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ASPECTS OF EMBRYONIC DEVELOPMENT IN TWO SOUTHWEST ATLANTIC GADIFORM FISH: TADPOLE CODLING, *SALILOTA AUSTRALIS* (MORIDAE), AND SOUTHERN BLUE WHITING, *MICROMESISTIUS AUSTRALIS* (GADIDAE)

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Background. Tadpole codling, *Salilota australis* (Günther, 1878), (known also as red cod) and southern blue whiting, *Micromesistius australis* Norman, 1937, are two commercially important species, which spawning grounds are situated in the Falkland waters. Nothing is known about duration of the embryonic development in these fish, whereas these data are necessary to study life cycle strategies as well as for stock management. Because of this, experiments with artificial egg fertilisation were carried out onboard a research boat that was the only way to obtain such an information.

Materials and Methods. Eggs from each species were taken from running females captured on their spawning grounds and then fertilised. Egg samples were collected every 6 hours and stage of embryonic development was assigned using a dissecting microscope.

Results. Tadpole codling eggs are of 1.20–1.55 mm, with an oil globule of 0.29–0.33 mm, incubation takes between 140–150 h at 6–8.5ºC, 40–45 degree-days. Larval size at hatching is ca 2.9 mm TL. Blue whiting eggs are of 1.40–1.55 mm with no oil globule. Development takes from 150 h at a mean temperature of 7.15ºC to 200 h at between 5.5 and 6ºC, 45–50 degree-days. Larval size at hatching is 2.8–3.0 mm.

Conclusion. Duration of embryonic development for commercial southwest Atlantic gadiform fish, tadpole codling, *Salilota australis*, and southern blue whiting, *Micromesistius australis*, is documented for the first time. It allows to draw some conclusions about possible mortality during this ontogenetic stage (assuming that daily rates are similar to those in other similar species) and to hypothesise about possible egg transport by currents and interannual spawning grounds' variability.

Keywords: blue whiting, tadpole codling, red cod, *Micromesistius*, *Salilota*, egg, embryogenesis, development

INTRODUCTION

The southern blue whiting, *Micromesistius australis* Norman, 1937, and the tadpole codling (red cod), *Salilota australis* (Günther, 1878), are two abundant commercial species distributed around southern South America. Southern blue whiting has a wide distribution in the southern hemisphere, including the Pacific Ocean near New Zealand and Chile, and the Southwest Atlantic between 37ºS and 65ºS and is found between the depth of 50–900 m (Froese and Pauly 2007). Tadpole codling inhabits waters of the South America between 41ºS and 59ºS and has a depth range of 30–1000 m (Froese and Pauly 2007). In the Atlantic Ocean reproduction of both species occurs in the austral spring in the shelf waters to the south and southwest of the Falkland Islands, from August to October in blue whiting (Macchi et al. 2005) and in September – October in tadpole codling (Wöhler et al. 2001). Both species are batch spawners with a determinate annual fecundity with a similar egg size (Pájaro and Macchi 2001, Macchi et al. 2005, Laptikhovsky and Brickle unpublished).

Tadpole codling belongs to the family Moridae, in which reproduction, and particularly egg size and embryonic development are poorly known. There is also a paucity of data on some species of confamiliar genera *Physiculus* and *Laemonema*, however, the few studies conducted indicate that these genera have pelagic buoyant eggs of 0.93–1.16 mm in diameter with an oil globule of 0.19–0.30 mm and larvae TL at hatching is of about 2–3 mm (Bertolini et al. 1956, Kitagawa et al. 1985, Okiyama 1988, Fahay 2007). Nothing is known about duration of the different stages of embryogenesis in fish of this family, including tadpole codling. In the case of *S. australis*, only the late stages of egg development and postlarvae have been

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captured in oblique hauls from 100 m to the surface and described (Weiss 1975, Ciechomski and Bowman 1981).

Oogenesis, late stages of egg development from planktonic samples, and larval morphology were described for southern blue whiting (Weiss 1974, Ciechomski and Bowman 1981, Pájaro and Macchi 2001, Balbontin et al. 2004, Macchi et al. 2005) but early stages as well as duration of embryogenesis are not known.

The objectives of this paper are to describe the eggs and newly hatched larvae of both species as well as to estimate the duration of their embryonic development.

MATERIALS AND METHODS

Seven batches of eggs (five of tadpole codling and two of blue whiting) were sampled from running females of the two species captured during a research cruise on the *R/V DORADA* within 4–11 October 2006. The vessel employed a bottom trawl equipped with polyvalent doors, a tickler chain and a 40-mm codend mesh size. The typical vertical opening was between 6 and 10 m with a towing speed 3.4–4.2 knots. Hauls were conducted between 149 and 328 m depth. Adult fish was already dead or dying because of everted stomach and liver damage caused by change in water pressure. Together with the rest of catch they were delivered into fish hold and sorted on the conveyor belt. Spawning males and females were easily recognisable because of sexual products shed at a slight pressure on the abdomen.

For each experiment, a volume of 200 mL of eggs were collected from 2–3 running females and approximately 100 mL of sperm from 2–3 running males. They were mixed and seawater added approximately 10 min later. Fertilised eggs were then transferred into 25-L barrels situated in the wet laboratory on board of the research vessel. The last portion of blue whiting eggs was incubated in the lab in continuous flow of the subsurface water of Stanley Harbour. The eggs were sieved and water was exchanged four times a day and several dozen were sampled. The subsurface water pumped onboard from approximately 2 m depth was also exchanged and its temperature measured within 0.5ºC. Embryonic stages were assigned using the Thompson and Riley (1981) scale for Atlantic cod, *Gadus morhua*: Stage I—Includes the period from fertilisation to the formation of the gastrula; Stage II—starts with the first sign of the primitive streak until the embryo is half way round the circumference of the egg; Stage III—the embryo tail grows from half way to three quarters of the way round the circumference of the egg; Stage IV—the embryo tail grows from three-quarters of the way round the circumference of the egg until the full circumference; Stage V—describes the growth of the embryo from when the tail is past the head and until the time of hatching. The eggs and larvae sampled were preserved in 4% BFS (Buffered Formal Saline) for further investigation and measurements ashore.

To monitor oceanographic conditions, a logging CTDO (SBE-25, Sea-Bird Electronics Inc., Bellevue, USA) was deployed from the surface to 1–20 m above the bottom to obtain profiles of temperature (ºC), salinity (PSU), and dissolved oxygen (mL · L^{-1}). Temperature was measured directly whereas the other variables were calculated using Seasoft v. 4.326 software (Sea-Bird Electronics Inc.).

RESULTS

Oceanographic features of water layers above the spawning grounds and temperatures experienced during incubation. Water temperatures on the spawning grounds (150–220 m depth) varied from 5.40 to 6.15ºC depending position of sampling station, salinity varied between 33.60–33.96 psu (practical salinity units), and oxygen content was 5.9–6.2 mL · L^{-1}. Incubation temperatures varied from 6 to 8.5ºC averaging between 6.64 and 7.22ºC in the tadpole codling samples. One of blue whiting samples was incubated onboard of the research vessel at the mean water temperature 7.15ºC. Another sample of blue whiting eggs was incubated in the onshore incubator tank with the temperature of running seawater 5.5–6ºC.

Embryonic development. Tadpole codling eggs were spherical with a smooth chorion, and homogenous yolk containing a single oil globule on the vegetative pole. The diameter of unfertilised eggs was 1.20–1.55 mm (Fig. 1). During embryogenesis the egg size not vary. Oil globule size was 0.29–0.33 mm. Perivitelline space was small, amounting to 0.07–0.14 mm.

At Stage I, the first division of the blastodisk resulted in two equal blastomers that were visible at 2 h 15 min after fertilization. Four blastomers were evident after 3 h 30 min. Stage II was apparent during the second and third days of incubation and was complete approximately 75 hours after insemination. At Stage III, some pigment spots were seen to develop on the embryo. This stage began approximately three and a half days, and finished four and a half days after insemination. Stage IV started roughly 110 h after insemination and lasted about 20 h. Stage V began in the order of 130 h after insemination; hatching occurred during nighttimes when the embryo was about 140–150 h old. The entire development took as much as 40–45 degree-days.

After hatching tadpole codling larvae were ca. 2.9 mm in total length (2.7 mm standard length). The digestive tract was not evident. The yolk sac was large and ovoid, 1–1.2 × 0.6 mm in length. The oil globule (ca. 0.3 mm) was located in the posterior region of the yolk sac. There were distinct melanophores on the body, mostly on the dorsal and ventral parts but also on the lateral surface. Melanophores began to form three distinctive pigment areas; one on the rear part of the body and two on the tail. Myomeres were clearly visible. The gut was evident at about 10–12 h post hatching (156 h after insemination) as an extension into the ventral fin fold. When the larvae were of 3.5 mm TL (85–90 h), their heads were completely free of the yolk sac. A diameter of eyes was of 0.25 × 0.20 mm. Considerable enlargement of occipital region developed at a size of 3.8 mm TL (160 h), at this size the myomeres were still visible on the rear part of the tail. Larvae grew

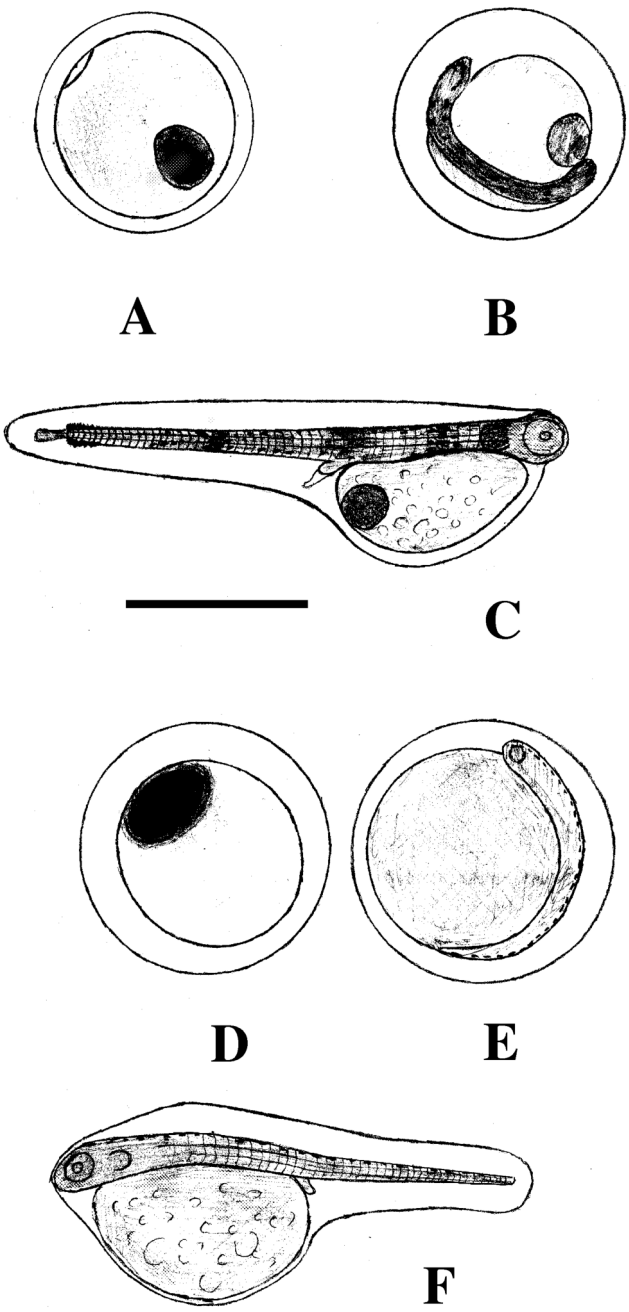

Fig. 1. Eggs and larvae of tadpole codling (*Salilota australis*) (A–C) and blue whiting (*Micromesistius australis*) (D–F); A: Eggs 22 min after insemination; B: Eggs at stage III, 83 h 30 min after insemination; C: Hatchling ca. 12 h after hatching; D: Eggs at stage I, gastrula, 51 h after insemination; E: Eggs at stage III, 102 h; F: Hatchlings, ca 20 h after hatching (about 220 h after insemination); scale bar = 1 mm

steadily attaining 4.4 mm after approximately 9 days after hatching (about 15 days after fertilization) using only yolk sac reserves. By that time the yolk sac was reduced to 0.7 × 0.4 mm in size, the size of oil globule did not change. Eye size did not change either. The gut slightly extended over the yolk sac but did not reach the edge of the ventral fin fold. Attempts at rearing the larvae further were unsuccessful, probably because of failure to provide the appropriate food onboard the vessel.

Blue whiting eggs are spherical. Their size was 1.40–1.55 mm (Fig. 1). The yolk was homogenous with no oil globule. It took approximately 150 h for the eggs to develop in the tanks onboard the vessel (mean temperature 7.15ºC) and in the region of 200 h in running seawater, on land, with temperature ranging 5.5–6ºC, a total of 45–50 degree-days. The total length of hatched larvae was 2.8–3.0 mm. Further larval growth was not observed, larvae died in spite of attempts to feed them with *Artemia*.

DISCUSSION

Our data show that egg size in both tadpole codling and blue whiting is similar, slightly less than 1.5 mm, and egg development in both species takes approximately 6 days at 6.5–7.5ºC. In a colder natural environment (about 6ºC) embryogenesis would likely be extended somewhat compared to our data measured herein, but does not exceed 8 days (duration of blue whiting embryogenesis at 5.5–6ºC). This temperature is also similar to that in which larvae of the blue whiting were collected west of the Falkland Islands in October–November 1969: 5.2–6.5ºC, depth range 140–274 m (Weiss 1974).

Eggs and embryonic development of *Salilota australis* are generally similar to those in other gadoid fish families Merluccidae, Phycidae, and Lotidae (see: Bertolini et al. 1956, Okiyama 1988). However, egg development in these species is faster than in the tadpole codling because of higher spawning temperatures, and takes 3–7 days at 10–18ºC (Patchell et al. 1987, Bustos and Landaeta 2005, Fahay 2007).

Eggs of blue whiting, *Micromesistius australis* are different from those of tadpole codling because they have homogenous yolk without an oil globule as in *Boreogadus saida*, *Gadus morhua*, *Melanogrammus aeglefinus*, *Micromesistius poutassou*, and *Pollachius virens* (Gadidae). Both eggs and larvae of the southern blue whiting are slightly larger than those of its northern sibling, *Micromesistius poutassou*. In the latter species eggs are of 0.99–1.15 mm and hatchling length is between 2.1–3.2 mm (Seaton and Bailey 1971, Coombs and Hiby 1979, Fahay 2007). The duration of embryonic development in *M. australis* is very similar to that in *M. poutassou* (205 h at 6ºC) although in the latter species it could be as short as 70 h at 15ºC (Coombs and Hiby 1979).

In this study, the size of eggs sampled in both tadpole codling and blue whiting was slightly larger than those given in the paper by Ciechomski and Booman (1981). Unfortunately, the authors did not indicate what preservative they used for their samples so it is difficult to draw any conclusions regarding possible reasons for the differences. However, the most likely explanation is different techniques of preservation.

Because no eggs at early stages of development of both species were captured in water layers <100 m depth

(Weiss 1974, 1975, Ciechomski and Bowman 1981), probably they gradually float up from the bottom layer, where they were released and fertilised. Similar floating up of fertilised pelagic eggs is particular for other gadoid fish—grenadiers family Macrouridae. Eggs of some species of this family are even ornamented by hexagonal sculpturing, which inhibits ascent rate upon fertilization and development (Merrett and Haedrich 1997).

Described differences in the egg morphology make possible to distinguish spawning products of both species in planktonic samples. It allows us to conduct egg surveys to estimate spawning biomass and to monitor stock dynamics in both species simultaneously.

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