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Reproduction of *Heterodontus portusjacksoni* in Victoria, Australia: evidence of two populations and reproductive parameters for the eastern population

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Abstract. Significant differences in the length–frequency composition of catch samples, mass–length relationships and length-at-maturity found between *Heterodontus portusjacksoni* from western and far-eastern Victoria, suggest the presence of at least two separate breeding populations off south-eastern Australia, with some mixing between these regions. In each region females attain a larger size than males, and collectively those sharks in western Victoria are smaller, present higher mass-at-length, and lower length-at-maturity than those in far-eastern Victoria. These regional differences might be a result of sampling bias, length-selective fishing mortality, environmental conditions, or genetic differences. However, tagging evidence from previous studies and the presence of two bio-geographic provinces in the region support the hypothesis of two separate breeding populations. The essential reproductive parameters for *H. portusjacksoni* population analysis in far-eastern Victoria were determined, using a novel method to estimate fecundity and the ovarian cycle of an egg-laying species. In this region, females have between 6 and 20 pre-ovulatory oocytes (average $= 14$, $n = 29$, standard deviation $= 3.71$) before the reproductive season. The species has an annual reproductive cycle correlated with water temperature, with ovulation starting during late winter to early spring and a $\sim$6 month egg-laying period. The period from the onset of vitellogenesis to ovulation of oocytes is $\sim$18 months.

Additional keywords: chondrichthyans, Port Jackson shark.

Introduction

Fishery stock assessment, ecological risk assessment, and assessment of species extinction risk all require quantitative information on a species' reproductive potential. The key reproductive parameters for these assessment methods include: (*1*) the sex ratio at birth; (*2*) the relationship between maternal age or size and the number of offspring (litter size or number of laid eggs per female); and (*3*) the relationship between the proportion of females contributing to annual recruitment and their age or size (maternity ogive). In some cases, the relationship between the proportion of mature females in the population and their age or size (maturity ogive) is also important. Unfortunately, differences in the reproductive strategies of some chondrichthyan species prevent the determination of some of these reproductive parameters (Walker 2005). For example, litter size can be investigated in viviparous species by counting the number of embryos at a full term stage *in utero*. The same method, however, cannot be applied to egg-laying species because oocytes appear to develop continuously and are laid during a protracted reproductive season or all year around. Thus alternative approaches are necessary, and we have developed a novel approach in this paper.

The Port Jackson shark, *Heterodontus portusjacksoni* (Meyer 1793), is an oviparous demersal species endemic to southern Australia (Last and Stevens 1994). It is commonly caught as by-catch by demersal otter trawl, shark gill-net and shark longline fisheries. In terms of mass and number of individuals, the species is the fifth top chondrichthyan species caught in the shark gill-net and longline fisheries in Bass Strait and South Australia (Walker *et al*. 2005), and the twelfth most important species in the trawl fisheries (Walker *et al*. 2007). However, little is known about the impact of these fisheries on its populations.

Several studies of *H. portusjacksoni* have focussed on the structure of the reproductive tract (Jones *et al*. 2005), reproductive physiology (Jones and Jones 1982; Jones and Lin 1992, 1993), and embryonic development (Rodda 2000). Some information about its reproductive biology off New South Wales (NSW) is available (McLaughlin and O'Gower 1971), but tagging studies (O'Gower and Nash 1978) suggest the presence of more than one breeding population off south-eastern Australia. Such separate populations of *H. portusjacksoni* might correspond to bio-geographic provinces, associated with oceanographic conditions and the distribution of dominant flora and fauna (Knox 1963). The aim of the present study was to investigate potential population differences by comparing length–frequency distributions, length–mass relationships and reproductive parameters, such as length-at-maturity, for

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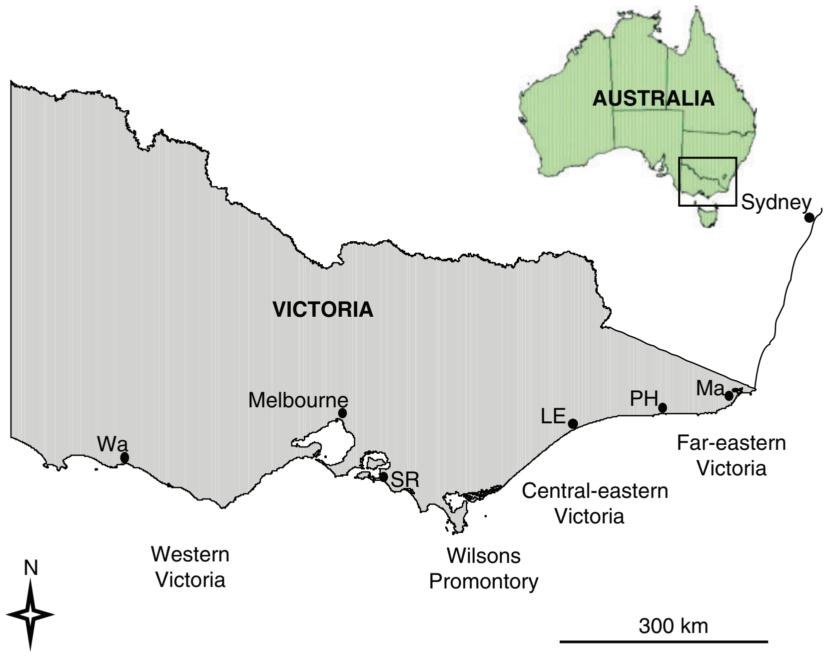

Fig. 1. Fishing ports sampled and regions adopted for *Heterodontus portusjacksoni*. LE, Lakes Entrance; Ma, Mallacoota; PH, Point Hicks; PPB, Port Phillip Bay; SR, San Remo; Wa, Warrnambool.

H. portusjacksoni from three broad regions off Victoria, and to determine the essential reproductive parameters required for population analysis for the putative population in far-eastern Victoria.

Materials and methods

Data collection

A total of 722 *Heterodontus portusjacksoni* was collected (355 females and 367 males) from commercial catches landed in several fishing ports in Victoria, Australia. Samplings were carried out monthly during August 2002–December 2004 and capture methods included demersal otter trawl (mainly in western Victoria), gill-nets and longlines (mainly in eastern Victoria) in depths up to ~80 m. The sharks were kept on ice after capture and transported to the laboratories of Primary Industries Research Victoria in Queenscliff, Victoria, where they were measured, weighed and dissected. Total length (TL) was measured to the nearest 1 mm as a straight line from the tip of the snout to the distal end of the tail, while allowing the shark to take a natural position without being stretched. Total body mass (TW) was obtained using a spring balance (±25 g). Usually there was negligible material in the gut, so stomach content mass was ignored.

Sex and regional comparisons of length, mass and maturity

Preliminary analysis of the data indicated regional differences among some of the relationships between biological variables, such as maturity condition and TL. Hence, data were grouped and analysed separately for three regions designated as: western Victoria (Warrnambool, Port Phillip Bay and San Remo; 21% of samples), central-eastern Victoria (Lakes Entrance; 17% of samples), and far-eastern Victoria (Point Hicks and Mallacoota; 62% of samples) (Fig. 1) to coincide with the two bio-geographic provinces and an overlapping zone (Knox 1963). However, regional comparison was not undertaken for other reproductive relationships, such as those for fecundity and TL, and the ovarian and reproductive cycle of *H. portusjacksoni*, because they could not be determined in western and central-eastern Victoria where only small numbers of mature sharks were collected during each month. Such information is presented only for far-eastern Victoria, where sampling was more intense, allowing estimation. Owing to sexual dimorphism in size, regional analysis was undertaken for each sex separately. Length–frequency distributions were compared among the regions using χ^2 tests.

The power model $TW = acTL^b$ was used to describe the relationships between TW and TL. In this equation c is a factor correcting for the effects of logarithmic transformation of the data (Beauchamp and Olson 1973), and a and b are parameters estimated for the linear regression equation $ln(TW) = a + b\,ln(TL)$. Differences in this relationship among regions for $ln(TL)$ and their interaction in $ln(TW)$ were tested by analysis of covariance (ANCOVA) using the generalised linear model $ln(TW) = c' + Rg + ln(TL) + Rg \times ln(TL) + \epsilon$, where c' is constant, Rg is the region factor, $ln(TL)$ is a covariate, and $Rg \times ln(TL)$ is an interaction term. Stepwise backward elimination was used to determine the significant terms in this model. Non-significant interactions and then other non-significant terms ($P > 0.05$) were sequentially excluded from the model, until only significant terms remained (Walker 2005, 2007).

Table 1. Maturity indices for *Heterodontus portusjacksoni* using the condition of reproductive structures

Maturity condition	Females			Males		
	Ovaries	Oviducal glands	Uteri	Testis	Claspers	Seminal vesicles
1) Immature	Undifferentiated from the epigonal organ, without visible oocytes	Indistinct from anterior oviduct	Indistinct from anterior oviduct	Undifferentiated from the epigonal organ	Short and non-calcified	Thin strip with translucent walls
2) Maturing	Whitish oocytes ≤3 mm in diameter	Differentiated from anterior oviduct, longer than wider	Narrow tubulus	Differentiated from the epigonal organ, neither lobular nor vascularised	Elongated and partly calcified	Thin strip with translucent walls
3) Mature	Vitellogenic (yellowish) oocytes >3 mm in diameter	Heart shaped, wider than longer	Narrow tubulus, with eggs or distended walls	Elongated, lobular and vascularised	Elongated and fully calcified	Thickened opaque walls, with or without seminal fluids

The ANCOVAs were undertaken by using the general linear modelling (GLM) procedure of the SAS statistical software package (SAS Institute, North Carolina, USA). Comparison between the sexes was carried out to explore potential differences in mass at any given length using a similar model.

Female maturity was assessed using three indices based on macroscopic examination of the ovaries, oviducal glands and uteri (Table 1). Females were considered as mature if their ovaries had vitellogenic oocytes >3 mm in diameter, as this represents the approximate time of the onset of vitellogenesis in other species (Walker 2005, 2007). The length and width of the oviducal glands, as well as the length and width at the central regions of the uteri, were measured to the nearest 1 mm using callipers. Male maturity was assessed using three indices based on macroscopic examination of the testis, claspers and seminal vesicles (Table 1). Clasper length (CL) was measured to the nearest 1 mm, from the join in the skin near the pelvic fin to the distal end of the clasper, and plotted against the TL of the shark.

Length-at-maturity ogives for each sex were determined using each maturity index separately. However, oviducal glands and uteri indices did not produce good shaped ogives, therefore only those based on gonad index are presented for females. The ogives were determined by applying logistic regression to the proportion of mature sharks (P) as a function of TL in the equation $P = c/[1 + e^{-(a+b\,TL)}]$, where P is given by a random dichotomous variable taking the value of 1 for mature animals and the value of 0 for immature animals, and, a, b and c are parameters. This equation was reformulated to express the relationship in terms of more biologically meaningful parameters as $P = P_{max}[1 + e^{-ln(19)(L-L_{50}/L_{95}-L_{50})}]^{-1}$, where P_{max} equals c and is the maximum proportion of mature animals, and L_{50} and L_{95} are lengths at which 50% and 95% of the maximum proportion of animals mature respectively. L_{50}, L_{95} and their 95% confidence intervals were estimated using the Probit procedure of the SAS statistical software package.

The effects of region on the relationship between P and TL were tested by logistic regression using the model $P = (n/N) = C + Rg + TL + Rg \times TL$, where n is the number of mature animals and N is the total number of animals, C is a constant and Rg is region (Walker 2005).

Fecundity, and ovarian and reproductive cycles in far-eastern Victoria

The average size of oocytes classed as ovulatory (~40 mm diameter and ~40 g mass) was determined from eggs inside freshly laid egg cases (Rodda 2000; Tovar-Ávila 2006). Oocytes that had reached ~75% of the ovulation size (≥35 mm) before the beginning of the egg-laying period were considered as pre-ovulatory eggs that would be ovulated during the following egg-laying period. Annual fecundity was defined as the number of eggs laid per female per season (year) and was estimated by counting the number of pre-ovulatory oocytes in females caught during the months before the onset of egg-laying. Oocytes showing any degree of atresia were not considered, irrespective of their size, because they were considered as non-ovulated oocytes from previous or current egg-laying seasons. Linear regression analysis was used to determine the relationship between the number of pre-ovulatory oocytes and maternal TL.

Seasonal variation in the reproductive organs was examined by comparing the month of collection with: (*1*) gonadosomatic index (GSI); (*2*) hepatosomatic index (HSI) for mature females and males; (*3*) oviducalsomatic index (OSI) for mature females; and (*4*) fullness of the seminal vesicles for mature males. The liver, gonads and oviducal glands were weighed with an electronic balance (±0.005 g). Gonad mass included the epigonal organ mass because of difficulties in separating these tissues, particularly when the gonads were not fully developed. Each index was then calculated as the mass of the organ divided by total body mass. As the right and left oviducal glands were of similar mass, only the right oviducal gland mass was used to calculate OSI. GSI, OSI and HSI data were multiplied by 100 to derive percentages and avoid small numbers. Seminal vesicle fullness was ranked as 0, 1, 2, 3 and 4 quarters, and converted to values of 0.10, 0.25, 0.50, 0.75 and 1.00 respectively (Y). This method avoided zero to allow data transformation. The decimal values Y were then transformed to $\sqrt{\sqrt{Y}}$. The effects of month and region on GSI, OSI, HSI and seminal vesicle fullness, and the possible effect of shark TL were tested by ANCOVA and stepwise backward elimination (Walker 2005, 2007).

The process of oocyte development from the onset of vitellogenesis to ovulation was examined using two methods. The first method was to examine seasonal trends in oocyte size. The diameters of the ovarian oocytes in the 24 largest follicles were measured to ensure that all possible pre-ovulatory oocytes were included. Oocyte diameter was then plotted against the rank of the follicle for each mature female separately as a line graph. These line graphs were plotted together for each season to identify temporal changes in the size of oocytes and number of pre-ovulatory oocytes. The second method was to plot as a scattergram the maximum oocyte diameter (MOD) against day of year (t). Where females had a full range of oocyte sizes in the ovaries, the oocytes could be grouped into three cohorts. These groups or cohorts included the large pre-ovulatory oocytes (used to estimate fecundity), a second group of intermediate-size oocytes, and a third group of small oocytes (immediately following the onset of vitellogenesis). Two or three cohorts were present depending on the time of the year. t was adjusted to separate females with a MOD associated with each of these cohorts. Hence, adjusted t (t_{adj}) was calculated by adding 730 (two extra years) to t for females in which the largest oocyte cohort (pre-ovulatory oocytes or MOD > 35 mm) was present, and adding 365 (one extra year) to t for females with a medium size oocyte cohort (MOD 16–34 mm). t was not adjusted for females with the smallest oocyte cohort (MOD < 15 mm). For those females having more than one oocyte cohort only the largest was considered for this analysis. Linear regression analysis was used to determine the parameters a and b of the equation $\text{MOD} = a + t_{adj}b$. Only data with t_{adj} <942 were used for the regression analysis to avoid bias caused by the continuous ovulation of the largest oocytes after this date.

The proportion of females with pre-ovulatory oocytes for each month and the average number of pre-ovulatory oocytes of females per month were also related to the ovarian and egg-laying cycles. The presence and diameter of *corpora lutea* and *corpora atretica* in the ovary were recorded and related to the reproductive cycle as well. Because of the difficulty in separating these two structures by their macroscopic characteristics at a certain degree of atresia (Hisaw and Hisaw 1959), only yellowish structures were considered as *corpora atretica*, whereas all flat whitish structures were considered *corpora lutea*.

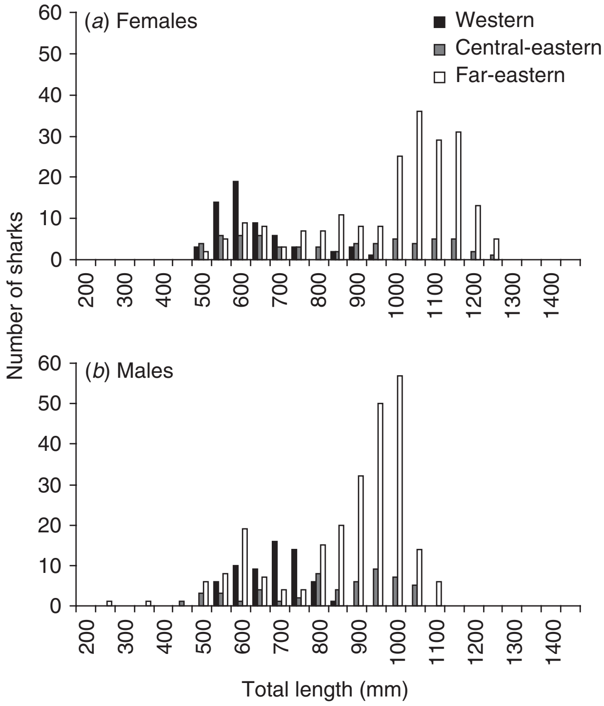

Fig. 2. Length–frequency distribution of *Heterodontus portusjacksoni* (*a*) females and (*b*) males collected in three broad regions off Victoria.

Table 2. Total length (TL) and total body mass (TW) ranges of *Heterodontus portusjacksoni* sampled from each region in Victoria

Region	Females			Males		
	n	TL (mm)	TW (g)	*n*	TL (mm)	TW (g)
Western	60	458–920	750–9100	62	500–816	510–4500
Central-eastern	63	476–1222	700–1450	54	439–1103	650–4150
Far-eastern	207	490–1225	840–1420	244	250–1090	110–9850

Results

Sex and regional comparisons of length, mass and maturity

Significant differences in length–frequency distributions of *Heterodontus portusjacksoni* were found among the three regions for both females ($\chi^2_{30} = 138.92$, $P < 0.0001$) and males ($\chi^2_{30} = 177.07$, $P < 0.0001$) (Fig. 2). Females reached a larger size than did the males in each region, and those sharks of both sexes captured in western Victoria reached a smaller size than those captured in far-eastern Victoria, with a wide range of lengths in central-eastern Victoria (Table 2).

The TW–TL relationships were significantly different between regions for both females (ANCOVA, $F_{2,326} = 7.71$,

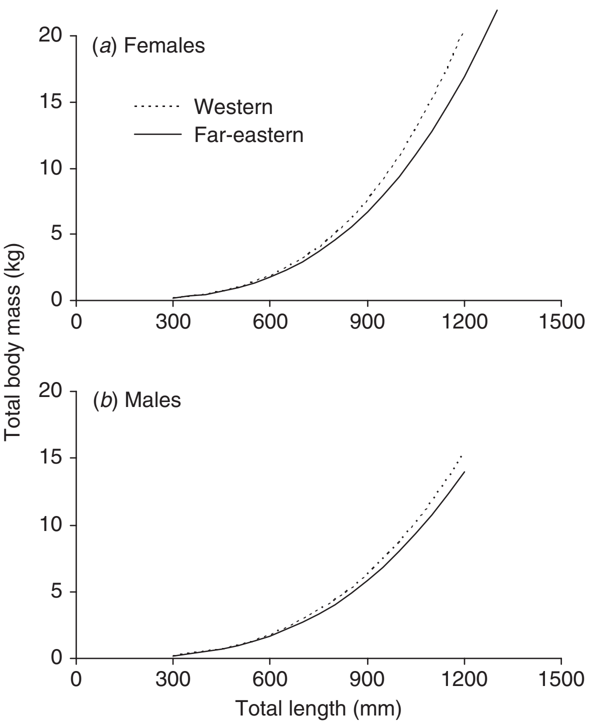

Fig. 3. Relationships between total body mass (TW) and total length (TL) for (*a*) females and (*b*) males from western Victoria (- - - - -) and far-eastern Victoria (——).

$P < 0.0005$) and males (ANCOVA, $F_{2,356} = 10.56$, $P < 0.0001$) indicating mass increases at different rates (Fig. 3). Sharks from western Victoria presented higher mass at any given length than those sharks from far-eastern Victoria, with intermediate mass in central-eastern Victoria (Table 3). Significant differences were also found in the TW–TL relationship between sexes for far-eastern Victoria (ANCOVA, $F_{1,447} = 13.04$, $P < 0.0003$). Sharks of both sexes of *H. portusjacksoni* from all the regions below 600 mm TL presented similar body mass, but differences between females and males and among regions increased with TL. The mass of the heaviest recorded male (10.5 kg, 1050 mm TL) was less than two-thirds the mass of the heaviest female (18 kg, 1210 mm TL).

Mature females had a single functional ovary (right) consisting of oocytes at various stages of development, *corpora lutea* and *corpora atretica* (Fig. 4). The ovary mass of mature females ranged 0.7–6.9% of TW. Mature males had both gonads functional. The testis mass ranged 0.08–1.35% of TW, whereas CL ranged 6.2–13.5% of the TL.

Significant differences in length-at-maturity were found between *H. portusjacksoni* from western and from far-eastern Victoria for each sex (Table 4). Length-at-maturity was smaller in western Victoria than in far-eastern Victoria, with intermediate maturity estimates in central-eastern Victoria. Though maturity ogives in western and far-eastern Victoria presented narrow confidence intervals for both sexes, those from central-eastern Victoria had wide confidence intervals. The relationship between CL and TL showed the same pattern of regional variation. Similar lengths-at-maturity for males were calculated when each of the

Table 3. Values of parameters and statistical quantities for the equation $TW = ca(TL^b)$ for female and male *Heterodontus portusjacksoni* from each region in Victoria

TW, total body mass; *TL*, total length; *c*, factor correcting for the effects of logarithmic transformation of the data (Beauchamp and Olson 1973); *a* and *b*, parameters; r^2, square of correlation coefficient; *rsme*, root mean square error; *n*, sample size; *P*, probability of statistical significance

Sex and region	*a*	*b*	r^2	*rsme*	*n*	*P*
Females						
Western	4.26 E–10	3.468	0.953	0.0155	60	<0.001
Central-eastern	7.80 E–10	3.308	0.987	0.0122	63	<0.001
Far-eastern	1.71 E–9	3.245	0.972	0.0143	207	<0.001
Males						
Western	3.69 E–9	3.123	0.937	0.0097	62	<0.001
Central-eastern	2.29 E–9	3.181	0.979	0.0125	54	<0.001
Far-eastern	5.52 E–9	3.053	0.978	0.0105	244	<0.001

three maturity indices (claspers, gonads and seminal vesicles) were used (Table 4).

Fecundity, and ovarian and reproductive cycles in far-eastern Victoria

Reproductively capable females in far-eastern Victoria contained between six and 20 (mean = 14, standard deviation = 3.71, $n = 29$) pre-ovulatory oocytes before the start of the ovulation period. A significant relationship was found between the number of pre-ovulatory oocytes (Y') and maternal TL ($Y' = -14.08TL + 0.0256$, $r = 0.57$, $n = 29$, $P < 0.01$) (Fig. 5).

In this region *H. portusjacksoni* displayed a degree of synchrony in its reproductive cycle, because the start of ovulation and mating (late winter–early spring) and the egg-laying period (late winter–summer) appears to occur at the same time for most of the population based on the analyses of reproductive organs. GSI for mature females increased from summer to autumn (southern hemisphere), peaking during May–June, and decreased during the spring (Fig. 6). In the case of mature males, highly significant differences were detected among months for GSI (ANCOVA, $F_{11,190} = 7.88$, $P < 0.0001$) and seminal vesicle fullness (ANCOVA, $F_{7,153} = 25.18$, $P < 0.0001$). GSI decreased during winter and spring (Fig. 7*a*) and seminal vesicles were full during winter (Fig. 7*b*). The interaction between month and TL was highly significant for both OSI (ANCOVA, $F_{8124} = 5.09$, $P < 0.0001$) and HSI in mature females (ANCOVA, $F_{9,164} = 2.97$, $P < 0.0027$), and for HSI in mature males (ANCOVA, $F_{8,176} = 3.77$, $P < 0.0004$); hence, seasonal variation could not be tested in terms of these indices.

Several cohorts of oocytes were distinguished in the ovary throughout the year. The differences among these cohorts were more evident during winter and autumn (Fig. 8*a*, *d*), when most of the pre-ovulatory oocytes reached the ovulation size. Pre-ovulatory oocytes were found first before the beginning of the ovulation period (autumn). The number of pre-ovulatory oocytes decreased during spring and summer (Fig. 8*b*, *c*), and the sizes of the oocytes of the medium-size oocyte cohort

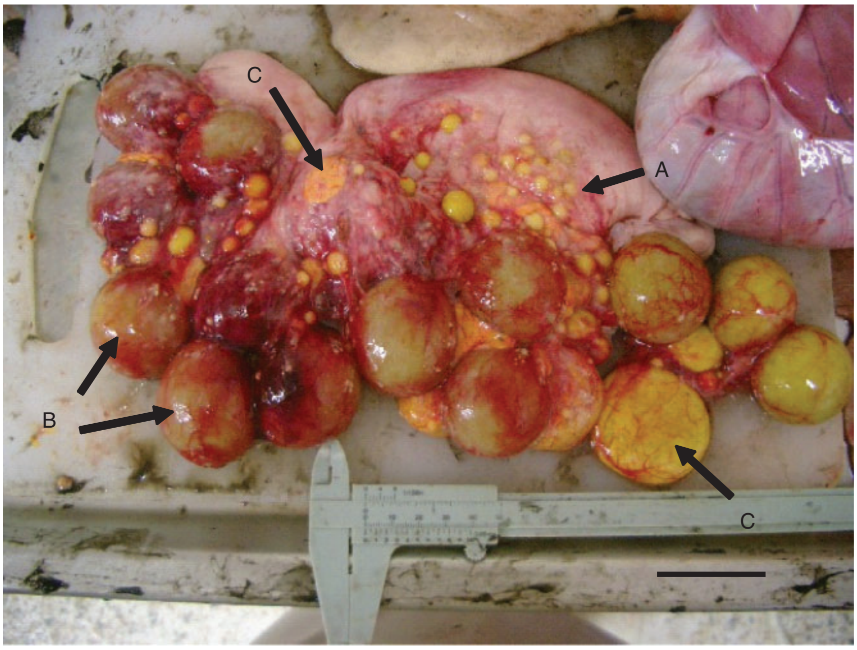

Fig. 4. Mature ovary of *Heterodontus portusjacksoni* showing (A) developing vitellogenic oocytes, (B) pre-ovulatory oocytes and (C) oocytes in different stage of atresia. Scale bar: 40 mm.

Table 4. Mean lengths (mm of total length) at which 50% (L_{50}) and 95% (L_{95}) of the population are mature for female and male *Heterodontus portusjacksoni* from each region in Victoria

CI, 95% confidence intervals; *n*, number of mature animals; *N*, total number of observations; *ML*, maximum likelihood; *P*, probability of statistical significance for differences among regions

Sex	Index	Region	L_{50} (*CI*)	L_{95} (*CI*)	*n*	*N*	*ML*	*P*
Females	Gonad index	Western	858 (812, 933)	1021 (942, 1223)	9	77	−18.706	<0.001
		Central-eastern	919 (854, 978)	1110 (1036, 1287)	26	67	−18.039	<0.001
		Far-eastern	990 (971, 1005)	1178 (1149, 1221)	106	208	−232.967	<0.001
Males	Gonad index	Western	672 (650, 693)	788 (754, 859)	31	71	−47.992	<0.001
		Central-eastern	750 (676, 801)	907 (843, 1112)	25	45	−15.001	<0.001
		Far-eastern	820 (802, 834)	963 (948, 982)	150	231	−249.899	<0.001
	Clasper index	Western	661 (639, 679)	736 (734, 820)	34	71	−43.388	<0.001
		Central-eastern	747 (677, 791)	888 (836, 1027)	36	56	−15.537	<0.001
		Far-eastern	822 (812, 830)	881 (871, 895)	171	243	−80.156	<0.001
	Seminal vesical index	Western	678 (655, 700)	793 (756, 875)	29	68	−43.447	<0.001
		Central-eastern	786 (704, 853)	1031 (937, 1307)	21	45	−21.889	<0.001
		Far-eastern	815 (800, 827)	905 (893, 921)	157	226	−123.344	<0.001

began increasing during spring, and reached ovulation size the following winter (Fig. 8*b*–*d*).

The period from the onset of vitellogenesis to ovulation of oocytes is ~18 months in far-eastern Victoria. A strong relationship was found between the MOD and t_{adj} for sharks from this region ($MOD = -32.2t_{adj} + 0.0835$, $r^2 = 0.74$, $n = 131$, $P < 0.05$) (Fig. 9). The highest proportion of mature females with pre-ovulatory oocytes, and the largest average number of pre-ovulatory oocytes in the ovary was found during autumn–winter (May–August) (Fig. 10). Some females (~25%), however, that had reached L_{50} (955–1077 mm TL) and had expanded uteri and expanded oviducal glands, did not have pre-ovulatory oocytes (10–19 mm of MODs) during autumn–winter as expected. *Corpora lutea* were present throughout the ovulation period, whereas *corpora atretica* were present mainly during summer–autumn, and they increased in number throughout this period. Atresia of a follicle began with the vascularisation of its granulosa and then an increase in its size. These oocytes filled with liquid and then changed their colour and lost the spherical shape to become flattened.

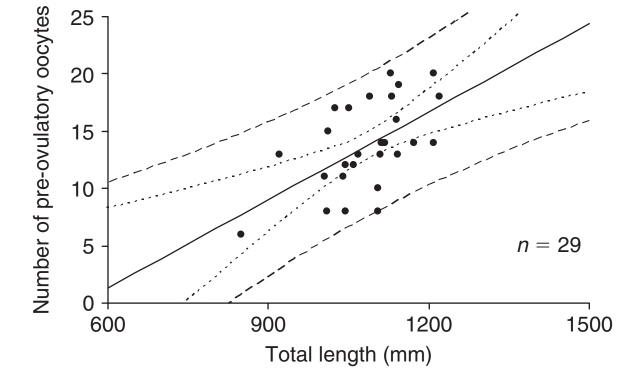

Fig. 5. Relationship between number of pre-ovulatory oocytes and female total length before the egg-laying period (May–June) in far-eastern Victoria. Linear regression (——), 95% confidence limits (– – – –) and 95% prediction intervals (- - - - -) are shown.

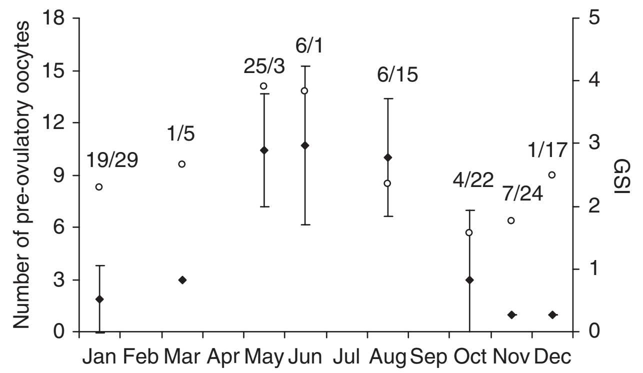

Fig. 6. Mean number of pre-ovulatory oocytes (>35 mm diameter) in the ovary (♦) and average gonadosomatic index (GSI) (O) of mature females from far-eastern Victoria. Standard error bars are shown for number of pre-ovulatory oocytes and the ratios of females with pre-ovulatory oocytes/mature females observed are presented above the bars.

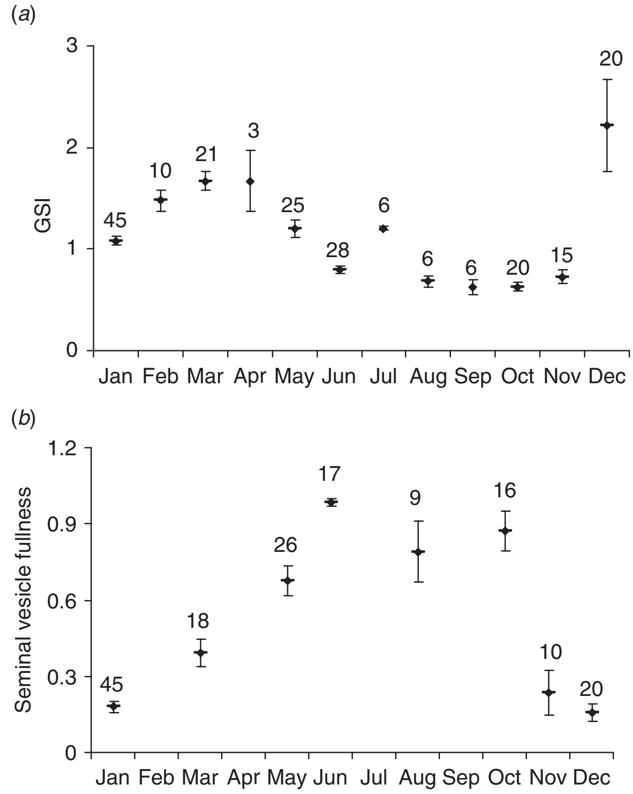

Fig. 7. Seasonality of (*a*) gonadosomatic index (GSI) and (*b*) seminal vesicle fullness for males in far-eastern Victoria. Observed mean monthly values (–), standard error bars (±1 s.e.), least-squares means corrected for effect of shark length (●), and number of observations above the bars are presented.

Discussion

Sex and regional comparisons of length, mass and maturity

Sexual dimorphism in the length–frequency distributions, length–mass relationship and length-at-maturity, such as that found for *Heterodontus portusjacksoni* in the present study, is a common feature for many chondrichthyan species; males often reach smaller sizes and mature earlier than females (Ford 1921; Cortés 2000; Walker 2007).

Length-at-maturity ogives for *H. portusjacksoni* are well determined by MOD for females, but indices of oviducal glands and uteri did not produce useful ogives, probably owing to the variation of such structures during the reproductive season. The three male maturity indices provide similar shaped ogives, as has been found in other species (Walker 2005, 2007; Braccini *et al.* 2006).

Estimates of L_{50} in the present study were different from estimates of length-at-maturity in previous studies. In far-eastern Victoria, female and male L_{50} estimates (based on all the three indices used) were considerably higher than length-at-maturity ranges in New South Wales (700–860 mm TL for females based on MOD >9–18 mm; 700–800 mm TL for males based on clasper and testis condition) (McLaughlin and O'Gower 1971), and in another study (Last and Stevens 1994) in which regions and maturity criteria were unspecified (800–950 mm TL for females; 750 mm TL for males). In western Victoria in contrast, female L_{50} was within those length-at-maturity ranges previously reported, whereas male L_{50}'s were all lower than the respective ranges.

The differences in length–frequency distribution composition of catch samples, mass–length relationships and length-at maturity among regions and in previous studies could be the result of apparent or real differences in the population parameters. Four hypotheses are advanced; the fourth appears to best explain the differences.

(1) The differences are the result of sampling bias caused by the length-selectivity of the fishing gears. Differences in the fishing gear used to catch *Squalus megalops* from different regions have been found to cause distortions of its maturity ogives (Braccini *et al.* 2006). Although different fishing methods were used to collect *H. portusjacksoni* in the present study, specimens encompassing a wide range of sizes around the length-at-maturity were collected, making it unlikely that sampling bias would have had any marked effect on the maturity ogives.

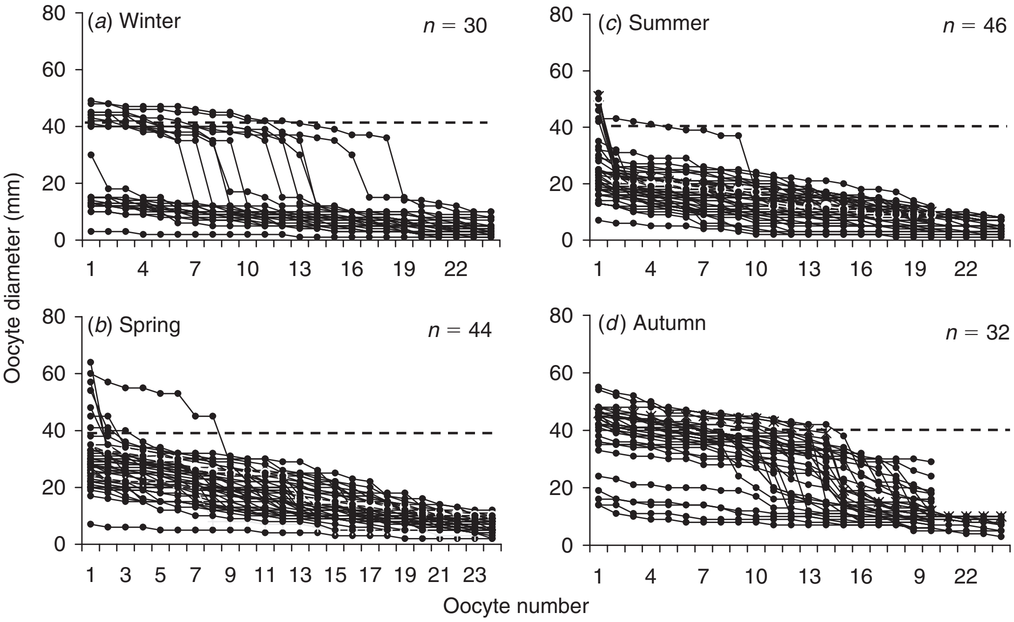

Fig. 8. Plots of the 24 largest oocyte diameters in the ovary of each mature female sampled in far-eastern Victoria. Dashed line (- - - - -) represents estimated average size of oocytes at ovulation (40 mm).

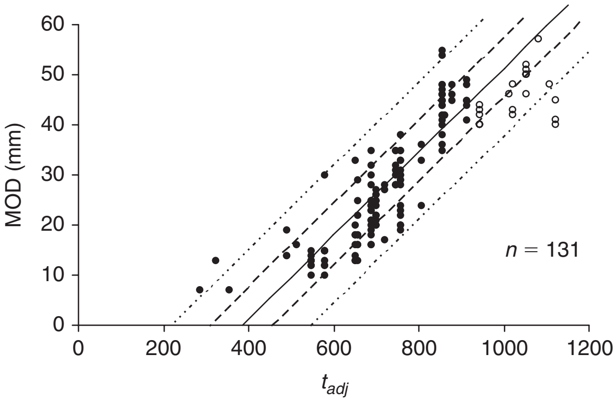

Fig. 9. Relationship between maximum oocyte diameter (MOD) and adjusted day of year (t_{adj}) (<942) for far-eastern Victoria. Linear regression (——), 95% confidence limits (– – – –) and 95% prediction intervals (- - - - -) are shown. MODs with t_{adj} >942 (○) are not used in regression analysis.

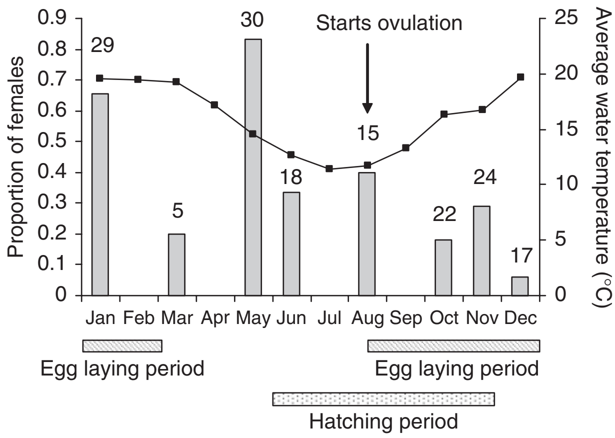

Fig. 10. Proportion of mature females with pre-ovulatory oocytes (bars) and average water temperature during each month in far-eastern Victoria. Numbers of mature females observed are presented above the bars.

(2) The differences are the result of length-selective fishing mortality caused by commercial gill-net fishing. The length-selectivity of gill-nets produced an apparent increase in length-at-maturity of *Mustelus antarcticus* populations during rising fishing mortality, with a subsequent decrease when fishing mortality declined (Walker 2007). In all the studied regions of Victoria however, the current level of fishing on *H. portusjacksoni* is low (Walker *et al*. 2005); so that the effects of length-selective fishing mortality from gill-nets is expected to be low.

(3) The differences are the result of different environmental conditions. Differences in length-at-maturity detected for other species, e.g. *Galeus eastmani*, *G. nipponensis*, *Sphyrna tiburo*, *Mustelus manazo* and *M. antarcticus*, have been related to environmental conditions such as water temperature (Horie and Tanaka 2000; Yamaguchi *et al*. 2000; Lombardi-Carlson *et al*. 2003; Walker 2007). Although western Victoria is mostly under the influence of the cold-deepwater Flinders Current and far-eastern Victoria is mostly under the influence of the warm-surface-water East Australian Current, central-eastern Victoria experiences intermediate conditions, and therefore is expected to have intermediate parameter values. However, central-eastern Victoria showed a wide range of sizes and wide confidence intervals in some estimated parameters, such as length-at-maturity, suggesting a mix of animals from western

and far-eastern populations rather than intermediate animals from a single intergrading population. Nevertheless, environmental conditions probably play a part, as these are likely to work in conjunction with genetic differences to produce differences between populations.

(4) The differences are the result of two separate breeding populations of *H. portusjacksoni* inhabiting Victorian waters, with different gene pools. This hypothesis is consistent with a conjecture, based on tagging experiments, that there are at least two separate stocks in south-eastern Australia (O'Gower and Nash 1978). One population may extend from mid NSW to central-eastern Victoria. *Heterodontus portusjacksoni* tagged near Sydney, NSW, have been recovered ~40 km south-east of Wilsons Promontory, Victoria, travelling up to 760 km (O'Gower and Nash 1978). A separate population may be present in western Victoria, with a mixing region located between Wilsons Promontory and Point Hicks.

The putative mixing region for *H. portusjacksoni* is consistent with the overlapping zone of the Peronian (Queensland 25°S, NSW and eastern Victoria) and Maugean (Victorian and Tasmanian coasts) bio-geographic provinces (Knox 1963). Overlapping zones of bio-geographic provinces depend on hydrological conditions and paleo-geographic factors involved in the evolution and distribution of species (Knox 1963, 1994). Land connection between Victoria and Tasmania, caused by changes in the sea level during glaciations, has been an important geographic and biological barrier in this region (Knox 1994), suggesting that the range of *H. portusjacksoni* has been divided and connected several times. Further studies are needed to determine the level of separation of the populations in Victoria and whether or not they interbreed in the mixing region.

Fecundity, and ovarian and reproductive cycles in far-eastern Victoria

In this region, pre-ovulatory oocytes of *H. portusjacksoni* attain a large size as compared with other species. *Galeus eastmani* and *G. nipponensis* oocytes reach a MOD of 20 and 25 mm, respectively (Horie and Tanaka 2000), whereas *Scyliorhinus retifer* reach a MOD of 18 mm (Castro *et al*. 1988). Reported MODs at ovulation for species with annual ovarian cycles are mostly <30 mm (Walker 2007). Species with MOD ~40 mm have biennial or triennial cycles, e.g. *Galeorhinus galeus* and *Squalus megalops* (Walker 2005; Braccini *et al*. 2006).

The number of pre-ovulatory oocytes is consistent with the number of eggs laid per season reported previously: 10–16 eggs (McLaughlin and O'Gower 1971), 16 eggs (Last and Stevens 1994), and 18 eggs (Gomon *et al*. 1994). These are also consistent with the number of eggs laid during a reproductive season (18) by a *H. portusjacksoni* (920 mm TL) caught in far-eastern Victoria and kept captive (Tovar-Ávila 2006). Fecundity of the species is low compared with other oviparous chondrichthyan species (e.g. *Raja brachyura*, *R. clavata*, *R. montagu*, *Scyliorhinus canicula and S. retifer*) with up to 40–150 eggs laid per year (Dodd 1983; Castro *et al*. 1988).

Difficulties in determining the seasonality of OSI and HSI in mature females could be related to the protracted ovulation and egg-laying periods, and the concurrent development of several cohorts of oocytes in the ovary. In contrast, female and male GSI and seminal vesicle fullness appear to be good indicators of the reproductive seasonality of the species in southern Australia, as found for *Mustelus antarcticus* (Walker 2007). The decrease in female GSI during spring could be related to the continuous ovulation process. The decrease in male GSI during winter–spring and the presence of semen in the seminal vesicles during winter suggest mating occurs before or during the first months of ovulation (late winter–early spring). It also suggests a need for sperm storage in the oviducal glands to ensure egg fertilisation during the long egg-laying period.

The novel method used in the present study to estimate the ovarian cycle could be useful for other species possessing seasonal reproductive cycles with oocytes at different vitellogenic stages simultaneously. The ovulation period and the egg-laying season appear to occur concurrently within individuals and with a degree of synchrony between individuals in the population. Oocytes that have reached the pre-ovulatory size before the egg-laying period are retained in the ovary until conditions are favourable for being ovulated at the end of winter–early spring. The number of pre-ovulatory oocytes then decreases during the spring and summer months because of the continuous ovulation process. Pre-ovulatory oocytes that have not reached the ovulation size by the end of winter continue growing during spring–summer, and may be ovulated if they reach ovulation size before the egg-laying period ends. Egg-laying may continue as long as water temperatures are favourable for the development of the eggs. It was observed that changes in water temperature altered the ovulation process (indicated by egg laying) in captive sharks (Tovar-Ávila 2006).

It has been stated that in oviparous species the period between first maturity (the onset of vitellogenesis) and first reproduction (the time when the animal first lays eggs) is short (Conrath 2004). However, the oocytes of *H. portusjacksoni* from far-eastern Victoria take up to 1.5 years to reach ovulation size. Furthermore, the interval between the onset of vitellogenesis and the time when free swimming animals are produced (eggs hatched) is longer. Embryonic development takes 9–12 months in this species, depending on water temperature (McLaughlin and O'Gower 1971; Rodda 2000).

Despite having a long oocyte development period, the presence of several oocyte cohorts in the ovary suggests that *H. portusjacksoni* has an annual reproductive cycle. However, the presence of some mature females lacking pre-ovulatory oocytes during the months previous to the reproductive season suggests that there may be a seasonal resting period for a proportion of the female population, as reported for other shark species, such as *Carcharhinus acronotus* (Hazim *et al*. 2002).

The presence of *corpora lutea* and *corpora atretica* may be good indicators of completion of ovulation (Bragdon, in Hisaw and Hisaw 1959). Although oocyte atresia can occur at any stage of oocyte growth (Hisaw and Hisaw 1959), the increase in the number of *corpora atretica* following the egg-laying period in *H. portusjacksoni* appears to be related to the end of ovulation and the absorption of large non-ovulated oocytes.

We have presented a novel approach to determine the ovarian cycle of an oviparous chondrichthyan species, which may be useful for other such species. The regional differences found in some reproductive and morphometric parameters of

H. portusjacksoni provide evidence of two populations off Victoria, but other methods, such as genetic techniques and tagging studies in Victorian waters, are needed to confirm this. If the populations are distinct, the reproductive parameters need to be determined separately for western Victoria for population modelling purposes. This paper provides those parameters for the population in far-eastern Victoria.

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