Deep-Sea Research II 115 (2015) 55–63

Contents lists available at ScienceDirect

Deep-Sea Research II

journal homepage: www.elsevier.com/locate/dsr2

Preliminary age, growth and maturity estimates of spotted ratfish (*Hydrolagus colliei*) in British Columbia

J.R. King*, R.P. McPhie

Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, British Columbia, Canada V9T 6N7

ARTICLE INFO

Available online 21 November 2013

Keywords:
Age determination
Tooth plates
Tritors
Maturity
Growth modeling

ABSTRACT

The spotted ratfish (*Hydrolagus colliei*) is a chimaeroid ranging from southeast Alaska to Baja California and found at depths of up to 1029 m. Despite being widespread and ubiquitous, few biological parameter estimates exist for spotted ratfish due to a lack of suitable ageing structures to estimate age and growth. We present preliminary results of age, growth and maturity estimates based on a new method in which tritor ridges are counted on the vomerine tooth plate. We also provide a method for estimating the number of worn tritor ridges based on tooth plate diameter measurements for the spotted ratfish. The tritor ridges are distinct bumps that are easy to identify and precision estimates between readers suggests that this method is transferable. Tritor ridges are a potential structure for estimating age in *H. colliei* and we provide recommendations for future research to improve the method. We sampled 269 spotted ratfish captured in trawl surveys off the coast British Columbia ranging in size from 74 to 495 mm in precaudal length (PCL). The estimated ages ranged from 2 to 16 years for males and from 2 to 21 years for females. The von Bertalanffy, von Bertalanffy with known size at birth, Gompertz and logistic growth models were fitted to the data. Based on Akaike information criterion corrected for sample size and number of parameters estimated, the logistic growth curve was selected as most suitable. The logistic growth model yielded the following parameter estimates: L_{inf}=407.22 mm (PCL), k=0.23 year^{-1}, t_0=−7.06 years for males; L_∞=494.52 mm (PCL), k=0.26 year^{-1}, t_0=−8.35 years for females. Estimated ages at 50% maturity were 12 and 14 years for males and females, respectively. Correspondingly, the size at 50% maturity estimates was smaller for males (302 mm, PCL) than females (393 mm, PCL). Both estimates are larger than those made for spotted ratfish off of California indicating regional differences in life history traits for this species. Our preliminary results suggest that *H. colliei* is a late maturing species with a moderate growth rate and is moderately long-lived compared to teleosts.

1. Introduction

The spotted ratfish (*Hydrolagus colliei*) is a chimaeroid (Class: Chondrichthyes; Subclass: Holocephali) distributed throughout the northeast Pacific, from southeast Alaska (Wilimovsky, 1954) down through Baja California and into the Gulf of California, Mexico (Grinols, 1965). Within the northern extent of its range, spotted ratfish are found in bays, sounds and inland seas. It is however, considered to be a deep-water, benthic chimaera found throughout the continental shelf and slope, down to reported depths of 913 m (Alverson et al., 1964). In British Columbia, observed commercial bottom trawl tows at depths of 1029 m have captured spotted ratfish (J. King, unpub. data).

The spotted ratfish is oviparous, with individual embryos contained in an egg case deposited onto the ocean bottom. Gestation is estimated to be 9–12 months (Dean, 1906), and peak parturition occurs from May to October (Barnett et al., 2009a). Hatchlings emerge at about 96 mm in precaudal length (PCL; estimated from Didier and Rosenberger, 2002).

Maximum reported length (location and sex unspecified) is 718 mm (PCL; estimated from Clemens and Wilby, 1961). A recent study off California, Washington, and Oregon observed maximum sizes of only 377 mm (PCL) for males and 498 mm (PCL) for females (estimated from Barnett et al., 2009a) which is much smaller than those observed in British Columbia: 670 mm PCL for males and 690 mm PCL for females (J. King, unpub. data).

Aggregates of spotted ratfish are common (Quinn et al., 1980; Barnett et al., 2012). There is some indication that spotted ratfish aggregate by size, with larger fish occupying shallower waters (Quinn et al., 1980) which may account for apparent sex segregation (Mathews, 1975) since females are larger than males. Spotted ratfish undergo seasonal movement into shallower waters in winter and spring and deeper waters in summer and fall (Mathews, 1975; Quinn et al., 1980). In Puget Sound, they have

* Corresponding author. Tel.: +1 250 756 7176; fax: +1 250 756 7053.
E-mail address: Jackie.King@dfo-mpo.gc.ca (J.R. King).

0967-0645/$ - see front matter
http://dx.doi.org/10.1016/j.dsr2.2013.11.002

been observed to undergo diel movement, occupying deeper waters during the day and moving into shallower waters at night (Quinn et al., 1980).

The spotted ratfish is most abundant between British Columbia and northern California (Ebert, 2003). Historically there have been commercial fisheries for spotted ratfish in British Columbia (Clemens and Wilby, 1961). From the late-1930s to the mid-1940s, a spotted ratfish liver fishery produced Vitamin A and a high quality oil for lubrication of machinery and guns during the war years. Periodically they have been exploited for reduction, with small landings occurring intermittently in the 1950s, 1980s, and 1990s ranging from 2 to 53 t annually. Currently spotted ratfish is not commercially exploited, but it is frequently encountered as bycatch by the groundfish trawl fishery. On average, 540 t of spotted ratfish are encountered annually with annual catches sometimes as high as 800 t, placing this species as the fourth ranked bycatch species by weight in the groundfish trawl fishery (J. King, unpub. data). Despite its wide distribution and high abundance, little is known about spotted ratfish life history parameters such as age and growth. Only recently did Barnett et al. (2009a) provide estimates of size at maturity, fecundity and reproductive cycle. They also estimated that size at 50% maturity off California was approximately 290 mm (PCL) for males and 360 mm (PCL) for females, with an increase in size at maturity with increasing latitude. Annual fecundity estimates ranged from 19.5 to 28.9 egg cases based on a discrete reproductive cycle, i.e. parturition season between May to October (Barnett et al., 2009a). There are no other life history parameter estimates for spotted ratfish in other areas of its geographic range.

The high level of fishery bycatch of spotted ratfish and their tendency to form aggregations, might make this species vulnerable to high depletion. Typically, chondrichthyans are considered to be vulnerable to overexploitation due to their life history traits, namely low fecundity, slow growth and late maturity (Stevens et al. 2000; Musick et al., 2000). However, Barnett et al. (2012) suggest that spotted ratfish off the west coast of the United States have exhibited the ability to rebound from low levels of abundance once fishing mortality (i.e. bycatch) is reduced. Regardless, determining sustainable levels of bycatch requires estimates from stock assessment approaches, which require information on age and growth for estimation of mortality and production rates. Hoff and Musick (1990) pointed to the lack of age and growth information as a limiting factor in the development of chondrichthyan management. Since spotted ratfish, like all chondrichthyan fishes, have a cartilaginous skeleton, suitable structures for age determination have been difficult to find. The dorsal spine has been used for other chimaeroids: *Callorhinchus capensis* (Freer and Griffiths, 1993), *Chimaera monstrosa* (Moura et al., 2004; Calis et al., 2005) and for *Callorhinchus milii* (Sullivan, 1977). Barnett et al. (2009b), using light microscopy, observed systematic banding patterns in the inner dentine layer of spotted ratfish dorsal spines. However, after assessment of the spine structure using radiological imaging they concluded that the spine was not a suitable structure for age determination since they did not observe mineral density gradients.

Chimaeroid fishes possess three pairs of tooth plates that grow at the posterior margin and are usually partially hypermineralized (Didier et al., 1994). The lower jaw has a pair of large mandibular tooth plates, and the upper jaw along with the roof of the mouth has two pairs of tooth plates, the palatine tooth plate (posteriorly) and the vomerine tooth plates (anteriorly). Johnson and Horton (1972) noted that the number of tritor ridges on the inner side of the vomerine plates increased with increasing body length and that they may be suitable structures for age determination. The vomerine tooth plate grows at the posterior edge (Patterson, 1992) and wears away on the convex, anterior edge inside the mouth (i.e. occlusal surface) over time. Juvenile spotted ratfish typically have vomerine plates without this wear. Johnson and Horton (1972) noted the amount of wear over time was not known and was a limiting factor for age determination. However, Tseng (2011) used back-calculated tritor ridge counts for worn vomerine tooth plates based on measurement and counts from unworn vomerine tooth plates in *Hydrolagus mitsukurii* in waters off Taiwan. A similar approach has been used successfully in estimating missing bands on worn dorsal spines in the validated age determination method for Pacific spiny dogfish, *Squalus suckleyi* ([previously *S. acanthias*], Ketchen, 1975; McFarlane and King, 2009).

We report on spotted ratfish biological data available from groundfish survey bottom trawl tows to quantify regional length and weight conversions, including the first published total length (TL) to PCL conversion. We also provide size at maturity estimates for spotted ratfish in British Columbia, thereby extending the northern extent of estimates for this life history parameter. In addition, we examine the tritor ridge counts on vomerine tooth plates as an alternate structure for age determination for this species. We develop a relationship between vomerine tooth plate measurements and tritor ridge counts in juvenile spotted ratfish with unworn tooth plates as a means of estimating the number of eroded tritor ridges in older specimens.

2. Materials and methods

All analyses were conducted using R (R Core Team, 2012).

2.1. Study area and biological samples

Biological data were obtained from research bottom trawl surveys (2003–2012) conducted throughout British Columbia waters (Fig. 1). These synoptic surveys were random, depth stratified with the primary objective to provide indices of abundance and biological samples for groundfish species throughout

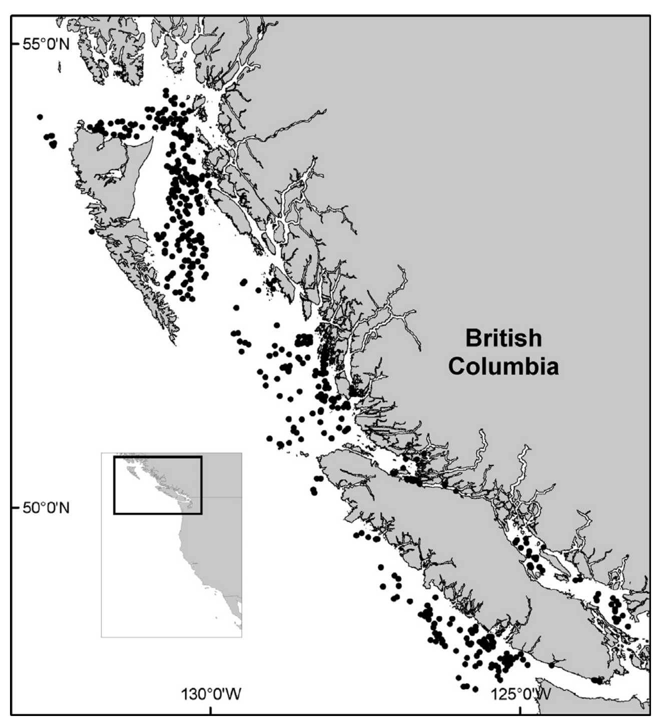

Fig. 1. Geographic extent of research bottom trawl tows conducted in British Columbia with spotted ratfish biological data used in this study. Inset shows study area (box) off the west coast of North America.

British Columbia. Fishing occurred on trawlable habitats from depths of 15 m–660 m. Sex was determined externally by the presence (males) or absence (females) of claspers. PCL (as per Didier and Rosenberger, 2002) was measured to the nearest mm and weight (W) was measured to the nearest g. Weight was not measured for each specimen. A weight–length relationship for each sex was estimated with non-linear least squares estimation:

$$W = aPCL^{b} \tag{1}$$

where W is weight (g) and PCL is precaudal length (mm). In May–August of 2010 and 2011, spotted ratfish were collected and frozen at sea and brought back to the laboratory for further age determination and maturity analyses. These specimens had TL, PCL and snout-vent length (SVL as per Didier and Rosenberger, 2002) recorded in mm and used in Length–length conversion estimates based on linear regression with intercept set to zero.

2.2. Age estimation

In the lab, spotted ratfish jaws were thawed and tooth plates were separated and removed from the surrounding tissue using a scalpel and forceps. For the smallest spotted ratfish, tooth plates were removed from the jaw tissue under a dissecting microscope. All tooth plates were then soaked in warm water for 2–3 min to loosen the remaining threads of connective tissue, which were picked off with forceps. Tooth plates were dried in separate envelopes for 24–48 h. After drying, vomerine tooth plates were imaged using a Leica MZ75 microscope with attached Leica DFC320 R2 digital camera and associated Leica Application Suite 3.1.0 software.

Tritor ridges were counted on the right vomerine tooth plate by a primary reader twice, with mean count used when discrepancies in counts occurred. White bands were visible, and represented the illuminated, elevated ridges. A ridge was assumed to be the start of the growth period, and taken together with material posterior to each white band, was assumed to represent one year of growth. Counting began anteriorly at the first complete tritor ridge evident and included the last complete tritor ridge formed at the posterior edge (Fig. 2). A tritor ridge was defined as complete if it extended from each side of the tooth plate and was distinct along its entire length (Fig. 2). To test for systematic bias in tritor ridge counts the number counted in the first reading was compared to the number counted in the second reading using Wilcoxon paired-sample test (Cailliet and Goldman, 2004).

Approximately 50% of the samples were selected at random to be read by a second reader. To test for systematic bias in tritor ridge counts between readers, the number counted by the first reader was compared to the number counted by the second reader using Wilcoxon paired-sample test (Cailliet and Goldman, 2004). Intra-reader and inter-reader precision was evaluated using the average percent error index (APE) of Beamish and Fournier (1981) and mean coefficient of variation (CV) as per Chang (1982). Campana (2001) suggested that acceptable levels of APE and mean CV were 5.5% and 7.6% respectively.

Each plate was assessed as having tritor ridges with either evidence of wear (W) or no wear (NW) near the anterior edge of the plate (Fig. 2). For NW plates, the number of tritor ridges were counted and the width (in mm) of the last complete tritor ridge at the posterior edge was measured at the widest point (ridge width: RW; Fig. 2A). The relationship between number of tritor ridges and the RW was estimated from the best fitting function using least squares linear regression and non-linear least squares estimation with the intercept set to zero. All measurements were made on the right vomerine tooth plate with Image Pro 6.1 software. For W plates, the number of complete tritor ridges were counted, and the width of the first complete tritor ridge (RW) at the anterior edge (after wear) was measured (Fig. 2B). The RW was used to estimate the number of missing, or worn, ridges based the estimated relationship between the two variables (see above) from NW plates.

2.3. Growth curve estimation

Growth curves were fitted using non-linear least squares estimation for each sex using each of the following growth equations:the von Bertalanffy growth equation (Beverton and Holt, 1957),

$$L_t = L_{inf}(1 - e^{-k(t-t_0)}) \tag{2}$$

the von Bertalanffy growth equation with a known size at birth (Fabens, 1965),

$$L_t = L_{inf} - (L_{inf} - 96)e^{-kt}, \tag{3}$$

(size at birth, 96 mm PCL, estimated from Didier and Rosenberger, 2002)

the Gompertz growth equation (Ricker, 1975),

$$L_t = L_0(e^{\ln(L_{inf}/L_0)(1-e^{(-kt)})}) \tag{4}$$

the logistic growth equation (Ricker, 1975),

$$L_t = L_{inf}/(1 + e^{-k(t-t_0)}) \tag{5}$$

where L_t is the PCL (mm) at age t (years), L_{inf} the maximum theoretical PCL (mm), k the growth coefficient, t_o is the theoretical age at zero length, L_0 is the length at birth.

Differences between male and female growth curves were tested with analysis of residual sum of squares (ARSS; Chen et al., 1992; Haddon, 2011). Growth model selection was based on Akaike's Information Criterion scores corrected for sample size and number of parameters estimated for least-squares non-linear estimation (AICc; Kimura, 2008). The model for each sex with the minimum AICc socre was selected if the difference in AICc scores (ΔAIC) to other models was greater than 4 units (Anderson, 2008).

2.4. Size and age at maturity

Each spotted ratfish was measured for PCL (mm) and weight (g). Maturity stage for each specimen was determined based on a macroscopic assessment of reproductive organ development in males and females. Following established survey protocol, individual specimens were classified into one of six maturity stages, adapted from Stanley (1961), Gorman (1963), Johnson and Horton (1972), and Barnett et al. (2009a) (Table 1). Ovaries were assessed by counting all oocytes ("ova" *sensu* Barnett et al. (2009a)) greater than 6 mm diameter, and classifying them all as undeveloped (white) or developing (yellow). The number of developing oocytes (>6 mm diameter and yellow) and fully-developed oocytes (>20 mm diameter, i.e. estimated ovulatory size; Barnett et al., 2009a) was recorded along with the diameter of the largest oocyte (mm).

Lengths-at-50% maturity (PCL_{50}) was estimated for males and females separately based on proportion mature at 10 mm length intervals. Likewise, age-at-50% maturity (age_{50}) was estimated for each sex using the proportion mature at yearly intervals assumed from the tritor ridge counts. All curves were estimated by binomial logistic generalized linear regression and differences between males and females were tested with sex included as a factor.

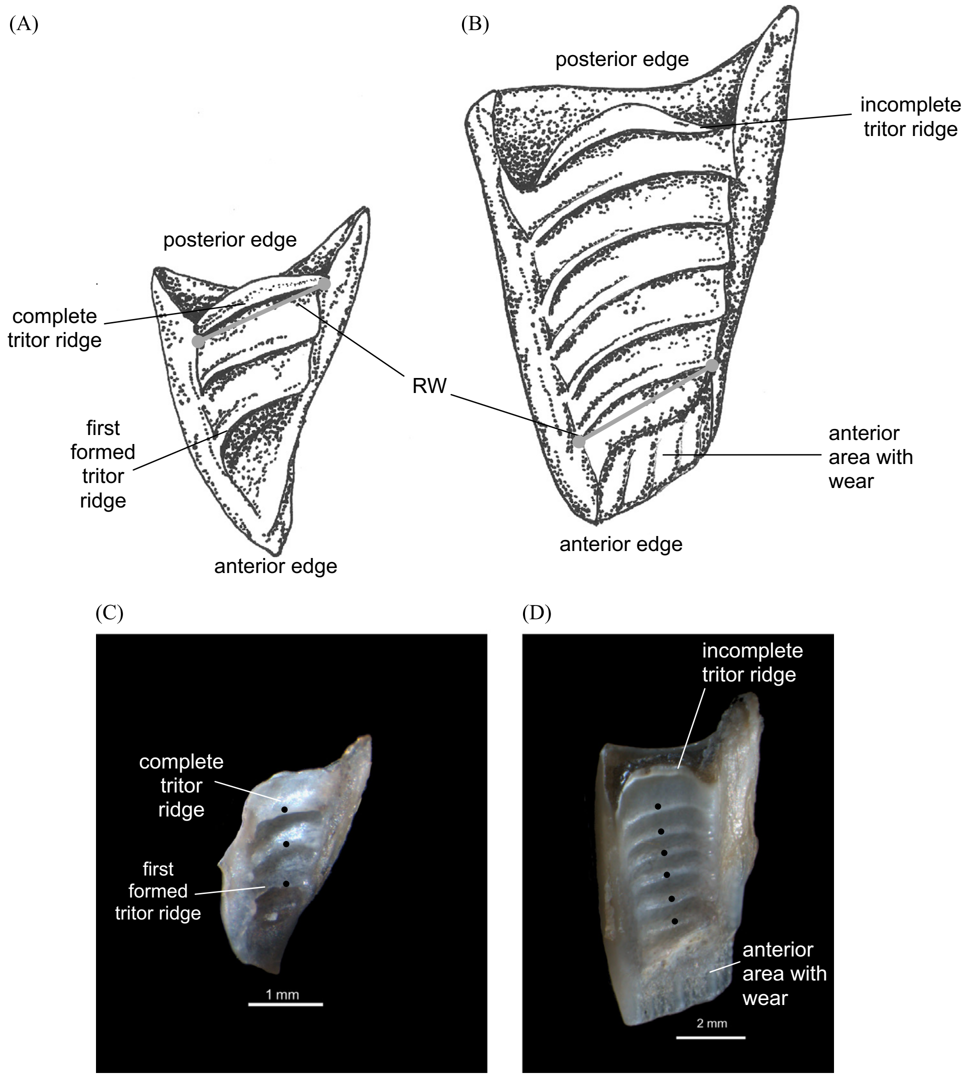

Fig. 2. (A and B) Schematic diagrams of spotted ratfish right vomerine tooth plates. (A) A tooth plate with no wear at anterior edge, depicting a new complete tritor ridge at the posterior edge and displaying three complete ridges which correspond to an assumed age of three years. (B) A tooth plate with wear at anterior edge, depicting an incomplete tritor ridge at the posterior edge, with five complete tritor ridges assumed to represent five years which would be combined with the estimated number of missing ridges when assigning an age. The location of the tritor ridge width (RW) measurement is indicated and was used from: (A) no wear tooth plates to estimate the relationship between number of tritor ridges and width and; (B) tooth plates with wear to estimate the number of missing, or worn away tritor ridges. (C and D) Photos of spotted ratfish vomerine right tooth plates. Ridges appear as highlighted white bands in photos and the dark bands are shadows cast by the ridges. (C) A tooth plate from an immature female, (PCL=132 mm) with no wear at the anterior edge and three complete tritor ridges indicated by black dots including a complete tritor ridge at the posterior edge. (D) A tooth plate from an immature female, (PCL=273 mm) with wear on the anterior edge, an incomplete tritor ridge at the posterior edge, and with six complete tritor ridges indicated by black dots.

3. Results

3.1. Biological samples

Length and weight data ($n=5948$) were available from 495 bottom trawls. There was no significant difference in weight–length relationship estimated for males ($n=3074$) and females ($n=2873$, $F_{20,1}=-10.15$, $p=0.998$). Sexes were combined to estimate the weight–length relationship, $W(\text{g})=2.493\times10^{-4}\cdot PCL\ (\text{mm})^{2.465}$ ($R^2=0.9044$, $n=5948$). Spotted ratfish ($n=123$ males; $n=150$ females) were collected from 32 trawls (2010–2012) for age and maturity estimation and to establish relationships to estimate conversions between TL, PCL and SVL by sex (Table 2).

3.2. Age estimation

Tritor ridges were visible as distinct bumps, and appeared in photographs as highlighted, white bands with an associated shadow anterior to each (Fig. 2C and D). A total of 57 spotted ratfish vomerine plates were assessed as NW. All of these fish were immature, and ranged in size (PCL) from 76 to 194 mm with number of complete tritor ridges ranging from 1 to 4. Overall, the number of complete tritor ridges counted (TRC) increased with the RW (Fig. 3). Linear regression estimation between TRC and RW was significant ($F_{1,50}=1309.43$, $p<0.001$, $R^2=0.96$):

$$TRC=2.1995RW \tag{6}$$

Tritor ridges were counted for an additional 212 spotted ratfish, and the RW was measured and used in Eq. (6) to estimate the number of worn (missing) tritor ridges (subtracting one for the tritor ridge measured). When these estimates are combined with counted tritor ridges (open triangles, Fig. 4) to estimate total tritor ridges (open circles, Fig. 4), the estimated ages for male and female spotted ratfish ranged from 2 to 16 years and from 2 to 21 years respectively (Fig. 4). The number of missing tritor ridges estimated for tooth plates with wear ranged from 1 to 10 (Fig. 4). New, incomplete tritor ridges on

Table 1
Criteria used to determine maturity stage of male and female spotted ratfish (*Hydrolagus colliei*).
Adapted from Stanley (1961), Gorman (1963), Johnson and Horton (1972), and Barnett et al. (2009a).

Maturity stage	Male	Female
Embryo	• Developing within egg case	• Developing within egg case
Immature (Neonate)	• Epididymis (“ductus deferens” *sensu* Jones et al., 2005) thin, with no coiling • Testes thin and soft with no development • Post-pelvic claspers extremely short, uncalcified and soft; white in colour • Frontal tenaculum not yet erupted	• Uterus extremely narrow, straight • Oviducal gland thin with no widening • No oocytes visible • No paired oviducal openings
Immature (Juvenile)	• Very slight coiling of epididymis • Testes thin and soft with no development • Claspers starting to calcify but still soft (do not extend to 1/2 pelvic fin) • Frontal tenaculum erupted but small, soft and smooth with no hooks	• Uterus narrow with thin epithelial walls • Oviducal gland marked by minor widening of the oviduct • Ovaries granular • Oocytes barely visible; <1 mm; translucent to whitish • No paired oviducal openings
Maturing (Adolescent)	• Epididymis enlarged, with few coils • Testes thin, straight with only some development • Post-pelvic claspers beginning to elongate but not completely calcified; extend to 1/2 length of pelvic fin, not yet perforated at their free ends • Frontal tenaculum not fully developed, with uncalcified hooks	• Oviducal gland slightly swollen and differentiated from uterus but without visibly contrasting tissue zones • Uterine wall thin (only starting to thicken) • Oocytes small (<6–10 mm diameter); whitish to cream-coloured • Small paired oviducal openings visible
Mature (Adult)	• Epididymis with many tight coils (esp. at anterior end) • Testes large, firm and well-developed • Vas deferens highly developed (white anterior, greenish posterior) • Post-pelvic claspers elongated, completely calcified and rigid; extend almost to or to the tip of the pelvic fin; perforated, bulbous/ expanded at their free ends • Pre-pelvic tenacula hard and protruding • Frontal tenaculum fully developed with calcified hooks	• Oviducal gland fully developed (bulbous and bullet-shaped) with sharply contrasting tissue zones; whitish in colour • Uterine wall thick, especially proximal to uterine openings where it is muscular and resistant to compression • Presence of small, white oocytes ($<$ 6–10 mm) and larger light yellow to yellow, vascularized oocytes (>6–10 mm diameter) • Some large, vitellogenic oocytes >20–25 mm diameter • Oviducal openings large, elongated and swollen
Mature (Ripe or gravid)	• Ripe with running sperm	• Gravid with fully or partially developed egg case present in uteri

Table 2
Length–length (mm) conversions (intercept set to zero) for spotted ratfish males ($n=123$) and females ($n=150$) and sexes combined for total length (TL), precaudal length (PCL) and snout-vent length (SVL).

Dependent variable	Independent variable	Sex	Slope	R^2
TL	PCL	Males	1.39	0.992
		Females	1.33	0.995
		Combined	1.35	0.994
TL	SVL	Males	2.49	0.993
		Females	2.35	0.994
		Combined	2.40	0.993
PCL	SVL	Males	1.79	0.993
		Females	1.77	0.997
		Combined	1.77	0.995

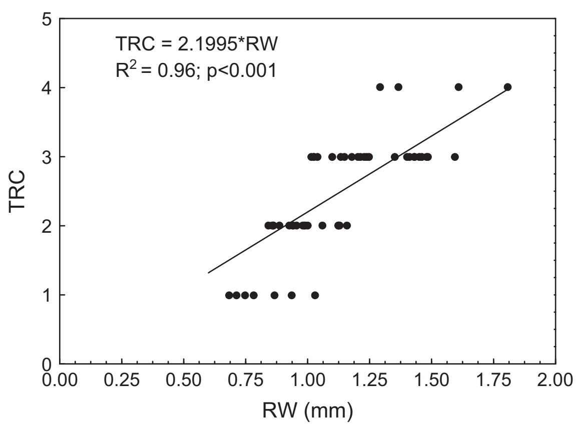

Fig. 3. Tritor ridge width (RW; mm) versus tritor ridge count (TRC) from spotted ratfish vomerine tooth plates with no wear ($n=57$).

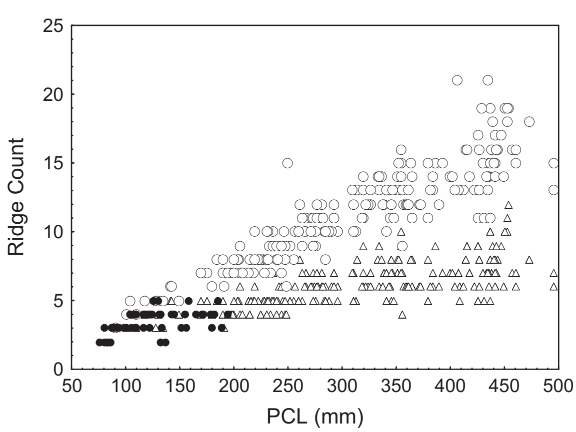

Fig. 4. Tritor ridge count vs. precaudal length (mm). Closed circles denote total counts made on vomerine tooth plates with no wear; open triangles denote total counts made on vomerine tooth plates with wear; open circles denote estimated total counts for vomerine tooth plates with wear, with missing tritor ridges estimated based on Eq. (6).

the posterior edge were most evident in May with the frequency complete tritor ridges increasing throughout the summer (Fig. 5). By August fewer vomerine tooth plates had an incomplete tritor ridge at the posterior edge indicating that tritor ridge growth begins to slow down by fall (Fig. 5).

Wilcoxon paired-sample test indicated there was no systematic bias between the first and second readings made by the primary reader ($p=0.08$). The intra-reader APE was 3.3% and mean CV was 4.6%, indicating an acceptable level of precision between readings. Tritor ridge counts by a second reader were done for 138 (51%)

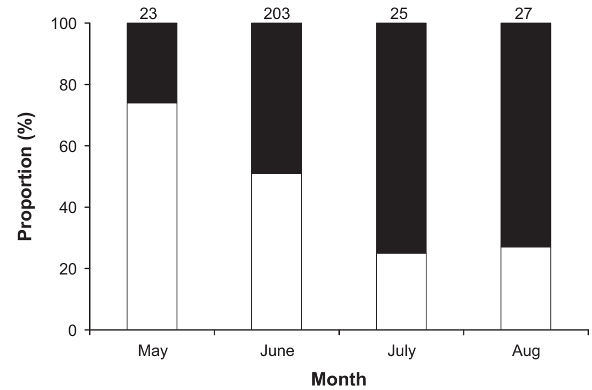

Fig. 5. The proportion of spotted ratfish vomerine tooth plates with complete tritor ridges (white) and incomplete tritor ridges (black) at the posterior edge by month. Numbers on top of columns indicate sample sizes.

Table 3
Growth model parameter estimates (L_{inf}=maximum theoretical precaudal length in mm; k=growth coefficient; t_0=theoretical age at zero length), total variation explained (R^2), Akaike Information Criteria (AIC) score, and differences in AICc scores from minimum (ΔAICc), for von Bertalanffy (including form with known length at birth (L_0), Gompertz and logistic growth curves estimated for male ($n=118$) and female ($n=151$) spotted ratfish. Asterisks denote significant ($p<0.05$) parameter estimates. Model comparison by sex was based on ΔAICc, where zero indicates minimum AICc score.

	L_{inf}	k	t_0	R^2	AICc	ΔAICc
von Bertalanffy						
Males	823.53*	0.03	−1.46	0.87	5203.3	33.1
Females	820.57*	0.04*	−0.26	0.90	7151.9	221.8
von Bertalanffy with known L_0						
Males	461.90*	0.08*	–	0.92	6844.9	1674.8
Females	764.51*	0.04*	–	0.92	7627.2	697.2
Gompertz						
Males	474.25*	0.13*	–	0.88	5183.0	12.8
Females	552.52*	0.15*	–	0.95	7024.1	94.1
Logistic						
Males	407.22*	0.23*	−7.06*	0.87	5170.2	0
Females	494.52*	0.26*	−8.35*	0.96	6930.0	0

randomly selected spotted ratfish. Wilcoxon paired-sample test indicated no systematic bias in tritor ridge counts between the first and second reader ($p=0.20$). The inter-reader APE was 4.5% and mean CV was 7.7%, indicating an acceptable level of precision between readers based on APE but slightly above the acceptable 7.6% CV threshold.

3.3. Growth curve estimation

The four growth curve models applied to males and females all explained a high proportion of variance (R^2) in the dataset (Table 3). The logistic model (Fig. 6) for both males and females had the lowest ΔAICc score (Table 3). Residuals were normally distributed with no excessive skew or kurtosis. The logistic growth curve estimate of L_{inf} was higher for females, than for males, but both functions estimated a similar k value (Table 3). The estimate for t_0 parameter was higher for females (Table 3). There was a significant difference between male and female logistic growth curves (ARSS: $F_{6,260}=49.11$; $p<0.001$).

3.4. Size and age at maturity

Maturity stage was determined in the laboratory for 273 spotted ratfish ($n=124$ males; $n=151$ females; Fig. 7); a subset of 269 also had age estimates. Four spotted ratfish were inadvertently discarded

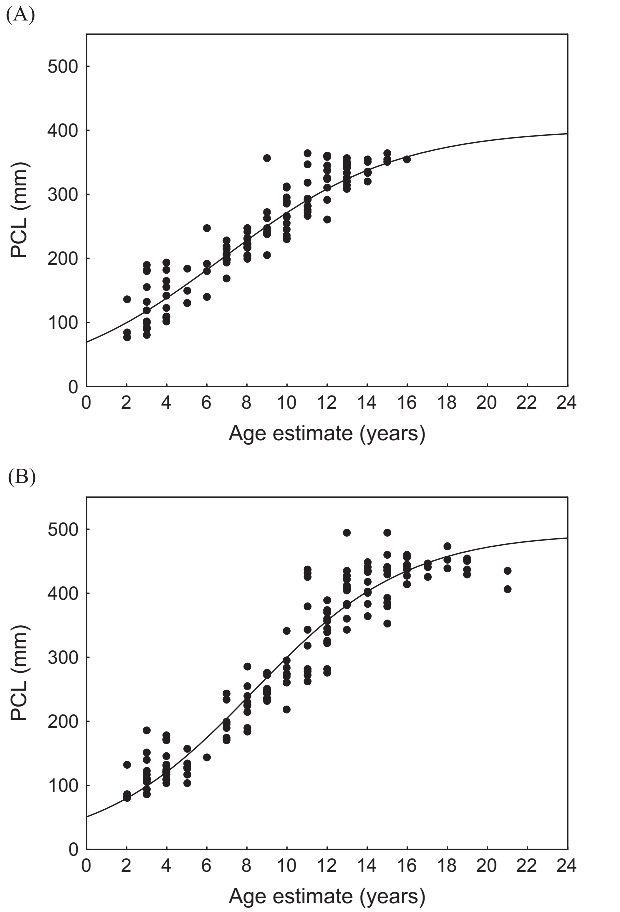

Fig. 6. Precaudal length (PCL, mm) as a function of estimated age (years) from tritor ridge counts for (A) male ($n=118$) and (B) female ($n=151$) spotted ratfish. Fitted curves are logistic growth model (Table 3).

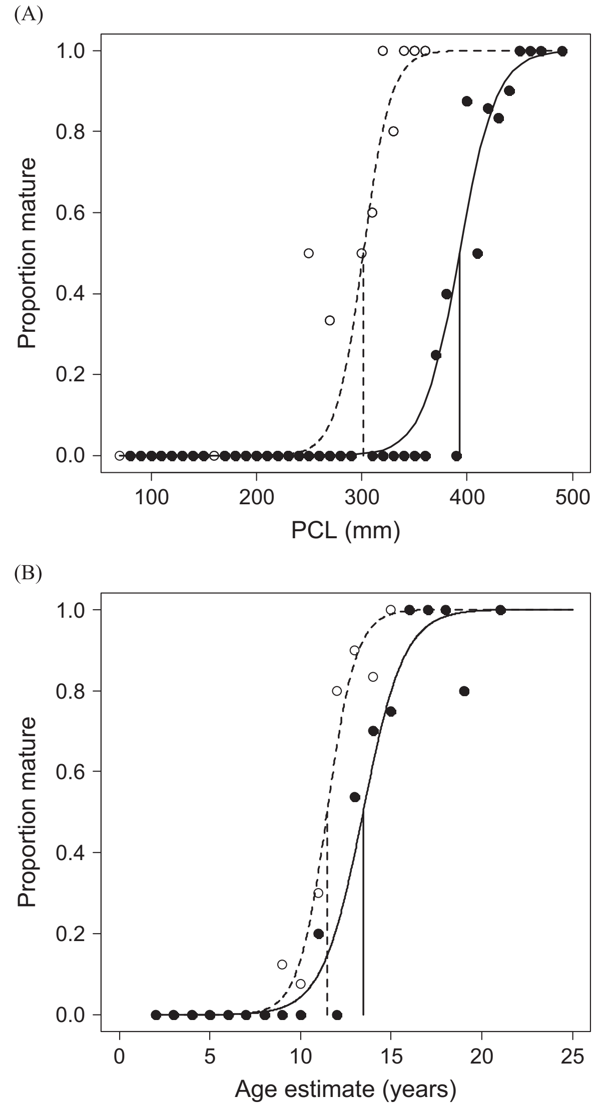

Fig. 7. Proportion of mature male (open circles; dotted lines) and female (solid circles; solid lines) spotted ratfish by: (A) 10 mm precaudal length (PCL) intervals ($n=123$ males; $n=150$ females); (B) age estimate (year) intervals ($n=119$ males; $n=150$ females). Length or age at 50% maturity determined from fitted logistic curves for males (length = 302 mm; age = 12 years) and females (length = 393 mm; age = 14 years) indicated by vertical lines to *x*-axis.

before tooth plates were extracted. For male ratfish, the smallest mature specimen was 250 mm PCL and the largest immature specimen was 333 mm PCL. The smallest mature female specimen was 379 mm PCL, and the largest immature female specimen was 442 mm PCL. The youngest mature male was estimated to be 9 years, and the oldest immature male was estimated to be 14 years. The youngest mature female was estimated to be 11 years, and the oldest immature was estimated to be 14 years. Overall, males mature at a smaller size (z-value for sex factor = 5.34, $p < 0.001$; Fig. 7A) and younger age (z-value for sex factor = 3.66, $p < 0.001$; Fig. 7B) than females. Accordingly, the PCL_{50} and age_{50} estimated from maturity gives for males were 302 mm PCL (standard error, S.E. = 5.5 mm) and 12 years (S.E. = 0.3 years) and for females were 393 mm PCL (S.E. = 6.4 mm) and 14 years (S.E. = 0.3 years) respectively.

Fifty-two female spotted ratfish had developing oocytes (>6 mm in diameter and yellow). There was no consistent difference between number of oocytes, or diameter of the largest oocyte in the left and right ovaries (t-tests: $p=0.99$ and $p=0.87$ respectively), therefore the total number of oocytes per female was used for analysis. Overall, the total number of developing oocytes, fully-developed oocytes (>20 mm), and the diameter of the largest oocyte increased with size ($developing\ oocytes = 0.15 \cdot PCL - 51.02$; $R^2=0.27$, $p<0.001$; $fullydev\ oocytes = 0.05 \cdot PCL - 19.63$; $R^2=0.29$, $p<0.001$; $diameter = 0.16 \cdot PCL - 50.18$; $R^2=0.22$, $p<0.001$) and with weight ($developing\ oocytes = 0.03 \cdot weight - 12.53$; $R^2=0.36$, $p<0.001$; $fullydev\ oocytes = 0.01 \cdot weight - 5.29$; $R^2=0.27$, $p<0.001$; $diameter = 0.02 \cdot weight - 4.42$; $R^2=0.19$, $p<0.001$). Although all of these relationships were significant, none explained greater than 36% of the variation in the data. We only observed fully-developed oocytes in spotted ratfish larger than 406 mm in PCL ($n=33$).

4. Discussion

We propose the vomerine tooth plates in the spotted ratfish are potential ageing structures and present here the first age estimates for this species based on tritor ridge counts. Overall, the number of tritor ridge counts increased with the size of the fish. The tritor ridges are distinct bumps which are easy to identify even without magnification. Precision estimates between readers suggest that this method is easy to transfer between readers. Tritor ridge counts have been suggested previously as a potential means of estimating age in this species, but issues regarding missing, or worn away, ridges (Johnson and Horton, 1972), and continuous growth of tooth plates along the posterior edge (Didier and Rosenberger, 2002) have been noted as possible drawbacks. We have provided a method for estimating the number of worn tritor ridges based on tooth plate diameter measurements for the spotted ratfish. This assumes that the tooth plate retains the same general shape as it grows and that erosion of the anterior edge of the tooth plate is continuous, occurring at a similar rate over the lifetime of the fish. Over the size range of our samples we did not observe any change in the general shape of the tooth plate. However, if the rate of erosion is not linear, then the estimated ages of older individuals in this study would be overestimated.

The peak frequency of new tritor ridges in spring suggests that there is a seasonal component to tooth plate growth, i.e. ridges are formed during a specific period each year. In the temperate waters of British Columbia, ecosystem productivity is dominated by spring plankton bloom and productivity declines by fall, with lower productivity in winter (Ware and McFarlane, 1989; McFarlane et al., 1997). As a consequence, fish species do not feed as much in winter and grow very little during that period (Chilton and Beamish, 1989). With this background, we expect new tritor ridges to form only in spring, but we are currently limited in this hypothesis since monthly samples were not available. Seasonal tooth plate growth has also been observed in *H. mitsukurii*, with new tritor ridges developing in March and again in October (Tseng, 2011), so it is possible that tritor ridges are formed twice a year in *H. colliei*. Here we have provided growth and maturity estimates based on one tritor ridge per year, but if *H. colliei* grows two tritor ridges per year the implications for the growth curve estimates and age-at-50% maturity estimates are easy to quantify. There would not be an impact on L_{inf} estimates, but k estimates would be expected to double and estimates of age-at-50% maturity would drop by half.

The logistic growth curve underestimated L_{inf} for males and females (407 mm and 494 mm PCL respectively) compared to observed maximum sizes (670 mm PCL for males and 690 mm PCL for females; J. King, unpub. data), perhaps limited by the lack of larger individuals (>495 mm PCL) in our data. Previous reported maximum size was 718 mm (PCL, estimated from Clemens and Wilby, 1961). The growth coefficients (k) estimated by the logistic growth model for either sex suggest moderately slow-growth per year.

The published length at hatch for spotted ratfish was reported as TL (130 mm; Didier and Rosenberger, 2002). In the absence of published estimates to convert TL to PCL we used the relationship estimated in this study to do so, and therefore estimate PCL at hatch to be 96 mm. In our samples we had 10 spotted ratfish with PCL length less than this estimated PCL (96 mm). It is known that spotted ratfish embryos continuously beat their tails in order to create water flow through the egg capsule for gas and waste material exchange (Didier and Rosenberger, 2002). This same behaviour has been observed in other chondrichthyans such as big skate (*Raja binoculata*), which have different growth rates of tails as embryos than as adults (Ford, 1971). Big skate embryos have relatively longer tails (compared to their bodies) than adults which translates into different conversion relationships (Ford, 1971). The result of applying our TL to PCL conversion estimated from adults is that we have likely overestimated the PCL length at hatch, although to what extent we cannot provide. As such we made no assumption on whether these 10 small spotted ratfish were hatched within the year or within the previous year of their capture. All of these 10 spotted ratfish had 2 tritor ridges, and were assigned age 2 based on the assumption that tritor ridges are formed after hatch. To date there are no published observations on the number of tritor ridges present on the tooth plates of embryonic spotted ratfish. Such observations would clarify if they hatch with any tritor ridges already formed, or if tritor ridges are formed after hatch.

It is important to note that the relationship between number of tritor ridges and ridge width was estimated with the intercept forced through zero (Fig. 3). This decision was based on the lack of spotted ratfish with zero tritor ridges, i.e. it is unknown what ranges of widths are observed when no tritor ridges are present. The tritor ridge count data are discrete, therefore forcing the intercept through zero ensures that the estimated relationship attempts to capture all possible widths from toothplate formation up until one tritor ridge is formed. Given the lack of toothplates with no tritor ridges, and the overall low sample size of small specimens, further investigation into this relationship is warranted and may prove that the assumption regarding the intercept and the overall linear nature of the relationship are incorrect. However, rejection of the assumption regarding estimation of the intercept would likely only change estimated ages in specimens with worn tritors ridges by 1–2 years (older).

The use of PCL rather than, or in addition to, TL in future observations of spotted ratfish would help to standardize methodology across studies. We have provided conversion estimates for TL, PCL and SVL. Barnett et al. (2009a) used SVL in size at maturity estimates; however this measurement can be impractical for at-sea observations, such as those made by on-board observers for commercial bycatch or those made on multi-species surveys where the priority may not be spotted ratfish. Often in large trawl catches the condition of the tail of the spotted ratfish is poor, with broken tips that preclude TL measurements. In British Columbia, the standard protocol since 2003 for at sea length measurements of spotted ratfish are PCL.

As with all age estimation methods, the use of tritor ridge counts requires validation. Barnett et al. (2009b) did not observe oxytetracycline (OTC) uptake in the dorsal spine of three spotted ratfish specimens held in captivity for one year. However, they did not examine the tooth plates for OTC uptake. Additional chemical markers such as alizarin, calcein or strontium (Campana, 2001) could be investigated for tooth plates or alternate validation methods, such as bomb radiocarbon analyses could be conducted if suitable archived specimens could be located.

The size at maturity estimates that we observed for spotted ratfish in British Columbia were higher than the estimates provided for California through Washington (Barnett et al., 2009a). Females mature at a larger size (PCL at 50% maturity = 393 mm, S.E. = 6.4 mm) than males (PCL at 50% maturity = 302 mm, S.E. = 5.5 mm), which corresponded to a later age (3 years later than males). Since classification of maturity did not include signs of mating, such as pelvic scars or broken hymen, reliance on presence of oocytes near the size of ovulation (>20 mm) to classify females as mature may overestimate the proportion mature by including maturing individuals that had yet to mate. This would result in an underestimate of size at maturity estimates, the degree to which would likely be small given that maturing females with oocytes near the size of ovulation would likely mate in the near future.

As per Barnett et al. (2009a) we also observed increasing number of oocytes (and diameter of largest oocyte) with increasing size of the fish, suggesting increased fecundity with size. In addition, the number of fully developed oocytes (>20 mm) per female spotted ratfish in this study fell within the same range (1–8) observed by Barnett et al. (2009a). However, neither study explained a high percentage of the variation in the data. In British Columbia, fully-developed oocytes were only observed in spotted ratfish greater than 406 mm (PCL), which is a larger size than that from more southern specimens (estimated PCL = 334 mm; Barnett et al., 2009a). It does appear that size at maturity increases with latitude, and it would therefore be useful to obtain estimates at the most southern (Baja) and northern (southeastern Alaska) extent of their range.

Our preliminary age, maturity and growth curve estimates suggest that *H. colliei* is a late-maturing and moderately slow-growing chondrichthyan that is long-lived compared to teleosts. Estimation of similar parameters for other chimaeroids have been published for *Callorhinchus milii* (Sullivan, 1977; Francis, 1997), *C. capensis* (Freer and Griffiths, 1993) and *Chimaera monstrosa* (Moura et al., 2004; Calis et al., 2005). Our maximum estimated ages were 16 for males and 21 for females; older than those recorded for *C. milii* (males = 4 years, females = 6 years; Sullivan, 1977) yet younger than those for *C. monstrosa* (males = 30 years, females = 26 years; Calis et al., 2005). Moura et al. (2004) estimated late ages at maturity for *C. monstrosa* (11 years for males; 13 years for females) that are similar to the estimates made in this study for *H. colliei* (12 years for males and 14 years for females). However, age at maturity estimates in Sullivan (1977) for *C. milii* is younger: 3 and 4.5 years for males and females respectively. The estimates of the von Bertalanffy growth coefficient (k) have varied from slow growing, 0.06 (Francis, 1997) to faster growing, 0.52 (Freer and Griffiths, 1993). Our estimates of k for *H. colliei* based on logistic growth curve estimation were 0.23 for males and 0.26 for females, indicative of moderately slow-growth. The present study suggests that spotted ratfish have the characteristics of an equilibrium strategist, common to many deep-water chondrichthyans, and therefore likely necessitate low or moderate harvest rates (King and McFarlane, 2003).

Acknowledgements

Dr. H.H. Hsun provided the protocol and results of age determination in *Hydrolagus mitsukurii* which formed the impetus of this study. P. Morrison assisted in the laboratory morphometric measurements. G.A. McFarlane, A.M. Edwards, C. Cotton and three anonymous reviewers provided insightful comments on this manuscript.

References

Alverson, D.L., Pruter, A.T., Ronholt, L.L., 1964. A study of demersal fishes and fisheries of the northeastern Pacific Ocean. In: MacMillan Lectures in Fisheries. Institute of Fisheries, University of British Columbia, Vancouver. 190pp.

Anderson, D.R., 2008. Model Based Inference in the Life Sciences: A Primer on Evidence. Springer, New York p. 183.

Barnett, L.A.K., Earley, R.L., Ebert, D.A., Cailliet, G.M., 2009a. Maturity, fecundity and reproductive cycle of the spotted ratfish, *Hydrolagus colliei*. Mar. Biol. 156, 301–316.

Barnett, L.A.K., Ebert, D.A., Cailliet, G.M., 2009b. Assessment of the dorsal fin spine for chimaeroid (Holocephali: Chimaeriformes) age estimation. J. Fish Biol. 75, 1258–1270.

Barnett, L.A.K., Ebert, D.A., Cailliet, G.M., 2012. Evidence of stability in a chondrichthyan population: case study of the spotted ratfish *Hydrolagus colliei* (Chondrichthyes: Chimaeridae). J. Fish Biol. 80, 1765–1788.

Beamish, R.J., Fournier, D.A., 1981. A method for comparing the precision of a set of age determinations. Can. J. Fish. Aquat. Sci. 38, 982–983.

Beverton, R.J.H., Holt, S.J., 1957. On the Dynamics of Exploited Fish Populations, 19. UK Ministry of Agriculture and Fisheries, London, UK. p. 533. (Fisheries Investigations (Series 2).

Cailliet, G.M., Goldman, K.J., 2004. Age determination and validation in Chondrichthyan fishes. In: Carrier, J.C., Musick, J.A., Heithaus, M.R. (Eds.), Biology of Sharks and their Relatives. CRC Press, United Kingdom, pp. 399–447.

Campana, S.E., 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J. Fish Biol. 59, 197–242.

Calis, E., Jackson, E.H., Nolan, C.P., Jeal, F., 2005. Preliminary age and growth estimates of the rabbitfish, *Chimaera monstrosa*, with implications for future resource management. J. Northwest Atl. Fish. Sci. 35, 15–26.

Chang, W.Y.B., 1982. A statistical method for evaluating the reproducibility of age determination. Can. J. Fish. Aquat. Sci. 39, 1208–1210.

Chilton, D.E., Beamish, R.J., 1989. Age Determination Methods for Fishes Studies by the Groundfish Program at the Pacific Biological Station, 60. Canadian Special Publication of Fisheries and Aquatic Sciences, Ottawa, Canada. p. v + 109.

Chen, Y., Jackson, D.A., Harvey, H.H., 1992. A comparison of von Bertalanffy and polynomial functions in modelling fish growth data. Can. J. Fish. Aquat. Sci. 49, 1228–1235.

Clemens, W.A., Wilby, G.V., 1961. Fishes of the Pacific coast of Canada, 68. Fisheries Research Board Canada, Bulletin p. 443.

Dean, B., 1906. Chimaeroid Fishes and their Development. Carnegie Institute Publication, Washington, DC.

Didier, D.A., Rosenberger, L.J., 2002. The spotted ratfish, *Hydrolagus colilei*: notes on its biology with a redescription of the species (Holocephali: Chimaeridae). Calif. Fish Game 88, 112–125.

Didier, D.A., Stahl, B.J., Zangerl, R., 1994. Development and growth of compound tooth plates in *Callorhinchus milii* (Chondrichthyes, Holocephali). J. Morphol. 222, 73–89.

Ebert, D.A., 2003. Sharks, Rays and Chimaeras of California. University of California Press, Berkeley, CA.

Fabens, A.J., 1965. Properties and fitting of the von Bertalanffy growth curve. Growth 29, 265–289.

Ford, P., 1971. Differential growth rate in the tail of the Pacific big skate (*Raja binoculata*). J. Fish. Res. Board Can. 28, 95–98.

Francis, M.P., 1997. Spatial and temporal variation in the growth rate of elephantfish (*Callorhinchus milii*). NZ J. Mar. Freshwater Res. 31, 9–23.

Freer, D.W., Griffiths, C.L., 1993. Estimation of age and growth in the St Joseph *Callorhinchus capensis* (Dumeril). S. Afr. J. Mar. Sci. 13, 75–81.

Gorman, T.B.S., 1963. Biological and Economic Aspects of the Elephant Fish *Callorhynchus milii* Bory in Pegasus Bay and the Canterbury Bight. Department of Fisheries Technical Report 8, New Zealand.

Grinols, R.B., 1965. Check-List of the Offshore Marine Fishes Occurring in the Northeastern Pacific Ocean, Principally off the Coasts of British Columbia, Washington, and Oregon (MS thesis). University of Washington, Washington.

Haddon, M., 2011. Modelling and Quantitative Methods in Fisheries. Chapman & Hall, USA p. 449.

Hoff, T.B., Musick, J.A., 1990. Western North Atlantic shark-fishery management problems and informational requirements. In: Pratt, H.L., Gruber, S.H., Taniuchi, T. (Eds.), Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics and Status of the Fisheries, 90. US Department of Commerce, Washington, D.C., pp. 455–472. (NOAA Tech. Rep. NMFS).

Johnson, A.G., Horton, H.F., 1972. Length-weight relationship, food habits, parasites, and sex and age determination of the ratfish, *Hydrolagus collei* (Lay and Bennett). Fish. Bull. 70, 421–429.

Jones, C.J.P., Walker, T.I., Bell, J.D., Reardon, M.B., Ambrosio, C.E., Almeida, A., Hamlett, W. C., 2005. Male genital ducts and copulatory appendages in chondrichthyans. In: Hamlett, W.C. (Ed.), Reproductive Biology and Phylogeny of Chondrichthyes. Science Publishers, Inc., Enfield, New Hampshire, pp. 361–394.

Ketchen, K.S., 1975. Age and growth of dogfish (*Squalus acanthias*) in British Columbia waters. Can. J. Fish. Aquat. Sci. 32, 43–59.

Kimura, D.K., 2008. Extending the von Bertalanffy growth model using explanatory variables. Can. J. Fish. Aquat. Sci. 65, 1879–1891.

King, J.R., McFarlane, G.A., 2003. Marine fish life history strategies: applications to fishery management. Fish. Manage. Ecol. 10, 249–264.

Mathews, C.P., 1975. Note on the ecology of the ratfish, *Hydrolagus collei*, in the Gulf of California. Calif. Fish Game 61, 47–53.

McFarlane, G.A., King, J.R., 2009. Re-evaluating the age determination of spiny dogfish using oxytetracycline and fish at liberty up to 20 years. In: Gallucci, V.F., McFarlane, G.A., Bargman, G.G. (Eds.), Biology and Management of Dogfish Sharks. American Fisheries Society, Bethesda, Maryland, pp. 153–160.

McFarlane, G.A., Ware, D.M., Thomson, R.E., Mackas, D.L., Robinson, C.L.K., 1997. Physical, biological and fisheries oceanography of a large ecosystem (west coast of Vancouver Island) and implications for management. Oceanol. Acta 20, 191–200.

Moura, T., Figueiredo, I., Machado, P.B., Gordo, L.S., 2004. Growth pattern and reproductive strategy of the holocephalan *Chimaera monstrosa* along the Portuguese continental slope. J. Mar. Biol. Assoc. UK 84, 801–804.

Musick, J.A., Burgess, G.M., Cailliet, G., Camhi, M., Fordham, S., 2000. Management of sharks and their relatives (Elasmobranchii). Fisheries 25, 9–13.

Patterson, C., 1992. Interpretation of the toothplates of chimaeroid fishes. Zool. J. Linn. Soc. 106, 33–61.

Quinn, T.P., Miller, B.S., Wingert, R.C., 1980. Depth distribution and seasonal and diel movements of ratfish, *Hydrolagus colliei*, in Puget Sound, Washington. Fish. Bull. 78 (3), 816–821.

R Core Team, 2012. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ⟨http://www.R-project.org⟩.

Ricker, W.E., 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. 191, 1–382.

Stanley, H.P., 1961. Studies on the Genital Systems and Reproduction in the Chimaeroid Fish *Hydrolagus colliei* (Lay and Bennett) (Ph.D. thesis). Oregon State Univ., Corvallis, 94p.

Stevens, J.D., Bonfil, R., Dulvy, N.K., Walker, P.A., 2000. The effects of fishing on sharks, rays and chimaeras (chondrichthyans), and the implications for marine ecosystems. Int. Counc. Explor. Sea J. Mar. Sci. 57, 476–494.

Sullivan, K.J., 1977. Age and growth of the elephant fish *Callorhinchus milii* (Elasmobranchii: Callorhynchidae). NZ J. Mar. Freshwater Res. 11, 745–753.

Tseng, C.H., 2011. Estimates of age and Growth of *Hydrolagus mitsukurii* in Gueishan Island Waters off Taiwan using Tooth Plate (MSc Thesis). Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University, Keelung, Taiwan, Republic of China. 79pp (in Chinese).

Ware, D.M., McFarlane, G.A., 1989. Fisheries production domains in the northeast Pacific Ocean. In: Beamish, R.J., McFarlane, G.A. (Eds.), Effects of Ocean Variability on Recruitment and an Evaluation of Parameters used in Stock Assessment Models, 108. Canadian Special Publication of Fisheries and Aquatic Sciences, Ottawa, Canada, pp. 359–379.

Wilimovsky, N.J., 1954. List of fishes of Alaska. Stanford Ichthyol. Bull. 4, 279–294.