Reproductive biology of blue marlin (*Makaira nigricans*) in the western Pacific Ocean

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Abstract—The reproductive biology of blue marlin (*Makaira nigricans*) was assessed from 1001 fish (ranging from 121 to 275 cm in eye-to-fork length; EFL) caught by Taiwanese offshore longliners in the western Pacific Ocean from September 2000 to December 2001 and from 843 gonad samples from these fish, The overall sex ratio of the catch was approximately 1:1 during the sampling period, but blue marlin are sexually dimorphic; females are larger than males. Reproductive activity (assessed by histology), a gonadosomatic index, and the distribution of oocyte diameters, indicated that spawning occurred predominantly from May to September. The estimated sizes-at-maturity (EFL_{50}) were 179.76 ±1.01 cm (mean ±standard error) for females and 130 ±1 cm EFL for males. Blue marlin are multiple spawners and oocytes develop asynchronously. The proportion of mature females with ovaries containing postovulatory follicles (0.41) and hydrated oocytes (0.34) indicated that the blue marlin spawned once every 2–3 days on average. Batch fecundity (BF) for 26 females with the most advanced oocytes (≥1000 μm), but without postovulatory follicles, ranged from 2.11 to 13.50 million eggs (6.94 ±0.54 million eggs). The relationships between batch fecundity (BF, in millions of eggs) and EFL and round weight (RW, kg) were $BF = 3.29 \times 10^{-12}\ EFL^{5.31}$ (r^2=0.70) and $BF = 1.59 \times 10^{-3}\ RW^{1.73}$ (r^2=0.67), respectively. The parameters estimated in this study are key information for stock assessments of blue marlin in the western Pacific Ocean and will contribute to the conservation and sustainable yield of this species.

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Blue marlin (*Makaira nigricans*) are widely distributed throughout the tropical and subtropical waters of the Pacific and Indian oceans (Nakamura, 1985). In the Pacific, blue marlin are harvested mainly by longline fisheries targeting tunas. Genetic studies (Buonaccorsi et al., 1999) and fishery data (Kleiber et al., 2003) indicate that there is a single stock of blue marlin in the Pacific Ocean. Annual landings of blue marlin in the western and central Pacific over the past decade have been stable at about 13,000 metric tons. However, stock assessments of Pacific blue marlin are uncertain, with results ranging from the stock being close to fully exploited (Kleiber et al., 2003), overfished (Yuen and Miyake, 1980), or in a healthy state (Hinton, 2001).

Quantifying the reproductive potential of blue marlin is important for understanding the population dynamics of this species and for stock assessment purposes. For example, estimates of the size- and age-at-sexual-maturity are necessary inputs for age- and size-structured stock assessments models (Quinn and Deriso, 1999). Despite the size of the fisheries for the stock of blue marlin in the Pacific Ocean, there have been few published studies on the reproductive biology of this stock. In the eastern Pacific, Kume and Joseph (1969) estimated the size-at-maturity of blue marlin from data from the Japanese longline fishery, and Hopper (1990) described spawning activity around the Hawaiian Islands. In the western Pacific, Nakamura (1944) reported that blue marlin spawned off Taiwan. However, none of these studies provided detailed information on ovarian development, even though knowledge of gonad development in individual fish is needed to establish the spawning season, the size- and age-at-maturity, and the spawning pattern. The reproductive biology of blue marlin has been studied more extensively in the Atlantic Ocean than in the Pacific. For example, La Monte (1958) first described the gonad of blue marlin in the Atlantic Ocean, Erdman (1968) observed the reproductive cycle off Puerto Rico, Cyr (1987) defined the gonad development and spawning cycle in the northwestern Atlantic Ocean, and Arocha and Marcano (2008) estimated the size-at-maturity of this species in the western central Atlantic. The objectives of this study were to evaluate the reproductive biology of blue marlin in the western Pacific Ocean. We determine reproductive activity and describe ovarian development using histological techniques. Key parameters required for stock assessments including sex ratio, reproductive season, and size-at-maturity are also estimated.

Materials and methods

Collections from fish markets and measurements of fish

Samples from 1001 blue marlin were collected randomly from September 2000 to December 2001 at the Tungkang fish market in southwest Taiwan. All samples were caught by offshore longliners operating between 16–23°N latitude and 115–135°E longitude. Sex (determined from the macroscopic characteristics of the gonads, and from histological sections for small individuals), eye-to-fork length (EFL; the posterior margin of eye's bony orbit to the distal end of the central ray of the caudal fin, cm), and round weight (RW, kg) were recorded for each fish. A tissue sample was randomly collected from the anterior (females) and middle (males) of either the right or left lobe of the gonad and immediately fixed in 10% buffered formalin for later oocyte measurement and histological analysis. Three ovary pairs were collected in July 2004 to evaluate the synchronicity of egg development within, and between, ovary pairs. The left and right lobes of these ovaries were each divided into anterior, central, and posterior portions, and each portion further divided into outer, middle, and central layers. A total of 54 0.05-g subsamples (3 ovaries × 2 lobes × 3 portions × 3 layers) were collected randomly and the number of whole oocytes and the mean oocyte diameter (MOD) for each subsample was estimated. More specifically, the MOD was estimated as the average of the diameters of the most advanced group of oocytes calculated with the Image-Pro Plus software (Media Cybernetics, Silver Spring, MD), after calibration against an optical micrometer. However, histological sectioning deforms the oocyte from its sphere-like shape, and three types of measurements were therefore used to obtain reliable results (Arocha, 2002). Early developed oocytes were measured at the major axis crossing the nucleus; maturing oocytes were measured across the nucleus from well-formed spheres; and the diameters of fully mature oocytes were calculated from the circumference of the oocyte divided by π.

The condition factor, C, was determined for each fish by using the relationship

$$C = (100{,}000 \times RW) / EFL^b, \quad (1)$$

where b = the slope of the length-weight relationship (King, 1995).

The value for b was estimated by using linear regression after the data were log-transformed. The parameter b was not significantly different from 3 for females (t=0.53, df=172, P >0.05) and males (t=–0.99, df=210, P >0.05). The condition factor was related to the gonadosomatic index (GSI), which was determined as follows (Uosaki and Bayliff, 1999):

$$GSI = (GW / EFL^3) \times 10^4, \quad (2)$$

where GW = gonad weight (g).

Sex ratio in each month or size class was calculated as the ratio of the number of females to the total numbers of females and males. Chi-square tests were used to test for significant differences in sex ratio among months and sizes. Sex ratios were also regressed on length by using logistic regression (DeMartini et al., 2000).

Reproductive activity

Microscopic characteristics of histological sections and the most advanced group of oocytes were used to assign ovaries to stage (Hunter and Macewicz, 1985; West, 1990; Arocha, 2002; Arocha and Bárrios, 2009). For males, the classification of testicular development was based on the degree of spermatogenesis, the development of the vas deferens, and the composition of germ cells (Grier, 1981; Ratty et al., 1990; deSylva and Breder, 1997). Each 150-mm^3 preserved tissue sample was embedded in paraffin, sectioned at 7 μm, and stained with Mayer's haematoxylin and eosin. The dynamics of the ovarian maturation process were evaluated by examining the modes in size-frequency distributions of whole oocytes (after Hunter and Macewicz, 1985, Arocha, 2002; Arocha and Bárrios, 2009). The 95% confidence intervals for the proportion of oocytes of each diameter by ovarian maturity stage were obtained by a bootstrap procedure in which each pseudo data set was constructed by selecting whole oocytes at random and with replacement. Changes over time in the mean diameter of the most advanced group of oocytes, the GSI values (for individuals larger than ≥180 cm EFL to enhance temporal variation), and the composition of ovarian development stages were evaluated to determine the spawning season.

Size-at-maturity

The proportion of mature fish of all assessed fish classes, defined as a maturity ogive, was developed from samples caught during the spawning season (Murua et al., 2003). Females were defined as sexually mature if they had early yolked, advanced yolked, migratory nucleus, or hydrated oocytes (Hunter and Macewicz, 2003; Arocha and Bárrios, 2009), whereas males were defined to be sexually mature if they had secondary spermatocytes, spermatids, or spermatozoa. The presence of postovulatory follicles (POF) in ovaries and spermatozoa in the vas deferens were respectively taken as evidence of recent spawning of females and males. Atresia of yolked oocytes was also noted for mature but reproductively inactive females. The probability that the i^{th} fish was mature (P_i) was modeled with a logistic curve:

$$P_i = 1 / \left(1 + e^{-\ln(19)\left[(EFL_i - EFL_{50})/(EFL_{50} - EFL_{95})\right]}\right), \quad (3)$$

where EFL_i = the EFL of fish i; and
EFL_{50} and EFL_{95} = the EFLs at which 50% and 95% of the assemblage reached maturity.

EFL_{50} and EFL_{95} were estimated by maximizing a log-likelihood function, and by assuming a binomial error distribution with AD Model Builder (Fournier, 2000).

Batch fecundity

Annual fecundity was estimated from the number of oocytes released per spawning (batch fecundity), the percentage of females spawning per day (spawning fraction), and the duration of the spawning season (Hunter and Macewicz, 2003; Murua et al., 2003) because blue marlin spawn multiple times during the season and have indeterminant fecundity (see *Results* section). There are two methods for estimating the batch fecundity: 1) the hydrated oocyte method; and 2) the oocyte size-frequency method (see Hunter et al., 1985). The oocyte size-frequency method was employed because the sample size for the batch fecundity estimates from the hydrated oocyte method is insufficient for later analysis of the relationship between batch fecundity and EFL or RW (fewer samples have migratory nucleus and hydrated oocytes been observed). The most advanced yolked, migratory nucleus, and hydrated oocytes that were destined to be spawned were identified for individuals on the basis of size-frequency distributions of whole oocytes (larger than 1000 microns). Batch fecundity was then back-calculated gravimetrically by the product of gonad weight and oocyte density, where oocyte density was the mean number of oocytes per gram of three ovarian tissues with no early postovulatory follicles (Hunter et al., 1985). Relative batch fecundity was expressed as batch fecundity divided by the round weight of the fish.

Spawning frequency

The spawning frequency of blue marlin was estimated indirectly by the inverse of the spawning fraction because direct monitoring of the spawning frequency of pelagic fish during the spawning season is difficult. The spawning fraction was calculated as the proportion of fish that spawned each day during the spawning season. There are two approaches to determine this average (Hunter and Macewicz, 1985): 1) the postovulatory follicle method; and 2) the hydrated oocyte method. Indirect methods for estimating spawning frequency are based on four assumptions: 1) females are spawning asynchronously throughout the spawning season; 2) fishes do not immigrate to or emigrate from the spawning ground; 3) the POFs of blue marlin are histologically detectable for no more than 24 hours or all hydrated oocytes are spawned in less than 24 hours (as observed for yellowfin tuna; Schaefer, 1996); and 4) the POFs do not degenerate continuously if a fish is caught and put into the refrigerator immediately.

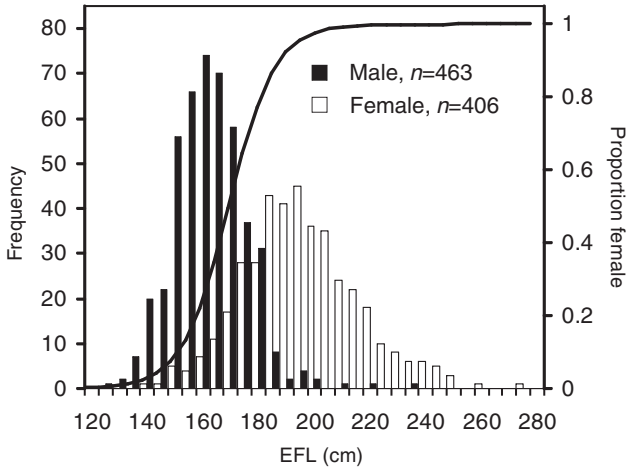

Figure 1

Length-frequency distribution of blue marlin (*Makaira nigricans*) sampled at the Tungkang fish market between September 2000 and December 2001. The solid line is the fit of a logistic curve to the proportion of females as a function of eye-to-fork length (EFL).

Results

Gonad samples, size distribution, and sex ratios

No significant differences in MOD were found between each lobe or layer within each individual blue marlin (split-plot ANOVA; F=0.41, df=1 and 52, P>0.05; F=1.30, df=2 and 51; P>0.05), although there were differences in MOD between the ovaries (F=49.97, df=2 and 52, P<0.05). Similar results were found in the analysis of oocyte number. Thus the gonad samples were collected from the random location of ovaries throughout this study (most from the posterior portion). Of the 1001 sampled fish, 406 were female (size range: 124.1–275 cm EFL), 463 were male (size range: 121–232 cm EFL), and sex could not be determined for the remaining 132 fish. Most of the sampled fish were between 140 and 220 cm EFL (Fig. 1). Blue marlin exhibit sexual dimorphism in growth. Specifically, almost all sampled blue marlin larger than 180 cm EFL were female (Fig. 1). The relationship between the female proportion (P_f) and *EFL* can be described by the logistic function (Fig. 1):

$$P_f = 1/\left(1+e^{-\ln(19)\left[(EFL-175.16)/23.59\right]}\right).$$

The sex ratio for the entire study deviated from the expected 1:1 ratio (χ^2=4.93, df=1, P<0.01) and varied among months (more males in the September 2000 [0.32], October 2000 [0.23], and May 2001 [0.31] collections, and more females in the November 2001 collection [0.67]).

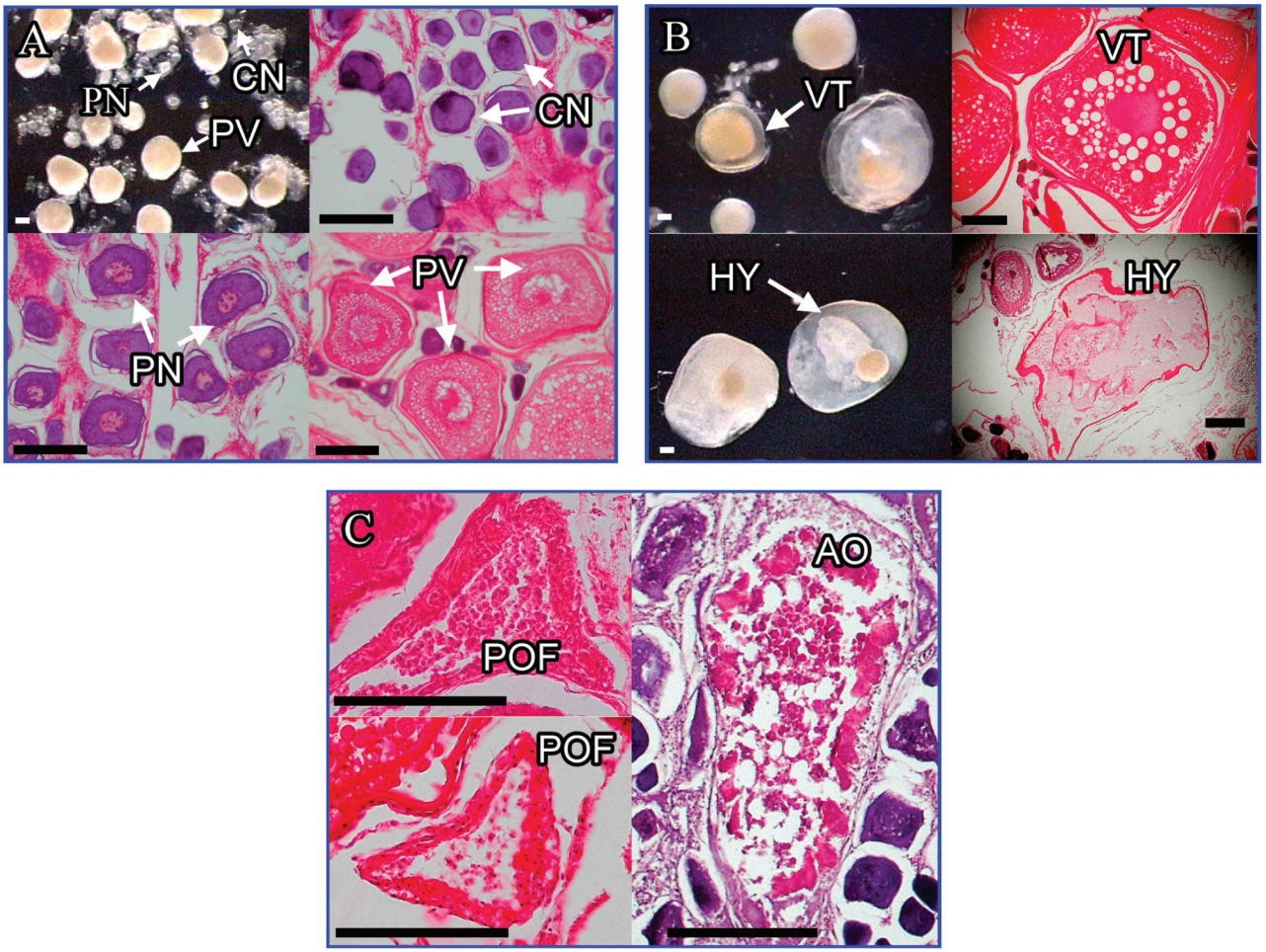

Figure 2

Micro-images of whole oocytes and sectioned oocytes of blue marlin (*Makaira nigricans*) from dissecting and light microscopes, respectively. (**A**) CN, chromatin nucleolar oocytes; PN, perinucleolar oocytes; PV, previtellogenic oocytes; (**B**) VT, vitellogenic oocytes; HY, hydrated oocytes; (**C**) POF, postovulatory follicles; AO, atretic oocyte. Scale bars: 100 microns.

Oogenesis and ovarian development

The most advanced group of oocytes for each ovary were classified as 1) unyolked—oocytes that had not begun vitellogenesis (chromatin nucleolar oocytes and perinucleolar oocytes, Fig. 2A); 2) early yolked—oocytes in the early vitellogenic stage (previtellogenic oocytes, Fig. 2A); 3) advanced yolked—oocytes in an advanced vitellogenic stage (vitellogenic oocytes, Fig. 2B); and 4) hydrated—unovulated hydrated oocyte stage (hydrated oocytes, Fig. 2B). Furthermore, the presence or absence of postovulatory follicles and atresia of yolked oocytes were identified and recorded for the determination of ovarian maturation (Fig. 2C).

The ovaries of 394 females could be staged into six ovarian development stages based on microscopic characteristics and the most advanced group of oocytes (Fig. 2 and Table 1). Ovaries were classified as immature (OM1) if they were packed with unyolked oocytes, maturing (OM2) when the unyolked oocytes were growing to early yolked oocytes, and mature (OM3) if they contained advanced yolked oocytes, but did not exhibit atresia. Ovaries that contained a migratory nucleus and hydrated oocytes, but for which no POFs were found, or for which there were unovulated hydrated oocytes and POFs, were classified as spawning-spawned (OM4). Ovaries with early yolked, advanced yolked, and atretic oocytes were assigned to the recovery stage (OM5). For resting ovaries (OM6), the unyolked oocytes were not packed as orderly as those in immature ovaries, and a few atretic oocytes were observed occasionally.

Table 1

Classification of ovary maturity stage, macroscopic and microscopic descriptions, gonadosomatic index (GSI), and mean diameter of the most advanced group of oocytes (MAGO) of ovary maturity stages of the blue marlin *Makaira nigricans*. n_h is the number of ovaries sampled for histological examination, and n_d is the number of samples for which the mean diameter of MAGO was estimated.

Ovary maturity stage	Macroscopic characteristics	Microscopic characteristics	GSI (mean ± standard error)	Mean diameter (± standard error) of MAGO
Immature (OM1)	Ovaries were solid or resilient, pinkish, with smooth streamline shape.	Ovaries are packed with unyolked or perinucleolar oocytes (Fig. 2A). No vitellogenic oocytes.	0.26 ±0.19 (n_h=66)	43.92 ±27.08 (n_d=15)
Maturing (OM2)	Ovaries became yellowish or orange with the ova developing. Oocytes were visible by eye.	Early yolked or previtellogenic oocytes were present (Fig. 2A). Some advance yolked oocytes were also observed.	0.56 ±0.24 (n_h=24)	83.91 ±53.85 (n_d=17)
Mature (OM3)	Ovary became firm and large, and the color turned orange or reddish. The surface of ovary was vascularized and translucent.	The MAGO was advanced yolked oocytes (vitellogenic oocytes, Fig. 2B). Unyolked, early yolked oocytes were also observed. No atresia of yolked oocytes were observed.	0.98 ±0.34 (n_h=31)	314.19 ±73.83 (n_d=30)
Spawning–spawned (OM4)	Ovaries reached the maximum size before spawning, and the hydrated oocytes were visible by eye. After spawning, the ovaries were flaccid. The wall of the ovary was thick.	The MAGO of the spawning fish were composed of the migratory nucleus and hydrated oocytes (Fig. 2B). Unspawned hydrated oocytes and the postovulatory follicles (Fig. 2C) were observed for spawned fish. Some early yolked oocytes were present.	4.65 ±2.78 (n_h=45)	759.01 ±173.68 (n_d=45)
Recovery (OM5)	Ovaries become small and smooth. A few yolked oocytes and hydrated oocytes were still visible.	The MAGO were composed of unyolked oocytes. Some of the yolked and the atresia of advanced yolked oocytes were present.	3.13 ±1.12 (n_h=101)	729.30 ±135.28 (n_d=31)
Resting (OM6)	Ovaries were flaccid and threadlike. Oocytes could not be observed by the naked-eye.	No vitellogenic oocytes were observed, and unyolked oocytes appeared loosely in the lumen of the ovary. A few atretic yolked oocytes (Fig. 2C) were observed.	0.98 ±0.5 (n_h=127)	304.92 ±123.37 (n_d=52)

Spermatogenesis and testicular development

The testes of 442 males could be staged into five testicular development stages by microscopic characteristics and the composition of germ cells. The five cellular stages were based on 1) spermatogonia (Fig. 3A); 2) primary spermatocytes (Fig. 3B); 3) secondary spermatocytes (Fig. 3B); 4) spermatid (Fig. 3C); and 5) spermatozoa (Fig. 3C). Testes were classified as immature (TM1) if they contained spermatogonia and primary spermatocytes, and no spermiogenesis was observed. The testes were regarded as maturing (TM2) when they contained spermatogonia, secondary spermatocytes, spermatid, and spermatozoa (<50% of total number). Testes were staged as mature (TM3) if the lobular lumens contained more than 50% spermatozoa, and the vas deferens was full of spermatozoa. Males were classified as having spawned (TM4) if the numbers of spermatozoa were decreasing in the lobular lumens and a few unspawned spermatozoa were observed in the vas deferens. Resting testes (TM5) were similar to immature testes, although a few unspawned spermatozoa were observed in the vas deferens.

Spawning season

GSI (gonadosomatic index) was relatively high between May and September for females, and between April and September for males (Fig. 4; boxplots). The condition factor, an index reflecting the interaction between biotic and abiotic factors on physiological condition, exhibited a pattern that was roughly the inverse of that of the GSI of males (Fig. 4; points-lines). There was a significant relationship between the MOD (mean oocyte diameter) and the GSI: MOD = 291.80+327.90 Ln(GSI) (r^2=0.79, n=191), and we propose that MOD

is a valid index for determining reproductive activity. Few ovaries containing vitellogenic oocytes (excluding the samples with atretic oocytes) were observed in January, February, and November. However, there was evidence for yolk accumulation between March and September based on the monthly variation of the percentage of vitellogenic ovaries and the MOD (Fig. 5). Mature ovaries were first seen in March, and females with spawning stage ovaries were observed from May to September (Fig. 6A). Postspawning females (i.e., the recovery stage) were observed from May to December. For males, mature testes were observed throughout the sampling period, and males with spawned testes were observed from March to December (Fig. 6B). The information in Figures 4, 5, and 6 together imply that the major spawning season for blue marlin in western Pacific is from May to September.

Spawning pattern

That oocytes of various developmental stages were present at the same time in an ovary was based on histological examination and is also evident from Figure 7, which shows that there were several modes in the distributions of oocyte diameter. There was a single distribution mode for diameters of early-stage oocytes: 25–35 μm for chromatin nuclear oocytes and 45–165 μm for perinuclear oocytes of immature fish. Two modes (corresponding to CN and PN) were evident in the oocyte distribution for resting fish (Fig. 7B). The number of previtellogenic oocytes (PV; 190–330 μm) began increasing with the development of the ovary for maturing fish (Fig. 7C). Vitellogenic oocytes (VT; 250–800 μm) and hydrated oocytes (800–1200 μm) appeared in the oocyte distribution when fish matured, and ocytes of several stages were present in the oocyte diameter distribution for these fish (Fig. 7D). However, there was a gap at an oocyte diameter at roughly 1000 μm. This gap indicates that oocytes larger than this size may be spawned soon (Fig. 7D). Modes corresponding to vitellogenic and unovulated hydrated oocytes were observed with the appearance of postovulatory follicles after spawning (Fig. 7E). PNs were most abundant for fish in the recovering stage, although there were also some PVs and VTs in their ovaries (Fig. 7F). Overall, Figure 7 indicates that oocytes grew in an asynchronous manner and that individual female blue marlin spawn multiple times during the spawning season.

Size-at-maturity

Maturity ogives were estimated for females and males caught during the spawning season (May to September). The relationship between the fraction mature and size can be described by a logistic curve with lengths at 50% and 95% maturity (EFL_{50} and EFL_{95}) of 179.76 ±1.01 cm EFL (estimate ±standard error, SE) and 194.2 ±1.01 cm EFL (n=394), respectively. For males, EFL_{50} and EFL_{95} were 130 ±1 and 130.13 ±46.56 cm EFL (n=442, Fig. 8).

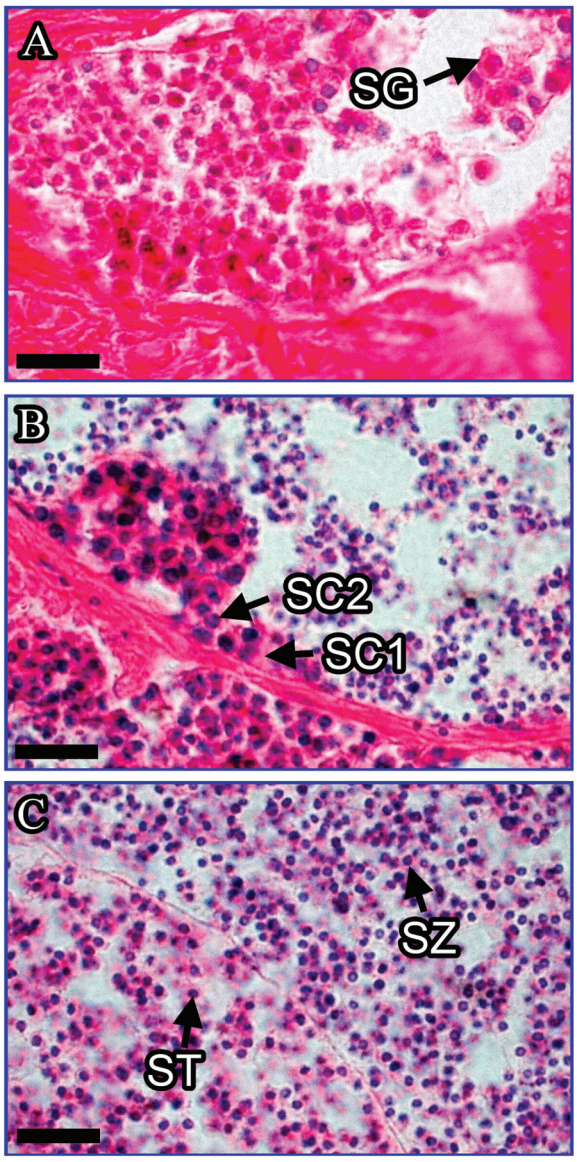

Figure 3

Developmental stages and spermatogenic cells observed in blue marlin (*Makaira nigricans*) testes. (**A**) SG, spermatogonia; (**B**) SC1, primary spermatocytes; SC2, secondary spermatocytes; (**C**) ST, spermatids; SZ, spermatozoa. Scale bars: 100 μm.

Batch fecundity

There was a clear gap in the oocyte distribution for mature ovaries at 1000 μm (Fig. 7D), and the oocytes of most advanced mode (composed of the most advanced yolked, migratory nucleus, and nonovulated hydrated oocytes) were considered to be those of the spawning batch. Batch fecundity, estimated for the 26 mature ovaries with no early postovulatory follicles, ranged from 2.11 to 13.50 million eggs (6.94 ±0.54; mean ±SE).

The relationships between batch fecundity (BF) and EFL, and between BF and RW were BF = 3.29×10^{-12} $EFL^{5.31}$ (r^2=0.70; Fig. 9A) and BF = 1.59×10^{-3} $RW^{1.73}$ (r^2=0.67; Fig. 9B), respectively. The batch fecundity of blue marlin was size related, and fecundity increased nonlinearly with body size. The relative fecundity of blue marlin ranged from 115 to 25 mature eggs per gram of female body weight (55.45 ±3.36).

Spawning frequency

The spawning fractions were estimated as the proportion of mature females with POFs or hydrated oocytes during the spawning season (May to September, 150 days). There was no significant difference in the spawning fraction among months within the spawning season for the postovulatory follicle and hydrated oocyte methods (χ^2=3.62, df=4, P>0.05; χ^2=1.97, df=4, P>0.05). There was also no significant difference in the spawning fraction between different size groups (<200 and ≥200 cm EFL) for these methods (χ^2=0.29, df=1, P>0.05; χ^2=0.03, df=1, P>0.05). Finally, there was no difference between the two methods in terms of the monthly spawning fraction (chi-squared independence tests; χ^2=3.54, df=4, P>0.05). The spawning fraction of mature females based on the postovulatory method was 0.41 (n=164; data combined over months), which indicates that each female would have spawned on average once every 2.4 days, or 62 times during the spawning season (Table 2). In contrast, 34% of the mature females had hydrated oocytes during the spawning season, which is equivalent to a mean spawning interval of 2.9 days, or a spawning frequency of 51 times (Table 2). Annual fecundity was estimated as 120–769 million eggs based on the product of batch fecundity and the average spawning frequency from the two methods (57 times).

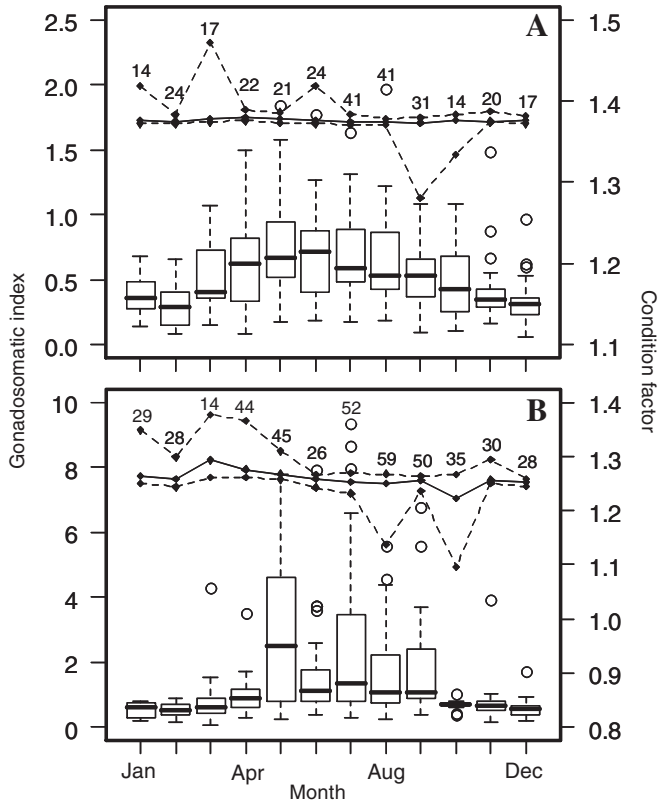

Figure 4

Monthly variation in the gonadosomatic index (GSI; boxplots, left axis) and condition factor (mean=solid-line diamonds, and interquartile range=dashed-line diamonds, right axis) for (**A**) female and (**B**) male blue marlin (*Makaira nigricans*) collected at the Tungkang fish market between September 2000 and December 2001 (monthly data combined over years). The numbers indicate how many individuals were examined.

Discussion

Size distribution and sex ratio

The sizes of blue marlin caught by Taiwanese offshore longliners in the western Pacific Ocean were primarily between 140 and 220 cm EFL. However, blue marlin are sexually dimorphic; animals 170 cm and less are generally males and those larger than 180 cm are generally female. Shung (1975) obtained similar results for blue marlin in the South China Sea (Pratas Islands). Reproductively active male blue marlin in the eastern Pacific Ocean are often smaller than 220 cm EFL, but all animals 230 cm EFL and larger are female (Kume and Joseph, 1969). Several billfish species, such as sailfish (Chiang et al., 2006b), blue marlin (Wilson et al., 1991) and swordfish (DeMartini et al., 2000; Wang et al., 2003) have been shown to exhibit sexually dimorphic growth. Several hypotheses have been proposed to explain this, including 1) sex change or a sex-specific mortality rate (deSylva, 1974); 2) sex-specific growth rates (Wilson et al., 1991; Sun et al., 2002); and 3) sex-specific natural mortality rates (Skillman and Yong, 1976; Sun et al., 2005). Histological analyses indicated that sex-change does not occur in blue marlin. Furthermore, Chen (2001) showed that growth of blue marlin differs between females and males. Thus, this dimorphism is most likely caused by sex-specific growth and mortality rates. However, there is a need to examine the degree to which sexual dimorphism is due to each of these latter two factors. Departure from a 1:1 sex ratio is not expected for most fish species even though females dominate the large size classes. The sample was male-biased for the nonspawning season. However, the sex ratio during the putative spawning season (May–September) was more balanced (χ^2=0.07, df=1, P>0.05). This

Table 2

Spawning fraction and spawning frequency during the spawning season determined using the postovulatory follicle (POF) and hydrated (HY) oocyte methods for female blue marlin (*Makaira nigricans*) in the western Pacific Ocean. EFL is the eye-to-fork length measurement.

		POF method			HY method		
Month	*n*	Ovary with POFs	Spawning fraction	Spawning frequency	Ovary with HY oocytes	Spawning fraction	Spawning frequency
May	20	5	0.25	4.00	8	0.40	2.50
Jun	26	12	0.46	2.17	6	0.23	4.33
Jul	39	17	0.44	2.29	17	0.44	2.29
Aug	46	25	0.54	1.84	16	0.35	2.88
Sep	33	9	0.27	3.67	9	0.27	3.67
Size							
<200 cm EFL	86	39	0.45	2.21	28	0.33	3.07
≥200 cm EFL	78	29	0.37	2.69	28	0.36	2.79
Total	164	68	0.41	2.41	56	0.34	2.93

may imply that mature females migrate to the spawning grounds during the spawning season. The exact spawning area of blue marlin in the western Pacific Ocean needs to be identified in the future in order to fully evaluate this hypothesis.

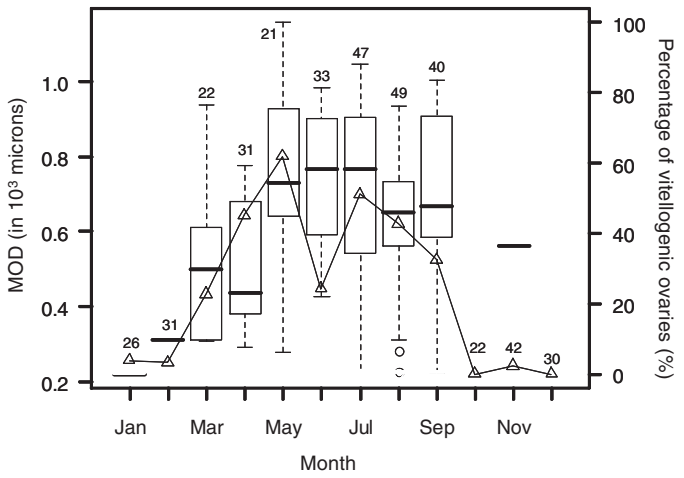

Figure 5

Monthly variation in the mean diameter of the most advanced group of oocytes (MOD; boxplots, left axis) and the percentage of vitellogenic ovaries (excluding the samples with atretic oocytes) (triangles, right axis) for blue marlin (*Makaira nigricans*) collected at the Tungkang fish market between September 2000 and December 2001 (monthly data combined over years). The numbers indicate how many ovaries were examined.

Maturity classification and gonad maturation

Misclassification of whether a female is mature will contribute error to the estimation of the maturity ogive, spawning season, batch fecundity and spawning frequency, and hence to uncertainty when estimating size-at-maturity and egg production. In this study, the use of histological techniques to study gonad maturation provided a more precise outcome than have traditional macroscopic techniques. The spawning season for females was estimated by using GSI and histology and by identifying oogenesis as well as the temporal variation of the mean diameter of the most advanced group of oocytes. The size-frequency distributions of whole oocytes (cross calibrated with histological characteristics) for the different maturation stages provided a fuller understanding of the dynamics of ovarian maturation and the spawning pattern of a fish that produces multiple batches. The size-frequency distributions of whole oocytes, which can be constructed by using a dissecting microscope, can serve as a quick way to determine the ovarian development stage when histological data are not available. The condition factor is usually related to fish health, and a roughly inverse pattern between the temporal variation of the GSI and condition factor was found in male blue marlin, which may imply a lower feeding activity during the spawning season as has been argued for some other pelagic migratory fishes (i.e., school mackerel, Begg and Hopper, 1997).

Size-at-maturity

The EFL_{50} for female blue marlin was estimated to be 179.76 cm, and the smallest size at which any female was mature was 157.8 cm EFL. For males, there was considerable uncertainty regarding EFL_{50} owing to a

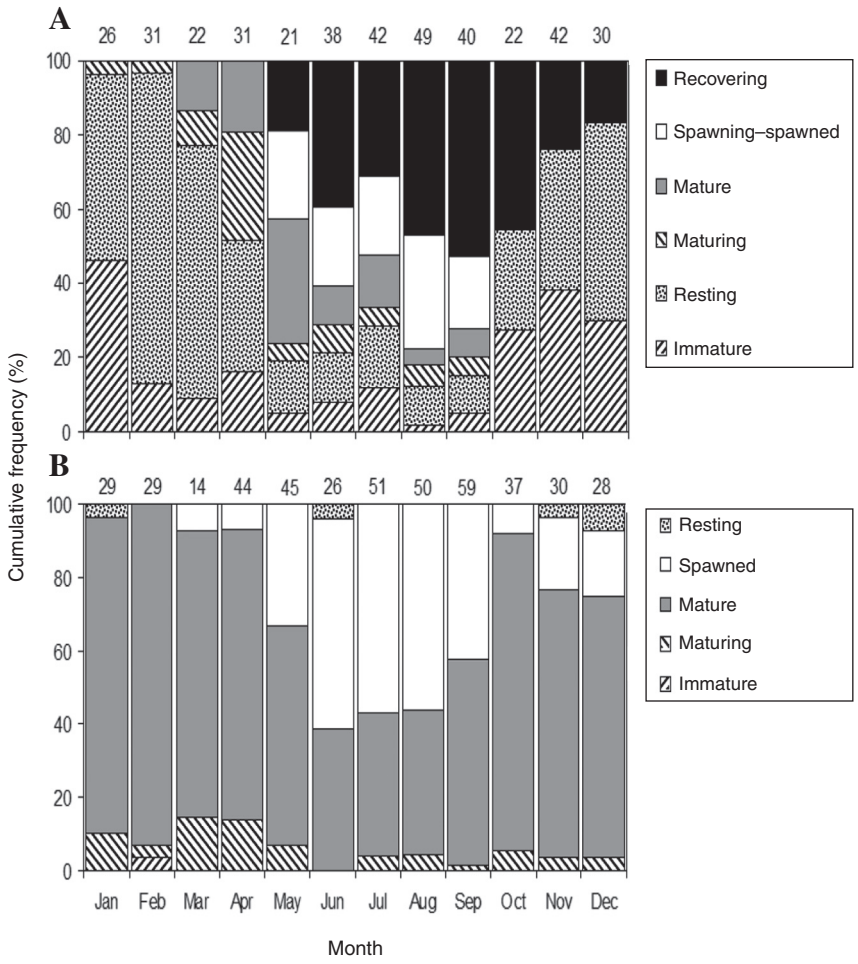

Figure 6

Percentage of blue marlin (*Makaira nigricans*) collected from the Tungkang fish market between September 2000 and December 2001 in each gonad development stage by sex and month: (**A**) females, and (**B**) males (monthly data combined over years). The numbers indicate how many animals were examined.

lack of samples in the size range when male blue marlin are maturing (i.e., within the transition from immature to mature) (Fig. 8). However, male blue marlin larger than 131 cm EFL (size-at-onset-of-maturity) were all mature. The size-at-maturity of blue marlin appears to vary across different regions of the Pacific Ocean. For example, the size-at-onset-of-maturity for male blue marlin was roughly 130–140 cm EFL in the western Pacific Ocean (Nakamura, 1985), and females 171–180 cm EFL have GSI values larger than three (i.e., have reached sexual maturation) in the eastern Pacific Ocean (Uosaki and Bayliff, 1999). This variation among areas may be due to environmental or genetic effects or simply sampling error. In this study, EFL_{50} for females was based on large sample sizes and a broad size range of fish collected throughout the spawning season, and maturity was determined by histology. Thus, the results of this study should provide an accurate representation of the size-at-maturity of blue marlin in the western Pacific Ocean.

Spawning season

Blue marlin have indeterminate fecundity, exhibit an asynchronous oocyte development pattern, and spawn multiple times during the spawning season (Hunter and Macewicz, 2003; Murua et al., 2003). The major spawning season seems to be from May to September based on MOD, the GSI values, and staging of ovaries. Shung (1975) indicated that blue marlin spawn between February and November (with high activity in June and September) in the South China Sea, and Hopper (1990) argued that blue marlin spawn primarily between May and September in Hawaiian waters. Kume and Joseph (1969) indicated that blue marlin spawn from December to January in the South Pacific Ocean. In the Atlantic

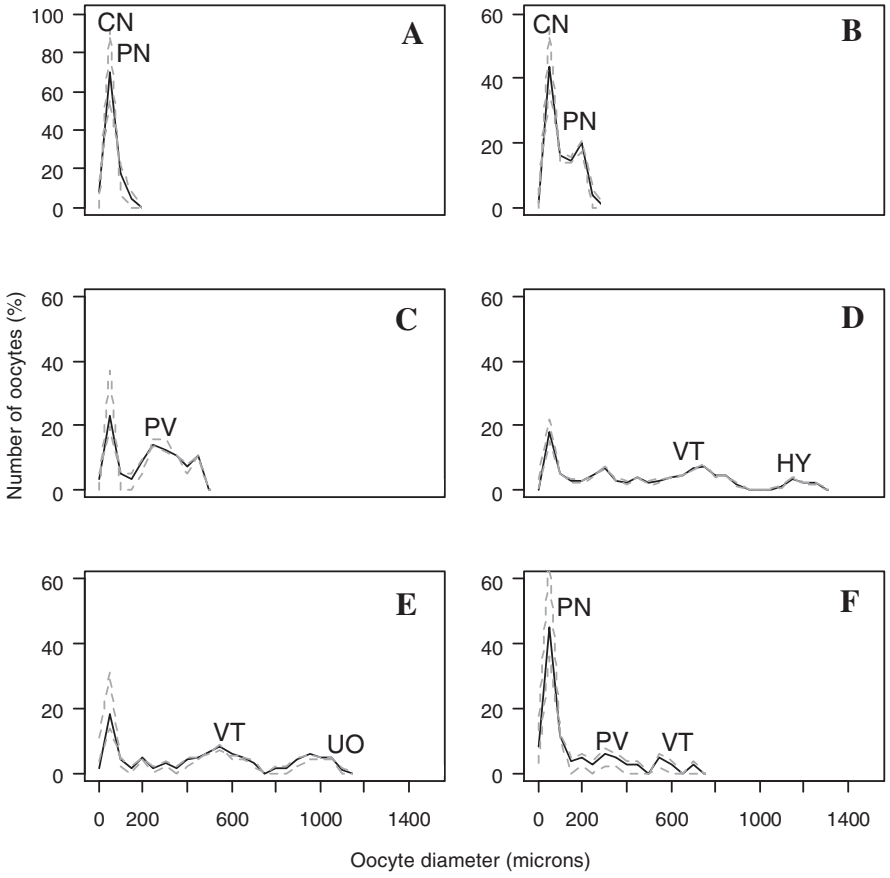

Figure 7

Frequency distributions (percentages) of oocyte diameter (black solid lines) with 95% bootstrapped confidence intervals (gray dashed lines) for six ovarian developing stages of blue marlin (*Makaira nigricans*): (**A**) immature; (**B**) resting; (**C**) maturing; (**D**) mature; (**E**) spawning–spawned; and (**F**) recovering. CN, chromatin nucleolar oocytes; PN, perinucleolar oocytes; PV, previtellogenic oocytes; VT, vitellogenic oocytes; HY, hydrated oocytes; UO, unovulated hydrated oocytes.

Ocean, the spawning season for blue marlin is May through September, but most activity is between July and August according to data collected near Puerto Rico (Erdman, 1968), although Yeo (1978) indicated that blue marlin spawn at temperatures of 26–29°C from April to September in the western North Atlantic. These estimates of spawning season would indicate that blue marlin have an extended spawning season and may be more reproductively active during summer, perhaps because of higher temperatures at that time.

Batch fecundity

It has been argued that more accurate estimates of batch fecundity can be obtained by using only oocytes in the migratory nucleus and hydrated stages (sailfish, *Istiophorus platypterus*; Chiang et al., 2006a). Unfortunately, few ovary samples with migratory nuclei or hydrated oocytes were observed in our study. However, the oocyte distributions exhibited clear modes, including oocytes at sizes that are ready to be spawned. The oocyte size-frequency method usually yields results similar to those based on counts of hydrated oocytes if females with highly advanced oocytes are used (Hunter et al., 1985). In this study, the gonad tissues were collected and preserved for further examination. Batch fecundity is usually back-calculated gravimetrically as the product of the oocyte density per gram of the preserved tissue and the total fresh weight of the ovary. However, Ramon and Bartoo (1997) indicated that preserved ovaries of mature albacore tuna (*Thunnus alalunga*) lost an average of 2% of their fresh weight. The effect of preservation on weight lost may bias the estimation of batch fecundity by the gravimetric method and may also bias identification of the most advanced-stage oocytes. Thus, gonad weights for fresh

and preserved samples should be compared in order to more fully evaluate the gravimetric and other (e.g., volumetric) methods.

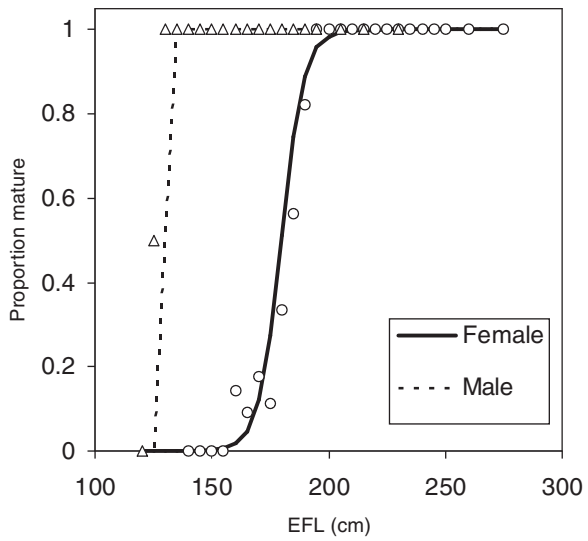

Figure 8

Maturity-at-length (eye-to-fork length [EFL]) relationships for female (solid lines) and male (dashed lines) blue marlin (*Makaira nigricans*). The open circles and triangles represent the observed proportions mature-at-age for females and males respectively (aggregated to 5 cm intervals).

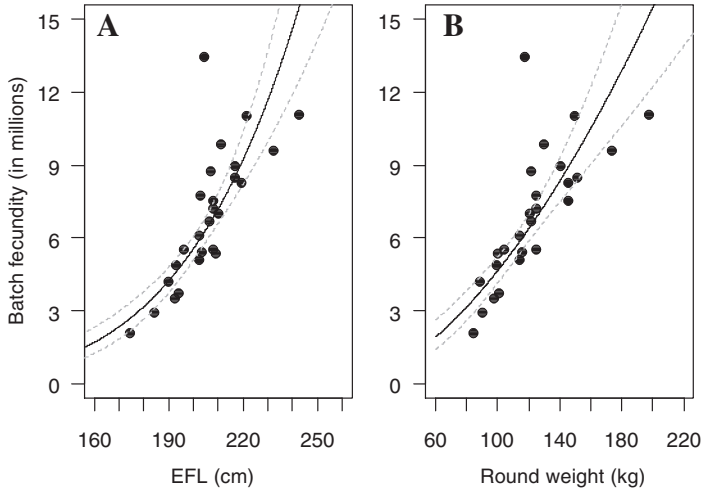

Figure 9

Batch fecundity as a function of (**A**) eye-to-fork length (EFL), and (**B**) round weight for blue marlin (*Makaira nigricans*) collected from the Tungkang fish market between September 2000 and December 2001. The solid lines denote the predicted fecundity, and the dashed lines the 95% confidence intervals for the mean relationship.

Individual estimates of batch fecundity ranged from 2.11 to 13.50 million eggs (6.94 ±0.54; size range 174–242 cm EFL). Batch fecundity of blue marlin is estimated to be larger than that of the black marlin (*Makaira indica*) in the waters off Taiwan (0.32–3.2 million eggs; Liu, 2007), than that of sailfish in eastern Taiwan waters (0.2–2.48 million eggs; Chiang et al., 2006a), and that of swordfish (*Xiphias gladius*) in the waters off eastern Australia (1.16–2.50 million eggs; Young et al., 2003).

Spawning frequency

We assumed that the hydrated oocytes of blue marlin were spawned in less than 24 hours (the hydrated oocyte method) and that the POFs were detectable for no more than 24 hours (the postovulatory follicle method), given observations for other pelagic fish (yellowfin tuna, *Thunnus albacares*; Schaefer, 1996). However, these assumptions need to be verified in the future. The mean time between consecutive spawning events was 2.4 days based on the postovulatory follicle (POF) method (2.9 days based on the hydrated oocyte method). There was no significant difference in spawning fraction among months, which indicates that females were spawning asynchronously throughout the spawning season. Furthermore, there is no relationship between the monthly spawning fraction and the two methods, which may indicate that the estimates of the spawning frequency are accurate. However, there are two caveats which need to be examined when applying the POF method. First, the degeneration of POFs varies among species and may be influenced by the preferred spawning temperature of a species (Chiang et al., 2006a). Second, the fish we collected from the fish market may have been caught a few days earlier. Because of that, it is possible that the POFs degenerated before we obtained the fish and therefore the fraction of ovaries with POFs may have been underestimated.

Conclusion and recommendations

This study provides reproductive parameters and their associated uncertainty as inputs for use in stock assessments of blue marlin in the western Pacific Ocean. The analyses were based on large samples from Taiwanese offshore longliners that cover broad areas in the western Pacific Ocean. Consequently, the estimates should be reliable. However, collecting by ships a greater number of specimens over the entire stock's spatial distribution (especially for hydrated ovaries, and testes during the spawning season) is recommended so that estimates of male size-at-maturity and egg production will be more robust. The spatial variation in some of the reproductive

parameters could be explored further by using data collected over a broader spatial domain and hence an exploration could be conducted to determine whether this variation is related to abiotic or biotic factors. We recommend that the methods used in this study be applied to estimate reproductive parameters for billfish and other pelagic fish.

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