Initial assessment of age, growth and reproductive parameters of the southern fiddler ray *Trygonorrhina fasciata* (Müller & Henle, 1841) from South Australia

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Abstract. We present preliminary age, growth and reproductive parameters of the southern fiddler ray (*Trygonorrhina fasciata*) from the South Australian gulf waters based on 43 specimens. Age estimates, based on counts of growth bands in the vertebrae, were used to calculate the parameters of the von Bertalanffy growth function: L_∞, = 1129 mm total length, $k = 0.13$, $t_0 = -2.55$ years (for both sexes combined). Based on the examination of internal and external morphology, males mature at 650 to 700 mm total length; with the smallest of three mature females examined being 1003 mm total length.

Key words: rhinobatid, fecundity, sexual maturity.

Resumen: Evaluación inicial de parámetros de edad, crecimiento y reproducción para la raya *Trygonorrhina fasciata* (Müller & Henle, 1841) del sur de Australia. Presentamos en este trabajo parámetros preliminares sobre edad, crecimiento y reproducción de la raya *Trygonorrhina fasciata* de las aguas del golfo del sur de Australia, basados en el análisis de 43 especímenes. Estimaciones de edad, basadas en el conteo de las bandas vertebrales, fueron usadas para calcular los parámetros de la función de crecimiento de von Bertalanffy: L_∞, = 1129 mm de longitud total, $k = 0.13$, $t_0 = -2.55$ años (para ambos sexos combinados). Con base en el examen de la morfología interna y externa, los machos maduran con longitud total de 650 a 700 mm; y la menor hembra madura de tres hembras examinadas medía 1003 mm de longitud total.

Palavras clave: rinobatídeo, fecundidad, madurez sexual.

Introduction

Chondrichthyans in general possess life histories characterized by low fecundity, slow growth rates, low intrinsic rates of population increase and rebound potentials, coupled with long generation times and high embryonic mortality, which render these fishes highly susceptible to the impacts of fishing and other coastal developments (Stevens *et al.* 2000). Thus, determinations of rates of growth and reproduction are becoming increasingly more important in identifying chondrichthyan species that are susceptible to fisheries impacts (Dulvy *et al.* 2004, Walker 2004). This is especially true for species that are taken as by-catch, as catch rates generally go unquantified and population impacts are unknown (Diamond 2003).

The southern fiddler ray, *Trygonorrhina fasciata*, is an endemic Australian rhinobatid, common along the southern coastline, from the eastern Bass Strait (including the northern Tasmanian coast) to Lancelin, Western Australia (Last & Stevens 1994). Characteristic of rhinobatids, *T. fasciata* possesses a demersal nature and benthic feeding habit, which has resulted in this species becoming a common by-catch species in bottom trawl fisheries and being observed in catches of gillnet and long-line fisheries (Walker *et al.* 2005). Current catches of the species and the fate of discards are unquantified; thus, posing serious questions over the sustainability of *T. fasciata*. Whilst there have been no appreciable declines in

catch rates of *T. fasciata* in the south east trawl fishery between 1992 and 2002 (Reardon 2003), this species was ranked the eighth most abundant among benthic chondrichthyan by-catch species caught in trawl operations in the lower west coast of Australia (Hyndes *et al.* 1999). Trawl fishing has been shown to significantly alter the relative abundances of chondrichthyans caught as both target and by-catch species (Graham *et al.* 2001, Kennelly 1995).

Marshall *et al.* (2007) previously described the dietary composition and reproductive cycle of *T. fasciata* in Western Australia; however, to date, there have been no previous investigations into the growth of *T. fasciata*, and the time to sexual maturity still remains unknown. Thus, here we determine rates of growth and reproduction which will be valuable in order to assess the susceptibility of *T. fasciata* to fishing impacts.

Materials and Methods

Specimens of *T. fasciata* were collected from the by-catch of commercial prawn trawl vessels undertaking normal fishing operations in the gulf waters of South Australia. Specimens were sexed and the total length (TL) and disc width (DW) of all specimens were measured to the nearest millimetre. TL and DW had a significant linear relationship:

$$TL \ (mm) = 2.4578 \times DW - 9.6382$$
$$(n = 43, \ r^2 = 0.95, \ p < 0.001)$$

$$DW \ (mm) = 0.3863 \times TL + 16.19$$
$$(n = 43, \ r^2 = 0.95, \ p < 0.001)$$

Specimens were also weighed (BW: body weight) to the nearest gram. Body weight increased predictably with TL:

$$BW \ (g) = 7 \times 10^{-6} \times TL^{2.96}$$
$$(n = 43, \ r^2 = 0.99, \ p < 0.001)$$

Age estimates were based on counts of bands in the vertebrae of specimens. Vertebral centra were cleaned of excess tissues, placed in a 5% sodium hypochlorite solution for 10 – 20 min, followed by a rinse in freshwater and dried in an oven. Individual vertebral centra were then embedded in a clear setting epoxy resin and cut into (approximately) 300 μm sections with a diamond tipped lapidary saw. These sections were then mounted onto a microscope slide and examined under a dissecting microscope with a transmitted light source (Fig. 1).

Marginal increment ratio (MIR) analysis was attempted in order to determine the time of band formation (Liu *et al.* 1998). However, growth band

formation could not be fully validated as specimens were obtained between November and April each year. Therefore, age estimates presented from hereon are based on counts of growth bands, with the explicit assumption that the periodicity of band formation in *T. fasciata* is annual (i.e., one band = one year). In order to verify the suitability of the vertebral centra as an ageing structure for *T. fasciata* (i.e., the vertebral centra developed in proportion to body size), the centrum diameter (CD) was measured to the nearest millimetre using digital calipers for each specimen before sectioning.
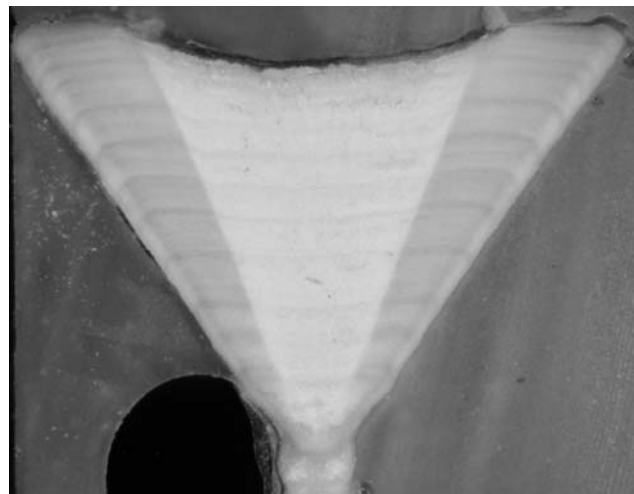

Figure 1. Vertebral centra section of an 860 mm total length male southern fiddler ray (*Trygonorrhina fasciata*) showing 11 growth bands.

Counts of bands were made from two vertebrae from each specimen. Growth bands were defined as a pair of opaque (outer) and translucent (inner) circuli observed in the corpus calcareum of the sectioned vertebrae (Fig. 1). Counts were made without prior knowledge of the size, sex or previous count of the specimen. If the counts varied between the two vertebrae, a third was sectioned and counted. If the count of the third vertebra matched either of the previous two it was taken as the count. If there was no agreement between any of the three samples then that specimen was excluded from the analysis. This counting procedure was repeated and the estimated ages between readings compared. Errors in the counts of growth bands in the vertebral centra were analyzed using the Index of Average Percentage Error (IAPE) as described by Beamish and Fournier (1981).

Age estimates based on counts of growth bands were fitted to the von Bertalanffy (1938) growth function. Growth parameters were calculated using FISHPARM version 3.0S (Prager *et al.* 1987).

We scored the male reproductive status of *T. fasciata* on three criteria: (1) the degree of clasper calcification (none, partial, or fully calcified); (2) the degree of vas deferens coiling (none, partial, or complete coiling); and (3) the presence, or absence of semen. Males were considered sexually mature when the claspers were rigid and the vas deferens showed partial or complete coiling and semen was present. Inner clasper length (CL) was also measured to the nearest millimetre.

The female reproductive status of *T. fasciata* was based on two criteria: (1) ova diameters; and (2) uterine width and development. Females were considered mature when gravid or with ova > 10 mm in diameter in their ovaries, and if the uteri were differentiated from the oviducts and measured > 10 mm in width at their widest point (Neer & Cailliet 2001).

Results and Discussion

In total, 43 specimens (26 males and 17 females) of *T. fasciata* were examined to provide preliminary age and growth estimates. Males ranged in length from 275 to 877 mm TL, whereas females were slightly longer, ranging from 251 to 1084 mm TL (Fig. 2).

Monthly changes in the vertebral MIR of *T. fasciata* appeared to peak in April, which coincides with the approximate time of birth, followed by an abrupt decrease in May (Fig. 3). These limited data suggest that growth band formation occurs once a year between April and May.

Vertebral centra were verified as suitable ageing structures as CD had a significant linear relationship with TL:

$$\text{TL (mm)} = 89.553 \times \text{CD} + 150.58$$
$$(n = 86, r^2 = 0.95, p < 0.05)$$

CD also showed a significant linear relationship with DW:

$$\text{DW (mm)} = 37.72 \times \text{CD} + 71.41$$
$$(n = 86, r^2 = 0.91, p < 0.05)$$

The IAPE for counts of growth bands in the vertebral centra of *T. fasciata* was 3.56%, well below the accepted 5% error thresholds (Campana 2001). Growth bands in sectioned vertebral centra were easily identifiable in individuals greater than 360 mm TL, and as a result no individuals were excluded from the study. The calculated von Bertalanffy growth function parameters for males, females and combined sexes are shown in Table I. Females and males possessed similar growth rates ($k = 0.16$ and $k = 0.18$, respectively); however, females attained greater maximum lengths than males ($L_\infty = 1157$, $L_\infty = 930$ mm TL, respectively; Fig. 4). The agreement of the band count (age)-at-length data to the calculated growth curve suggests that the von Bertalanffy growth curve accurately forecasts growth in South Australian *T. fasciata*. The smallest specimens which displayed signs of fresh umbilical scarring were 251 to 289 mm TL, indicating that this is the approximate size range at birth of *T. fasciata* (Table I). This is similar to the estimated size at birth (*Y*-axis intercept) derived by the von Bertalanffy growth curve (Fig. 4).

The oldest *T. fasciata* specimen determined from counts of growth bands in vertebrae was a 1084 mm TL female that was 15 years old. This specimen provides an initial, conservative estimate for longevity. Using the equation for theoretical longevity (t_{max}) (Skomal & Natanson 2003) we estimated that the t_{max} for both sexes combined was 26.6 years (Table I).

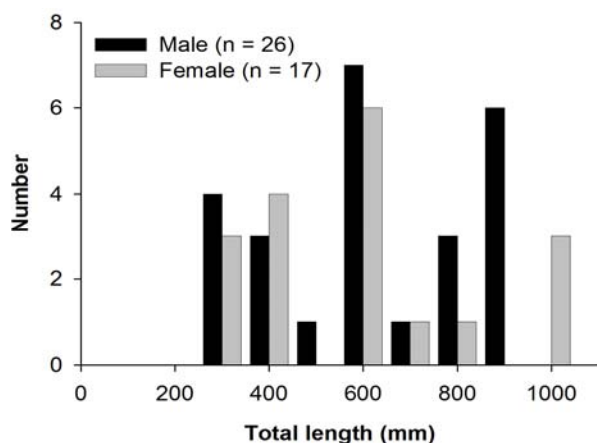**Figure 2.** Length-frequency distribution of the southern fiddler ray (*Trygonorrhina fasciata*) specimens collected from South Australia.
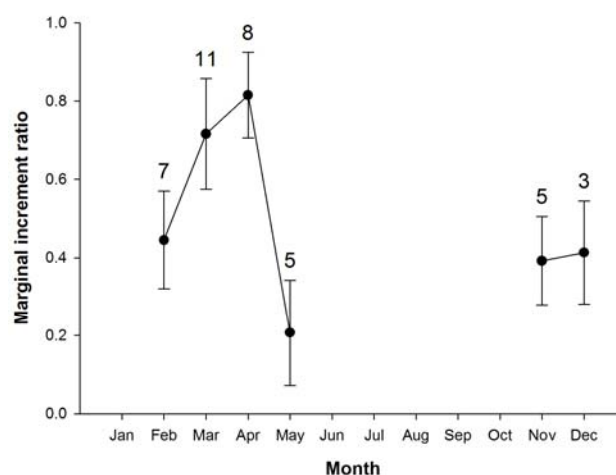

Figure 3. Monthly changes in the marginal increment ratio (MIR) of the southern fiddler ray (*Trygonorrhina fasciata*). Where the numbers indicate sample size and vertical bars indicate ± the standard error values.

Table I. von Bertalanffy growth function parameters for the southern fiddler ray (*Trygonorrhina fasciata*). All (total) lengths given in millimeters with asymptotic standard errors shown in parenthesis.

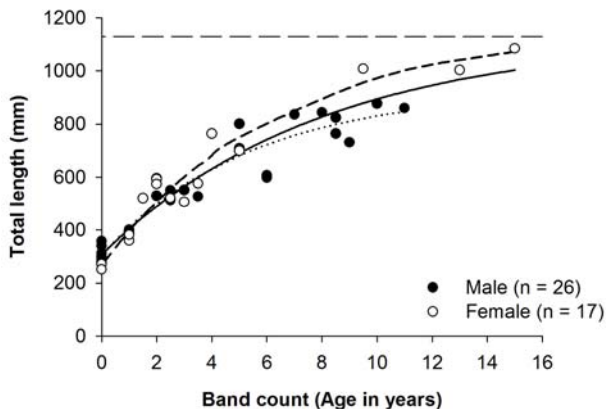| VBGF parameter	Males	Females	Combined
n	26	17	43
r^2	0.92	0.96	0.92
k: the growth coefficient	0.18 ($\pm$ 0.05)	0.16 ($\pm$ 0.03)	0.13 ($\pm$ 0.03)
L_∞: maximum theoretical length	930 ($\pm$ 87.3)	1157 ($\pm$ 82.7)	1129 ($\pm$ 107.3)
t_0: age at length 0 mm	–2.19 ($\pm$ 0.51)	–1.69 ($\pm$ 0.44)	–2.55 ($\pm$ 0.47)
Estimated size at birth	279.8 ($\pm$ 6.3)	261 ($\pm$ 14.1)	273.5 ($\pm$ 12.5)
t_{max}: theoretical longevity	19.3	21.6	26.6

Figure 4. von Bertalanffy growth curves for the southern fiddler ray (*Trygonorrhina fasciata*) fitted to the band count (age)-at-total length data for: male (dotted line), female (thick dashed line) and for both sexes combined (solid line). The line (– – –) is the estimated L_∞ (Table I).

Clasper lengths and the degree of calcification increased rapidly between 500 and 600 mm TL (Fig. 5) with male *T. fasciata* attaining sexual maturity between 650 and 700 mm TL. The claspers of sexually mature *T. fasciata* were characterized as being long, rigid and very slender, terminating in a bulbous head. Inner clasper length (CL) showed a significant increase with increased TL:

$$CL \ (mm) = e^{0.0062 \ TL}$$
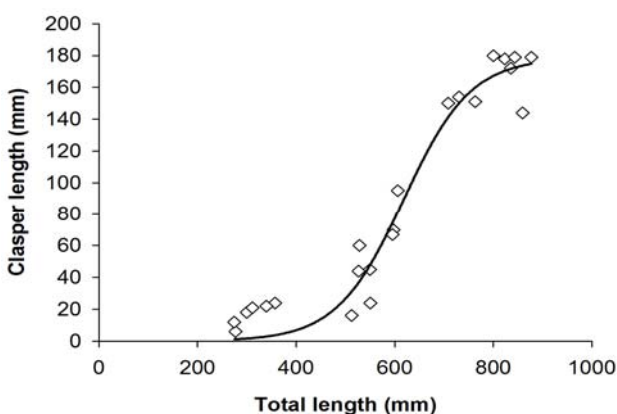$$(n = 26, \ r^2 = 0.75, \ p < 0.0001)$$

Figure 5. Relationship between total body length and inner clasper length in the southern fiddler ray (*Trygonorrhina fasciata*).

Morphological examinations of female specimens of *T. fasciata* confirmed that this species is aplacental yolk-sac viviparous, with both uteri being functional and symmetrical. Of the three mature females examined, all were pregnant with near term embryos present in both uteri. Litter sizes ranged from 4 to 7 embryos per female, with a mean litter size of 5.33 embryos ($\pm$ 1.53: standard deviation), with both the left and right uteri having on average 2.67 embryos each ($\pm$ 1.54 and $\pm$ 0.57: standard deviation, respectively). Litter sizes showed a trend of increase with an increase in the TL of the three pregnant females. The embryonic sex ratios of each of the three litters were: 1:1, 4:1 and 2.5:1 for males:females, with the mean embryonic sex ratio of all litters 2.5:1 ($\pm$ 1.5: standard deviation) for males:females.

The largest immature female examined was 764 mm TL and displayed morphological characteristics, enlarged ova and differentiation between the uteri and the oviducts, implying that sexual maturity was imminent. All of the pregnant females examined were in excess of 1000 mm TL, with the smallest of three mature females being 1003 mm total length. These limited data suggest that females attain sexual maturity beyond 1000 mm TL.

Chondrichthyan fishes are characterized as possessing life history parameters that make them susceptible to population declines as a result of fisheries impacts, either as targeted or non-targeted species (Stevens *et al.* 2000). Thus, determination of rates of growth and reproduction in chondrichthyan species is essential in identifying those species that are "at risk" to anthropogenic factors (Walker 2007).

Our findings suggest that the southern fiddler ray is a relatively fast growing species, with a von Bertalanffy growth coefficient of $k = 0.13$ for both sexes combined, with a considerably extended longevity. The age range (0 – 15 years) of *T. fasciata* based on counts of growth bands was greater than the reported age range (0 – 11 years) for *Rhinobatos productus* (Timmons & Bray 1997) and *R. horkelii* (Lessa 1982), and was markedly greater

than the reported age range (0 – 6 years) in *R. annulatus* (Rossouw 1984) and (0 – 5 years) in *R. rhinobatos* (Ismen *et al.* 2007), all of which grow to comparable sizes (1200 mm TL). Comparisons of rates of growth in other rhinobatid species indicate that this group of rays possess a variety of modes of growth, with estimated rates of growth (*k*) ranging from a rapid *k* = 0.29 in the common guitarfish (*R. rhinobatos*) (Ismen *et al.* 2007), to a comparable to *k* = 0.19 in *R. horkelii* (Lessa 1982), to a sluggish *k* = 0.016 in the shovelnose guitarfish (*R. productus*) (Timmons & Bray 1997).

Vertebral centra were determined to be an appropriate ageing structure based on the positive linear relationship between centrum diameter and TL. However, due to the opportunistic nature of our sampling regime, we were unable to fully validate periodicity of growth band formation through MIR. The limited data presented here indicates annual band formation, and thus our estimates of age are based on the assumption that one growth band corresponds to one year of animal age. This assumption is supported by the validation of annual band formation in *R. horkelii* (Lessa 1982) and the partial validation of band formation in the shovelnose guitarfish (*R. productus*) (Timmons & Bray 1997). However, in *T. fasciata* the annual periodicity of growth band formation in all age classes still requires validation (Beamish & McFarlane 1983).

Based on our estimates of maximum theoretical length (L_∞) *T. fasciata* attains sexual maturity relatively late in their life, with males achieving sexual maturity between 650 and 700 mm TL, which is 70% – 75% of their expected maximum body size. Similarly, the smallest mature female examined was 1003 mm TL, which is 86% of their expected maximum body size. Size at sexual maturity in males *T. fasciata* is to similar to that reported in the banded guitarfish (*Zapteryx exasperata*) (Villavicencio-Garayzar 1995), the eastern Australian shovelnose ray (*Aptychotrema rostrata*) (Kyne & Bennett 2002), and in *R. rhinobatos* (Abdel-Aziz *et al*. 1993; Ismen *et al*. 2007). In females *T. fasciata* size at sexual maturity is comparable to *R. productus* (Timmons & Bray 1997) and the blackchin guitarfish (*R. cemiculus*) (Capapé & Zaouali 1994; Seck *et al*. 2004).

Male *T. fasciata* appeared to undergo three stages of maturity as described in *R. cemiculus* (Capapé & Zaouali 1994). In the first stage, from 200 to 500 mm TL, the juvenile slowly develops into sub-adulthood. This stage was followed by a rapid increase in clasper length and degree of clasper calcification between 500 and 600 mm TL. Finally,

the onset of sexual maturity occurred between 650 and 700 mm TL. Due to the small number of mature, or maturing female *T. fasciata* specimens, we concluded that females attained sexual maturity beyond 1000 mm TL. This estimated size at maturity is larger than that estimated for the west Australian population of *T. fasciata*, that undergo sexual maturity at 892 mm TL (Marshall *et al*. 2007). The larger estimated size at maturity of this study may be a result of the lack of female specimens between 800 and 1000 mm TL. Nevertheless, these findings do indicate sexual dimorphism in the onset of maturity, with females reaching sexual maturity at larger sizes than males, a trait shared in other rhinobatids and a general characteristic of chondrichthyan fishes (Capapé & Zaouali 1994; Marshall *et al*. 2007).

Macroscopic examination of three litters from pregnant female *T. fasciata* indicates that this species has a low reproductive potential, which is consistent with the general chondrichthyan reproductive strategy (Conrath 2004). Marshall *et al.* (2007) observed a mean ovarian fecundity of 3.0 embryos per female observed in the west Australian population of *T. fasciata* from 12 individuals examined; we observed a mean 5.33 embryos per female, and amongst other rhinobatids, *T. fasciata* has a low reproductive output (see Kyne & Bennett 2002). Whilst we did see a trend towards increasing fecundity with increasing TL in the female specimens examined, we sampled animals that were greater than the previously reported maximum lengths of the species (Last & Stevens 1994) and close to the maximum theoretical length (L_∞) calculated in this study, suggesting that the largest observed litter size (n = 7) may be representative of the species' maximum reproductive potential.

As we were unable to obtain specimens of *T. fasciata* for all months of the year, we were unable to determine the precise reproductive cycle of the species in South Australia. Marshall *et al*. (2007) showed that the Western Australian population of *T. fasciata* has a gestation period of 12 months, whereby primary embryonic growth occurs across a four to five month period, followed by a protracted period of embryonic diapause lasting 7 to 8 months, with birth occurring in April/May, coinciding with ovulation. We collected two of the pregnant females in March of 2006 and a third in March of 2007, supporting the notion of an annual reproductive cycle with birth occurring in April/May. In general, rhinobatids have variable gestational periods lasting from 3 – 5 months in *R. productus* (Márquez-Farías 2007), *A. rostrata* (Kyne & Bennett 2002) and in *Z. exasperata* (Villavicencio-Garayzar 1995) to 12 months in *R. hynnicephalus* (Wenbin & Shuyuan

1993); however, the incidence of embryonic diapause, which appears to be characteristic of this group, appears to form an annual breeding pattern. Kyne and Bennett (2002) suggest rhinobatids adopt embryonic diapause in an effort to provide seasonally favourable conditions for newborn rays. We suggest that embryonic diapause in *T. fasciata* may be a mechanisms to combat juvenile mortality. Diapause may facilitate in furthering embryonic development; therefore, *T. fasciata* produce few, larger offspring that are less vulnerable to predation and do not require rapid growth (Cortés 2004).

In conclusion, the southern fiddler ray is a relatively fast growing rhinobatid species that achieves maturity late in its life. Whilst displaying a low fecundity, *T. fasciata* appears to invest additional parental care, giving birth to large offspring, thus reducing juvenile mortality. These life history traits are not indicative of a species that would be considered "at risk" to fisheries induced population declines (Walker 2004). Whilst current catch rates indicate that relative species abundance remains stable throughout southern Australian, shifts in fishing pressure could put this species at risk. Thus, the need for assessing the effects of current by-catch rates of *T. fasciata* requires further investigations, with particular emphasis into the survival of discards.

Acknowledgments

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