Received 13 June 2017

Accepted 29 September 2017

Reproductive biology of female striped marlin *Kajikia audax* in the western Pacific Ocean

H.-Y. CHANG*†, C.-L. SUN*‡§, S.-Z. YEH*Q, Y.-J. CHANG*, N.-J. SU*Q AND G. DINARDO#

*Institute of Oceanography, National Taiwan University, No. 1, Sec. 4, Roosevelt Rd, Taipei 10617, Taiwan, ‡Institute of Fisheries Science, National Taiwan University, No. 1, Sec. 4, Roosevelt Rd, Taipei 10617, Taiwan and #NOAA Fisheries, Fisheries Resources Division, National Marine Fisheries Service, Southwest Fisheries Science Center, 8901 La Jolla Shores Drive, La Jolla, CA 92037-1508, U.S.A.

§Author to whom correspondence should be addressed at present address: Center of

Excellence for the Oceans, National Taiwan Ocean University, 2 Pei-Ning Road, Keelung, Taiwan 20224. Tel.: +886 224636954; fax.; email: chilu@ntu.edu.tw

† Present address: School of Marine Sciences, University of Maine, Orono, ME04469, U.S.A.

QPresent address: Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University. 2, Pei-Ning Road, Keelung, Taiwan 20224

Length and mass data for 1260 (536 females, 683 males, 41 sex unknown) striped marlin *Kajikia audax* were collected at the fish markets of Tungkang, Singkang and Nanfangao from July 2004 to September 2010. Of these samples, 534 gonads (236 females and 298 males) ranging from 95 to 206 cm in eye-to-fork length (L_{EF}) and 8 to 88 kg in round mass (M_R), were collected. Chi-square tests indicated sex ratios were homogeneous among months in 2004 and 2006–2008, but not in 2005, 2009 and 2010; and there were significant differences in sex ratio by size. The overall sex ratio (R_{S}) differed significantly from the expected 0.5. *Kajikia audax* are sexually dimorphic and the proportions of females increased with size between 140 and 210 cm L_{EF}. Reproductive activity was assessed using a gonado-somatic index (I_{G}), external appearance of the gonads and histological examination and results indicated that the spawning season occurred from April to August with a peak in June to July. Based on histological observations and the distribution of oocyte diameters, *K. audax* are multiple spawners and their oocytes develop asynchronously. The estimated length-at-50% maturity (L_{EF50}) was *c.* 181 cm (*c.* 4.8 years of age) for females. The proportion of reproductively active females in the spawning season with ovaries containing postovulatory follicles (0.27) indicated that they spawned every 3.7 days

on average. The hydrated oocyte method estimated mean ± S.D. batch fecundity (F_B) to be 4.4 ± 2.02 million eggs; average relative fecundity was 53.6 ± 13.9 oocytes g^{-1} M_R; and the average annual fecundity was 181.3 ± 48.3 million eggs. The parameters estimated in this study are key information for stock assessments of *Kajikia audax* in the north-western and central Pacific and will contribute to the conservation, management and sustainable yield of this species.

Key words: billfish; fecundity; sexual maturity; sex ratio; size-at-50% maturity; spawning season.

INTRODUCTION

Striped marlin *Kajikia audax* (Philippi 1887) is the most widely distributed istiophorid billfish occurring from 45° N to 30° S in the eastern Pacific, from 45° N to 45° S in the western Pacific and extending southward to 35° S and 45° S in the eastern and western Indian Ocean (Nakamura, 1985). *Kajikia audax* appear to prefer more temperate waters of 20–25° C (Nakamura, 1985) and are generally limited in their vertical movements to approximate 8° C change in water temperature relative to the surface layer (Brill *et al.*, 1993). The distribution pattern of *K. audax* is characterized as horseshoe shaped in the Pacific Ocean, with sparse occurrence at the equator between *c.* 20–30° N and S (Domeier, 2006; Sippel *et al.*, 2011).

Kajikia audax are caught in recreational fisheries in the eastern and western Pacific Ocean (Melo-Barrera *et al.*, 2003; Ortega-Garcia *et al.*, 2003) and captured commercially as by-catch (*i.e.*, non-targeted and incidental) in pelagic longline fisheries targeting tunas and swordfish *Xiphias gladius* L. 1758 (Jensen *et al.*, 2010). In the western Pacific Ocean, they are also captured as by-catch by drift gillnet, harpoon and set-net fisheries in nearshore areas. *Kajikia audax* are also a target species of artisanal fisheries in Latin America (Kume & Joseph, 1969) and directed

longline fisheries in Mexico and Australia (Jensen *et al.*, 2010).

Several studies have documented the reproductive biology of *K. audax* in the Pacific and Indian oceans. Kume & Joseph (1969) estimated size at maturity and spawning season by developing a gonado-somatic index (I_G) for *K. audax* in the northern and southern Pacific Ocean. Merrett (1970;1971) identified the gonad development and estimated size at maturity in the western Indian Ocean. The appearance of the gonads was described by Eldridge & Wares (1974) who also estimated size at maturity, fecundity and spawning season in the eastern Pacific Ocean. González-Armas *et al.* (2006) determined the spawning season and confirmed the spawning ground by the occurrence of larvae and mature females in the southern Gulf of California. In the south-western Pacific, Kopf *et al.* (2012) reported size at maturity, batch fecundity, spawning frequency and spawning season. Despite these early attempts to quantify the reproductive biology of *K. audax*, most studies were regional in scope (except for Kopf *et al.*, 2012), generally had small and unrepresentative samples (*i.e.*, not acquired evenly on a temporal basis) and only a few studies provided reliable estimates based on histological methods, *e.g.* Kopf *et al.* (2012).

Due to a paucity of studies, the reproductive biology of *K. audax* in the western

Pacific Ocean is enigmatic. By collecting samples on a temporal basis, this study aimed to determine: sex ratio of the sample; spawning seasonality; stage of ovarian maturation using histological techniques; size at maturity, fecundity and spawning frequency. These goals were estimated to provide the necessary biological input parameters for age and size structured models that are required for the regional stock assessment of *K. audax*.

MATERIALS AND METHODS

SAMPLE COLLECTION AND LABORATORY PROCESSING

Length and mass data of 1260 *K. audax* (536 females, 683 males, 41 sex-undetermined), including 236 pairs of ovaries, were collected by random sampling on a monthly basis at the Tungkang, Singkang and Nanfangao fish markets, Taiwan, from July 2004 to September 2010. Relatively low numbers of samples were collected in August to November, however, due to the switch of some Tungkang-based longliners from the fishing area off south-eastern Taiwan to the

South China Sea (Sun *et al.*, 2015). All samples were caught by offshore longline, gillnet and harpoon fisheries operating in the western Pacific Ocean (Fig. 1). Sex (determined by macroscopic appearance and histological observations of the gonad), eye-to-fork length (L_{EF}, measured to the nearest 1 cm), lower-jaw-fork length (L_{LJF}, measured to the nearest 1 cm) and round mass (M_{R}, measured to the nearest 0.1 kg) were recorded for each fish (L_{EF} data for eight fish were missing). Ovaries were weighed to the nearest 0.1 g and sub-samples of ovaries were preserved in 10% neutral buffered formalin (Cuellar *et al.*, 1996) for later histological examination.

Three of the most advanced ovaries containing hydrated oocytes were selected from samples (oocyte diameters ranged from 905.5–1395.5 μm, 915.1–1316.5 μm and 650.2–864.3 μm for the three fish) collected April–September 2009 for evaluating the synchronicity of oocyte development within and between ovaries (Arocha, 2002; Sun *et al.*, 2009; 2013). The maturity and development of ovaries were assessed mainly based on the development of the most advanced group of oocytes (MAGO), so the number and size of MAGO were counted and measured for each of the three samples. The left and right lobes of these ovaries were divided into three regions (anterior, middle and posterior). Three sub-samples of about 0.05 g (weighed to the nearest

0.001 g) were taken from each region, immersed in 33% glycerol solution for 15 minutes (Schaefer, 1987) and oocytes were teased apart with a blunt probe (Hunter *et al.*, 1985). The number and diameters of MAGO in each sub-sample were counted and measured with the Image-Pro Plus 6.0 software package (https://en.freedownloadmanager.org/users-choice/Image_Pro_Plus_6.0_Free_Download.html) in combination with a Leica MZ6dissecting microscope (www.leica.com) equipped with a charge-couple device (CCD) camera (Leica DFC420) and a high-resolution computer monitor (Friedland *et al.*, 2005). Mean oocyte diameter was calculated as the average of the MAGO. A split-plot ANOVA found no significant differences in mean oocyte diameter of MAGO between lobes of the ovary ($F_{1,16}$ = 1.24, $P > 0.05$) or within ovaries among regions ($F_{2,15}$ = 1.73, $P > 0.05$), although differences were found among ovaries ($F_{2,15}$ = 1059.4, $P < 0.05$). Similar results were found in the same tests using the number of oocytes (between ovary lobes: $F_{1,16}$ = 1.40, $P > 0.05$; within ovaries among regions: $F_{2,15}$ = 4.12, $P > 0.05$; among ovaries: $F_{2,15}$ = 27.83, $P < 0.05$). This analysis allowed for maximizing and streamlining of the experimental design to collect gonad samples from the posterior regions of ovaries throughout this study.

SIZE RELATIONSHIPS, SEX RATIO, GONADO-SOMATIC INDEX AND CONDITION FACTOR

Relationships between L_{LJF} and L_{EF} by sex were tested by linear regression and the relationships between L_{EF} and M_{R} by sex were determined using a power function. An analysis of covariance (ANCOVA) was used to test for differences in the slope between sexes by size. All statistical analysis was carried out in R 2.8.1 (www.r-project.org).

Sex ratio (R_{S}) was calculated as the proportion of females to the total sample size and χ^2-tests determined the homogeneity between sex ratios among months, years and 5 cm length classes (Sun *et al.*, 2009). Gonado-somatic index (I_{G}) for female *K. audax* was determined by the equation (Kume & Joseph, 1969; Eldridge & Wares, 1974; González-Armas *et al.*, 2006): $I_{\mathrm{G}} = M_{\mathrm{G}}(L_{\mathrm{EF}})^{-3}10^4$, where M_{G} is gonad mass (g). Based on the smallest size at maturity and average size of reproductively active females, monthly I_{G} was divided into three body length groups to see if there are differences in spawning season among different size groups. The condition factor (*K*)

was calculated for each fish by the equation (Bolger & Connolly, 1989): $K = M_{\mathrm{R}}(L_{\mathrm{EF}})^{b}10^{6}$, where b is the slope of length–mass relationship.

HISTOLOGICAL CLASSIFICATION AND SIZE-AT-MATURITY

Reproductive activity was assessed by macroscopic characteristics, I_{G}, mean diameter of MAGO and histological examination. Atresia and postovulatory follicles (POF) were identified and classified to evaluate ovarian maturity (Hunter & Macewicz, 1985*a*,*b*). Three stages of POFs were classified, but the exact degeneration rate of POFs has not been verified for *K. audax* (Kopf *et al.*, 2012). Assumptions were made for estimates of spawning frequency and the classification of POFs does not affect estimates of spawning frequency. According to ovarian development stage criteria from previous studies (de Sylva & Breder, 1997; Arocha & Bárrios, 2009; Kopf *et al.*, 2012; Sun *et al.*, 2009) and based on the MAGO in the ovaries, each ovarian sample was classified into one of six stages (immature, developing, maturing, mature, spawning and spent or resting stage). Ovaries classified as immature, developing, maturing were defined as sexually immature samples and those classified

as mature, spawning, spent–resting were defined as sexually mature samples (Table I). The probability that the *j*th fish was mature (P_j) was modelled with a logistic curve (Smith *et al.*, 2004, Norman and Stevens, 2007, Sun *et al.*, 2009): $P_j = \left\{1 + e^{-\ln(19)\left[\left(L_{\mathrm{EFj}} - L_{\mathrm{EF50}}\right)\left(L_{\mathrm{EF95}} - L_{\mathrm{EF50}}\right)^{-1}\right]}\right\}^{-1}$, where $L_{\mathrm{EF}j}$ = the L_{EF} of fish *j*; L_{EF50} and L_{EF95} = size at which 50% and 95% of the population reached maturity. L_{EF50} and L_{EF95} were estimated by maximizing a log-likelihood function and assuming a binomial error distribution with AD Model Builder (Fournier, 2000; Sun *et al.*, 2009).

Because the ovaries contained asynchronous oocytes, the mean oocyte diameter was calculated as the average of the MAGO. The diameters of each oocyte of the MAGO on a histological section (*c.* 1 cm^2) at the six maturation stages were measured from histological sections for 236 ovaries. Diameters of the MAGOs were calibrated with the Image-Pro Plus 6.0 software package in combination with an optical Leica DMLS microscope equipped with a Leica DFC420 CCD camera and a high-resolution computer monitor.

SPAWNING FREQUENCY

Two methods were used to estimate spawning frequency: the POF method (Hunter & Macewicz, 1985*a*) and hydrated oocyte method (Arocha & Bárrios, 2009). Spawning frequency was estimated by the inverse of the spawning fraction and the spawning fraction was calculated as the proportion of females with ovaries containing POFs or hydrated oocytes to the number of sexually mature females and reproductively active females during the spawning season. Tunas have been documented to spawn at temperatures > 24° C and the degeneration of POFs occurs *c.* 24 h after spawning (Hunter *et al.*, 1986; McPherson, 1991; Nikaido *et al.*, 1991; Schaefer, 1996) and degeneration has been reported to show a strong correlation with temperature (Fitzhugh & Hettler, 1995). The spawning of many large pelagic species appears to show concordance with temperatures of about 24° C (Schaefer, 2001; Young *et al.*, 2003; Arocha & Bárrios, 2009) and actively spawning *K. audax* have been documented at temperatures of 24.8–28.3° C (Kopf *et al.*, 2012). Therefore, using the degeneration rate of tuna POFs as an approximate guide, it was assumed that the POFs of *K. audax* were histologically detectable until 24 h after spawning. Spawning frequency estimated by the hydrated oocyte method depends on the sampling time and duration of the period of hydration, which can avoid the biases

caused by the onset of spawning or by failure to detect hydration (Hunter & Macewicz, 1985*a*). The best available data of sampling time, however, were sampling dates. Therefore, a few assumptions were made for estimating approximate spawning frequency. The assumptions for estimating spawning frequency are (Arocha & Bárrios, 2009): all females in spawning condition (with ovary containing POFs or hydrated oocytes) are vulnerable to the method of capture; the time of day for spawning is approximately fixed for all spawning females; the spawning stock is unchanged during the spawning season; fish do not immigrate to or emigrate from the spawning ground. Although *K. audax* is a highly mobile species, several studies have shown that *K. audax* exhibit some level of regional site fidelity with little to no mixing among regions and they may inhabit discrete habitats in the Pacific Ocean (Bromhead *et al.*, 2003; Gonzalez-Armas *et al.*, 2006; Kopf *et al.*, 2012; Su *et al.*, 2015). Therefore, it is reasonable to assume that the spawning stock is unchanged during the spawning season.

INDIVIDUAL BATCH FECUNDITY

The hydrated oocyte method was used to estimate individual batch fecundity (F_B). Ovarian samples were observed using a dissecting microscope to identify the number of hydrated oocytes present in the ovaries. Individual F_B was calculated as the number of fresh non-ovulatory hydrated oocytes in the ovary, which contained no new POFs (early stage POF) and three sub-samples were taken from each ovary that contained hydrated oocytes without new POFs (Schaefer, 1996). Each sub-sample was processed by the method described previously and only the number of hydrated oocytes were counted (fresh hydrated oocytes are sphere-shaped with large diameters, translucent and easily distinguishable from other oocytes). The estimate of individual F_B was back-calculated by the gravimetric method (Hunter *et al.*, 1992): $F_B = M_G n(m)^{-1}$, where *n* is the number of hydrated oocytes in the sub-sample and *m* is the mass of ovarian sub-sample (0.05 g).

Individual relative fecundity (F_R) was defined as F_B divided by M_R of females (Hunter *et al.*, 1992): $F_R = F_B \times {M_R}^{-1}$. Since *K. audax* have indeterminate batch fecundity, individual annual fecundity (F_A) was estimated from F_B, spawning frequency (F_S) and the duration of spawning season (S_S) (Arocha & Bárrios, 2009): $F_A = F_B(F_S)^{-1} \times S_S$.

RESULTS

SIZE DISTRIBUTION AND SEX RATIOS

Sampled fish ranged in size from 95 to 220 cm L_{EF} (mean ± S.D. L_{EF} = 167 ± 20) for females and 92 to 221 cm L_{EF} (mean ± S.D. L_{EF} = 155 ± 19) for males. Most (*c.* 90%) of the sampled fish were between 140 and 200 cm L_{EF} (Fig. 2). Relationships between L_{LJF} *v.* L_{EF} and M_R *v.* L_{EF} by sex were tested by ANCOVA and no significant differences were found ($P > 0.05$) and the data for sexes were combined: $L_{LJF} = 1.12L_{EF} + 7.08$ (r^2 = 0.98; n = 549) and $M_R = 5 \times 10^{-6} L_{EF}^{3.15}$ (r^2 = 0.93; n = 957).

The overall R_S (0.44; female:male = 1:1.27) was significantly different from the expected 0.5 ($Ç^2$ = 17.73, d.f. = 1, $P < 0.01$) and $Ç^2$-tests suggested that R_S was homogeneous among months in 2004 ($Ç^2$ = 2.99, d.f. = 5, $P > 0.05$), 2006 ($Ç^2$ = 18.63, d.f. = 11, $P > 0.05$), 2007 ($Ç^2$ = 13.31, d.f. = 10, $P > 0.05$) and 2008 ($Ç^2$ = 6.41, d.f. = 11, $P > 0.05$), but not in 2005 ($Ç^2$ = 33.66, d.f. = 11, $P < 0.01$), 2009 ($Ç^2$ = 27.42, d.f. = 11, $P < 0.01$) and 2010 ($Ç^2$ = 21.12, d.f. = 8, $P < 0.01$). The estimated yearly R_S was

not homogeneous across the years of the study period ($Ç^2$ = 15.12, d.f. = 6, P < 0.01). Chi-square tests indicated that the sex ratio by size was also not homogeneous ($Ç^2$ = 154.6, d.f. = 26, P < 0.01) among different length classes. The R_S at lengths < 140 cm L_{EF} fluctuated between 0.14 and 0.5 without a discernible pattern (Fig. 3). The proportion of females, however, increased linearly for L_{EF} > 140 cm and exceeded 0.5 when L_{EF} reached *c.* 165 cm. Only one male fish was > 210 cm L_{EF} in the entire sample. The relationship between sex ratio (R_S) and L_{EF} across the range of 140 to 210 cm was given by the following equation: $R_S = 0.01L_{EF} - 1.25$ (r^2 = 0.98; n = 14). The overall R_S (0.37; female:male = 1:1.69) in the spawning season (April to August) was significantly different from the expected 0.5 ($Ç^2$ = 30.45, d.f. = 1, P < 0.01), yet the overall R_S (0.49; female:male = 1:1.05) in the non-spawning season was significantly different from the expected 0.5 ($Ç^2$ = 0.46, d.f. = 1, P > 0.05).

Female *K. audax* caught by longliners ranged from 95 to 219 cm L_{EF} (mean ± S.D. = 165.9 ± 19.3, n = 210) and males from 92 to 212 cm L_{EF} (156.4 ± 20.0, n = 296); females caught by gillnet ranged from 103 to 220 cm L_{EF} (169.3 ± 20.7, n = 279) and males from 107 to 221 cm L_{EF} (153.5 ± 18.0, n = 347); and females caught by harpoon ranged from 131 to 214 cm L_{EF} (170.7 ± 18.7, n = 44) and males from

127 to 190 cm L_{EF} (156.6 ± 15.0, n = 38). The relationships between R_{SL} *v.* L_{EF} (5 cm class intervals) by three fishing gears were tested by ANCOVA and no significant differences were found ($F_{2,31}$ = 0.02, P > 0.05). Similarly, the R_S showed a trend such that the proportions of females gradually increased between 140 and 210 cm L_{EF} in the three fisheries where males dominated when L_{EF} < *c.* 165 cm.

OOGENESIS AND CLASSIFICATION OF OVARIAN DEVELOPMENT

Seven stages of oogenesis were classified (Fig. 4): primitive oogonia stage; chromatin-nucleolar oocyte stage; perinucleolar oocyte stage; previtellogenic oocyte stage; vitellogenic oocyte stage; migratory nucleus oocyte stage; hydrated oocyte stage. Atretic oocytes [Fig. 4(h)] and POFs (Fig. 5) were also identified for classifying ovarian development.

Six stages of ovarian maturation were identified for 236 ovarian samples on the basis of macroscopic and microscopic examination (Table I) and the percentage distribution of diameter of MAGO at each stage is shown in Fig. 6: immature stage; developing stage; maturing stage; mature stage; spawning stage; spent or resting stage.

The histological sections and size distribution of oocyte diameters show that the ovaries contain asynchronously developed oocytes, which indicates that *K. audax* are multiple spawners in the spawning season with indeterminate annual fecundity.

SIZE-AT-MATURITY

The smallest sexually mature female fish sampled was 150 cm L_{EF}. The relationship between the proportion (p) of mature fish and L_{EF} for female *K. audax* was described by the logistic function (Fig. 7; r^2 = 0.85; n = 228). The L_{EF50} and L_{EF95} estimated for female *K. audax* was *c.* 181 cm L_{EF} (95% C.I.: 177.0–184.4 cm) and *c.* 217 cm L_{EF} (95% C.I.: 210.0–229.9 cm), respectively. According to the age and growth study of *K. audax* by Sun *et al.* (2011), the estimated age at 50% maturity (A_{50}) was 4.8 years for females.

SPAWNING SEASON

In order to identify whether the spawning season and spawning activity differed

with respect to female size, monthly variation (years pooled) in I_G and the proportion of ovarian maturing stage, females were grouped into three body-length classes: d 150 cm L_{EF} (*i.e*. the smallest sexually mature female was *c.* 150 cm L_{EF}); 151–175 cm L_{EF} (*i.e.* the smallest female in spawning condition was 151 cm) and > 175 cm (*i.e.* average size of reproductively active females was *c.* 173 cm).

The monthly mean I_G [Fig. 8(a)] for smaller females (d 150 cm L_{EF}) varied below 1 throughout the year without a discernible pattern. The I_G values for females 151 and 175 cm L_{EF} increased to 2.60 in April, reached a peak of 3.56 in June and then slightly decreased to 2.45 in August before sharply decreasing to 0.29 in September. A similar pattern was found for females > 175 cm L_{EF}, but the I_G values reached higher levels. The monthly mean I_G for females > 175 cm L_{EF} increased rapidly from less than 1 in March to 4.83 in April, followed by a slight decrease to 3.73 in May, but stabilized around 4.45 until August and then suddenly decreased to 0.61 in September.

The monthly mean K for females [Fig. 8(b)] decreased from 5.3 in April to a minimum of *c.* 4.7 in August and then gradually increased to peak at around 5.3 in December. Mean *K* from January–April was stable at around 5.1-5.3.

Females d 150 cm L_{EF} were all immature. The most advanced ovarian samples of this length class were classified in the maturing stage and appeared in May–June (Fig. 9). Fish in the mature stage for medium sized females (151–175 cm L_{EF}) occurred from April to August, but fish classified in the spawning stage occurred only from June to July. Most of the females > 175 cm L_{EF} were sexually mature and fish in the spawning stage at this size occurred in April through August, with a higher proportion from June through August.

The monthly mean diameter of MAGOs (Fig. 10) gradually increased from December to March with an average of *c.* 65 μm; followed by a dramatic > three-fold increase to 234 μm in April and later reaching a peak of 284 μm in June. The mean diameter of MAGOs from April to August was 234 μm. This suddenly decreased to 60 μm in September and stayed at this size until November. The mean diameter of MAGOs were large with high variability in April to August, the high variability implying that there were several samples at different stages of maturity in a given month.

Information on monthly changes (years pooled) in mean I_{G}, proportion of fish in the spawning stage, mean diameter of MAGOs and mean *K* together indicated that the

major spawning season for *K. audax* in the western Pacific Ocean is from April to August with a peak season from June to July. The patterns of monthly mean I_G and percentage of fish in the spawning stage for females at different sizes indicates that female *K. audax* > 175 cm L_{EF} have an extended spawning season of about three months longer than females *K. audax* < 175 cm L_{EF} (April, May and August). This implies that larger females may have a longer spawning season than smaller females in the population.

SPAWNING FREQUENCY

The spawning frequency estimated by the POF method for sexually mature and reproductively active females was 6.2 and 3.7 days during the spawning season (April–August), respectively. The spawning frequency estimated by the hydrated oocyte method for sexually mature and reproductively active females was 10.0 and 6.7 days during the spawning season, respectively.

The spawning frequency estimated by the POF method for sexually mature and reproductively active females between 151 and 175 cm L_{EF} was 12.5 and 9.5 days

during the spawning season, respectively. The spawning frequency estimated by the POF method for sexually mature and reproductively active females > 175 cm L_{EF} was 3.57 and two days during the spawning season, respectively. The hydrated oocyte method was not available for females < 175 cm L_{EF}, because no fish < 175 cm L_{EF} with hydrated oocytes were collected.

INDIVIDUAL BATCH FECUNDITY

Individual fecundity estimated from fresh ovarian samples of three fish (190–191 cm; 77–79 kg) was 2.4–6.4 million eggs, with a mean ± S.D. of 4.4 ± 2.02 million eggs. The estimates of individual relative fecundity ranged between 30.3–78.3 oocytes g^{-1} M_R, with a mean ± S.D. of 53.6 ± 24.0 oocytes g^{-1} M_R. The individual annual fecundity estimated based on batch fecundity, spawning frequency and spawning duration (spawning season), ranged between 98.9 and 266.1 million eggs, with a mean ± S.D. of 181.3 ± 83.6 million eggs.

DISCUSSION

SIZE DISTRIBUTION AND SEX RATIO

The R_S in a population is important to fisheries population dynamics and may have evolutionary significance (Hutchings & Rowe, 2008). In this study, there was a higher proportion of males and the overall R_S was 0.44. Similar results of male-skewed sex ratios have been reported for samples of *K. audax* in the southern Gulf of California (0.43; González-Armas *et al.*, 2006) and the south-western Pacific Ocean (0.49; Kopf *et al.*, 2012). The R_S of *K. audax* in the western Pacific Ocean has been reported to be around 0.5 during the spawning season (Nakamura, 1949), but misidentification of marlin species may have compromised the accuracy of this estimate (*i.e.* other marlins were sometimes regarded as *K. audax* in this report). Kume & Joseph (1969) also observed a predominance of male *K. audax* on spawning grounds in the north-eastern and south-eastern Pacific Ocean.

The R_S increased gradually for L_{EF} between 140 and 210 cm and most fish > 210 cm were females (only one male was found in this size range). The increase in the proportion of females in samples was noted by Kopf *et al.* (2012) at lengths > 234 cm

L_{LJF} and a similar observation was noted by Kume & Joseph (1969) in the eastern Pacific Ocean. Sexual dichotomy by size in billfishes has been previously documented by numerous authors: *X. gladius* (Wang *et al.*, 2003; Wang *et al.*, 2006); blue marlin *Makaira nigricans* Lacépède 1802 (Shimose *et al.*, 2009; Sun *et al.*, 2009); black marlin *Istiompax indica* (Cuvier 1832) (Sun *et al.*, 2015.); sailfish *Istiophorus platypterus* (Shaw 1792) (Chiang *et al.*, 2006*b*; and white marlin *Kajikia albida* (Poey 1860) in the Atlantic Ocean (Arocha & Bárrios, 2009).

The differences in R_{S} could be attributed to several hypotheses. 1: Sexually dimorphic growth patterns or different mortality; *K. audax* is a sexually dimorphic species and according to Sun *et al.* (2011), no statistical difference was found in growth rates and life spans between sexes. Kopf *et al.* (2011) reported however, that the growth rates of males and females were significantly different. 2: Sex-change (*e.g.* protogynous hermaphrodites). Based on histological examination, the presence of both male and female gametes in a gonad was never found and has never been reported in the literature. Therefore, a sex-change model does not account for the differences in sex ratios in *K. audax*. 3: Selectivity of fishing gears. The relationships between R_{SL} *v.* L_{EF} were not significantly different in the three gears (longline,

gillnet and harpoon fisheries) which basically covered different depth strata and captured fish of a wide size range. Hence, fishing gear probably had no effect on the composition of R_{SL}. 4: Temperature-induced or temperature-stress induced sex determination. There is a growing literature that suggests temperature changes, especially at critical times during ontological development, may greatly influence the sex of some gonochoristic fish species (Ospina-Alvarez & Piferrer 2008; Hattori *et al.*, 2009), but this hypothesis has never been tested in istiophorod billfish. 5: Animal behaviour during the spawning processes. Higher concentration of males were observed in the spawning area due to courting and coupling behaviour for fertilizing the released eggs during the spawning processes for *X. gladius* (Neilson *et al.*, 2013). Higher proportions of males (0.63) were also observed in the spawning season in this study, yet the R_S at size had similar patterns in the spawning season and non-spawning season. 6: A biological characteristic intrinsic to the species with its environmental requirements and habitats. Studies of *X. gladius* (Neilson *et al.*, 2013) indicated that females seemed to have a higher probability of covering a broader geographic area to meet their energy requirements in order to support the high production of eggs. The first three hypotheses above could not completely explain

the difference in R_{SL} for *K. audax* and proximate reasons [*e.g.* temperature, stochastic environmental events, such as El Niño–Southern Oscillation (ENSO), ocean salinity and acidity, and population genetic factors] warrant further investigation.

Kume & Joseph (1969) and Squire (1987) indicated that *K. audax* in the Pacific Ocean could be divided into two stocks separated by the equator based on size differences (larger southern stock: 160–200 cm L_{EF}; smaller northern stock: 140–180 cm L_{EF}). Ueyanagi & Wares (1975) reported that, in the eastern Pacific Ocean, the sizes of *K. audax* at the spawning grounds in the southern hemisphere were significantly larger than those in the northern hemisphere. These observations imply that there is geographic variation in body size for *K. audax,* which could be tested by examining distribution patterns and historical patterns of the temperature of mixed-layer depths (*i.e*. *K. audax* spend the majority of time in the mixed layer; Brill *et al.*, 1993).

SIZE AT MATURITY

The estimated L_{EF50} for female *K. audax* was *c.* 181 cm (*c.* 210 cm L_{LJF}), similar to that estimated by histological methods in the south-western Pacific Ocean (Kopf *et al.*, 2012). The present estimate appeared however, to be larger than the estimates of previous studies (Kume & Joseph, 1969; Eldridge & Wares, 1974). Based on I_G and mean oocyte diameter (fish were classified as mature when I_G > 1.0 and mean oocyte diameter > 300 μm), size at maturity was estimated at 155–165 cm L_{EF} for the *K. audax* caught in the eastern Pacific Ocean (Eldridge & Wares, 1974). Nevertheless, ovaries with mean oocyte diameter of MAGOs at *c.* 300 μm were classified as sexually immature in this study and most fish with I_G *c.* 1 were also sexually immature. Furthermore, the size at maturity estimated by Kume & Joseph (1969) ranged from *c.* 140 to 160 cm L_{EF} based on I_G (fish were classified as mature when $I_G \geqq 3$) for *K. audax* in the eastern Pacific Ocean and was smaller than the range determined in this study. The mean L_{EF} of mature females was *c.* 177 cm with a mean I_G of *c.* 4.0 in this study. Therefore, oocyte diameters or I_G might not be able to provide accurate estimates of size at maturity. In the present report, a combination of complimentary techniques histological methods, measuring oocyte diameters, I_G and macroscopic observation) was used to provide accurate criteria for

determining the sexual maturity of *K. audax*.

SPAWNING SEASON AND SPAWNING GROUNDS

The results f from this and previous studies indicate that the spawning season for *K. audax* occurs mainly in late spring and summer for the southern and northern hemispheres. The impetus for spawning is probably related to changes in sea temperature (González-Armas *et al.*, 2006) and the lowest temperature limit of larval distributions was around 24° C in the Indian and Pacific Ocean (Ueyanagi & Wares, 1975; Nakamura, 1985). The pattern of *K* exhibited roughly the reverse pattern of that of I_G values of females, suggesting that fish might decrease or suspend feeding activities to direct their energy for spawning (Begg & Hopper, 1997; Shimose *et al.*, 2009).

Compared with tropical tunas, *K. audax* are thought to form geographically disparate spawning aggregations (Kopf *et al.*, 2012). The occurrence of hydrated oocytes and POFs provided conclusive evidence that the western Pacific Ocean is a spawning ground for *K. audax*. The potential spawning grounds and spawning season

were suggested by several previous studies based on the occurrence of larvae or mature females (Table II). These areas need to be delimited so that important spawning habitat can be protected.

SPAWNING FREQUENCY

Kajikia audax are multiple spawners and their ovaries contain asynchronously developed oocytes and the frequency distributions of oocyte diameters are continuous and multimodal (Schaefer, 1987; Murua & Saborido-Rey, 2003). Spawning frequency obtained from POFs or hydrated oocytes is necessary for estimates of annual fecundity (Arocha & Bárrios, 2009). There is little empirical evidence however, for quantifying spawning frequency for *K. audax*. The spawning frequency estimated with the POF method was 3.7 days for reproductively active females, similar to 3.3 days estimated by the hydrated oocyte method for south-western Pacific female *K. audax* (Kopf *et al.*, 2012). Nevertheless, the estimated spawning frequency was longer (6.7 days) than that estimate by Kopf *et al.* (2012) if the hydrated oocyte method was used. Additionally, both Kopf *et al.* (2012) and this study observed the presence of

degenerated POFs with residual hydrated oocytes in the ovary. This is an important observation and suggests that the spawning frequency might be higher than the results suggest. In addition to sea temperature (Fitzhugh & Hettler, 1995), the differences in the estimates of spawning frequency may be due to the differences in method employed (the POF and hydrated oocyte method could lead to different estimates) and bias in fishing methods (it is possible the sampling area was at the fringe of the main spawning ground) (Shimose *et al.*, 2009).

Sampling time is required for using the hydrated oocyte method to estimate spawning frequency, which can be used to estimate the time of ovulation and spawning and also the duration of time that hydrated oocytes stay in the ovaries (Hunter and Macewicz, 1985*a*). Sampling time data, however, were not available for this study. Therefore, the estimates of approximate spawning frequency were based on the assumptions of spawning time and relative situations. Nevertheless, the spawning frequency estimated by the hydrated oocyte method were apparently longer than the estimates using the POF method for both sexually mature and reproductively active females. Hunter & Macewicz (1985*a*) indicated that spawning frequency estimated by the hydrated oocyte method varies due to short period from ovulation to spawning,

different vulnerability to fishing gears from hydrated females and different fish behaviours for hydrated females. Considering the above points, spawning frequency estimated by the POF method may be more accurate than the hydrated oocyte method.

The patterns of monthly mean I_G and percentage of fish in the spawning stage for females at different sizes indicated that female *K. audax* > 175 cm L_{EF} had an extended spawning season of about three months longer than females < 175 cm L_{EF} (April, May and August). This implies that larger females may have a longer spawning season than smaller females in the population.

The results of this study indicated that females with larger body sizes are more likely to have an extended spawning season than females with smaller body sizes. Furthermore, previous studies reported that batch fecundity tends to be related to female body sizes, with larger females of several species of billfishes having higher batch fecundity (Chiang *et al.*, 2006; Sun *et al.*, 2009; Sun *et al.*, 2015). Although there were only nine samples available for spawning frequency analysis in this study, which was not considered to be sufficient for estimating spawning frequency by body length classes, it seems reasonable to assume that spawning frequency may also relate to female body size, *i.e.* larger females are likely to have a shorter spawning interval

than smaller females. No significant difference was found however, in spawning frequency between different size groups for *Thunnus obesus* (Lowe 1839) (Sun *et al.*, 2013). More evidence is needed to support this assumption.

BATCH FECUNDITY

A length-stratified sampling approach should be used to estimate batch fecundity for improving the precision of the overall estimate. The estimate of batch fecundity was however, based on only three females, which was neither sufficient to provide the relationship between fecundities and body size nor sufficient to use a length-stratified sampling approach due to the small sample size of hydrated females. Ovaries containing hydrated oocytes were rarely sampled in this study. Possible factors for this observation might include: oocytes were rapidly ovulated after being hydrated [*i.e*. the duration that hydrated oocytes persisted on the ovaries was short (Schaefer, 1996)]; females spawned at specific times and locations that did not coincide with the geographical or depth-temperature strata covered by the commercial fishing fleet targeting tunas (Hunter *et al.*, 1986; Schaefer, 1987; McPherson, 1991; Nikaido *et al.*,

1991; Schaefer, 1996); oocytes might be rapidly ovulated when fish are undergoing stress associated with capture, confinement or temperature [*e.g. Katsuwonus pelamis* (L. 1758) (Kaya *et al.*, 1982)]. Although the relationship between body size and fecundity was not available in this study, the estimates of individual batch fecundities are provided to compare with estimates of fecundities in other areas. Owing to the small sample size and narrow range of body size of hydrated females, care should be taken in the use of estimates of batch fecundity. More hydrated ovaries from females of a wide range of body sizes should be collected and analysed in the future for estimates of batch fecundity for the population.

Comparisons of fecundity between species are best done based on relative fecundities, rather than a range of eggs, but estimates of relative fecundity were not provided by previous studies. Therefore, the ranges of batch fecundity were used here for comparing fecundities between different studies. The mean ± S.D. F_{B} was 4.4 ± 2.02 million eggs, which was similar to the 3.1 million eggs estimated by Kopf *et al.* (2012). Fecundity was estimated at 12 million eggs for a female *K. audax* of 182 cm L_{EF} in the western Indian Ocean with a gonad mass of 1532 g and the mean diameter of MAGO was 470 μm (Merrett, 1971). The fecundity estimated from three females

(155–180 cm L_{EF}) in the eastern Pacific Ocean ranged 11.3 to 28.6 million eggs; ovaries weighed 2580–3550 g and the mean diameter of MAGO was 600 μm (Eldridge & Wares, 1974). The mean diameter of oocytes selected by Merrett (1971) and Eldridge & Wares (1974) for estimating fecundity were merely 470 μm and 600 μm, respectively, these were probably vitellogenic oocytes (or migratory nucleus oocytes which were less developed than hydrated oocytes). Fecundities were overestimated if the counts of oocytes included vitellogenic oocytes and that is wrong for fecundity estimates.

Various authors have attempted to account for geographical differences in fecundities. Some of the observed patterns of variability might be attributed to: environmental factors such as sea temperature or food supply (Wootton, 1990); geographical variation [Schaefer (1987; 1998) indicated that the F_B of tunas increased with latitude]; semantic issue, *i.e* different criteria for oocytes are sometimes used for estimates of F_B (Hunter *et al.*, 1985; Schaefer, 1996); the method employed and the different methods of preservation, *i.e.* the oocyte size-frequency method takes the most advanced mode as the oocytes which are about to spawn, but it is widely accepted that oocytes shrink in preservation fluid (Ramon & Bartoo, 1997),

which could bias the estimate. Additionally, batch fecundity estimated by the hydrated oocyte method is generally back-calculated by gravimetric methods. Nevertheless, preserved samples lose mass in comparison with fresh samples (West, 1990; Ramon & Bartoo, 1997; White *et al.*, 2003), which may bias the estimate.

For istiophorid billfishes in the Pacific Ocean, the batch fecundity was estimated for *M. nigricans* at 2.11–13.5 million eggs (Sun *et al.*, 2009) and 0.98–5.85 million eggs (Shimose *et al.*, 2009); *I. indica* at 3.2–32 million eggs (Sun *et al.*, 2015); and *I. platypterus* at 0.29–2.49 million eggs (Chiang *et al.*, 2006*a*), 1.8–5.1 million eggs (Eldridge & Wares, 1974) and 0.42–2.5 million eggs (Hernández-Herrera *et al.*, 2000). Nakamura (1949) indicated that the fecundities of billfishes ranged between 1 and 1.2 million eggs and varied by species and size. Compared with *M. nigricans* and *I. indica*, in the Pacific Ocean, the body of *K. audax* is more laterally compressed, which might limit the body cavity in accommodating larger gonads and batch fecundity might be highly correlated with body size (Chiang *et al.*, 2006*a*; Sun *et al.*, 2009, 2015). Sample sizes obtained from previous studies however and the present report were probably too small to describe the relationship between batch fecundity and body size for *K. audax* with great precision.

In summary, *K. audax* are sexually dimorphic and the proportion of females increased with size between 140 and 210 cm L_{EF}. The spawning season occurred from April to August with a peak in June to July. *Kajikia audax* are multiple spawners and their oocytes develop asynchronously. The estimated length at 50% maturity (L_{EF50}) was *c.* 181 cm [*c.* 4.8 years of age, based on Sun *et al.* (2011)] for females. The reproductively active females spawned every 3.7 days on average. Mean ± S.D. F_B was 4.4 ± 2.02 million eggs by the hydrated oocyte method; average relative fecundity was 53.6 ± 13.9 oocytes g^{-1} M_R; and the average annual fecundity was 181.3 ± 48.3 million eggs.

The parameters estimated in this study are key information for stock assessments of *K. audax* in the north-western and central Pacific and will contribute to the conservation, management and sustainable yield of this species. In the near future, the vertical and horizontal movements and spawning dynamics of *K. audax* in the Indian and Pacific Oceans must be determined to aid in the conservation and protection of the spawning-stock biomass from various fisheries interactions. Genetic studies using appropriate nuclear markers to examine gene flow in real time (*e.g.* single nucleotide polymorphisms) among populations and locations, combined with

surveys for larval distribution and information from tagging data, needs to be undertaken to understand the temporal and spatial distribution better of spawning activity in *K. audax*.

This study was financially supported by the Ministry of Science and Technology (MOST) (former the National Science Council, NSC) and the Fisheries Agency of Council of Agriculture, Taiwan, through the grants MOST102-2313-B-002-019-MY3, NSC96-2313-B-002-040-MY3, 95AS-14.1.1-FA-F1(3) and 94AS-14.1.1-FA-F1(3) to C.-L. Sun. We thank S. Flores in the Southwest Fisheries Science Center for English editing of the manuscript.

References

Arocha, F. (2002). Oocyte development and maturity classification of swordfish from the north-western Atlantic. *Journal of Fish Biology* **60**, 13–27.

Arocha, F. & Bárrios, A. (2009). Sex ratios, spawning seasonality, sexual maturity and

fecundity of white marlin (*Tetrapturus albidus*) from the western central Atlantic. *Fisheries Research* **95**, 98–111.

Begg, G. A., & Hopper, G. A. (1997). Feeding patterns of school mackerel (*Scomberomorus queenslandicus*) and spotted mackerel (*S. munroi*) in Queensland east-coast waters. *Australian Journal of Marine &* Freshwater *Research* **48**, 565–571.

Bolger, T. & Connolly, P. L. (1989). The selection of suitable indices for the measurement and analysis of fish condition. *Journal of Fish Biology* **34**, 171–182.

Brill, R. W., Holts, D. B., Chang, R. K. C., Sullivan, S., Dewar, H. & Carey, F. G. (1993). Vertical and horizontal movements of striped marlin (*Tetrapturus audax*) near the Hawaiian Islands, determined by ultrasonic telemetry, with simultaneous measurement of oceanic currents. *Marine Biology* **117**, 567–574.

Chiang, W. C., Sun, C. L., Yeh, S. Z., Su, W. C. & Liu, D. C. (2006*a*). Spawning frequency and batch fecundity of the sailfish (*Istiophorus platypterus*) (Istiophoridae) in waters off Eastern Taiwan. *Zoological Studies* **45**, 483–491.

Chiang, W. C., Sun, C. L., Yeh, S. Z., Su, W. C., Liu, D. C. & Chen, W. Y. (2006*b*).

Sex ratios, size at sexual maturity and spawning seasonality of sailfish *Istiophorus platypterus* from eastern Taiwan. *Bulletin of Marine Science* **79**, 727–737.

Cuellar, N., Sedberry, G. R. & Wyanski, D. M. (1996). Reproductive seasonality, maturation, fecundity and spawning frequency of the vermilion snapper, *Rhomboplites aurorubens*, off the southeastern United States. *Fishery Bulletin* **94**, 635–653.

de Sylva, D. P. & Breder, P. R. (1997). Reproduction, gonad histology and spawning cycles of north Atlantic billfishes (Istiophoridae). *Bulletin of Marine Science* **60**, 668–697.

Domeier, M. L. (2006). An analysis of pacific striped marlin (*Tetrapturus audax*) horizontal movement patterns using pop-up satellite archival tags. *Bulletin of Marine Science* **79**, 811–825.

Fitzhugh, G. R. & Hettler, W. F. (1995). Temperature influence on postovulatory follicle degeneration in Atlantic menhaden, *Brevoortia tyrannus*. *Fishery Bulletin* **93**, 568–572.

Fournier, D. A. (2000). *An Introduction to AD Model Builder, vers. 4: for Use in*

Nonlinear Modelling and Statistics. Sydney, BC: Otter Research Ltd.

Friedland, K. D., Ama-Abasi, D., Manning, M., Clarke, L., Kligys, G. & Chambers, R. C. (2005). Automated egg counting and sizing from scanned images. Rapid sample processing and large data volumes for fecundity estimates. *Journal of Sea Research* **54**, 307–316.

González-Armas, R., Klett-Traulsen, A. & Hernandez-Herrera, A. (2006). Evidence of billfish reproduction in the southern Gulf of California, Mexico. *Bulletin of Marine Science* **79**, 705–717.

Hattori, R. S., Fernandino, J. I., Kishii, A., Kimura, H., Kinno, T., Oura, M., Somoza, G. M., Yokota, M., Strüssmann, C. A. & Watanabe, S. (2009). Cortisol-induced masculinization: does thermal stress affect gonadal fate in pejerrey, a teleost fish with temperature-dependent sex determination? *PLoS ONE* **4**, e6548.

Hernández-Herrera, A., Ramírez-Rodríguez, M. & Muhlia-Melo, A. (2000). Batch fecundity and spawning frequency of sailfish (*Istiophorus platypterus*) off the Pacific coast of Mexico. *Pacific Science* **54**, 189–194.

Hunter, J. R. & Macewicz, B. J. (1985*b*). Rates of atresia in the ovary of captive and wild northern anchovy, *Engraulis mordax*. *Fishery Bulletin* **83**, 119–136.

Hunter, J. R., Macewicz, B. J. & Sibert, J. R. (1986). The spawning frequency of skip jack tuna, *Katsuwonus pelamis*, from the South-Pacific. *Fishery Bulletin* **84**, 895–903.

Hunter, J. R., Macewicz, B. J., Lo, N. C. H. & Kimbrell, C. A. (1992). Fecundity, spawning and maturity of female Dover sole *Microstomus pacificus*, with an evaluation of assumptions and precision. *Fishery Bulletin* **90**, 101–128.

Hutchings, J. A. & Rowe, S. (2008). Consequences of sexual selection for fisheries-induced evolution: an exploratory analysis. *Evolutionary Applications* **1,** 129–136.

Hyde, J. R., Humphreys, R., Musyl, M., Lynn, E. & Vetter, R. (2006). A central north Pacific spawning ground for striped marlin, *Tetrapturus audax*. *Bulletin of Marine Science* **79**, 683–690.

Jensen, O. P., Ortega-Garcia, S., Martell, S. J. D., Ahrens, R. N. M., Domeier, M. L., Walters, C. J. & Kitchell, J. F. (2010). Local management of a "highly migratory species": the effects of long-line closures and recreational catch-and-release for Baja California striped marlin fisheries. *Progress in Oceanography* **86**, 176–186.

Kaya, C. M., Dizon, A. E., Hendrix, S. D., Kazama, T. K. & Queenth, M. K. K. (1982).

Rapid and spontaneous maturation, ovulation and spawning of ova by newly captured skipjack tuna, *Katsuwonus pelamis*. *Fishery Bulletin* **80**, 393–396.

Kopf, R. K., Davie, P. S., Pepperell, J. & Bromhead, D. (2011). Age and growth of striped marlin (*Kajikia audax*) in the southwest Pacific Ocean. *ICES Journal of Marine Science* **68**, 1884 – 1895.

Kopf, R. K., Davie, P. S., Bromhead, D. B. & Young, J. W. (2012). Reproductive biology and spatiotemporal patterns of spawning in striped marlin *Kajikia audax*. *Journal of Fish Biology* **81**, 1834–1858.

Kume, S. & Joseph, J. (1969). Size composition and sexual maturity of billfish caught by the Japanese longline fishery in the Pacific Ocean east of 130°W. *Bulletin Far Seas Fisheries Research Laboratory* **2**, 115–162.

McPherson, G. R. (1991). Reproductive biology of yellowfin tuna in the eastern Australian fishing zone, with special reference to the northwestern Coral Sea. *Australian Journal of Marine and Freshwater Research* **42**, 465–477.

Melo-Barrera, F. N., Felix-Uraga, R. & Quinonez-Velazquez, C. (2003). Growth and length–weight relationship of the striped marlin, *Tetrapturus audax* (Pisces: Istiophoridae), in Cabo San lucas, Baja California Sur, Mexico. *Ciencias Marinas*

29, 305–313.

Merrett, N. R. (1970). Gonad development in billfish (Istiophoridae) from the Indian Ocean. *Journal of Zoology* **160**, 355–370.

Merrett, N. R. (1971). Aspects of the biology of billfish (lstiophoridae) from the equatorial western Indian Ocean. *Journal of Zoology* **163**, 351–395.

Murua, H. & Saborido-Rey, F. (2003). Female reproductive strategies of marine fish species of the North Atlantic. *Journal of Northwest Atlantic Fishery Science* **33**, 23–31.

Nakamura, I. (1949). The tunas and their fisheries. *United States Bureau of Sport Fisheries and Wildlife Research Report* **82**, 115.

Nakamura, I. (1985). Billfish of the world. An annotated and illustrated catalogue of marlins, sailfishes, spearfishes and swordfishes known to date. In *FAO species catalogu: FAO Fisheries Synopsis* **125**. Rome: FAO.

Neilson, J., Arocha, F., Cass-Calay, S., Mejuto, J., Ortiz, M., Scott, G., Smith, C., Travassos, P., Tserpes, G. & Andrushchenko, I. (2013). The recovery of Atlantic swordfish: the comparative roles of the regional fisheries management organization and species biology, *Reviews in Fisheries Science* **21**, 59–97. doi:

10.1080/10641262.2012.754842

Nikaido, H., Miyabe, N. & Ueyanagi, S. (1991). Spawning time and frequency of bigeye tuna, *Thunnus obesus*. *Bulletin of National Research Institute of Far Seas Fisheries* **28**, 47–73 (in Japanese with English abstract).

Norman, B. & Stevens, J. (2007). Size and maturity status of the whale shark (*Rhincodon typus*) at Ningaloo Reef in Western Australia. *Fisheries Research* **84**, 81–86.

Ortega-Garcia, S., Klett-Traulsen, A. & Ponce-Diaz, G. (2003). Analysis of sportfishing catch rates of striped marlin (*Tetrapturus audax*) at Cabo San Lucas, Baja California Sur, Mexico and their relation to sea surface temperature. *Marine and Freshwater Research* **54**, 483–488.

Ospina-Álvarez, N. & Piferrer, F. (2008). Temperature-dependent sex determination in fish revisited: prevalence, a single sex ratio response pattern and possible effects of climate change. *PLoS ONE* **3**, e2837.

Ramon, D. & Bartoo, N. (1997). The effects of formalin and freezing on ovaries of albacore, *Thunnus alalunga*. *Fishery Bulletin* **95**, 869–872.

Schaefer, K. M. (1987). Reproductive biology of black skipjack, *Euthynnus lineatus*,

an eastern Pacific tuna. *Inter-American Tropical Tuna Commission Bulletin* **19**, 169–260.

Schaefer, K. M. (1996). Spawning time, frequency and batch fecundity of yellowfin tuna, *Thunnus albacares*, near Clipperton Atoll in the eastern Pacific Ocean. *Fishery Bulletin* **94**, 98–112.

Schaefer, K. M. (1998). Reproductive biology of yellowfin tuna (*Thunnus albacares*) in the eastern Pacific Ocean. *Inter-American Tropical Tuna Commission Bulletin* **21**, 205–221.

Schaefer, K. M. (2001). Reproductive biology of tunas. In *Tuna: Physiology, Ecology and Evolution*. (Block, B. A. & Steven, E. D., eds), pp. 225–270. New York, NY: Academic Press.

Shimose, T., Fujita, M., Yokawa, K., Saito, H. & Tachihara, K. (2009). Reproductive biology of blue marlin *Makaira nigricans* around Yonaguni Island, southwestern Japan. *Fisheries Science* **75**, 109–119.

Sippel, T., Holdsworth, J., Dennis, T. & Montgomery, J. (2011). Investigating behaviour and population dynamics of striped marlin (*Kajikia audax*) from the Southwest Pacific Ocean with Satellite Tags. *PLoS ONE* **6**, e21087.

Smith, K. D., Hall, N. G., de Lestang, S. & Potter, I. C. (2004). Potential bias in estimates of the size of maturity of crabs derived from trap samples. *ICES Journal of Marine Science* **61**, 906–912.

Squire, J. L. (1987). Striped marlin, *Tefrapfurus audax,* migration patterns and rates in the northeast Pacific Ocean as determined by a cooperative tagging program: its relation to resource management. *Marine Fisheries Review* **43**, 26–43. URL: http://spo.nmfs.noaa.gov/mfr492/mfr4925.pdf

Sun, C. L., Wang, S. P., Porch, C. E. & Yeh , S. Z. (2005). Sex-specific yield per recruit and spawning stock biomass per recruit for the swordfish, *Xiphias gladius*, in the waters around Taiwan. *Fisheries Research* **71**, 61–69.

Sun, C. L., Chang, Y. J., Tszeng, C. C., Yeh, S. Z. & Su, N. J. (2009). Reproductive biology of blue marlin (*Makaira nigricans*) in the western Pacific Ocean. *Fishery Bulletin* **107**, 420–432.

Sun, C. L., Yeh, S. Z., Chang, Y. J., Chang, H. Y. & Chu, S. L. (2013). Reproductive biology of female bigeye tuna *Thunnus obesus* in the western Pacific Ocean. *Journal of Fish Biology* **83**, 250–271.

Sun, C. L., Chang, H. Y., Liu, T. Y., Yeh, S. Z. & Chang, Y. J. (2015). Reproductive

biology of the black marlin, *Istiompax indica*, off southwestern and eastern Taiwan. *Fisheries Research* **166**, 12–20.

Wang, S. P., Sun, C. L. & Yeh, S. Z.. (2003). Sex Ratios and Sexual Maturity of Swordfish (*Xiphias gladius L.*) in the Waters of Taiwan. *Zoology Studies* **42**, 529–539.

Wang, S. P., Sun, C. L., Yeh, S. Z., Chiang, W. C., Su, N. J., Chang, Y. J. & Liu, C. H. (2006). Length distributions, weight–length relationships and sex ratios at lengths for the billfishes in Taiwan waters. *Bulletin of Marine Science* **79**, 865–869.

West, G. (1990). Methods of assessing ovarian development in fishes – a review. *Australian Journal of Marine and Freshwater Research* **41**, 199–222.

White, G. G., Munroe, T. A. & Austin, H. M. (2003). Reproductive seasonality, fecundity and spawning frequency of tautog (*Tautoga onitis*) in the lower Chesapeake Bay and coastal waters of Virginia. *Fishery Bulletin* **101**, 424–442.

Wootton, R. J. (1990). *The Ecology of Teleost Fishes*. London: Chapman Hall.

Young, J., Drake, A., Brickhill, M., Farley, J. & Carter, T. (2003). Reproductive dynamics of broadbill swordfish, *Xiphias gladius*, in the domestic longline fishery off eastern Australia. *Marine and Freshwater Research* **54**, 315–332.

Electronic References

Eldridge, M. B. & Wares, P. G. (1974). Some biological observations of billfish taken in the eastern Pacific Ocean 1967–1970. In: *Proceedings of International Billfish Symposium* Part 2 (Shomura R.S. & Williams, F., eds), 89–101. *NOAA Technical Report NMFS SSRF* **675**. Available at https://swfsc.noaa.gov/publications/CR/1974/7408.PDF

Hunter, J. R., Lo, N. C. & Leong, R. J. (1985). Batch fecundity in multiple spawning fishes. *NOAA Technical Report NMFS* **36**, 67–78. Available at https://swfsc.noaa.gov/publications/CR/1985/8547.PDF

Hunter, J. R. & Macewicz, B. J. (1985*a*). Measurement of spawning frequency in multiple spawning fishes. In: *An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy,* Engraulis mordax (Lasker, T., ed.), pp. 79–94. *NOAA Technical Report NMFS* **36**. Available at https://swfsc.noaa.gov/publications/CR/1985/8544.PDF

Jones, S. & Kumaran, M. (1964). Distribution of larval billfishes (Xiphiidae and Istiophoridae) in the Indo-Pacific with special reference to the collections made by the Danish Dana Expedition. In *Proceedings of the Symposium on Scombroid*

Fishes, Part 1, pp. 483–498. Mandapam Camp: The Marine Biological Association of India. Available at http://eprints.cmfri.org.in/2418/1/Article_25.pdf

Sun, C. L., Hsu, W. S., Su, N. J., Yeh, S. Z., Chang, Y. J. & Chiang, W. C. (2011). Age and growth of striped marlin (*Kajikia audax*) in the waters off Taiwan: A revision. Working document submitted to the Intercessional Workshop of the Billfish Working Group of the International Scientific Committee for Tuna and Tuna-like Species in the North Pacific Ocean: ISC/11/BILLWG-2/07. Available at http://isc.fra.go.jp/pdf/BILL/ISC11_BILL_2/ISC11BILLWG2_WP07.pdf

Ueyanagi, S. (1964). Description and distribution of larvae of five istiophorid species in the Indo-Pacific. In *Proceedings of the Symposium on Scombroid Fishes*, Part 1, pp. 499–528. Mandapam Camp: The Marine Biological Association of India. Available at http://eprints.cmfri.org.in/2418/1/Article_25.pdf p.

Ueyanagi, S. & Wares, P. G. (1975). Synopsis of biological data on striped marlin, *Tetrapturus audax* (Philippi), 1887. I In: *Proceedings of International Billfish Symposium* Part 2 (Shomura R.S. & Williams, F., eds), 132–159. *NOAA Technical Report NMFS SSRF* **675**. Avaiable at

https://swfsc.noaa.gov/publications/cr/1974/7447.pdf

TABLE I. Macroscopic and microscopic characters of the gonad maturity stages of female *Kajikia audax*

Maturity classification	Sexual reproductive state	Macroscopic description of gonad appearance	Oogenesis condition	MAGO	I_G (mean ± S.D.)	Diameter of MAGO (μm, mean ± S.D.)	*n*
Immature stage	Immature, inactive	Gonads are small, finger-like and firm; oocytes are not visible.	No vitellogenesis; only primitive oogonia or chromatin-nucleolar oocytes; no atresia.	Chromatin-nucleolar oocytes	0.2 ± 0.2	37 ± 11	36
Developing stage	Immature, inactive	Gonads are larger than the previous stage; tubular, firm, and pinkish in colour; oocytes are not yet detectable with the naked eye.	No vitellogenesis; mainly chromatin-nucleolar oocytes and perinucleolar oocytes; no atresia.	Perinucleolar oocytes	0.4 ± 0.2	64 ± 17	122

Maturing stage	Immature, inactive	Gonads are vascularized, orange in colour; ovigerous folds are clearly visible when the ovary is cut.	Vitellogenesis begins; previtellogenic oocytes present, with perinucleolar oocytes and some chromatin-nucleolar oocytes; no atresia.	Previtellogenic oocytes	1.7 ± 1.1	243 ± 94	22
Mature stage	Mature, active	Gonads are tumescent, highly vascularized, reddish orange in colour; opaque oocytes can be obviously seen when ovary is cut.	Vitellogenesis ongoing. Vitellogenic oocytes present, with few previtellogenic oocytes; sometimes atretic oocytes are present.	Vitellogenic oocytes	4.9 ± 1.9	416 ± 86	21
Spawning stage	Mature, active	Gonads are swollen, highly vascularized and bloodshot in appearance, reddish	Hydrating. Hydrated oocytes, migratory nucleus oocyte, and vitellogenic oocytes;	Hydrated oocytes	7.6 ± 2.6	626 ± 206	12

		orange in colour, transparent (hydrated) oocytes are easily detectable.	sometimes postovulatory follicles were also present.				
Spent or resting stage	Mature, inactive	Gonads are much smaller than spawning stage, flaccid, without resilience, greyish pink in colour, no vascularization; few or no oocyte can be detectable.	Vitellogenesis ceasing; ± and [2] atretic oocytes frequently observed, vitellogenic oocytes, previtellogenic oocytes, and perinucleolar oocytes also present.	Vitellogenic oocytes or perinucleolar oocytes	1.3 ± 0.9	139 ± 102	23

I_G, gonado-somatic index; MAGO, most advanced group of oocytes.

TABLE II. The potential spawning grounds and spawning seasons estimated for *Kajikia audax* in different areas by different authors

Author	Sampling area	Spawning season	Peak season	Based on
This study	Western Pacific Ocean	April–August	June–July	Mature females (by histological techniques, I_G, and diameters of MAGO)
Nakamura (1949)	Taiwan waters		April–May	Mature female (with ripe ova)
Jones & Kumaran (1964)	Western Indian Ocean	December–January		Appearance of larvae
	Eastern Indian Ocean	October–November		Appearance of larvae
Ueyanagi (1964)	North-western Pacific Ocean	May–June		Appearance of larvae
	South-western Pacific Ocean	October–January		Appearance of larvae
Kume & Joseph (1969)	North Pacific Ocean		May–June	Mature females (by I_G)
	South Pacific Ocean		November–December	Mature females (by I_G)
Eldridge & Wares (1974)	Eastern Pacific Ocean		June–July	Mature females (by I_G)
Ueyanagi & Wares (1975)	Waters off Socorro Island in eastern Pacific Ocean	June–October		Mature females (with ripe and running ripe ovaries)
Armas *et al.* (2006)	southern Gulf of California,		July–August	Appearance of larvae and mature females (by

	Mexico			histological techniques and I_G)
Hyde *et al.* (2006)	Central North Pacific Ocean		May (samples were collected only in May)	Appearance of larvae
Kopf *et al.* (2012)	South-west Pacific Ocean	October–January	November–December	Mature females (by histological techniques)

I_G, gonado-somatic index; MAGO, most advanced group of oocytes (μm).