Reproductive biology of the blue shark (*Prionace glauca*) in the western North Pacific Ocean

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Abstract. The reproductive biology of the blue shark (*Prionace glauca*) in the western North Pacific Ocean was investigated to contribute to future stock assessments because of limitations of recent studies and the lack of information about the reproductive cycle. Reproductive data were obtained from 490 males (precaudal length (PCL), 33.4–252.0 cm) and 432 females (PCL, 33.4–243.3 cm). Size at 50% maturity was estimated to be 160.9 cm for males and 156.6 cm PCL for females. Litter size varied from 15 to 112 (mean 35.5) and was positively correlated with maternal PCL. Parturition, ovulation and mating occurred sequentially from spring to summer. The gestation period was estimated to be 11 months. The ovarian follicles of pregnant females developed synchronously throughout the gestation period along with embryonic growth, indicating that females reproduce annually. Our results showed that the productivity of North Pacific blue sharks is higher than previously thought, based on larger fecundity and a shorter reproductive cycle. These new findings will improve future stock assessments and provide management advice.

Additional keywords: fecundity, gestation, maternity, maturity, reproductive cycle.

Introduction

The blue shark (*Prionace glauca*) is the most abundant pelagic carcharhinid shark and has a circum-global distribution in tropical and temperate oceans, ranging from ∼60°N to 50°S latitude (Nakano and Stevens 2008). This species is capable of large-scale migrations (e.g. Stevens *et al.* 2010; Campana *et al.* 2011; Block *et al.* 2011) involving complex movement patterns (Mucientes *et al.* 2009; Vandeperre *et al.* 2014; Queiroz *et al.* 2016). Blue sharks are mainly captured by tuna longline and drift-net fisheries as target or by-catch species (Nakano and Stevens 2008). Their fresh meat, liver-oil, cartilage, skin and fins are used in many countries (Nakano and Seki 2003; Camhi *et al.* 2008); thus, they are considered an important fishery resource. Therefore, stock assessments of this species have been conducted by several regional fishery management organisations for sustainable exploitation of this species in each region.

Two stocks of blue shark are thought to exist in the Pacific Ocean; one stock is in the North Pacific and the other is in the South Pacific, divided by the Equator (ISC 2012). Although there has been a lack of support by genetic studies (Taguchi *et al.* 2015), several tagging studies have shown that blue sharks tagged in the North and South Pacific move widely, but never cross the Equator (e.g. Weng *et al.* 2005; Stevens *et al.* 2010; Block *et al.* 2011). In addition, the mating grounds of the North

Pacific blue shark are limited to subtropical areas (Nakano 1994), suggesting that the North Pacific and South Pacific populations do not associate with each other.

According to the latest blue-shark stock-assessment results in the North Pacific, stock abundance of this species has changed since the 1980s. Stock biomass was high in the 1970s, but decreased to its lowest level between the 1980s and early 1990s, and then increased thereafter (ISC 2014; Hiraoka *et al.* 2016; Ohshimo *et al.* 2016). An assessment report recommended improved monitoring of the blue shark fishery and researching their biology, owing to uncertainty of the data and biological parameters (ISC 2014).

Knowledge of the current reproductive aspects of exploited species is essential for sustainable fisheries and conservation management. Reproductive parameters, such as size and age at maturity, fecundity, and the length of the reproductive cycle, are used in assessment models to estimate productivity and the rebound potential of a fish stock (Baremore and Passerotti 2013). Data and samples that include the entire stock should be analysed to estimate these parameters; however, few biological studies have included all blue shark habitats in the North Pacific because of their wide distribution. Nakano (1994) reported a representative biological study on North Pacific blue shark, which has been used as the basis for stock assessment and

modelling in the North Pacific, because the samples were collected from a broad area and during all seasons from 1978 to 1987; however, the length of the reproductive cycle was not estimated. Subsequently, Joung *et al.* (2011) reported the reproductive biology of this species in northern and south-eastern Taiwan. However, their samples were collected in a limited area. Thereafter, most of the biological parameters estimated by Nakano (1994) were used for a blue shark stock assessment in the North Pacific (ISC 2014). However, owing to a shift in stock abundance, the reproductive parameters of blue shark may have changed from those reported by Nakano (1994) and they should be re-estimated using more recent samples. Moreover, it is necessary to estimate the reproductive periodicity of the blue shark for future assessments, because it is an important parameter to evaluate productivity. In the present study, we updated the reproductive parameters and elucidated the reproductive cycle of blue shark collected from a wide area in the western North Pacific Ocean.

Materials and methods

Sampling and data collection

Blue shark samples were collected between 2010 and 2016 by Japanese research (long-line, driftnet, and trawl) and commercial vessels (long-line and set net) operated in the western North Pacific Ocean (Fig. 1). Long-line fisheries caught nearly all of the sharks (95%). The sharks were sexed and several length (precaudal length, PCL; total length, TL; fork length, FL; dorsal length, DL; length from the origin of the first dorsal fin to that of the second dorsal fin) and bodyweight (BW) measurements were recorded. The head and viscera had been removed from fish caught by commercial vessels; therefore, only DL was measured to the nearest centimetre. DL was converted to PCL with a conversion formula estimated using a linear regression model.

To determine the difference in size frequency of sharks by the sampling area, the sampling area was divided into four sites. The boundary at 30°N was determined to be the subtropical front boundary for the Kuroshio–Oyashio transition zone at ~32°N. The boundary for the east–west direction (155°E) was determined arbitrarily by the longitude of the mid-point in the survey area.

Male size at maturity

Left inner clasper length (from the tip of the clasper to the anterior margin of the cloaca) was measured and the degree of calcification of the clasper was recorded (uncalcified, partly calcified or fully calcified). The weight of both testes combined was measured, and the presence of semen was investigated by applying pressure to the seminal vesicle. Because calcification of the claspers in male blue sharks is a more gradual process than it is in other carcharhinids (Pratt 1979), the present study classified male maturation into the following three stages: (1) immature–juvenile, (2) immature–adolescent and (3) mature–adult, and calcification of the claspers was prioritised (see Table S1, available as Supplementary material to this paper). The
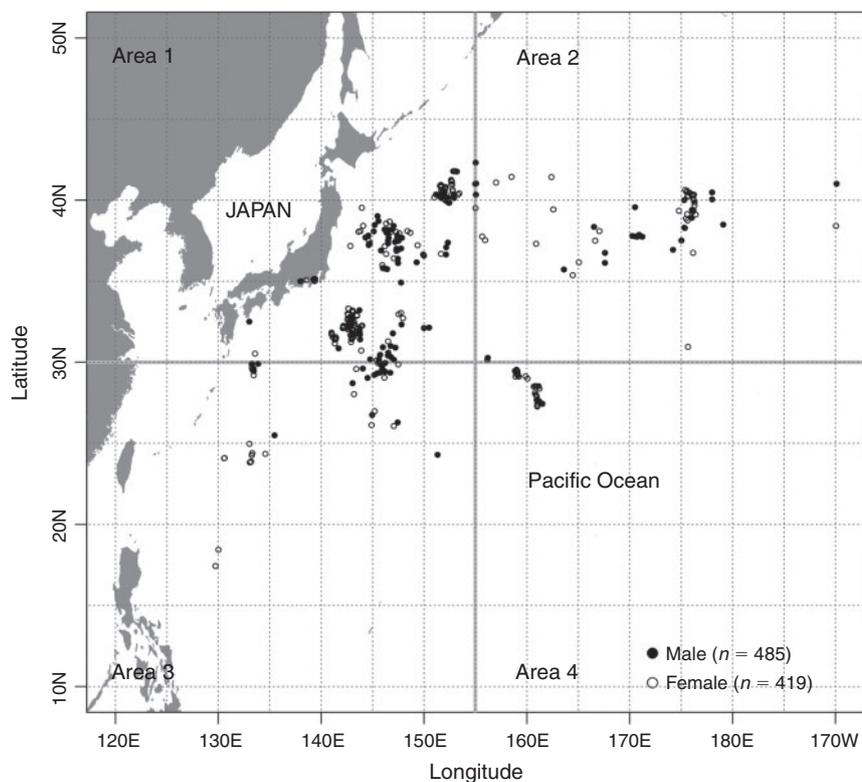

Fig. 1.   Blue shark (*Prionace glauca*) sampling locations in the western North Pacific. Black and white circles indicate males and females respectively.

maturation stage of each individual was converted into binary data (immature = 0, mature = 1) at 5-cm intervals for the statistical analysis. A logistic regression model was fit to the binomial maturity data, to determine male sizes at 50 and 95% maturity. This model is described as follows:

$$Y = 1/[1 + \exp\{-(\alpha + \beta X)\}]$$

where Y is the proportion of mature individuals in each interval, X is PCL, and α and β are coefficients. A generalised linear model with a binomial error structure and logit-link function was used to estimate the α- and β-coefficients using R statistical software, ver. 3.3.0 (R Foundation for Statistical Computing, Vienna, Austria, see https://www.R-project.org/).

Female size at maturity and maternity

The left and right uterine widths at the widest point and the largest diameter follicle, which was determined as the largest of five large oocytes chosen randomly, were measured to the nearest millimetre, and ovarian weight was measured to the nearest 0.1 g. The presence or absence of embryos, fertilised eggs, placenta and an umbilical cord in the uterus were recorded.

Sexual maturation in females was classified into the following five stages: (1) immature–juvenile; (2) immature–adolescent; (3) mature–adult; (4) mature–pregnant; and (5) mature–post partum, from the uterine and ovarian development observations (see Table S1). The maturation stage of each female was converted to binary data (immature = 0, mature = 1) for the statistical analysis. Female sizes at 50 and 95% maturity were estimated using the same equation as that used for males. Size at 50% maturity was estimated on the basis of Montealegre-Quijano *et al.* (2014). Data of pregnancy (mature–pregnant or mature–post partum) or non-pregnancy (immature or mature–adult) were converted to binary data (pregnancy = 1, non-pregnancy = 0). The logistic function was fit to these data in the same way as for estimating size at maturity.

Fecundity

Litter size was estimated by counting the number of embryos. We omitted females that delivered pre-term from the analysis. The criteria used to judge pre-term delivery were (1) embryos only on one side of the uterus and (2) <10 embryos present, and the placenta was not retained in the uterus. To prevent underestimating litter size owing to early delivery or abortion, the number of placentas without embryos was counted. When the embryos without placentas and only placentas were found, the litter size was adjusted by adding the larger number of those to the number of embryo with placenta. The relationship between litter size and PCL of pregnant females was estimated using a linear regression model.

Reproductive cycle

Sex and PCL (to the nearest 0.1 cm) were recorded for all embryos. Parturition period was estimated from the mean size of near-term embryos per litter and the size of neonates. A near-term embryo was defined as one with developed teeth and external proportions similar to the adult and a girth that equals or exceeds head circumference (Pratt 1979). Free-swimming neonates were identified by an open, fresh umbilical scar.

The mating period was judged from the monthly trend in gonadosomatic index (GSI) of mature males and females. The GSI was calculated as:

$$\text{GSI} = (\text{gonad weight} \div \text{BW}) \times 10^2$$

where gonad weight (g) is testis weight for males and ovarian weight for females, and BW is bodyweight (g). Mean GSI was calculated for all months. The ovulation period was estimated from the monthly changes in follicle diameter in mature females. The values reported by Nakano (1994) and Joung *et al.* (2011) were used to fill missing months for these analyses. The gestation period was estimated as the approximate length in months between the mean ovulation date and the mean parturition date.

The reproductive cycle of females comprised the following three phases: (1) vitellogenesis, (2) gestation and (3) resting (Castro 2009). We verified whether development of the ovarian follicle and embryonic growth occurred synchronously or asynchronously and estimated the duration of the reproductive cycle on the basis of the gestation period and resting phase. The occurrence of a resting phase was evaluated on the basis of temporal changes in the largest-follicle diameter in pregnant females and the mean embryo size per litter. In addition, the relationships between uterine width and the largest-follicle diameter according to reproductive condition (non-pregnant, pregnant and post partum) were analysed to understand the morphological changes related to reproduction throughout life and to estimate the proportion of individuals in the resting phase.

Results

Sample collection and conversion factors

In total, 1408 individuals were collected. Samples for reproductive parameters were obtained from 922 individuals (490 males and 432 females) between 2011 and 2016 (see Tables S2, S3, available as Supplementary material to this paper). Most of the samples were collected in Area 1 (72%) as the main fishing grounds for commercial vessels (≥30°N and <155°E) during spring (April–June) and autumn (October–December). Immature males and females were collected during the same times. Mature males were collected year-round. Most of the post-partum females were captured in April and June. Males and females ranged from 33.4 to 252.0 cm PCL, and from 33.4 to 243.3 cm PCL respectively (Fig. 2). The sizes (62.2–252.0 cm PCL, mean 143.0) of the male and female blue sharks caught in Area 1 varied widely. Sharks caught in Areas 3 (128.7–214.0 cm PCL, mean 166.4) and 4 (143.7–219.7 cm PCL, mean 175.4) were relatively larger than those captured in Area 2 (33.4–224.0 cm PCL, mean 119.4).

The relationships among the four lengths (PCL, TL, FL and DL) and between PCL and BW are presented in Table 1. No differences in PCL–TL or PCL–FL were observed between the sexes (ANCOVA, PCL–TL, $P = 0.10$; PCL–FL, $P = 0.41$); however, significant differences were observed in the PCL–DL and PCL–BW between males and females (ANCOVA, PCL–DL, $P = 0.002$; PCL–BW, $P = 0.022$).
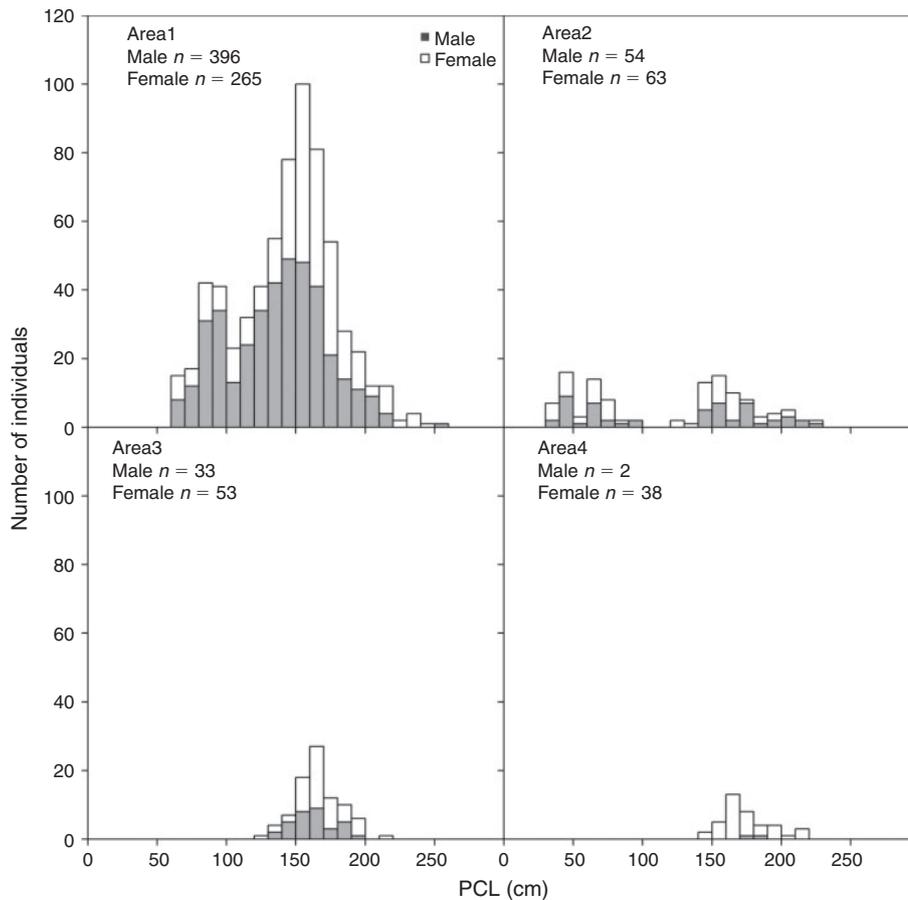
Fig. 2. Length–frequency distribution of male and female blue sharks caught in the western North Pacific.

Table 1. Length–length and length–weight relationships for blue sharks collected in the western North Pacific Ocean

PCL, TL, FL, BW denote precaudal length, total length, fork length and bodyweight respectively

Conversion	Equation	Sex	n	r^2	P
TL to PCL	$PCL = 0.78\,TL - 3.75$	Combined	396	0.994	0.10
FL to PCL	$PCL = 0.92\,FL - 0.22$	Combined	338	0.998	0.41
DL to PCL	$PCL = 2.51\,DL + 12.33$	Male	587	0.961	0.02
DL to PCL	$PCL = 2.62\,DL + 7.48$	Female	275	0.983	
PCL to BW	$BW = 1.21 \times 10^{-5} \times PCL^{2.94}$	Male	756	0.954	0.02
PCL to BW	$BW = 5.86 \times 10^{-6} \times PCL^{3.09}$	Female	283	0.985	

Male size at maturity

Clasper-length and clasper-condition data were obtained from 490 individuals (PCL, 33.4–252.0 cm). Clasper length increased gradually with PCL (see Fig. S1*a*, available as Supplementary material to this paper). All individuals >181.0 cm PCL had fully calcified claspers. The presence or absence of semen in the seminal vesicle was assessed in 288 individuals (PCL, 33.4–252.0 cm). Approximately half of the 150.0-cm-PCL individuals had semen (Fig. S1*a*), indicating that males can produce semen before their claspers are fully calcified. Considerable variation in testis weight was found in individuals >150.0 cm

PCL (Fig. S1*b*). Male sizes at 50 and 95% maturity were 160.9 cm PCL (CI: 158.8–163.3 cm) and 179.4 cm PCL (CI: 175.3–185.5 cm) respectively (Fig. 3*a*).

Female sizes at maturity and maternity

All reproductive organs (uterus, ovary and ovarian follicles) tended to increase with PCL (see Fig. S2*a*–*c*, available as Supplementary material to this paper). No differences in the width of the right and left uteri were detected (Wilcoxon rank-sum test, $P = 0.39$). Uterine width for individuals <145.0 cm PCL increased gradually (0.8–23.8 mm), whereas a rapid increase in uterine width was observed from 8.2 to 180.0 mm in individuals sized 140.0–160.0 cm PCL (Fig. S2*a*). The uteri of pregnant females ranged from 62.4 to 261.5 mm. Post-partum females also possessed thickened uteri of 78.1–126.9 mm. Ovarian weight of individuals <150.0 cm PCL was 0.1–58.9 g, whereas that for individuals >150.0 cm PCL varied from 5.5 to 236.5 g (Fig. S2*b*). Follicle diameter also showed an increasing trend with PCL, similar to uterine width and ovarian weight (Fig. S2*c*).

In total, 139 pregnant females were caught, with a size range of 143.7–243.3 cm PCL. Post-partum females were 159.5–219.7 cm PCL ($n = 22$). The estimated sizes at 50 and 95% maturity in females were 156.6 cm PCL (CI: 154.4–158.6 cm) and 175.4 cm PCL (CI: 171.8–180.7 cm) respectively (Fig. 3*b*). Sizes at 50 and
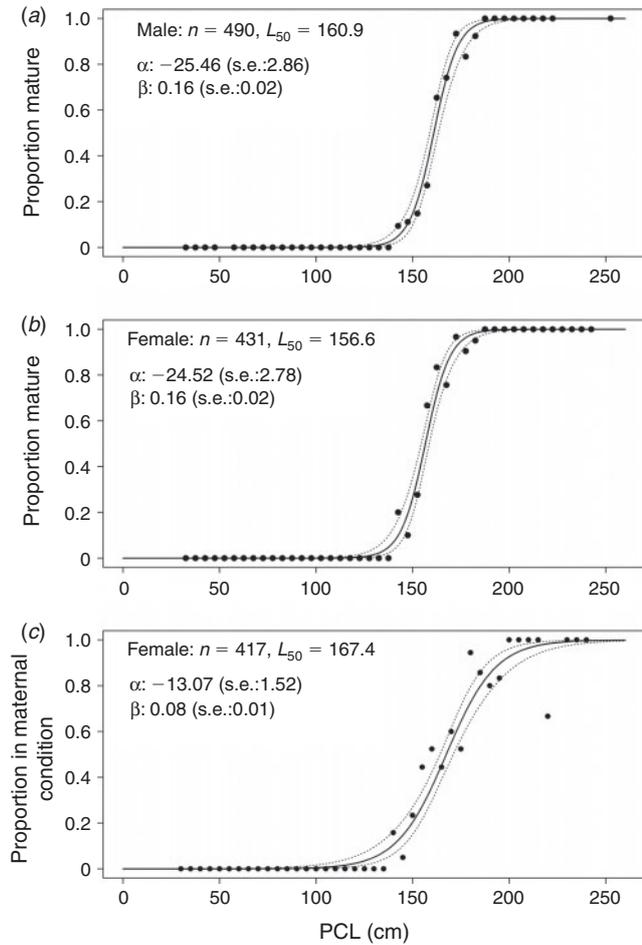
Fig. 3. Maturity and maternity ogives for blue shark. (*a*) Maturity ogives for males and (*b*) females, and (*c*) maternity ogive for females. Size-class interval is 5 cm for precaudal length. L_{50} indicates size at 50% maturity or maternity. Dotted lines indicate 95% confidence intervals. α and β are coefficients. s.e. denotes standard error.

95% maternity were 167.4 cm PCL (CI: 164.1–171.0 cm) and 205.1 cm PCL (CI: 196.9–217.8 cm) respectively (Fig. 3*c*). Size at 50% maternity was 10.8 cm larger than the estimated size at 50% maturity.

Fecundity

Litter size (127 pregnant females) estimated only on the basis of the number of embryos ranged from 1 to 112 (mean ± standard deviation (s.d.), 33.1 ± 15.9), whereas litter size based on the sum number of embryos and placentas (124 pregnant females, three females removed for preterm delivery) ranged from 15 to 112 (mean ± s.d., 35.5 ± 14.8). The latter litter size was positively correlated with maternal PCL (Fig. 4) and the relationship between litter size and maternal PCL was statistically significant ($P < 0.01$). The linear regression was as follows:

$$\text{litter size} = 0.46\,\text{PCL} - 45.54$$

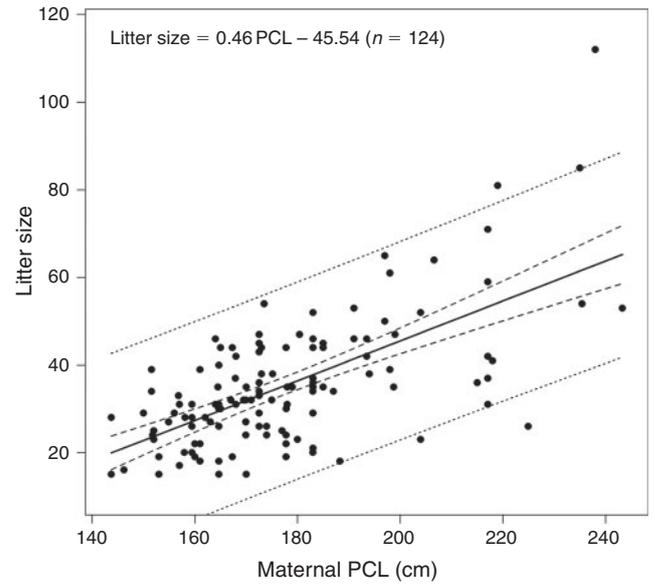where $n = 124$ and $r^2 = 0.412$.

Fig. 4. Relationships between precaudal length (PCL, cm) of 124 pregnant females and litter size on the basis of the number of embryos and placenta in uteri. Solid line indicates the fitted linear regression, broken lines indicate 95% confidence intervals, and dotted lines indicate 95% prediction intervals respectively.
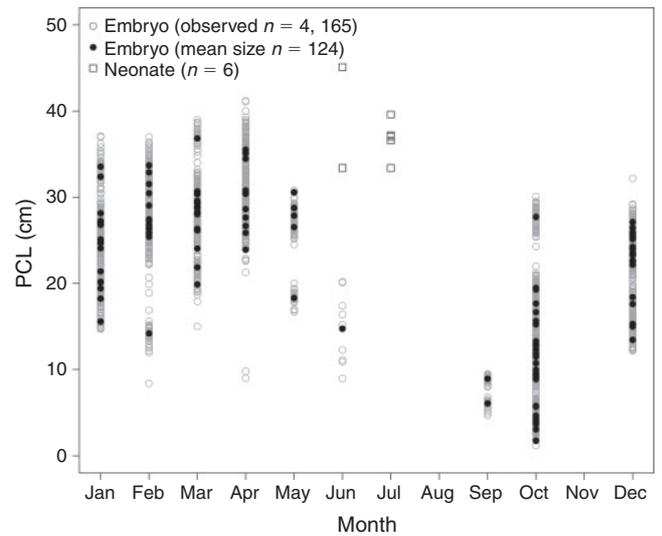

Fig. 5. Monthly changes in precaudal length (PCL, cm) of embryos (circles) and neonates (squares). Gray circles indicate the size of each embryo. Black circles indicate mean PCL of embryos per litter.

Reproductive cycle

Parturition period and size at birth

In total, 4165 embryos were observed (1908 males and 1967 females). The sex of 290 individuals was unknown. The ratio of male to female embryos was not different from 1 : 1 (Chi-square test, $P = 0.34$). The embryos ranged in size from 1.2 to 41.2 cm PCL, and monthly embryo size varied widely. The largest embryos were observed from females caught in April, whereas the smallest were observed in October (Fig. 5). In total,

559 near-term embryos (16 litters) were observed from January to April, and their size range was 30.2–41.2 cm PCL (mean ± s.d., 34.3 ± 2.11 cm). Six free-swimming neonates (PCL, 33.4–39.6; mean ± s.d., 36.2 ± 2.41 cm) were observed in late June and July. Therefore, size at birth was estimated to be 34.0–36.0 cm PCL, and parturition was estimated to occur between April and July.

Gestation period

Monthly changes in GSI showed an opposite trend between the sexes (Fig. 6). Mean GSI of mature males tended to increase from summer to winter and then decrease in spring. GSI was lowest in July and highest in December (Fig. 6a). Although mature female data were lacking for July, August and November, mean GSI was highest in June and lowest in January and showed an increasing trend from winter to summer (Fig. 6b).
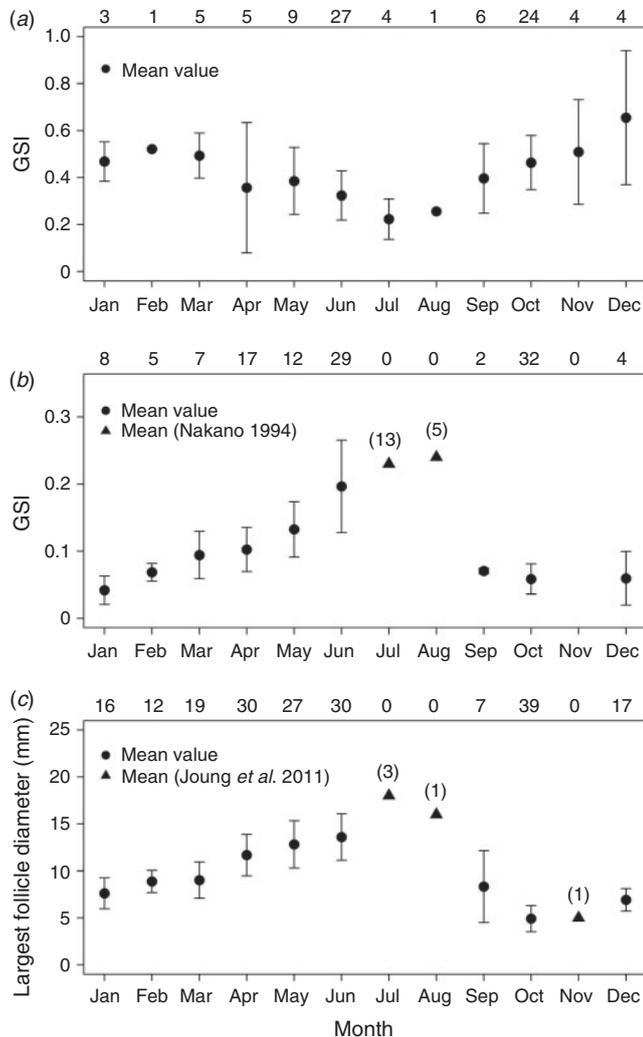

Fig. 6. Monthly change in gonadosomatic index (GSI) for mature (*a*) males and (*b*) females, and (*c*) the largest-follicle diameter (mm) in mature females. Values from Nakano (1994) and Joung *et al.* (2011) are used for months without data. Error bars are standard deviations. Numbers in the margin represent monthly sample sizes and those in parentheses represent sample sizes of previous studies.

A similar trend was observed for the mean largest-follicle diameter (Fig. 6c). The ovarian follicles began to increase in size in March. Females showed a high GSI and a large mean follicle diameter in the boreal summer (June–August); hence, ovulation was presumed to occur in summer. On the basis of these results, mean parturition occurred in May and the mean ovulation period was July, indicating an 11-month gestation period.

Reproductive periodicity

The ovarian follicles of pregnant females developed synchronously with embryonic growth throughout the gestation period, and pregnant females carried developing follicles and embryos at the same time (Fig. 7). As a result, the development of ovarian follicles and embryonic growth occurred synchronously. The relationship between uterine width and largest-follicle diameter during different reproductive stages is shown in Fig. 8. For the first time, ovarian follicles matured along with uterine width, and developed follicles (12.0–17.0 mm) are ovulated. The follicles developed throughout gestation synchronously with embryonic growth, and these females had enlarged uteri (73.8–271.0 mm) with variably sized follicles (2.6–15.0 mm). Following parturition, the uterus contracted to ~80.0 mm, but the follicles enlarged. Non-pregnant females with a thickened uterus (uterine width: ≥40.0 mm) and mature follicles (≥12.0 mm) were identified as ovulating (PCL 156.0–196.1 cm). Five females (PCL, 164.7–222.3 cm), which possessed thickened uteri (56.8–78.7 mm) but had only small developing follicles (7.4–9.0 mm), were identified as resting phase shark. These females appeared during March, April, June and September.

Discussion

Size at maturity and maternity

Male size at 50% maturity estimated in the present study (PCL, 160.9 cm) was slightly greater than that reported in other studies
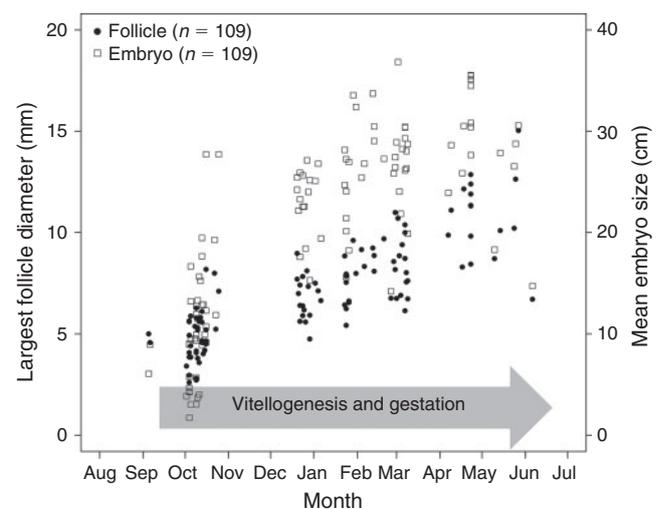

Fig. 7. Temporal changes in the largest-follicle diameter (mm) and mean embryo size (cm) in pregnant female blue sharks (*n* = 109). Circles represent follicles, and squares represent embryos.

for blue sharks in the North Pacific (e.g. Carrera-Fernández *et al.* 2010; Joung *et al.* 2011; Table 2). The most important factor associated with this difference was the use of different maturation criteria. Our results showed that claspers and testis developed similarly, but males stored semen in the seminal vesicles before clasper calcification. Carrera-Fernández *et al.* (2010) and Joung *et al.* (2011) used the presence of semen or spermatozeugmata as an indicator of maturation; thus, their

Fig. 8. Relationship between the largest-follicle diameter (mm) and uterus width (mm) of female blue sharks in different reproductive conditions (non-pregnant, pregnant or post partum).

estimates were smaller than our estimates. Natanson and Gervelis (2013) suggested that clasper calcification is the most accurate way to determine male maturation. Sizes at 50% maturity reported in the south-western Atlantic (Jolly *et al.* 2013; Montealegre-Quijano *et al.* 2014) and Mediterranean (Megalofonou *et al.* 2009) are similar to our results (Table 2), which were mainly determined using clasper calcification as a maturation indicator. Therefore, the size estimate of mature males in our study could be the most reasonable value for blue sharks in the North Pacific Ocean.

Estimated sizes at 50% maturity and maternity for females were 156.6 and 167.4 cm PCL respectively. These values are similar to those reported by other studies in the northern and southern hemispheres (Table 2), which may be due to the similar criteria used for female maturity. The present study suggested little regional difference in size at maturity or maternity in female blue sharks. In addition, the size of these fish has not changed in the North Pacific since the 1980s because our results are similar to those reported by Nakano (1994).

Using length at maturity estimated in the present study, ages at 50% maturity estimated by the growth equation reported by Nakano (1994) were 5.5 years for males and 6.3 years for females, and age at 50% maternity was estimated to be 7.2 years. These values are not remarkably different from those reported by Nakano (1994; 4.0–5.0 years for males and 5.0–6.0 years for females). Although male and female blue sharks mature at similar sizes as reported by other studies (Pratt 1979; Nakano 1994), males reach maturity slightly earlier than do females according to the growth model of Nakano (1994). Furthermore, females became pregnant ~1 year after reaching maturity. This time gap was reported by Pratt (1979) and suggests that a maternity ogive might be more appropriate than a maturity ogive for quantifying productivity of this species in stock assessments.
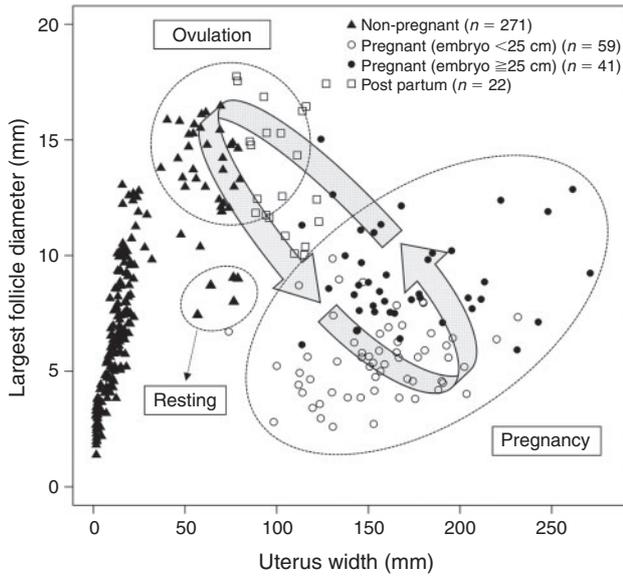
Table 2.   Size at sexual maturity, maternity and birth, and litter size of blue sharks from previous studies

Original total length (TL) and fork length (FL) data were converted to precaudal length (PCL) using the estimated conversion factors, as follows: $(PCL = 0.78 \times TL - 3.75, PCL = 0.92 \times FL - 0.22)$

Region	Size at maturity (cm)		Size at maternity (cm)	Size at birth (cm)	Litter size (mean value)	Reference
	Male	Female				
North Pacific			150.0	30.0–35.0	(30.0)	Suda (1953)
North Pacific	130.0–160.0	140.0–160.0		30.0–35.0	1–62 (27.6)	Nakano (1994)
North-eastern Pacific	139.8[A]	149.1[A]			7–64	Carrera-Fernández *et al.* (2010)
North-western Pacific	140.2[A]	147.1[A]		27.5	2–52 (25.2)	Joung *et al.* (2011)
North-western Pacific	160.9[A]	156.6[A]	167.4[A]	34.0–36.0	15–112 (35.5)	The present study
Central Pacific			158.4–188.9	22.7–33.6	4–38	Strasburg (1958)
South-western Pacific	174.6–179.2	156.2–174.6	152.5–231.6			Francis and Duffy (2005)
South-eastern Pacific			154.4–226.1		13–68 (35)	Zhu *et al.* (2011)
North Atlantic			162.4–186.5		28–54 (41)	Bigelow and Schroeder (1948)
North-western Atlantic	168.1	170.0		23.5–30.5		Pratt (1979)
South-western Atlantic	153.3[A]	147.8[A]				Jolly *et al.* (2013)
South-western Atlantic	165.6[A]	157.3[A]	178.2[A]		9–74 (33.5)	Montealegre-Quijano *et al.* (2014)
South-eastern Atlantic			165.4		4–75 (37)	Castro and Mejuto (1995)
Indian Ocean		>136.7		27.5–35.3	10–135 (56)	Gubanov and Grigor'yev (1975)
Mediterranean	*154.5	163.7[A]				Megalofonou *et al.* (2009)

[A]Size at 50% maturity or maternity.

Fecundity

In the present study, the number of embryos ranged from 1 to 112 (mean ± s.d., 33.1 ± 15.9), which is wider than that reported by Nakano (1994; range, 1–62, mean ± s.d., 25.6 ± 8.9; Table 2). The difference in mean litter size was likely to be due to the larger females sampled in the present study than in that by Nakano (1994) because litter size increases with maternal size. In addition, we considered litter size to be the total number of embryos with a placenta and placentas without embryos in uteri to prevent underestimating litter size. Adjusted litter size ranged from 15 to 112, with a mean of 35.5 per litter. Minimum litter size of blue sharks using this method was relatively higher than that reported previously (Table 2). Some authors have reported that females in the terminal phase of pregnancy abort when brought on-board (e.g. Strasburg 1958; Nakano 1994; Carrera-Fernández *et al.* 2010, Montealegre-Quijano *et al.* 2014), suggesting that recorded litter size might be less than the actual litter size. Analysing the number of placenta remaining in the uterus or excluding sharks with near-term embryos may be necessary to reliably estimate fecundity. This is the first study showing a reliable blue shark litter size adjusted by the number of placentas.

Reproductive cycle

Our estimated birth size was 34.0–36.0 cm PCL and did not remarkably differ from that in previous studies (e.g. Suda 1953; Pratt 1979; Nakano 1994; Table 2). The parturition period in the present study was estimated to be April–July. This result is similar to Pratt (1979; April–July) and Nakano (1994; peak in May–June) but different from that reported by Suda (1953; December–April). Samples of Suda (1953) were lacking for May, June, September and October; in addition, the number of embryos was only 100 individuals (4.165 individuals in the present study). In our study, embryo size varied widely within months, and most near-term embryos occurred in spring, whereas some were already present in January. As previously suggested (Suda 1953; Nakano and Seki 2003), blue sharks seem to have a broad mating and fertilisation period, which caused wide variations in embryonic development and prolonged the parturition period. However, our observations indicated that peak parturition occurred during spring and summer because most of the near-term embryos were observed at this time, and neonates with fresh umbilical scars appeared in early summer. Because of the large sample size and the wide coverage of sampling season and area, the estimated parturition period in the present study would represent the characteristics of this population.

Monthly changes in male GSI tended to be higher in winter and lower in summer. In general, male sharks possess small testis during the mating season (Teshima 1981) because spermatozoa are transported to the seminal vesicles and accumulate until the mating season. Monthly changes in female GSI showed a trend opposite to that in males, with high values from spring to summer and low values from autumn to winter. On the basis of previous reports (e.g. Stevens 1974; Pratt 1979; Nakano 1994) and our results, the mating season for blue sharks in the northern hemisphere is summer.

Monthly changes in the largest-follicle diameter were similar to those of GSI for females, and follicles were larger during spring and summer. Large yolky follicles indicated forthcoming ovulation; thus, ovulation occurred during summer. The ovulatory period has been reported to be summer (Nakano 1994) or July and August (Joung *et al.* 2011; Fig. 6c) in the North Pacific Ocean. Therefore, blue sharks ovulate and mate during the same season in the North Pacific. Although the fertilisation period could not be estimated precisely in the present study because of a lack of females with fertilised eggs, other studies have reported that those females are caught during June and July (Suda 1953) and from May to August (Joung *et al.* 2011) in the North Pacific. On the basis of these findings, parturition, mating, ovulation and fertilisation by blue sharks in the North Pacific occur sequentially from late spring to summer. In addition, on the basis of mean ovulation date and mean parturition date, the gestation period was estimated to be 11 months, which is similar to that reported previously (9–12 months) on the basis of monthly changes in embryo size (e.g. Suda 1953; Pratt 1979; Nakano 1994; Carrera-Fernández *et al.* 2010).

Our results suggested that female blue sharks have an annual reproductive cycle based on synchronous ovarian follicle development and embryonic growth, and an 11-month gestation period with no resting phase. Synchronous development indicates that the ovarian follicles are ready to be ovulated during late gestation and that ovulation and pregnancy occur after parturition. Several authors have reported that female blue sharks store semen in their oviducal glands (Pratt 1979; Joung *et al.* 2011), enabling females to breed consecutively. Some mature females (2.8%) possessed undeveloped small follicles, although uterine width in these females was similar to that of ovulating females, indicating that these females were unable to ovulate and were not ready for pregnancy after parturition. Thus, they may have undergone a resting phase, but their body size and timing of occurrence did not differ from those of other postpartum females. The reason why these females underwent a resting period is unknown, so further study is necessary. We conclude that the majority of female blue sharks reproduce annually but that a small portion of mature females may rest after parturition.

Although the blue shark is a member of the family Carcharhinidae, but not that of the genus *Carcharhinus*, the majority of sharks in *Carcharhinus* typically have a biannual reproductive cycle (Castro 1996). Joung *et al.* (2011) reported that the reproductive cycle of female blue sharks in the north-western Pacific is biannual because not all pregnant females have large oocytes in their ovaries. However, the number and monthly sample size of embryos observed in their study were insufficient, and most embryos were at an early developmental stage. Therefore, these sampling biases may have affected their gestation and reproductive-cycle estimates. In general, an adequate number of samples at different embryonic stages (from early to near-term) is necessary to accurately estimate the reproductive cycle (Castro 2009). Our samples included early and near-term embryos; thus, it seems reasonable to conclude that the reproductive cycle of female blue shark in the western North Pacific is annual.

Conclusions

The reproductive parameters in the present study differed little from those reported by Nakano (1994), except fecundity, and the present study extended the reproductive-parameter estimates

reported by Nakano (1994). We estimated size at 50% maturity using the Ogive model and evaluated the reproductive cycle quantitatively. Our results indicated that productivity of the blue shark in the North Pacific Ocean is higher than was previously thought, because fecundity was higher and the reproductive cycle was shorter than those reported previously. In addition, blue sharks have much higher fecundity than do other pelagic requiem sharks such as oceanic whitetip shark (*Carcharhinus longimanus*) (Seki *et al.* 1998) and silky shark (*Carcharhinus falciformis*) (Galván-Tirado *et al.* 2015). Moreover, those shark species have a biannual reproductive cycle. These biological characteristics are one of the main reasons why blue sharks maintain a high abundance in pelagic waters. Knowledge of the reproductive parameters provided in the present study will play a big part in fishery management and conservation assessments of blue sharks in the North Pacific Ocean. In a future study, more female samples must be collected during summer. Additionally, a biochemical analysis on the temporal changes in steroid hormones (e.g. 17β-oestradiol and progesterone) would help verify our estimates of the reproductive cycle, particularly vitellogenesis. Furthermore, nutritional status of pregnant females should be investigated to elucidate the mechanism of the resting phase.

Acknowledgements

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