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# **Reproductive biology of** *Zearaja chilensis* (Chondrichthyes: Rajidae) in the south-east Pacific Ocean

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Between 2000 and 2002, three artisanal landing sites were sampled in southern Chile, with data on population structure and reproductive development collected from 5477 yellownose skates *Zearaja chilensis*. Total length  $(L_T)$  ranged from 33 to 158 cm for females and 34 to 155 cm for males. No sexual dimorphism was evident in disc size (length or width) or in  $L_T$ -mass relationships. The smallest mature female was 95 cm  $L_T$  and the size at which 50% were mature  $(L_{T50})$  was 109 cm. Males matured between 80 and 90 cm  $L_T$  with a  $L_{T50}$  of 88 cm. Although the largest *Z. chilensis* captured by the artisanal fishery was 155 cm  $L_T$ , 89% of landings comprised relatively small, immature fish. This situation may compromise the stock integrity if intrinsic vulnerability and probable long-life span of *Z. chilensis* are considered. Consequences for the survival of the species and possible signs of a fishery collapse must be reviewed by management authorities by consideration of both artisanal and industrial landings in Chile. © 2011 The Authors

Key words: fishery management; size-at-maturity; yellownose skate.

### **INTRODUCTION**

Skates are cartilaginous benthic fishes that occupy the same spatial niche as demersal teleosts, such as flatfishes, hakes and eels (Walker & Hislop, 1998). Unlike the majority of bony fishes, however, skates have intrinsic life-history patterns that make them highly susceptible to commercial over-exploitation (Dulvy *et al.*, 2000; Dulvy & Reynolds, 2002; Frisk, 2010). Skates present unique challenges for fishery management (Stevens *et al.*, 2000) and conservation (Roberts & Hawkins, 1999; Dulvy & Reynolds, 2002) due to their low fecundity, late age at sexual maturity and relatively high longevity (Holden, 1977; Frisk *et al.*, 2001). Over the last decade, evaluation of the conservation status of skates and rays in several geographic areas has established that commercially exploited species could be seriously threatened (Dulvy &

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Reynolds, 2002) or even extirpated (Dulvy et al., 2003) from a given fishing area. For example, large declines in populations and local extinctions of North Atlantic Ocean skates, such as the common skate Dipturus batis (L. 1758), the barndoor skate Dipturus laevis (Mitchill 1818), the white skate Rostroraja alba (Lacépède 1803) and thornback ray Raja clavata L. 1758, have resulted from commercial fishing activities (Dulvy & Forrest, 2010). Resilience to fishing pressure varies among skate species, however, and while some populations have decreased dramatically (Brander, 1981; Iglésias et al., 2009), others have not, and, in some cases, may even have increased because of reorganization of ecological niches (Walker & Hislop, 1998; Dulvy et al., 2000; Dulvy & Forrest, 2010). The vulnerability of batoid populations is mostly assessed through the examination of fisheries catch trends (Dulvy et al., 2000), but in many South American countries, including Argentina (Massa & Hozbor, 2003), Brazil (M. C. Oddone, pers. obs.) and Chile (Lamilla et al., 2005), skates and rays have generally been recorded as a single unidentified category (e.g. rays, including rajoids and Myliobatiformes) in official fisheries records for several decades. From 2003, however, landings of the target species [the yellownose skate Zearaja chilensis (Guichenot 1848)] and by-catch (a combination of six other skate species) in the Chilean skate fishery have been recorded separately.

Zearaja chilensis is the most commercially valuable batoid that inhabits the continental slope of southern South America from Uruguay (south-western Atlantic Ocean) to central Chile (south-eastern Pacific Ocean) (Menni & López, 1984) and to the east of the Falkland Islands (Agnew et al., 2000). The first recorded landings of Z. chilensis in Chile were made in the early 1970s (Bahamonde et al., 1996). Since then, the catch effort for this species has gradually intensified especially in the small-scale coastal fleet located between Valdivia ( $40^{\circ}$  S) and Aisén ( $45^{\circ}$  S), which is considered an artisanal fishery by local management authorities (Licandeo et al., 2006). In addition to this target fishery, Z. chilensis is caught as by-catch in two industrial trawl fisheries: the southern hake Merluccius australis (Hutton 1872) fishery that operates south of  $41^{\circ}$  28.6' S (Quiroz *et al.*, 2009) and the common hake *Merluccius gayi* (Guichenot 1848) bottom trawl fishery that operates between  $34^{\circ}$ 28' S and 41° 28.6' S (Gálvez et al., 1998). These artisanal and industrial fishing grounds do not overlap due to local management measures which restrict the target catch of Z. chilensis for industrial vessels to south of  $41^{\circ}$  28.6' S and 9.26 km (5 nautical miles) out into Chilean waters. According to official records, the annual national landing (from artisanal and industrial fleets) of Z. chilensis has increased from 1317 in 1990 to 3010 in 2007, with an average landing of 2663 for this period (SERNAP, 2007). After a biological closure was imposed on the artisanal fisheries in 2008, the annual landing by industrial fleets has averaged 1727 for 2008 and 2009 (SERNAP, 2009). Despite this closure, a fishing quota of 2307 and 642 were granted to the artisanal fishery during 2009 and 2010.

Although size at maturity (Fuentealba *et al.*, 1990; Bahamonde *et al.*, 1996) and the life history of this species (Licandeo *et al.*, 2006; Quiroz *et al.*, 2009) have been determined previously, based on landings in southern bottom longline fishery grounds, there is a lack of information about the northern coastal artisanal fishery population. In order to provide additional information on the reproductive biology of *Z. chilensis*, sexual dimorphism was assessed and size at maturity was determined from the artisanal exploited stock. Further details of the sexual development of both males and females, as well as estimates of fecundity are also presented.

# **MATERIALS AND METHODS**

Zearaja chilensis landings were sampled from three localities in southern Chile: Queule (39·4° S), Corral (39·8° S) and Bahía Mansa (40·5° S) (Fig. 1), between September 2000 and December 2002. All specimens were caught by artisanal vessels of 6–15·5 m length using bottom-set longlines of 300–400 hooks (M/K No. 7). Total length ( $L_T$ ), disc width ( $W_D$ ) and length ( $L_D$ ) were recorded for each specimen following Hubbs & Ishiyama (1968) and Last *et al.* (2008), along with sex and left clasper post-cloacal length ( $L_C$ ). Measurements were made to the nearest cm. Total body mass ( $M_T$ ) and liver mass ( $M_L$ ) were determined for all Z. *chilensis* and recorded to the nearest gram. The hepato-somatic index ( $I_H$ ) was calculated as:  $I_H = 100 (M_L M_T^{-1})$ . Ovary and oviducal-gland mass and maximum ovarian-follicle diameters were measured for females, while testes and epigonal organ mass were determined for males. All measurements are presented as the mean  $\pm$  s.D.

The  $\chi^2$  goodness of fit (Sokal & Rohlf, 1987) was used to test whether the ratio of males to females varied significantly from a 1:1 ratio. Linear relationships between  $L_T$ , partial lengths ( $L_D$  and  $W_D$ ) and  $M_T$  were fitted separately for sex and tested for sexual dimorphism using ANCOVA. Regressions were fitted to raw data, except for  $L_T$  and  $M_T$ , which were  $\log_{10}$ 



FIG. 1. Map of Chile (a) showing location of study area (inset box), and (b) three Zearaja chilensis landings sites ( $\odot$ ).

transformed. A *t*-test was used to evaluate differences between the  $I_{\rm H}$  of mature and immature *Z. chilensis* according to sex. Paired *t*-tests were performed to explore possible differences in left *v*. right ovary mass, oviducal-gland mass and ovarian-follicle size and number, and differences between epigonal organ and testes mass. Relationships between ovary–oviducal-gland mass and  $L_{\rm T}$ , and between  $L_{\rm C}$  and  $L_{\rm T}$  were used to further assess the onset of maturity (Capapé & Quignard, 1974; Restrepo & Watson, 1991). One-way ANOVA was used to test for significant differences in mean maximum ovarian-follicle diameter and  $I_{\rm H}$  in available months (September to December).

Binomial maturity data (immature 0, mature 1) were determined by sex at 10 cm intervals. Size at maturity in both sexes were calculated by fitting the following logistic curve (by minimization of the least squares), to the relationship between the fraction of mature males or females as a function of  $L_{\rm T}$ ,  $Y = [1 + e^{-(a+bX)}]^{-1}$ , where Y is the fraction of mature individuals in  $L_{\rm T}$  class X, and a and b are the model coefficients. The ratio *a:b* represents the size at which 50% of the fish were mature (Mollet *et al.*, 2000; Oddone *et al.*, 2005). Females were considered mature when vitellogenic (yellow) ovarian follicles and egg capsules were present (Chen *et al.*, 1997; Oddone *et al.*, 2005). Individual males with sperm in their seminal vesicles and calcified, rigid claspers were considered mature (Sosebee, 2005). All data analyses were performed using the freeware software PAST (Hammer *et al.*, 2001), with significance accepted at P < 0.05.

### RESULTS

# CATCH COMPOSITION

A total of 5477 specimens were examined (2356 females and 3121 males). The catch composition sex ratio of 1:0.75 had a significant bias towards males ( $\chi^2 = 53.692$ , d.f. = 1, P > 0.001). Body size of females caught ranged between 33 and 158 cm  $L_T$  (75.4 ± 12.6 cm); males ranged between 34 and 155 cm  $L_T$  (85.4 ± 7.2 cm) (Fig. 2). The  $L_T$  and  $M_T$  relationship was influenced by sex ( $M_T = 1e.05 L_T^{2.9165}$  in females and  $M_T = 3e.05 L_T^{2.6614}$  in males), but no significant differences were observed between slopes (ANCOVA,  $F_{1,5202} = 0.293$ ; P > 0.05) [Fig. 3(a)]. Size-related sexual dimorphism was not obvious despite significant differences when comparing slopes between  $L_T$  and  $L_D$  [ANCOVA,  $F_{1,5254} = 12.14$ ; P < 0.001; Fig. 3(b)] or  $W_D$  [ANCOVA,  $F_{1,4848} = 4586$ ; P < 0.001; Fig. 3(c)]. The overall mass of catches was 24.6 tonnes with mean ± s.D. of 4530 ± 3625 g with a minimum of 800 g and maximum of 3800 g.

## LIVER MEASUREMENTS

The mean  $\pm$  s.D.  $I_{\rm H}$  was  $5 \cdot 135 \pm 1 \cdot 443$  for males, and  $5 \cdot 157 \pm 1 \cdot 254$  for females, with no significant differences observed between months ( $F_{4,244} = 10 \cdot 17$ ,  $P > 0 \cdot 05$ ) for either sex (Fig. 4). Significant differences were observed between  $I_{\rm H}$  of mature and immature males ( $t = -3 \cdot 524$ , d.f. = 2802,  $P < 0 \cdot 001$ ) and between mature and immature females ( $t = -3 \cdot 939$ , d.f. = 2064,  $P < 0 \cdot 001$ ), with relatively heavier livers present in mature specimens. There was no significant difference between overall mature male and female  $I_{\rm H}$  (t = 1.916, d.f. = 4074, P > 0.05).

# FEMALE REPRODUCTIVE BIOLOGY

Asymmetry of the reproductive tract was not observed in mature Z. *chilensis*, with no significant differences between the mass of the left  $(127.08 \pm 52.22 \text{ g})$  and right



FIG. 2. Total length ( $L_T$ ) absolute frequency distribution of Zearaja chilensis (n = 5477) [ $\blacksquare$ , males (n = 3121) and  $\Box$ , females (n = 2356)].

 $(130.57 \pm 48.03 \text{ g})$  ovaries (t = 0.588, d.f. = 2084, P > 0.05). Similarly, there were no significant differences between left  $(43.57 \pm 38.65 \text{ g})$  and right  $(44.44 \pm 40.24 \text{ g})$ oviducal-gland masses (t = 0.187, d.f. = 2132, P > 0.05). Significant differences were detected, however, in ovarian-follicle counts between left (142.91  $\pm$  48.73) and right (163.26  $\pm$  62.39) ovaries (t = 3.145, d.f. = 2368, P < 0.001). Ovarian follicles were 0.3-4.1 cm in diameter and 0.04-19.21 g in mass. No significant differences (t = 1.730, d.f. = 2110, P > 0.05) were observed between maximum diameters of ovarian follicles in left (3.38  $\pm$  0.35 cm) and right (3.50  $\pm$  0.43 cm) ovaries. The minimum size at first maturity occurred at c. 95 cm  $L_{\rm T}$  based on ovary and oviducal-gland mass [Fig. 5(a), (b)]. Substantial variations in ovarian-follicle mass were observed for follicles of the same diameter as a result of differences in yolk consistency [Fig. 5(c)]. Mean maximum ovarian-follicle diameter ranged from  $3.45 \pm 0.24$  cm in September to  $4.10 \pm 0.43$  cm in December, although no significant differences were observed between months [ANOVA,  $F_{3,273} = 4.0$ , P >0.05; Fig. 4(a)]. The size at which 50% of the fish were mature  $(L_{T50})$  was 109 cm  $L_{\rm T}$  [Fig. 5(d)], and mature females represented only 8% of the female catch at this size.

#### MALE REPRODUCTIVE BIOLOGY

The  $L_{\rm C}$  varied markedly between mature specimens with the smallest mature male recorded at 62 cm  $L_{\rm T}$ , whereas some individuals were immature at c. 95 cm  $L_{\rm T}$  [Fig. 6(a)]. No significant differences were found between left (11·2 ± 5·6 g, range = 0·08-26·5 g) and right (11·4 ± 4·8 g, range = 0·09-27·57 g) testis masses (t = 0·245, d.f. = 2214, P > 0·05). No significant differences were found between left (38·8 ± 14·2 g, range = 2·39-82·44 g) and right (38·7 ± 10·4 g, range = 1·83-81·28 g) epigonal-organ masses (t = 0·075, d.f. = 2214, P > 0·05). Based on



FIG. 3. Relationships between total length  $(L_T)$  and (a) total body mass  $(M_T)$ , (b) disc width  $(W_D)$  and (c) disc length  $(L_D)$  of male  $(\bullet)$  and female  $(\bigcirc)$  Zearaja chilensis. The curves were fitted by (a) male y = 2.706x - 1.572 and female y = 2.951x - 2.036, (b) male y = 0.596x - 1.263 and female y = 0.628x - 3.185 and (c) male y = 0.708x + 5.094 and female y = 0.748x + 2.641.

the calculated  $L_{T50}$  of 88 cm  $L_T$  for males [Fig. 6(b)], 13.6% of males in the catch were mature.

# DISCUSSION

The sex ratio in *Z. chilensis* was biased towards males, which suggests some sexual segregation in the study area although the reason for this is not known. Differences in sexual composition may be a consequence of behaviour, and many deep-sea skates are known to segregate by sex, size and maturity (Ebert *et al.*, 2008).



FIG. 4. Monthly variation of maximum ovarian-follicle diameter ( $\bullet$ ) and hepato-somatic index ( $I_{\rm H}$ ; O) of *Zearaja chilensis*. Values are mean  $\pm$  s.p.

In elasmobranchs, size is commonly regarded as a major secondary sexual characteristic, with females often larger and heavier than males (Cortés, 2000). This characteristic seems to be common in rajoid species (Lamilla *et al.*, 1984; Smith & Merriner, 1986; Martin & Cailliet, 1988), but while the size-frequency distribution



FIG. 5. Relationships between total length  $(L_T)$  and (a) ovary mass and (b) oviducal-gland mass, (c) ovum diameter and ovum mass and (d) logistic model (\_\_\_\_) fitted for the relationship between  $L_T$  and proportion of mature female *Zearaja chilensis*. ( $\bullet$ , immature; O, mature;  $\blacksquare$ , the observed proportion of mature individuals in each size interval) (d) The curve was fitted by:  $y = [1 + e^{-(8\cdot185+0\cdot075x)}]^{-1}$ .



FIG. 6. Relationships between (a) total length  $(L_T)$  and clasper length  $(L_C)$  and (b) logistic model (\_\_\_) fitted for the relationship between  $L_T$  and proportion of mature males of *Zearaja chilensis*. ( $\bullet$ , immature; O, mature;  $\blacksquare$ , the observed proportion of mature individual in each size interval. (b) The curve was fitted by:  $y = [1 + e^{-(11.649+0.132x)}]^{-1}$ .

in the current study indicated that Z. chilensis >100 cm  $L_T$  were predominantly female, in absolute terms, it appeared that males and females attained similar maximum sizes. There may be a selective advantage for female Z. chilensis to grow to a larger size than males (Licandeo *et al.*, 2006) in order to accommodate relatively large ova (that mature at c. 41 mm in diameter) and the associated reproductive tract. A large body size would in itself provide a large energy reserve to draw upon for egg production and may also influence the diet, by allowing larger and possibly different species of prey to be taken as seen in other skate species (Brickle *et al.*, 2003; Kyne *et al.*, 2008). In some species, however, such as the ovoviviparous apron ray *Discopyge tschudii* Heckel 1846, the length–mass relationship and body size (measured as disc width) indicate that males grow larger than females (García, 1984; Pequeño *et al.*, 1988). A similar situation has also been reported for *Bathyraja interrupta* (Gill & Townsend 1897), *Bathyraja minispinosa* Ishiyama & Ishihara 1977 and *Bathyraja parmifera* (Bean 1881) (Ebert, 2005), as well as for *Leucoraja wallacei*  (Hulley 1970), *Raja straeleni* Poll 1951, *Raja barnardi* (Norman 1935) and *Raja leopardus* (von Bonde & Swart 1923) (Ebert *et al.*, 2008). Pequeño *et al.* (1988) argued that this could be an adaptation to activities such as mating or swimming behaviour in those species that undergo significant migrations. It is also common to find that size-at-maturity varies according to sex, with males commonly reaching maturation at smaller sizes than the females (Wetherbee, 1996; Ellis & Shackley, 1997; Girard & Du Buit, 1999). In *Z. chilensis*, males matured at *c.* 80% of the size at maturity of females, but Mabragaña *et al.* (2002) suggest that this dimorphism could be due to local ecological characteristics rather than to biological peculiarity.

The  $I_{\rm H}$  was significantly larger in mature fish compared to immature specimens in both sexes, which is presumably correlated with larger energy reserves in animals in which growth has slowed. Interestingly, while female elasmobranchs often have larger livers than males, due to requirements for egg and yolk development (Quiroz *et al.*, 2011), these differences are not reflected in the present findings. Unlike mature males, the liver of mature females produces vitellogenin, the precursor protein molecule of vitellogenic material during the oocyte maturation. But according to the  $I_{\rm H}$ , there are not significant differences between both mature males and females despite females needing to store great amounts of lipids in their livers during previtellogenic and gestation phases. This is consistent with mature females of the Aleutian skate *Bathyraja aleutica* (Gilbert 1896) reported by Ebert (2005). Mabragaña *et al.* (2002) and Oddone & Velasco (2006) described similar observations in the smallnose fanskate *Sympterygia bonapartii* Müller & Henle 1841 and the eyespot skate *Atlantoraja cyclophora* (Regan 1903) from the south-west Atlantic ocean.

While many elasmobranchs have a single functional ovary (Dodd *et al.* 1983; Hamlett & Koob, 1999; Musick & Ellis, 2005), ovarian symmetry, as observed in *Z. chilensis*, is common in all skates (Oddone *et al.*, 2007). The symmetry of paired structures extended to other part of the reproductive system, which is also consistent with previous studies on skates that have shown no difference between oocyte number and oviducal-gland width (Mabragaña *et al.* 2002; Ebert 2005; Ebert *et al.* 2008). While male maturity occurred at *c.* 88 cm  $L_T$ , however, the relationship between  $L_C$  and  $L_T$  during the maturation phase suggested that there was an unusually high degree of plasticity in the size at maturity compared to other skates (Ebert, 2005).

Despite relatively large geographical differences between sampling locations, the results from the present study provide similar estimates of sexual maturity to those made previously in the south-western Pacific Ocean (Licandeo *et al.*, 2006) and the south-western Atlantic Ocean (Oddone *et al.*, 2005; Paesch & Oddone, 2008), suggesting that size at maturity is a conservative trait in this species as a compensatory dynamic over latitudinal gradients in the entire fishery and occurrences zones (Frisk, 2010).

Large batoids appear to be longer-lived, slower growing and have lower reproductive output among the elasmobranchs, which makes them highly susceptible to overexploitation (Dulvy *et al.*, 2000). Besides, fisheries may change abundance and distribution of skates as most of these fisheries are biased towards larger individuals (Jennings *et al.*, 1999; Frisk, 2010). Indeed, there is a negative correlation between skate body size and population trend (Walker & Hislop, 1998; Dulvy *et al.*, 2000), and *Z. chilensis* is no exception. According to Licandeo *et al.* (2006), the main targets of the *Z. chilensis* fishery in Chile are immature fish. The present results indicate that 106 cm  $L_{\rm T}$  females (c. 14 years-old) and 86 cm  $L_{\rm T}$  males (c. 11 years-old) represent 85% of total catch and, as small Z. *chilensis* (<35 cm  $L_{\rm T}$ ) are discarded, there is evidence of a fishery highly biased towards mid-sized Z. *chilensis* that may compromise the stock integrity.

Reproductive seasonality could not be determined given the scarcity of gravid females and the limited number of months sampled by this fishery. The monthly mean maximum ovarian-follicle diameter and  $I_{\rm H}$  did not, however, differ significantly across the 4 month study period which is consistent with the suggestion that deepwater skate species may not exhibit a defined reproductive or egg-laying season (Braccini & Chiaramonte, 2002; Mabragaña *et al.*, 2002; Ruocco *et al.*, 2006; Ebert *et al.*, 2008). It should be noted though that the data in the current study would also be consistent with a defined reproductive season that coincided with the sampling period, and further studies of this species across all months of the year are necessary to resolve the issue. Fishing records of *Z. chilensis* in the southern Falkland Islands demonstrated that an intense fishing operation culminated in a drastic stock collapse (Agnew *et al.*, 2000). The present results indicate that 89% of the females captured were below size at maturity, which may pose a serious threat to their population.

The size structure of the *Z*. *chilensis* catch in the Valdivia fishing zone  $(39.4^{\circ} \text{ S} \text{ to } 40.5^{\circ} \text{ S})$  differs considerably when compared to the reported catch from fishing grounds to the south: around Chiloé Island  $(41.5^{\circ} \text{ S to } 43.5^{\circ} \text{ S})$ , Moraleda Channel  $(43.8^{\circ} \text{ S to } 45.9^{\circ} \text{ S})$  and Castro-Magallanes Channel  $(51.0^{\circ} \text{ S to } 54.0^{\circ} \text{ S})$  (Quiroz *et al.*, 2009). In the case of females, mature individuals comprised only 8-10%



FIG. 7. Accumulated size frequencies of *Zearaja chilensis* (a) females and (b) males by fishing grounds (modified from Quiroz *et al.*, 2009): Valdivia 39.4° S to 40.5° S (\_\_\_\_), Chiloé Island 41.5° S to 43.5° S (\_\_\_\_\_), Moraleda Channel 43.8° S to 45.9° S (\_\_\_\_) and Castro-Magallanes Channel 51.0° S to 54.0° S (\_\_\_\_).

of the catch in the northern fishing grounds (Valdivia, Chiloé Island and Moraleda Channel), but closer to 50% in the most southerly fishing grounds [Fig. 7(a)]. This pattern is repeated for males, with mature individuals comprising c.75% of the catch in the Castro-Magallanes Channel zone compared with c. 14% in the Valdivia zone [Fig. 7(b)]. Evidence of an increasing median  $L_T$  ( $L_T$  of 50% of the catch) with respect to latitude can be observed in both sexes even though the same fishing gear is used in all zones. The reason for these differences in catch size structure may be the result of fishing effort moving south as the southernmost fishing grounds have been developed relatively recently compared to the Valdivia zone (Quiroz et al., 2011). If the southern fishery catch composition represents a relatively natural situation, then the catch size structure in the northern fisheries suggests that they are at particular risk of collapse if the tonnage landed is maintained, as mature Z, chilensis numbers appear to have been overfished. Growth rates and recruitment need to be evaluated in each fishery zone for a better understanding of the population ecology and dynamics of Z. chilensis in southern Chile. If the intrinsic vulnerability and long life spans of Z. chilensis are considered, the implications for survival and the signs of an imminent fishery collapse need to be reviewed by management authorities before local extirpation occurs, such as have been documented for other species of skates by Dulvy & Forrest (2010).

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