pm 4.1^{a,b}$	20.8 ± 1.4^{c}	$10.1 \pm 0.4^{a,b}$
September	62.2 ± 1.2^{b}	46.4 ± 5.2^{c}	36.1 ± 1.8^{c}	7.6 ± 0.6^{c}	$70.9 \pm 0.6^{b,c}$	$52.6 \pm 1.6^{b,c}$	$21.8 \pm 1.4^{b,c}$	$10.2 \pm 0.3^{a,b}$
October	63.4 ± 2.6^{b}	46.0 ± 1.8^{c}	37.8 ± 1.2^{c}	6.1 ± 0.2^{b}	$67.9 \pm 1.9^{a,b}$	$51.6 \pm 1.5^{a,b}$	15.2 ± 1.4^{a}	$11.0 \pm 0.5^{a,b}$
November	$63.7 \pm 0.8^{b,c}$	$44.6 \pm 3.4^{b,c}$	$38.0 \pm 1.1^{c,d}$	$4.8 \pm 0.6^{a,b}$	$70.5 \pm 0.7^{b,c}$	$51.8 \pm 1.0^{a,b}$	$18.9 \pm 1.3^{b,c}$	9.8 ± 0.6^{a}

3.7. Proximate composition

Proximate composition (Table 6) was determined for gonads and digestive glands of female blacklip abalone only. The moisture content of gonads of females ranged from 56.7% to 65.8% and protein, total lipid, and ash from 40% to 46%, 27.4% to 40.4%, and 4.7% to 7.6%, respectively. The digestive glands of females contained 67.7–77.2%, 49–57.8%, 15.2–24.8%, and 9.3–12.1% of protein, total lipid and ash, respectively (Table 6).

The moisture content in the ovary increased significantly from August to December 1998 ($P < 0.05$). An increase in moisture content from December 1998 to February 1999 ($P > 0.05$), was followed by a decrease towards June 1999. Except in July 1998, protein was present in high amounts in April 1998, and September and October 1999. The lowest protein and lipid content in the ovary was observed in February 1999 being 40.8 ± 1.0% and 27.4 ± 2.3%, respectively. On the other hand, total lipid was high in July, October and November 1999 (Table 6). Total lipid content increased significantly ($P < 0.05$) from August to November in the first (1998), and from July to November in the second year (1999) of sampling. In addition, there was an increase in protein from August to October 1998, and from June to September 1999. These increases were followed by a significant decrease towards February. Changes in the ash content do not show a clear trend as for others proximate constituents (Table 6).

There was variation in the moisture content of the digestive gland through the year (Table 6). However, changes were not always significant. Similarly, changes in the protein content of digestive gland were not always significant. A significant decrease ($P < 0.05$) in total lipid content occurred from July (24 ± 2.8 %) to November (16.1 ± 1.9%) in the first year, and from June (24.8 ± 1.8%) to October (15.2 ± 1.4%) in the second year (Table 6). It is also evident from Table 6 that there were variations in ash content of the digestive gland throughout the study. Ash content of the digestive gland was highest in December (12 ± 0.5%), and lowest in July (9.3 ± 0.3%).

4. Discussion

4.1. Sex ratio

The fishing season for blacklip abalone along the south east coast of Australia is all year around. However, harvesting of wild animals is restricted to a quota and a size limit. Minimum size limit for the study area was 12 cm. At that size, animals in the wild are considered mature and it is thought that this size limit allows the population to be sustained.

Throughout the study period, the sex ratio of wild-caught blacklip abalone did not deviate significantly from the expected ratio of 1:1, indicating an equal distribution of adult male and female abalone in the population. The results agree with findings on others abalone species elsewhere such as *H. midae*, *H. cracherodii*, *H. rufescens*, *H. tuberculata* (Crofts, 1937; Booloottian et al., 1962; Newman, 1967; Bussarawit et al., 1990).

4.2. Spawning season

It is generally accepted that marine invertebrates could be divided into those that spawn near maximum temperatures and those that spawn on falling or minimum temperatures. However, Kinne (1970) suggested that in many cases seasonal variations in temperature act in concert with variations of photoperiod and nutrition as well as endogenous factors that control the breeding cycle. Photoperiod and nutrient supply were considered to be important factors in the reproductive biology of *H. cracherodii* (Webber and Giese, 1969). In *H. laevigata* and *H. cyclobates*, the period of maximum gonad growth coincided with that of an abundant food supply (Shepherd and Law, 1974) and optimal feeding possibilities (Shepherd, 1973). More recently, Counihan et al. (2001) suggested that the spawning of *H. asinina* in the Heron Reef, Australia, was associated with an increase in water temperature (October to April), and that other environmental cues, such as the tidal regime, also appeared to play a role.

In the present study, we found that there was an inverse trend between GSI and HSI, when GSI increased towards the spawning season and declined after spawning, whilst HSI changes were the reverse. Furthermore, the Pearson product moment correlation showed a significant inverse correlation between GSI and HSI ($r = –0.577$, $P < 0.05$). The opposite trend between GSI and HSI may be an indication of utilization of energy resources during the reproductive cycle of the animal. The digestive gland is a nutrient store, and as maturation proceeds nutrients are drawn from the digestive gland, thereby depleting its resources, and hence the resulting lowering of the HSI.

GI has been widely used in several abalone species for determination of the spawning season (Ino and Harada, 1961; Booloottian et al., 1962; Newman, 1967; Shepherd and Law, 1974; Bussarawit et al., 1990). However, GI based on weight could give a misinterpreted result as variations in moisture could contribute an error to the analysis. GI based on gonad area may be the best alternative for studying reproductive seasonality, and it can be done on fresh, frozen or preserved samples.

It appears that even though different methods have been used in studies on reproductive biology, histological examination is considered as the most accurate way to clarify a maturity stage of animals. Histological examinations showed that ripe gonads were found throughout the study period even though its proportion in the population was relatively low outside the spawning season. This could be an explanation for unsynchronized spawning which occurs randomly in wild populations.

Shepherd and Law (1974) reported that the breeding cycle of *H. rubra* showed a perplexing variability in the timing and duration of its spawning season at different places. For ex-

ample, the spawning season of blacklip abalone at West Island occurred from spring to early summer and also from early fall to early winter; in Tipara Reef (South Australia), from early autumn to throughout winter (Shepherd and Law, 1974); at Beach Port, South Australia, from early to mid spring and mid summer to early fall (Shepherd, unpublished data in Shepherd and Law, 1974). On the other hand, the blacklip population in Tasmania was found to spawn from early winter to late spring (Harrison and Grant, 1971). Shepherd and Law (1974). stated that the propensity of *H. rubra* to have different spawning seasons at different places emphasized the importance of local environmental variables in regulating the reproductive periodicity of the species.

The present study indicates that the main spawning season for blacklip abalone, deduced using a number of criteria (proportion of ripe and spent females in the population, changes in GI, GSI and HSI) from Port Fairy waters was comparable with the results from previous studies (Fig. 4). The main spawning season in Port Fairy waters occurred from spring to summer, with two maxima in October and December, as indicated by a drop in GSI and a corresponding significant change ($P < 0.05$) in the GI.

4.3. Fecundity

In the present study the fecundity of wild-caught blacklip abalone was found to bear a linear relationship to length ($P < 0.001$) and weight ($P < 0.005$). In a previous study on hatchery production (Litaay and De Silva, 2001), fecundity was best described as a polynomial function of length. McShane (1990) and Nash et al. (1994) found that fecundity of blacklip abalone was related to length, non-linearly, and was described by a power function ($Y = aX^b$). Such exponential increase of fecundity with increasing length has been also reported for other abalone species (Newman, 1967; Hayashi, 1980; Wells and Kessing, 1989; Wells and Mulvay, 1995). It is possible that the increased sample size in this study indicating a linear relationship to both length and weight would be more reliable.

4.4. Proximate composition

Previous studies on other molluscs indicated that metabolism of energy resources from storage may differ between species. Energy resources are mainly stored in the digestive gland (Sastry and Blake, 1971; Vassalo, 1973; Berthelin et al., 2000) and the muscle (Pazos et al., 1997; Berthelin et al., 2000). Furthermore, previous studies also have shown that the digestive gland is implicated not only in nutrient storage but also in the transfer of assimilated food to body tissues in molluscs (Sastry and Blake, 1971). Carefoot et al. (1998) stated that because the digestive gland is the chief organ involved in these energy transformations in abalone, its greatest relative size and metabolic activity would be expected to occur concomitantly with active gametogenesis. In bivalves, lipid reserves are considered to be used principally in gametogenesis and lost during spawning (Gabbott, 1983). Soudant et al. (1999) studied the biochemical composition of the Pacific oyster through the reproductive cycle and found that the stable and high percentage of neutral lipids in the gonad plus mantle underscores the fact that lipid reserves were preferentially located in that organ. Seasonal variations in total lipid and different classes of lipid of the female gonad of *Pecten maximus* are dependent upon the reproductive cycle and their levels were generally higher in ripe gonads and decreased with spawning (Pazos et al., 1997). Maternally derived lipids are used for the formation of membranes and are a major source of energy for embryogenesis and early larval development of various molluscs (Caers et al., 1999; Soudant et al., 1999). Fluctuations of lipid content coincide with the reproductive cycle, and such trends have been also reported for other molluscs such as dog cockle *Glycymeris glycymeris* (Galap et al., 1997, 1999) and the osyter *Crassostrea virginia* (Chu et al., 1990; Berthelin et al., 2000).

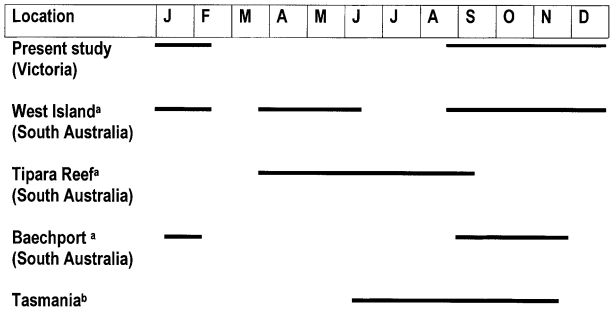

Fig. 4. Schematic diagram showing the spawning season of different abalone populations. Superscripts a and b represent data from Shepherd and Law (1974) and Harrison and Grant (1971), respectively.

Utilization of biochemical stores is dependent on the physiological status of the animal and the environmental condition (Martinez et al., 2000). In bivalve mussel, stress during winter condition result in the loss of 75% of body tissue and protein (Gabbott and Bayne, 1973). In the bivalve *Agropecten irradians*, during gametogenesis, digestive gland lipid is first metabolized, followed by muscle glycogen and finally protein reserves (Barber and Blake, 1981, 1991).

Gonadal development is an energy demanding process, as the mobilization of nutrients to the gonad occurs during gamete development. Development of gonads also depends on recently ingested food, storage reserves, or some degree of combination of the two (Barber, 1984). The relationships between food supply and gametogenesis have been examined experimentally for scallops (Soudant et al., 1996; Martinez et al., 2000) and oysters (Soudant et al., 1999). Under normal conditions, during oogenesis and spermatogenesis there is a rapid increase in lipid and protein in the gonad, and has been reported for scallop (Barber and Blake, 1991; Utting and Millican, 1998) and oyster (Soudant et al., 1996).

Proximate analyses of the digestive gland and the gonad of the blacklip abalone gave an indication of changes of energy resources during the reproductive cycle. Changes in proximate composition of gonad and digestive gland in blacklip abalone are in agreement with previous findings of other molluscs. It seems that for blacklip abalone, the main energy

used in the reproductive effort is lipid as indicated by a significant decrease in lipid content of the gonad after spawning. The inverse relationship between GSI and HSI may be an indication of a translocation of energy resources between the ovary and the digestive gland during the reproductive cycle of the animal. Storage metabolism in blacklip abalone as in other molluscan species, is closely associated with reproductive events and represents an important biochemical pathway.

Finally, the present study, apart from determining the spawning seasonality of blacklip abalone in Port Fairy waters, has shown the interactions that occur between the gonad and the digestive gland during maturation. It is evident that in blacklip abalone the digestive gland is a main provider of food reserves during maturation, and these reserves are provided mainly in the form of lipids, as in the case of early ontogeny (Litaay et al., 2001). The next step will be to determine the nature of the lipid reserves that are mostly involved in this translocation between the digestive gland and the maturing gonad in abalone.

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