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The egg capsule of *Rioraja agassizi* (Müller & Henle) (Elasmobranchii, Rajidae), endemic to the SW Atlantic

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Abstract. Freshly collected egg capsules of *Rioraja agassiz*i were light and bright brown in colour, laterally keeled and symmetrically convex with a smooth configuration of the surface. The base of the horns, the lateral margins and the ventral wall of the egg capsules were covered by sticky, shiny yellow attaching fibrils. The mean total length of the egg capsules was 47 mm and the mean width was 31 mm. Microscopically, the dorsal wall of the egg capsules was 0.21 mm thick and consisted of three layers, being the central one colourless and laminated. The ventral wall was 0.11 mm in thickness and presented the same layer's patterns of the dorsal wall. Length of the egg capsules positively increased with female's length.

Key words: oviparity, lateral keel, egg-bearing, microscopic structure.

Resumen. La cápsula ovígera de *Rioraja agassizi* (Müller & Henle, 1841) (Elasmobranchii, Rajidae), endémica del Atlántico SW. Las cápsulas ovígeras de *Rioraja agassiz*i recientemente colectadas eran de un color marrón claro brillante, presentaban una quilla lateral, eran simétricamente convexas y poseían superficies lisas. La base de los cuernos, así como los márgenes laterales y la pared ventral estaban recubiertos por filamentos pegajosos de adhesión de color amarillo brillante. La longitud total media de las cápsulas fue 47 mm y el ancho medio 31 mm. Microscópicamente, la pared dorsal de las cápsulas ovígeras tenía un grosor de 0.21 mm y consistía en tres capas, siendo la del medio descolorida y laminada. La pared ventral tenía un grosor de 0.11 mm y presentaba el mismo patrón laminar de la fase dorsal. La longitud de las cápsulas ovígeras aumentó positivamente con la longitud total de las hembras.

Palabras clave: oviparidad, quilla lateral, ovada, estructura microscópica

Introduction

The genus *Rioraja* is endemic to the austral region of South America (McEachran & Aschliman 2004) and comprises one single species, *R. agassizi* (Müller & Henle 1841), which occurs from Argentinean waters to Rio de Janeiro in Brazil and inhabits from coastal waters to depths of up to 130 m (Figueiredo 1977). In south Brazil is considered a constantly present species being found from 15 to 60 m of depth (Vooren 1997).

In oviparous elasmobranchs the egg is covered with a thick leathery membrane, the egg capsule (shell) with features that aid in their identification besides establishing possible relationships between genera and even between species; apart from throwing some light on adaptive differentiation of various species (Ishiyama 1958). Apart from providing taxonomic information (Hubbs & Ishiyama 1968), the morphology and external features of the skate's egg capsules can provide data on the distribution and reproductive biology of skates (Oddone *et al*. 2004). Furthermore, Ishiyama (1958) demonstrated that the microscopic structure of Japanese skates' egg capsules is an important source of systematic information on the different rajid species.

In this work, the morphological and microscopical description of the egg capsule of *R. agassizi* is presented.

Materials and Methods

A number of 119 egg capsules of *Rioraja agassizi* were collected by bottom trawler commercial vessels during March and May 2005, off Southeast Brazil. The fishing area is presented in Figure 1. Depth ranged between 13 and 52 m. Egg capsules were directly extracted from the females' uteri; fixed in 4% formalin and preserved in 70% ethanol (Fig. 2). Terminology on the egg capsule and methods of measurements followed Hubbs & Ishiyama (1968) Templeman (1982) and Gomes & Carvalho (1995).

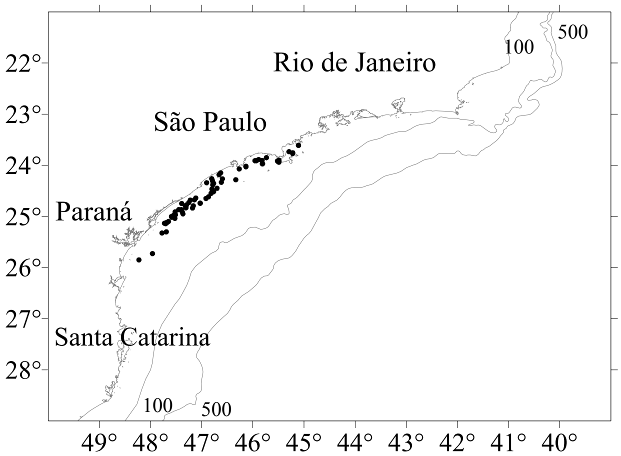

Figure 1. Map of the study area: Southeast Brazil, showing the trawling stations (dotted circles) from where samples of *Rioraja agassizi* came from.

Data recorded from each egg capsule were classified into measurable variables and features (Hubbs & Ishiyama 1968). The measurable variables were: total length; total width; total length of anterior and posterior horns, height (in the highest point) and thickness and width of the lateral keel. Features recorded were: colour; configuration of the surface or texture; basic morphology, presence of adhesion fibrils and microscopical structure of both sides of the capsule's wall. Horns were measured on each side, according to Hubbs & Ishiyama (1968).

All measurements were made with Vernier calipers with 0.1 mm precision. Differences were tested using a t-test (Sokal & Rohlf 1987) with a significance level of 0.05. The terms 'anterior'/'posterior' and 'ventral/'dorsal' refer to the position of the egg capsules in the uteri (Clark 1922, Hubbs & Ishiyama 1968).

To examine the microscopical structure of the egg capsules' wall, rectangular sections (5x5 mm) of the dorsal and ventral walls were carried out centrally on the egg capsules using a scalpel according to Hubbs & Ishiyama (1968). The latter were submitted to the following inclusion protocol in historesin to be afterwards cut with the aid of a microtome: a) sections were dehydrated for half an hour in alcohol 70%, 80 %, 90 % and finally 95 %; b) an embedding resin was prepared and reposed for 24 hours refrigerated; c) preparation protocol for inclusion resin consisted in mixing 15 ml of the embedding plus 1 ml of the hardener solution, shaking (in the shaker) for 3 minutes and depositing in mould, where the egg capsules' cuts were incorporated and hold with tweezers until the resin hardened to keep the vertical position to allow the transversal cut desired in the microtome; d) the samples were kept in heater for 24 hours and immersed in silica to extract humidity; e) moulds containing the sections were glued to wooden blocks to be cut in the microtome (LEICA RM 2145, at Histology Laboratory, Biology Department, UNESP); f) after discriminating between several thickness in the microtome and observing in the microscope, the ideal thickness was found to be 8 μ and this thickness was kept for all the cuts. Sections were placed in the slides and deposited in the heater for 24 hours; g) sections were embedded in Canadian Balsam, covered with cover slide, deposited in the heater for 48 hours for fixation; h) sections were observed under microscope (at 100X) for black and white photographing and assessing. Internal layer refers to the egg capsule's wall layer oriented to the capsule interior; external layer refers to the layer in contact to the exterior.

Figure 2. Complete posterior oviducts (*uteri*) recently extracted from a female of *Rioraja agassizi* bearing term egg capsules; n.g.=nidamental glands. Black bar represents 1 cm.

A linear regression was used to analyse the relationship between total length of the egg capsule and total length of the female.

Results

In all the analysed females, only one egg capsule per uterus was found. Freshly collected and fully formed egg capsules of *R. agassizi* were typically rectangular with a horny process in each corner and bright brown in colour. The base of the horns, the lateral margins and the ventral wall of the egg capsules were covered by sticky, shiny yellow adhesion fibrils. To the naked eye and touch, both dorsal and ventral walls were smooth. In lateral view, the egg capsules were keeled and symmetrically convex, with the highest point situated centrally (Fig. 3).

Figure 3. The term egg capsule of *Rioraja agassizi* in lateral view (above) and upper view (below). Black bar represents 1 cm.

The total length of the egg capsules of *R. agassizi* ranged from 41 to 56 mm (mean=47.34) and the width ranged from 22 to 36 mm (mean=30.53). The descriptive statistics for all the morphological variables recorded are presented in Table I. The anterior horns' length varied from 38 to 53 cm (mean=49.15) and posterior horns' length from 35 to 68. Posterior horns were significantly longer than anterior horns (t=-15.1, P>0.000, df=95). The ratio posterior/anterior horns was 1:4. The lateral keel was in mean 1.16 mm wide and 1.66 mm thick. The egg capsules had a mean height of 12.08 mm. In eight egg capsules, anterior velum was absent, and was variable in length when existing, with a mean length of 3.34 mm and the posterior velum of 6.33.

Microscopically, the dorsal wall of the egg capsule of *R. agassizi* was 0.21 mm thick and consisted of three layers: an external layer; dark brown and markedly darker than the rest, 0.023 mm thick; a middle layer; colourless, luminous and laminated, with a number of 28 superimposed lamina and 0.182 mm thick and an interior rather thin layer; dark brown and 0.007 mm thick (Fig. 4a). The ventral wall presented the same pattern of a light, middle uncoloured layer between two darker and rather thinner layers. In transverse section, the ventral wall of the egg capsule was 0.11 mm in thickness. The most external layer was dark brown and 0.022 mm thick, followed by a middle layer, colourless and laminar (consisting of about 20 lamina) and 0.084 mm thick, and finally, an interior layer, dark brown and 0.0044 mm wide (Fig. 4b).

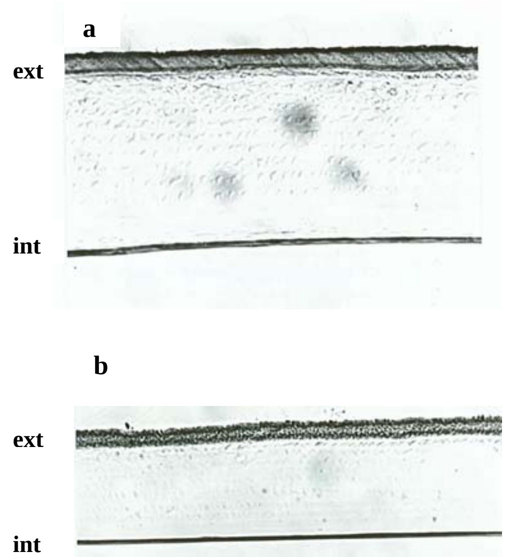

Figure 4. The microscopical structure of the dorsal (a) and ventral (b) walls of *Rioraja agassizi* (100X); ext.=external; int.=internal.

Larger females were found to produce larger egg capsules according to: ECL=0.0402*FTL+1.1632 (ECL=egg capsule length; FTL=female total length). The total length of the egg capsule as dependent variable had a positive and significant ($p < 0.000$, R=0,61) relationship with the female's total length (Fig. 5).

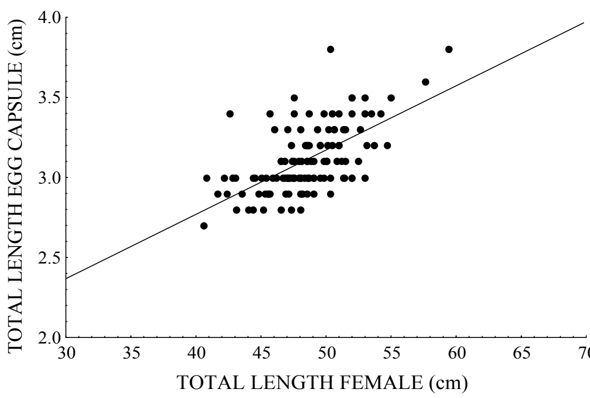

Figure 5. The relationship between female's total length (cm) and egg capsule's length (cm) for *Rioraja agassizi*.

Table I. Descriptive statistics for the variables measured (mm) on the egg capsules of *Rioraja agassizi*; number (n), mean, range, standard deviation (SD) and standard error (SE) for: total length, total width, height, width and thickness of the keel, length of anterior and posterior horns (A., P., horns), length of anterior and posterior velum (A., P., *velum*).

Variable	n	Mean	Range	SD	SE
Total length	119	47.34	41-56	3.09	0.28
Total width	118	30.53	22-36	2.32	0.21
Height	79	12.08	7-15	1.45	0.16
Keel width	110	1.16	1-2	0.33	0.03
Keel thickness	110	1.66	1-3	0.42	0.04
A. horns	112	35.93	15-53	1.06	0.45
P. horns	107	49.15	35-68	6.54	0.45
P. *velum*	114	6.33	3-10	1.13	0.11
A. *velum*	55	3.34	1-6	1.33	0.18

Discussion

Single oviparity (one embryo per egg capsule) is the only type of reproduction in the family Rajidae, being one egg deposited at a time from each oviduct usually in pairs during the spawning season (Musick & Ellis 2005).

The smooth surface of the egg capsule of *R. agassizi* distinguishes it from the egg capsules of the species of genus *Atlantoraja*, where a marked (and macroscopical) longitudinal striation exist, with the exceptional egg capsule of *A. castelnaui* which has a rather smooth surface that resemble the egg capsule's surface of *R. agassizi*, even in the microscopical composition of the egg capsule's wall (Oddone, unpublished data). In the Japanese rajids, there is a notorious difference in the configuration of the surface of the main portion of the capsules between the northern and the southern species; being the capsule somewhat roughened with minute prickles or tubercles over the entire surface in the northern forms, but it is, in general, very smooth in the southern ones (Ishiyama 1958).

Another difference with the genus *Atlantoraja* is the equally convex walls of the egg capsule of *R. agassizi,* that are also typical of *Sympterygia acuta, S. bonapartii*, and *Psammobatis* spp. (Oddone & Vooren 2002, Oddone *et al*. 2004). The egg capsule of *R. agassizi* is smaller than the egg capsules of *Atlantoraja* spp. (with a mean size of ~47x30 cm against 68x39 cm in *A. cyclophora*, the smallest egg capsule of the genus) and than the egg capsules of *S. bonapartii* with mean dimensions of 77x48 cm (Mabragaña *et al*. 2002), but somewhat larger than *S. acuta's*, with mean size of ~5x3 cm. The latter can be easily distinguished because of the coloration (green olive) and the presence of tendrils instead of posterior horns as in *R. agassizi* (Oddone & Vooren 2002).

The disposition of the adhesion fibrils in *R. agassizi* is the same than observed in *Atlantoraja* spp. (Oddone *et al*. 2004, Oddone, unpublished data). In *Callorhynchus milii* a convex surface is covered with sticky fibrils that attach sand thereby camouflaging the egg capsule, while the opposite side is smooth and acts as a suction cup to keep the capsule planted in the sand (Hamlett *et al*. 2005) that likely happens in *R. agassizi*. The degree of development of the fibrils' mass in some cases is a character of value in distinguishing one species from another (Ishiyama 1958).

Ishiyama (1958) recorded lateral keel in 20 of a total of 21 examined species. Egg capsules of *Rioraja* are typically laterally keeled as in *Atlantoraja* spp., unlike in genus *Symperygia* (at least in *S. acuta* and *S. bonapartii*) where capsules are laterally flanged (Mabragaña *et al.* 2002, Oddone *et al*. 2004). A striking external feature of the egg capsule of *R. agassizi* was the variability of the anterior *velum* length that varied from absent to 6 mm, in some cases being longer than the posterior velum.

The length of the horns shows rather large intraspecific variations and as a rule, the length of the posterior horns tends to appear a trifle longer than that of the anterior ones (Ishiyama 1958). In *R. agassizi*, the ratio posterior/anterior horns was 1.4. In *Atlantoraja cyclophora* and *A. platana* this ratio was 2.4 and 2.7 respectively (Oddone *et al*. 2004). In *S. acuta* the ratio is 15.7, particularly high because of the presence of tendrils instead of (posterior) horns (Oddone & Vooren 2002).

As observed by Ishiyama (1958) for Japanese rajid species, the main portion of the egg capsule in *R. agassizi* is made of an internal, 'pulpy' layer and an external layer darker than the rest. The author noted that, in general, the 'pulpy', middle layer (forming the lining of the capsule wall) is relatively soft and colourless, but the tissue becomes hard and the colour changes from yellow to brown in the external layer which covers the surface of the capsule and that the degree of development of this 'pulpy' layer seemed to be correlated with the distribution of the skates. In *A. cyclophora,* for instance, the external layer corresponds with about 0.33% of the egg capsule' wall in both ventral and dorsal walls (Oddone, 2005). In *R. agassizi*, this percentage is ~0.1-0.2. Ishiyama (1958) concluded that there is a close relationship between the differentiation of the capsule and the geographical distribution, as well as the breeding habits of the adult skates (though there are some examples that do not fit this conclusion) and the much smoother surface of an egg capsule's species, could have resulted from its adaptation to an environment where the temperature is relatively high. This can be related with the different areas inhabited by *R. agassizi* and *A. cyclophora*; while the former prefers coastal, shallow waters of up to 50 m deep, the latter is found at deeper shelf waters of up to 300 m deep (Vooren 1997, Oddone 2003, Oddone 2005).

Positive relationships between egg capsules length and female's total length have been observed for several Rajidae and also Scyliorhinidae (Ishiyama 1958, Templeman 1982, Braccini & Chiaramonte 2002, Iglesias *et al*. 2002). Such relationship, however, was not observed in *Atlantoraja cyclophora* (Oddone 2003).

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