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A N I N T E R N A T I O N A L J O U R N A L

Egg Development of *Paralichthys orbignyanus* (Valenciennes, 1839)

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ABSTRACT

Eggs of the Southern Brazilian flounder Paralichthys orbignyanus *were obtained through spawning induction and reared in laboratory. The eggs released were free-floating or pelagic. Other important characteristics were: spherical shape, smooth chorion, narrow perivitelline space, and a single oil globule. Egg and oil globule diameter were about 0.792 ± 0.008 mm and 0.114 ± 0.003 mm, respectively. The blastodisc was observed approximately 1 h after fertilization and followed by blastodisc cleavage. Blastula stage started after about 5 h and gastrula stage after 9 h. Approximately 20 h after fertilization, blastopore closure was observed. Neurula or early embryo occurred after 24 h. Cardiac beats and movements of the free embryonic tail were recorded after 40 h of incubation. Hatching occurred after an incubation period of 40-50 h at temperatures ranging from 18 to 20 ºC. Newly hatched larvae were about 2.04 ± 0.024 mm long and quite undeveloped, with a large yolk sac with a posterior oil globule and sparse pigmentation. These results were greatly similar to those obtained by previous studies regarding congener species. This is one of the first steps towards the artificial propagation of this species under controlled conditions.*

Key words: Flounder, *Paralichthys orbignyanus*, egg, embryo, development

INTRODUCTION

Flounders of the genus *Paralichthys*, belonging to the order Pleuronectiformes, have both eyes on the left side of the body, which is highly compressed (Ahlstrom et al., 1984). According to Lema et al. (1980), they pertain to the family Bothidae and subfamily Paralichthinae. However, Ahlstrom et al. (1984) and Figueiredo and Menezes (2000) placed this genus in the family Paralichthyidae. Several species of this order are of great commercial importance for aquaculture and are being reared worldwide (Ikenoue and Kafuku, 1992; Lavens and Remmerswaal, 1994; Alvial and Manríquez, 1999; Bengtson, 1999; Olsen et al., 1999).

There are many flatfish species commercially exploited by fisheries along the southern Atlantic coast of America (Brazil, Uruguay and Argentina), due to their high quality food value, but they are not produced through aquaculture yet. Lema et al. (1980) provided a list of pleuronectiforms inhabiting areas from Santa Catarina state (Brazil) to Rio de La Plata (Argentina), emphasizing the occurrence of the flounder *Paralichthys orbignyanus* (Valenciennes, 1839). In Rio Grande do Sul state (Brazil, lat. 29º S - 34º 40' S), *P. orbignyanus* and Patagonian Flounder *P. patagonicus* Jordan, 1889 are very important (Haimovici et al., 1996) for artisanal and industrial fisheries, respectively.

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There is scant information on the reproductive biology of south Atlantic flatfish species. Silveira et al. (1995) studied some aspects of *P. orbignyanus* reproduction in the estuary of Patos Lagoon and nearby coastal waters (lat. 32º S). Cerqueira et al. (1997) obtained artificial spawning of wild *P. orbignyanus* specimens, through hormonal induction. On the other hand, the rearing of juveniles of this species has succeeded experimentally (Sampaio et al., 2001).

The egg and larval development of two congener species inhabiting the Pacific coast of South America, *Paralichthys adspersus* (Steindachner, 1887) and *P. microps* (Gunther, 1881), has already been described (Silva, 1988; Silva and Flores, 1989; Zuñiga and Acuña, 1992). The early life history stages of some flounder species from the northern hemisphere, namely the bastard halibut *P. olivaceus* (Temminck and Schlegel, 1846), summer flounder *P. dentatus* (Linnaeus, 1766), the California flounder *P. californicus* (Ayres, 1859) (Ahlstrom et al., 1984), gulf flounder *P. albigutta* Jordan and Gilbert, 1882 and southern flounder *P. lethostigma* Jordan and Gilbert, 1884 (Powell and Henley, 1995), are well known.

Knowledge of early life history stages of fish is essential for rearing studies, but also for those regarding exploitation of wild stocks. Artificial propagation is an excellent tool for this purpose. The objective of this work was to study the main morphological characteristics of the egg development stages of the flounder *P. orbignyanus* under experimental conditions.

MATERIALS AND METHODS

The methodology employed in the artificial propagation was developed at the Marine Fish Culture Laboratory (Universidade Federal de Santa Catarina). Experiments were carried out during winter months (July to September) in 1995 and 1996, during natural spawning season. In this study, data obtained in some trials are summarized. The technique adopted in the artificial reproduction was the same described by Cerqueira et al. (1997), with slight adaptations.

Brood Stock and Spawning Induction

Adult flounders in advanced sexual maturity stage were caught by artisanal fishermen in Conceição Lagoon and Barra da Lagoa Beach (Florianópolis - Santa Catarina), using fishing nets. Sometimes, caught females were in process of ovulation and hormonal induction was unnecessary. Otherwise, the hormone human Chorionic Gonadotropin (hCG commercial grade) was injected intramuscularly at 250-500 IU/kg in males, and at 500-1,000 IU/kg in females, depending on sex maturity. Gametes were obtained through strip spawning. Artificial fertilization was performed by the dry method, spreading milt over the eggs, adding a small volume (10-20 mL) of saltwater (35 g/L) and mixing altogether. Some minutes later, the eggs were washed in a current of new water to eliminate debris, and finally placed into incubators.

Eggs Incubation

For the incubation, 35-L cylinder-conical tanks were placed inside a rectangular 1,000-L tank, with water exchange at 10-20%/h and continuous aeration provided by air stones. Stocking density varied from 1,000 to 3,000 eggs/L. Salinity was around 35 g/L and water temperature was 18-20 ºC.

Sampling and Description of Eggs and Larvae

In order to describe morphology and measure eggs and newly hatched larvae *in vivo*, periodic samples ($n \geq 30$) were taken directly from the incubators, by using a beaker. Observation intervals were at least 30 min apart, depending on the occurrence of main events. Observations were made under a light microscope with a 10x eyepiece and a 10x objective, coupled to a video camera and a color monitor. The most representative images were printed. Determination of developmental stages was based on description, and terminology proposed by Lagler et al. (1977) and Balinsky (1981). Morphometric data were collected, to the nearest 0.01 mm using an ocular micrometer, from eggs (total diameter and oil globule diameter) and from yolk-sac larvae (total length, yolk-sac length, and oil globule diameter). For each variable, the average, standard deviation, and confidence interval (significance level of 5%) were calculated.

RESULTS

Morphological Aspects and Size of Eggs and Larvae

Right after fertilization and placement into the incubators, eggs were pelagic, free, round and transparent. They had a single and small oil globule. Appearance of the yolk was homogeneous. The chorion was smooth and thin, whereas the

perivitelline space was narrow. Eggs had a mean diameter of 0.792 ± 0.008 mm, and mean oil globules diameter was 0.114 ± 0.003 mm (Table 1). Just after hatching, the yolk sac with its oil globule represented the largest volume of the larvae. Length of the yolk sac (0.972 ± 0.015 mm) was equivalent to approximately 50% of total length (2.04 ± 0.024 mm). The single oil globule had a mean diameter of 0.098 ± 0.006 mm (Table 1).

Egg Development Stages

Egg development was divided into eight stages, from fertilization to hatching, corresponding to the main events. Only the most important morphological characteristics of each stage were registered.

Table 1 - Measurements of eggs and yolk-sac larvae of southern Brazilian flounder, *Paralichthys orbignyanus.*

Measure	Mean (mm)	Standard deviation	Confidence interval (±)	Range (mm)	n
Eggs					
Diameter	0.792	0.025	0.008	0.744-0.860	40
Oil globule diameter	0.114	0.005	0.003	0.105-0.116	30
Larvae					
Total length	2.040	0.038	0.024	1.980-2.090	30
Yolk-sac length	0.972	0.024	0.015	0.930-1.000	30
Oil globule diameter	0.098	0.010	0.006	0.081-0.116	30

Fertilization: Approximately 60 min after fertilization, the cytoplasmic cap on the animal pole was formed that contained the nucleus, in opposition to the vegetal pole with the yolk and a thin layer of cytoplasm surrounding the yolk.

Cleavage: After successive divisions in the animal pole the blastoderm formed, following the typical pattern of telolecithal eggs. First division occurred 1 h 30 min after fertilization, and resulted in the formation of two cells or blastomeres (Fig. 1a). Following that, at 1 h 50 min after fertilization, were four cells (Fig. 1a), then eight cells (2 h 20 min), 16 cells (3 h 00 min) (Fig. 1b), 32 cells (3 h 30 min), and 64 cells (4 h 20 min). More than 64 small blastomeres were impossible to enumerate *in vivo*.

Blastula: In the beginning, between 5 h 10 min and 6 h 00 min after fertilization, the blastoderm had the shape of a convex disc on the animal pole (blastodisc). At the end, between 6 h 00 min and 7 h 00 min after fertilization, there was a flattening of the blastoderm and formation of a syncytial layer (periblast) at the interface of the animal and vegetal poles (Fig. 1c).

Gastrula: In the initial stage, 9 h 00 min after fertilization, there was a thickening of the marginal ridge of the blastodisc, forming an inner layer called the germ ring. A cellular growth occurred to cover the yolk mass, a movement of the blastodisc termed epiboly (Fig. 1d). In the advanced stage, between 15 h 00 min and 15 h 30 min after fertilization, the process of epiboly resulted in covering practically 2/3 of the yolk mass by mesodermic and ectodermic tissue. In the dorsal region the embryonic shield, forming since the initial gastrula, could be observed. At the end, 16 h 00 min after fertilization, the neural keel became more clearly visible in the midline of the embryonic shield. The yolk plug, a very restricted region of the yolk, was not covered by the blastoderm (Fig. 1e).

Blastopore Closure: The edges of the blastodisc surrounded the entire yolk, converged and closed at the posterior end of the embryo, approximately 20 h after fertilization. The cephalic region, with rudiments of the central nervous system, the first somites, and the caudal region became visible. An enlargement of the perivitelline space was obvious.

Embryo: In the initial stage, 24 h after fertilization, the process of neurulation was taking place. The cephalic and caudal edges of the embryo were well differentiated. There was an expansion of the forebrain. The neural plate was quite distinct (Fig. 1f). Following that, there was formation of the optic vesicles, brain and Kupffer's vesicles, and olfactory pits.

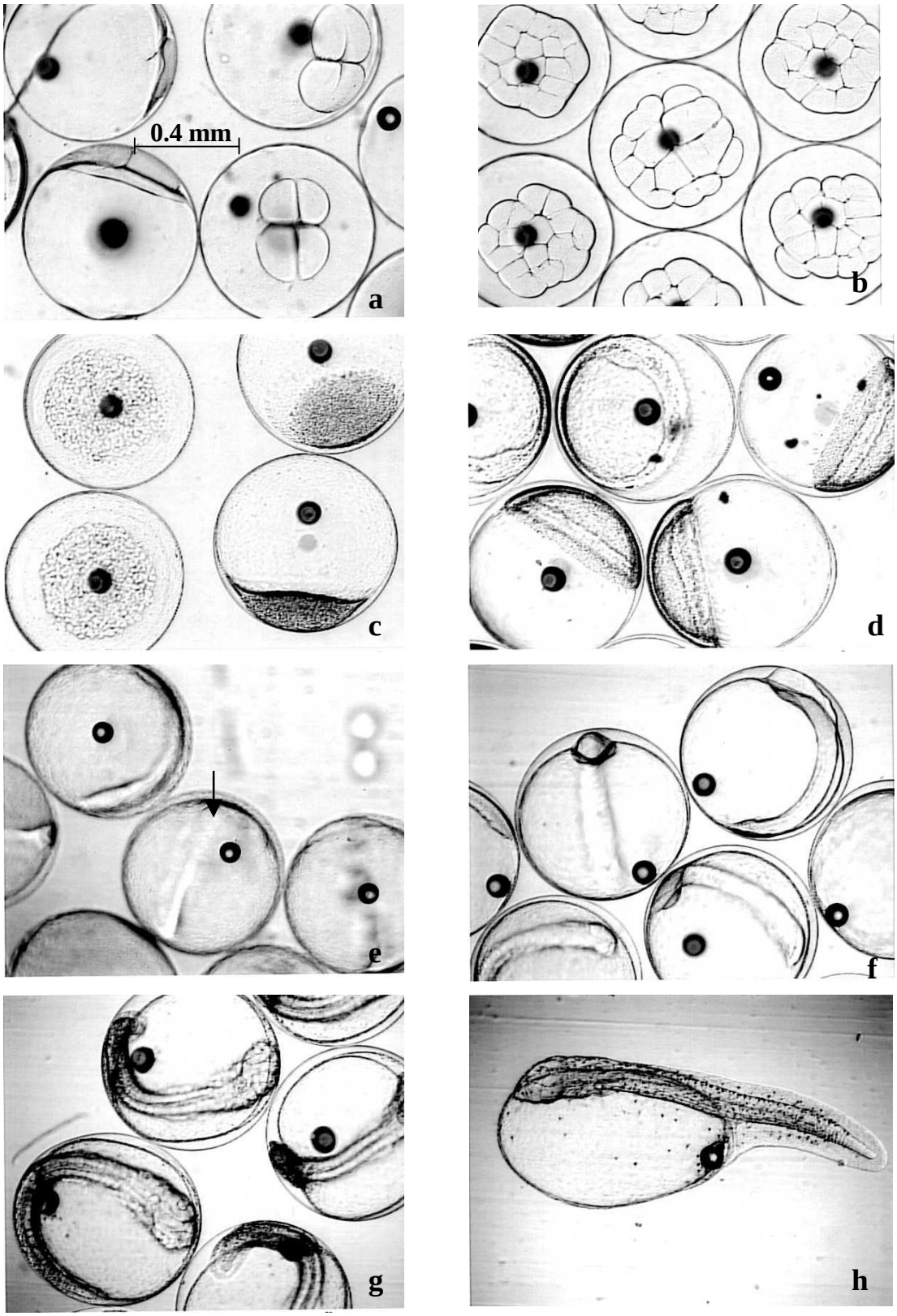

Figure 1 - Development of laboratory-reared eggs and newly hatched larvae of southern Brazilian flounder, *Paralichthys orbignyanus*. Developmental stages: **a**- Cleavage, 2 and 4 blastomeres; **b**- Cleavage, 16 blastomeres; **c**- Blastula (early stage); **d**- Gastrula (early stage); **e**- Gastrula (late stage) and blastopore closure (arrow); **f**- Embryo (early stage); **g**- Embryo (late stage); **h**- Newly hatched larva.

In the advanced embryo, 40 h after fertilization, melanophores were more evident over the body and yolk sac.
The number of somites was increasing, auditory placodes were visible, and lens of eyes were forming in the optic vesicles. There were cardiac beats, an increase in perivitellline space, and tail liberation inside the chorion, where it was free of movement (Fig. 1g).
From a ventral view of the head and trunk of the embryo, the neural tube and notochord, somites, eye lenses, olfactory placodes, cardiac region, and yolk sac pigmentation were clearly visible.

Hatching: Approximately 41 h after fertilization, there was vigorous activity of the embryo in some eggs, rupture of the chorion and liberation of the embryo. The hatching interval for larvae from a single batch could take a few hours. At water temperatures near 20 ºC, time to hatching was about 41 h, but at 18 ºC or lower it could be prolonged to about 50 h.

Yolk-sac larva: Newly hatched larva, or free-embryo according to Balon (1975), had a large elliptical yolk sac, with a single posterior oil globule. A continuous median finfold (dorsal, anal, and caudal) surrounded the trunk and the tail. Larvae lacked a functional mouth and digestive tract, which presented only rudiments. Iris of the eyes had no pigments. Otoliths, heart, and brain were clearly visible. Body pigmentation was still sparse, with few melanophores on the dorsal and anal finfolds. Pigment was more developed in the ventral and dorsal midlines and on the lateral surface, except at the tail end. Pigment was rare but present on the yolk sac, primarily on the dorsal portion and around the oil globule (Fig. 1h).

DISCUSSION

Eggs

Paralichthys orbignyanus eggs present the basic patterns of most marine fishes: they are pelagic and float individually near the surface, are spherical with a diameter around 1 mm, and hatch into undeveloped yolk-sac larvae (Kendall et al., 1984). *P. orbignyanus* eggs are quite similar to other congener flounder eggs. Ahlstrom et al. (1984) summarized data from different studies, including seven species of the family Paralichthydae among those pleuronectiforms having pelagic eggs with a single oil globule. Egg diameter in these species varied from 0.7 to 1.38 mm, and the oil globule between 0.10 and 0.31 mm. They were round, had a narrow to moderate perivitelline space, homogeneous yolk and smooth chorion. Among the congeners mentioned by Ahlstrom et al. (1984), *P. californicus* (0.74-0.82 mm), *P. dentatus* (0.90-1.10 mm), and *P. olivaceus* (0.83-1.03 mm) had egg diameters very close to *P. orbignyanus* (0.74-0.86 mm). On the same note, the egg and oil globule diameters of *P. albigutta* (0.84-0.90 mm and 0.17-0.19 mm) and *P. lethostigma* (0.84-0.96 mm and 0.16-0.20 mm) were comparable to *P. orbignyanus* (Powell and Henley, 1995).
In a laboratory study, Silva (1988) described a very similar pattern of egg development for *P. microps* (egg diameter 0.8 mm), compared to *P. orbignyanus* in the present study. First segmentation occurred in almost the same time interval after fertilization, despite a low temperature range (13-17 ºC). From gastrula stage (12 h 30 min) onwards, differences became more evident between the two species, likely due to temperature. Blastopore closure (24 h 30 min), appearance of optic vesicles (46 h), and the embryo with cardiac beats and tail movements (52 h) were also noted later than in *P. orbignyanus* (20 h, 25 h, and 40 h, respectively). Also, the time to hatching, occurred 57-68 h after fertilization for *P. microps*, quite longer than the time observed for *P. orbignyanus* (40-50 h). Silva and Flores (1989) reported an incubation time of 54-62 h for *P. adspersus* at 16-18 ºC. Gadomski and Caddell (1996) observed that temperature (12-20 ºC) strongly affected rate of development in *P. californicus* eggs and was inversely related to time to hatching. Time intervals to blastopore closure were 34, 24 and 17 h, and hatching time intervals were 74, 50 and 34 h, respectively, for 12, 16 and 20 ºC. If we consider only the 16 and 20 ºC interval values, they are quite close to the values observed in the present study. However, species-specific differences in egg-stage sequence, mainly of organogenesis in relation to germ ring migration and blastopore closure, were also found. The sequence of main events described for *P. californicus* and for the other congeners was basically the same observed for *P. orbignyanus*.

Yolk-Sac Larvae

Some morphological aspects of the newly hatched larva could be important in terms of systematics. For example, in the family Paralichthyidae, the single oil globule is usually in the rear of the yolk

mass (Ahlstrom et al., 1984). Moreover, other aspects of the *P. orbignyanus* larva, as the elliptical yolk sac, corresponding to approximately half of the larval length, single median finfold, undifferentiated mouth, rudimentary digestive tract, and unpigmented eyes are common to *P. microps*, *P. adspersus*, *P. albigutta* e *P. lethostigma* (Silva, 1988; Silva and Flores, 1989; Powell and Henley, 1995). Concerning pigmentation, there may be differences that are helpful in some cases in the identification of distinct species. Powell and Henley (1995) observed that *P. lethostigma* had pigment more concentrated in the middle of the body (dorsal and anal finfolds, and dorsal midline), and was less pigmented than *P. albigutta*. Zuñiga and Acuña (1992) verified that *P. adspersus* had melanophores in only some areas of the head, trunk and dorsal region of the yolk sac, whereas *P. microps* had melanophores also in the median finfold. *Paralichthys orbignyanus* larva had melanophores in almost all parts of the body as well.

Total length at hatching of *P. orbignyanus* larva was 2.04 mm. Ahlstrom et al. (1984) reported a total length of 2.0 mm for *P. californicus*, 2.4-2.8 mm for *P. dentatus*, and 2.6-2.8 mm for *P. olivaceus*. Silva (1988) observed a total length of 2.7 mm for *P. microps* larva, while Silva and Flores (1989) reported that *P. adspersus* larva was 1.9 mm in length. Powell and Henley (1995) described notochord length of *P. albigutta* (1.8-2.0 mm) and of *P. lethostigma* (2.0-2.2 mm). Among 26 species of the family Paralichthydae listed by Ahlstrom et al. (1984), only four had body lengths higher than 2.3 mm. Therefore, *P. orbignyanus* seemed to correspond to the body length pattern observed for this family, and could be considered a species with small larvae. This characteristic is important because the larval size define the kind of live prey that the larva will be fed when reared in laboratory (Hunter, 1984).

CONCLUSION

P. orbignyanus egg development was greatly similar, chronologically and morphologically, to other congeners. Results obtained in the present study demonstrated the feasibility of egg incubation and normal development in laboratory conditions, representing a very important step towards the evaluation of the species potential to be commercially reared in the future.

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RESUMO

Através do cultivo de ovos do linguado *Paralichthys orbignyanus*, obtidos de reprodução induzida em laboratório, foram descritos pela primeira vez seus estádios de desenvolvimento. O ovo era livre e flutuante, caracteristicamente pelágico. Além disso, tinha formato esférico, córion liso, espaço perivitelino estreito, vitelo homogêneo, e uma única gota de óleo. Seu diâmetro médio era de 0,792 ± 0,008 mm e o da gota de óleo de 0,114 ± 0,003 mm. Cerca de 1 h após a fecundação observou-se o blastodisco e em seguida a segmentação celular. A blástula iniciou após cerca de 5 h e a gástrula após 9 h. Com aproximadamente 20 h observou-se o fechamento do blastóporo. A nêurula ou embrião na fase inicial ocorreu após 24 h. Com cerca de 40 h havia batimentos cardíacos e movimentação da cauda do embrião. A eclosão ocorreu num período de 40 a 50 h após a fecundação, em temperaturas de 18 a 20 ºC. A larva tinha um comprimento total médio de 2,04 ± 0,024 mm, o saco vitelino bastante volumoso, a gota de óleo em sua porção posterior e pigmentação reduzida e esparsa. Estes resultados foram muito similares aos observados anteriormente em outras espécies congêneres.

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