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RESEARCH NOTE

Description of neonate of the freckled sand skate *Psammobatis lentiginosa* and observations on incubation period

Descripción del neonato de la raya lenticulada *Psammobatis lentiginosa* y observaciones sobre su período de incubación

Valeria Gabbanelli[1*], Diego M. Vazquez[1], Sergio M. Delpiani[1], Juan M. Díaz de Astarloa[1] and Ezequiel Mabragaña[1]

[1]Laboratorio de Biotaxonomía Morfológica y Molecular de Peces (BIMOPE), Instituto de Investigaciones Marinas y Costeras (IIMyC)-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Funes 3350, Mar del Plata 7600, Buenos Aires, Argentina
*Corresponding author: vgabbanelli@mdp.com.ar

Abstract.- Some of the characters used in description and identification keys for skates are mostly valid for adults, and may dramatically change through ontogeny. The lack of morphological description of the neonate stage prevents from identifying them in nature. Two neonates of *Psammobatis lentiginosa* born in captivity and three collected from the wild were used in this study. Morphometric differences were found, among neonates, juveniles and adults, especially, in distance between orbits, distance between first gill slits and epichordal lobe. Colour pattern and spinulation presented differences as well. Observations on incubation period were taken. Hatching occurred 130 days after egg case collection.

Key words: Egg cases, freckled sand skate, hatching, neonate morphometry, South West Atlantic

INTRODUCTION

Elasmobranchs, particularly skates (Rajiformes, Rajoidei), undergo pronounced changes in body shape throughout ontogeny (Bass 1973, Gutteridge & Bennet 2014). Although skates´ neonates resemble adults, they may differ in disc shape, spinulation, number of tooth rows, tooth shape and in possessing a somewhat longer tail (Bigelow & Schroeder 1953, Braccini & Chiaramonte 2002, Oddone & Vooren 2002). However, some of these characters have been used as taxonomical tools in descriptions and identification keys. Most of these features are based on adult and juvenile specimens (Stehmann 1979, McEachran 1983), and fail to identify newborn and young skate individuals in nature (Oddone & Vooren 2002, Mabragaña *et al*. 2015). The freckled sand skate *Psammobatis lentiginosa* McEachran, 1983, is an endemic species of the South West Atlantic Ocean (McEachran 1983, Mabragaña 2007, Mabragaña *et al*. 2012). Until recently, it was listed by the IUCN Red List of Threatened species as "Data Deficient" (Kyne 2007) but it was reassigned to "Least Concern" by Pollom *et al*. (2020). However, information about incubation period, size at hatching and neonate morphology is not available. Therefore, the aim of this paper was to provide the first description of neonates of *P. lentiginosa* and to provide information on its incubation period in captivity.

MATERIALS AND METHODS

Two egg cases of *P. lentiginosa* (UNMDP 4824 and UNMDP 4825, stored at the Colección Ictiológica of the Instituto de Investigaciones Marinas y Costeras, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina, containing live embryos, were collected with demersal bottom trawl pilot net (50 mm mesh in the wings, and 20 mm in the cod end; vertical height 1 m, horizontal opening 4 m) on the Argentine Continental Shelf (ACS) at 40°3´S and 60°14´W at 63 m depth on August 21 2014, by the RV Puerto Deseado (CONICET). Egg cases were identified following Mabragaña *et al*. (2011). At the moment of collection, they were inspected by transparency and stage of development was assigned according to Brant (2006).

Encapsulated embryos were maintained alive during the last three days of a five days long cruise and then kept until hatching in a 1,400 L tank with marine water and an airlift system, at room temperature between 10-24 °C from August to December, at Nágera Station (Universidad Nacional de Mar del Plata, Argentina). Neonates were fed with fish and squid. Parameters of water quality (ammonia NH_4^+, nitrite NO_2^-) were controlled every 15 days with a specific kit Tetra Test brand, and water replacement was made according to them.

After decease of neonates, a tissue sample from the tail was taken and preserved in 96% ethanol at -20 °C, for genetic analysis in order to corroborate the identification. DNA extraction, polymerase chain reaction (PCR) and sequencing of cytochrome oxidase subunit I (COI) gene were performed following standard DNA barcoding methods (Hebert *et al.* 2003). The DNA sequence was compared to the library of available sequences in BOLD (Barcode of Life Data Systems) and the relative placement of the complete sequence in the lineage cluster, or Barcode Index Number (BIN) assigned by BOLD, was assessed. The BIN system is an online framework that clusters barcode sequences algorithmically. Since clusters show high concordance with species, BINs can be used to verify species identifications (Ratnasingham & Hebert 2013).

In addition, two neonates (UNMDP 5207 and UNMDP 5208) and a young of the year (YOY) (UNMDP 5209) of *P. lentiginosa*, could be identified within a sample containing specimens of other species at the same life stages. These were subsequently kept frozen. These specimens were collected with a bottom trawl net on the ACS between 44°35.7´S-65°1.9´W and 46°1.1´S-65°49.3´W, by the RV "Dr. Eduardo Holmberg" (INIDEP) in 2005. Barcode analysis was carried out in these specimens as well. The identification of these three specimens as neonate or YOY was based in the comparison of total length within specimens born in captivity, since age of each individual is unknown.

Description of the four neonates (two collected in the wild and two born in captivity) and the YOY of the freckled sand skate was carried out considering colour pattern and spinulation following McEachran (1983). Measurements were obtained following McEachran (1983) and Last *et al.* (2008). Neonate UNMDP 4824 was measured 30 days after hatching and neonate UNMDP 4825 could not be included in the morphologic analyses (see Results and discussion section).

Results and discussion

The inspection by transparency at the moment of collection of the egg cases of *P. lentiginosa* allowed identifying the internal content of each of them. One of the egg cases (UNMDP 4824) had a visible embryo which characteristics (distinguishable trunk and tail movements) corresponded to an embryo close to stage 25 of development. The other egg case (UNMDP 4825) presented a distinguishable yolk indicating it was recently laid. Observations on the incubation period could be made as they were kept in captivity. Hatching occurred 100 days after collection for UNMDP 4824, and the neonate could be kept alive for 30 days before its decease (Fig. 1A). For UNMDP 4825, hatching occurred 130 days after collection, but the neonate could not be kept alive (Fig. 1B). These results suggest that the incubation period of the freckled sand skate could take a minimum of 130 days.

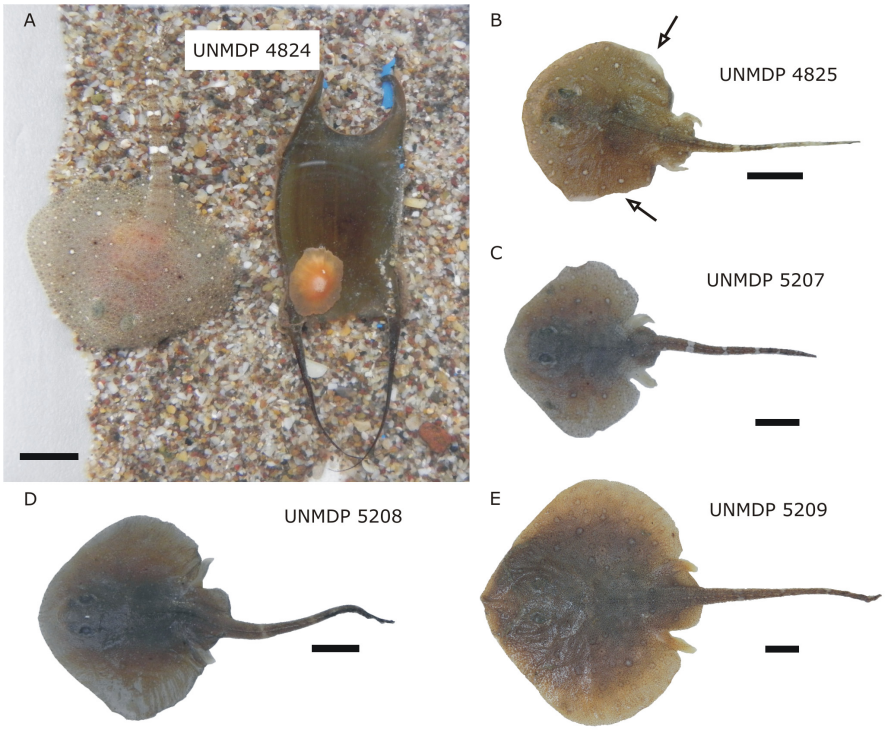

Figure 1. Neonates of *Psammobatis lentiginosa* born in captivity: A) alive after hatching and with egg case, B) when found in the aquarium, arrows indicate parts of the body deteriorated by high temperature; and neonates (C, D) and young of the year (E) collected in the wild. Scale bar (A-E)= 10 mm / Neonatos de *Psammobatis lentiginosa* nacidos en cautiverio: A) vivo luego de la eclosión junto a su cápsula de huevo, B) en el estado en que se halló en el acuario, las flechas indican las partes del cuerpo deterioradas por las altas temperaturas; y neonatos (C, D) y juvenil del año (E) colectados en la naturaleza. Barra de escala (A-E)= 10 mm

However, it is noteworthy that *P. lentiginosa* inhabits in the shelf from 50 to 164 m (Mabragaña *et al.* 2012) and water temperature ranges between 10 and 16 °C in the area where egg cases were collected (Cousseau *et al.* 1979). Therefore, considering that an increase in temperature may reduce the incubation period in elasmobranchs, and that egg cases were incubated at a range of temperature between 10 and 24 °C, incubation period is expected to be longer in nature (Capapé *et al.* 2006, Hoff 2008, Hume 2019). Size at hatching of UNMDP 4825 was 64.6 mm in total length, corresponding to 13.8% of the maximum length reported for this species, which is 467 mm (Cousseau *et al.* 2007, Mabragaña *et al.* 2012). It is important to note that this value was taken only for one neonate, and that size at hatching in elasmobranch oviparous species is related to egg case size, so the variability of this characteristic could not be addressed in this study (Capapé *et al.* 2007). Nevertheless, size at hatching constitutes the length at time zero, and is an important parameter needed in age and growth models for population assessment, therefore this information can be valuable in future studies (Ricker 1979).

For the barcode analysis, a complete DNA sequence of 553 base pairs of the COI gene was obtained from specimen UNMDP 4824. When compared to BOLD, the sequence presented a similarity range of 98.5 to 100% with other sequences of *P. lentiginosa*, and was included in a single BIN (BOLD: AAD8610) corresponding to this species, and confirming their identification.

Two newborns (UNMDP 5207, UNMDP 5208) and a YOY (UNMDP 5209) of *P. lentiginosa* were identified and taken into account for the description based on the characteristics of the neonates born in captivity and tail spinulation described by McEachran (1893) for juveniles (Fig. 1C, D and E). Sequences of UNMDP 5207, UNMDP 5208, and UNMDP 5209 produced a bright band on the gel of the PCR products, but yielded poor-quality sequences. Therefore, species identification was traditionally based on morphological data. Morphometric measurements and meristic values of specimens included in this analysis, and egg cases for the specimen born in captivity are presented in Table 1. It is noteworthy that the neonate UNMDP 4824 was measured 30 days after hatching, since it was not possible to take the measurements while the neonate was alive. On the other hand, neonate UNMDP 4825 was not included in the morphometric analyses. This neonate hatched during Christmas holidays in summer season in Argentina. Unfortunately, when returning after four days, the neonate was found death because of an important increase in the water temperature (up to 24 °C). Due to high temperature and the small body size, the neonate was found partially deteriorated, which prevented to take several measurements on this specimen. Neonates present a caudal filament which length and persistence after hatching can vary among individuals (Bigelow & Schroeder 1953). It is important to remark that the caudal filament was absent in the case of neonates analyzed in this study (Bigelow & Schroeder 1953). Therefore, measurements expressed in percentage of total length are provided, in order to be compared to the original description of the species. The total length range of the neonates and YOY was 71.12-111.5 mm and they had the following features and proportions: heart-shaped disc, margin of tip convex to level of the orbits, anterior margin of the pectoral fins almost straight with a slightly concave curvature, tip and posterior margin of pectoral fins rounded, anterior and posterior lobes of pelvic fins separated by a deep incision, anterior lobe tapering towards tip and the posterior one rounded; dorsal fins separated and curved backwards, tail more or less half of total length tapering towards tip, and caudal fin poorly developed; preorbital snout length 0.9 to 1 times preoral snout length; prenasal length 1.4 to 1.5 times distance between fifth-gill slits; interorbital distance 0.9-1.3 times orbit diameter; disc width 3.7-6.3 times distance between first-gill slits; epichordal lobe 1.5-2.6 times half the base of second-dorsal fin and 0.5-0.8 as high as second-dorsal fin; disc width 1.2-1.3 times disc length; distance from cloaca to caudal-fin tip 1.1 to 1.5 times distance from snout to cloaca; length of preoral snout 2.7 to 2.9 times the diameter of the orbit; preoral snout length 2.3 to 2.7 times distance between nostrils; orbit diameter 1.2 to 1.6 times spiracle length; distance between mouth and nostril 0.9 to 1.5 times distance between nostrils; distance between first-gill slits 2 to 2.6 times distance between nostrils; distance between fifth-gill slits 1.4 to 1.5 times distance between nostrils. McEachran (1983) described *P. lentiginosa* from juvenile and adult specimen's 119-433 mm total length. Differences in body proportions (included in the species diagnosis) among neonates, juveniles and adults were observed. Distance between orbits is less (0.7-0.9) than orbit diameter (McEachran 1983) in adults, while in neonates this distance is greater than the orbit diameter (0.9-1.3). Epichordal lobe is nearly as high as the second-dorsal fin in adults, while in neonates it can be about half as high (0.5-0.8) as the second-dorsal fin. Distance between first-gill slits in relation to disc width is somewhat greater (3.7-6.2) in neonates than in adults and juveniles (3.7-4.7). On the other hand, disc, pelvic fins and dorsal fins shape and number of tooth rows, are consistent with the characteristics described for juvenile and adult females.

Table 1. Measurements expressed as percentage of total length (TL) and meristic values of neonates and young of the year (YOY) of *Psammobatis lentiginosa* in comparison with those of adults and juveniles obtained from literature (McEachran 1983) (ht: holotype, pt: paratypes and $\bar{x}$: mean). Egg cases measurements are shown in mm / Medidas expresadas en porcentaje del largo total (TL) y valores merísticos de los neonatos y del juvenil del año (YOY) de *Psammobatis lentiginosa*, en comparación con aquellos correspondientes a adultos y juveniles, obtenidos de la literatura (McEachran 1983) (ht: holotipo, pt: paratipos y $\bar{x}$: media de los paratipos). Se muestran las medidas de las cápsulas de huevo en mm

	Neonates			YOY	McEachran 1983	
	UNMDP 4824	UNMDP 5207	UNMDP 5208	UNMDP 5209	ht.	pt. (n= 20)
Sex	2	2	2	1	1	
Total length (TL) (mm)	71.1	87.2	87.6	115.7	370	119-433
Disc width (DW) (mm)	40.9	44.4	48.1	70.8	-	-
	Percent of TL					
	UNMDP 4824	UNMDP 5207	UNMDP 5208	UNMDP 5209	ht.	$\bar{x}$
DW	57.5	50.9	54.9	61.2	56	53
Direct disc length	52.6	41.9	47.5	53.2		
Indirect disc length	48.9	38.8	44.9	49.3	45	46
Snout to maximum width	32.3	24.3	23.9	28.3	28	27
Direct preorbital snout	14.7	13.9	13.5	13.1		
Indirect preorbital snout	13.4	11.7	12.3	12.2	9.9	10.7
Orbit diameter	4.8	4.8	4.7	4.9	4.9	4.5
Orbit and spiracle length	7.6	6.5	5.8	5.5	5.7	5.8
Spiracle length	4.1	3.2	3.0	3.5	2.1	2.6
Distance between orbits	5.6	5.2	6.3	4.2	3.5	3.4
Snout to cloaca	47.9	40.0	42.0	46.8	41	41
Distance cloaca to caudal fin tip	53.0	60.0	59.4	54.3	58	59
Preoral snout length	14.0	11.9	13.5	13.4	10.1	11.3
Prenasal length	10.9	8.6	10.4	10.9	7.3	8.5
Mouth width	7.4	10.0	8.9	9.2	7	7.1
Distance between nostrils	5.0	5.3	5.6	4.9	4.7	4.3
Distance between mouth and nostril	7.3	5.3	4.9	5.2	5	4.4
Width of first gill slit	5.2	1.5	1.8	1.8	1.5	1.6
Width of fifth gill slit	2.3	1.8	2.5	1.1	1.2	1.2
Distance between first gill slits	10.3	13.9	13.2	9.8	13	13
Distance between fifth gill slits	7.2	7.5	8.0	7.2	6	7
Length of anterior pelvic lobe	12.0	11.9	15.2	14.2	12	11
Length of posterior pelvic lobe	14.1	11.8	13.9	15.4	21	17
Tail width at axil of pelvic fins	5.2	4.2	5.5	3.5	4.6	4.3
Length of first dorsal fin base	3.0	3.8	3.1	4.1		
Height of first dorsal fin	1.5	2.3	2.1	1.4		
Length of the second dorsal fin base	3.3	3.2	4.5	3.0		
Height of second dorsal fin	1.0	2.3	1.4	1.1		
Length of caudal fin	2.5	-	12.4	4.0		
Height of caudal fin	0.5	-	0.7	0.9		
Snout to start of second dorsal fin	88.7	81.4	81.7	93.5		
Number of tooth rows in the upper jaw	38	32	32	40	38	40
Egg case length	35.8					
Egg case width	23.9					

Regarding colouration, neonates and YOY presented dorsal surface brownish with indistinct paler circles surrounded with dark brown rings of various sizes scattered over the entire disc, pelvic fins and tail. The smallest spots were margined with open light brown-coloured rings, while the biggest ones had darker and closed margins. There were also small black spots scattered over the body (Fig. 2A). Four light-coloured transversal bands were present on the tail (Fig. 2A). Ventral surface whitish. It is remarkable to mention that colouration of specimens UNMDP 5207, UNMDP 5208, UNMDP 5209, and UNMDP 4825 was darker (Fig. 1B-E) because they had been stored in freezer, when collected from wild and from the aquarium. Meanwhile, in adult specimens the dorsal-colour pattern is light brown with numerous small black spots scattered over the disc and tail. Juveniles have the same colour pattern as adults but they may present the spots concentrated into small circles with tanned centers (McEachran 1983).

The dorsal surface of neonates and YOY was uniformly covered with dermal denticles, characteristic that is shared with juveniles of this species (McEachran 1983), but they lacked thornlets. Instead, adults have thornlets on both the anterolateral and posterior margins of each pectoral fin, and dermal denticles constitute a belt along the midline of the disc. Orbital thorns are absent in neonates, while up to four and seven can be found in juveniles and adults, respectively. Juveniles have one row of lateral-dorsal thorns flanking the single row of medial dorsal thorns along the midline of the disc, posterior to suprascapula. These were absent in neonates. Adults, instead, have two or three irregular rows of thorns in the mid dorsal zone of disc. In addition, the triangular patch of thorns in the nuchal and scapular region, characteristic in adults of this species is absent in neonates and YOY (McEachran 1983). In neonates and YOY, a single row of 17 to 21 strong backwardly curved thorns was present in the midline of the tail (Fig. 2B). Irregular rows of thorns were seen on each side of the midline of the tail from the fourth thorn. These thorns were smaller and thinner than those of the midline, starting at the level of posterior pelvic lobe (Fig. 2C). Towards the end of the tail a well-defined row of thorns was developed on either side, thus three rows of thorns were clearly noticeable (Fig. 2D). The ventral surface was smooth with no dermal denticles present. Juveniles had one to three irregular rows of caudal thorns, consistent with what was found in neonates and YOY in this study, but different to those of adults which have three to five rows (McEachran 1983).

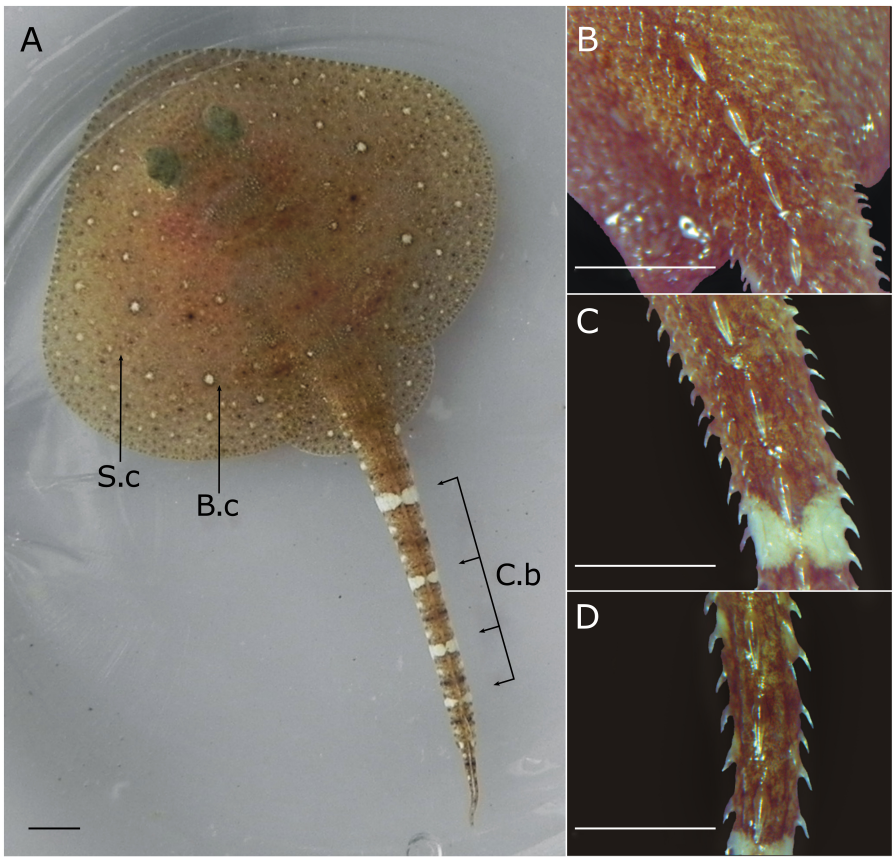

Figure 2. Details of dorsal colouration (A) of *Psammobatis lentiginosa* neonates and variation of their spinulation; from irregular rows at the beginning of tail (B), passing through a transition (C), to three regular rows at the end (D). C.b: clear bands, B.c: big circles, S.c: small circles. Scale bar (A-D)= 5 mm / Detalles de la coloración dorsal (A) de los neonatos de *Psammobatis lentiginosa* y variación de su espinulación; desde hileras irregulares al comienzo de la cola (B), pasando por una transición (C), hasta tres hileras regulares hacia el final (D). C.b: bandas claras, B.c: círculos grandes, S.c: círculos pequeños. Barra de escala (A-D)= 5 mm

A detailed description of neonates and YOY of *P. lentiginosa* was given herein. The results of the present work showed the similarities and differences among neonates, juveniles, and adults of *P. lentiginosa*, indicating the variation in morphology, spinulation, and colour pattern skates may go through during ontogeny. McEachran (1983) provided an identification key to distinguish species of *Psammobatis* based on adult specimens, in which most of the characteristics used to differentiate *P. lentiginosa* from its congeners, are those listed above. As expected, this identification key does not allow identifying neonates and juveniles in nature. Therefore, an update of identification keys including characters of neonates and juveniles of *Psammobatis* identification is needed in order to ensure adequate identification in the field.

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