Early development of artificially spawned southern mullet, *Liza richardsonii* (Smith, 1846)

J.A. Cambray*
Albany Museum, Grahamstown, 6140, Republic of South Africa

A.H. Bok
Cape Department of Nature and Environmental Conservation, P.O. Box 12043, Amalinda, 5252 Republic of South Africa

Received 2 August 1988; accepted 13 December 1988

The embryonic development of artificially spawned southern mullet, *Liza richardsonii,* eggs and the development of the larvae to 46 days are described and illustrated using drawings and photographs. The floating eggs hatched in sea water at 18–24°C after 46–60 h. Newly stripped eggs usually had more than one oil droplet (up to 16) which merged during development. Free embryos averaged 2,36 mm at hatching and had distinct yellow pigment.

Die embrionale ontwikkeling, eiers en ontwikkeling van die larwe tot 46 dae van kunsmatig gebroeide suiderharder, *L. richardsonii,* word beskryf en geïllustreer met tekeninge en foto's. Die drywende eiers het na 46–60 h teen 18–24°C in seewater uitgebroei. Nuutgestroopte eiers het gewoonlik meer as een olie druppeltjie (tot 16) wat gedurende ontwikkeling saamsmelt. Vry embrio's is by uitbroeiing gemiddeld 2,36 mm lank en is helder geel gepigmenteer.

*To whom correspondence should be addressed

In southern Africa, research into the early life history of marine fish species, including the Mugilidae has, to date, largely relied on material collected in plankton nets as artificially fertilized eggs were not available. Brownell (1979), for example, described the eggs and larvae of a species he collected off the Cape of Good Hope as the mugilid *Liza richardsonii.* In such studies the misidentification of eggs and larvae is a distinct possibility. The recent successful spawning of the southern mullet, *L. richardsonii,* by Bok & Jongbloed (1987) enabled a taxonomic developmental series to be studied, ensuring against misidentification.

L. richardsonii is endemic to southern Africa and is known only from the Kunene River on the west coast to St Lucia on the east coast (Smith & Heemstra 1986). Juveniles have been found in the Berg River (west coast) to Kwelega near East London (Wallace & van der Elst 1975). It has considerable commercial importance (de Villiers 1976) and being euryhaline, may have value in brackish and freshwater aquaculture (Bok 1983). An accurate description of the early developmental stages of this species, as attempted in this paper, would be of value to ichthyoplanktologists as well as facilitate any attempts to culture this species. Data on the larval rearing of this species are given elsewhere (Bok 1989).

Methods

Eggs were obtained from hormone-injected females which were manually stripped and fertilized using milt obtained from untreated male fish as described in detail by Bok & Jongbloed (1987). The information given here on egg and larval development is based on combined data obtained from three separate induced spawnings of southern mullet between November 1984 and November 1985. Further information on the induced spawning of *L. richardsonii* can be found in Bok & Jongbloed (1987).

Egg incubation and holding of larvae

The eggs were incubated in sea water and the larvae held under a variety of conditions at the East London Aquarium and at the Amalinda Hatchery (Bok 1989). In addition, approximately 50 newly hatched (approximately 36 h post-hatch) free embryos were sent to the Albany Museum, Grahamstown on 13 December 1984 for photographing and study by the first author. Here they were held in sea water (35‰ salinity) in all-glass aquaria (50 × 30 × 30 cm). One half of the tank water was replaced with untreated sea water every two days. Details of egg incubation and rearing of the *L. richardsonii* larvae are given in Bok (1989) and will not be discussed in this paper.

Larvae were sampled on a regular basis to observe growth and development. They were immobilized by a sudden temperature drop to *ca* 4°C (placing a petri dish with live larvae into a deepfreeze for a few minutes) or anaesthetized with Benzocain at *ca* 30ppm and examined under a stereomicroscope with a graduated eye-piece. In addition, eggs, free embryos and larvae were photographed with a camera attached to a stereomicroscope. Camera lucida drawings of the anaesthetized larvae were made. Specimens up to 34 days were then preserved in 5% phosphate-buffered formalin.

Specimens from this study are lodged at the Albany Museum, collection number AMG/P11700.

Results

Embryonic and larval development

The newly fertilized eggs are described in Table 1. They are pelagic, spherical, and transparent with a smooth surface (viewed at ×80 magnification). Scanning electron micrographs revealed a ragged, plate-like ultrastructure for the chorion of *L. richardsonii* eggs

Table 1 Embryonic development of *L. richardsonii* eggs at 18–24°C

Time (h) after fertilization	Description
At fertilization	Round, non-adhesive, transparent, positively buoyant in 35‰ sea water, mean diameter of eggs varied between individual fish from 884–915 µm; multiple oil droplets (1–16, usually 6–10). Size range of the oil globules varied between 250 µm and 400 µm (Figure 2a).
1	Two-cell stage. Perivitelline space represents 10-22% of total egg diameter (n=10 live eggs), equal cleavage. Blastomere slightly goldish-brown. Oil droplets start to coalesce, gastrulation complete.
13	Neural keel formed (Figure 2b).
13–24	Somites begin to form, head and tail clearly differentiated.
24	Embryo half-way around egg, optic vesicles and otic placodes well developed (Figure 2c).
31	Oil droplets have coalesced into one large, or one large and 1–3 very small droplets, stellate melanophores forming on chorion (Figure 2d).
34	Caudal finfold apparent, first jerky movement of embryo seen (Figure 2e).
37	Tail movement, heart beat apparent, pigmentation marked (Figure 2f).
44	Tail touching head, strong heart beat, regular body movements, some live eggs became negatively buoyant (Figure 2g).
46–60	Hatching, tail first.

(Figure 1). The yolk is unsegmented and the perivitelline space is narrow, and there are one or more oil globules (Figure 2a). When multiple oil globules are present these usually merge during the first 31 h of development and when the free embryo hatches the oil globule is still present (Figure 3a). Embryonic development is described in Table 1 and depicted in Figures 2 & 3.

The development of *L. richardsonii* free embryos and larvae is shown in Figures 3a & 3b and Figures 4a–4c.

The digestive tube and the mouth are undeveloped at hatching (Figure 3a) but the mouth develops rapidly (Figure 3b) and becomes functional 3–4 days after hatching. There are between 22–23 myomeres during the free embryo development period. The pectoral fin buds are present at hatching and become more developed and functional by the second day. The free embryos become more active at this time (2,75–3,0 mm NL fixed). The otic capsules are also present at hatching and one otolith could be seen in each. The gill clefts were first observed after three days (3,0 mm NL). Also by the third day after hatching the yolk sac and the oil droplet are greatly reduced in size and there were more free embryos near the surface. By the fourth day after hatching the larvae

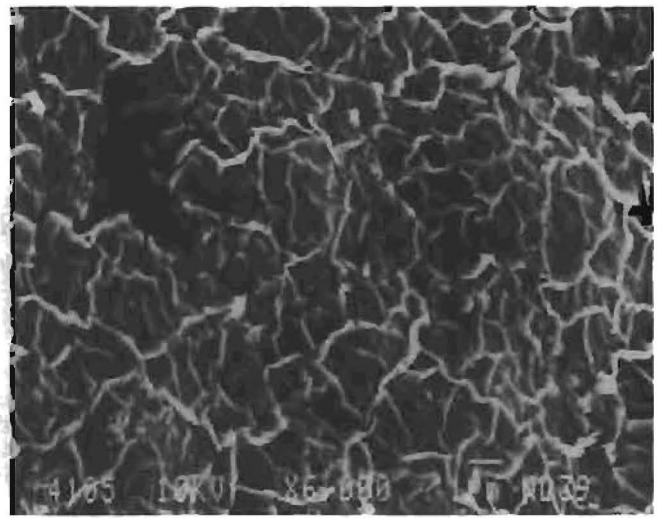

Figure 1 Scanning electron micrograph of the surface of the chorion of a *Liza richardsonii* egg. (Measurement in µm).

make active, darting movements with the mouth opening and closing. The lower jaw is well developed as is the digestive tube and the stomach is now forming and at this stage there is very little yolk left. The nostrils are also visible now. Feeding is assumed to begin at this time, although larvae with food in their guts were not sampled, possibly owing to low feeding incidence.

The yolk sac is completely absorbed by the fifth day after hatching, while the size of the oil droplet is reduced more slowly and is completely absorbed after fifteen days (Figure 4a). Yellow pigmentation is very noticeable by the fourth day after hatching, particularly on the dorsal surface and in the head area (Figure 5).

The swimbladder started to inflate after five days (3,0 mm NL fixed) and after seven days the swimbladder is well inflated. After 11 days the caudal area of the dorsal and ventral finfold started to indent (*ca* 3,7 mm NL fixed). The caudal area of the body is without pigment.

After 15 days the larvae became very active and their pigment pattern is shown in Figure 4a. The dorsal finfold is now retreating posteriorly. Flexion of the notochord was seen 19 days post-hatch at *ca* 5,3 mm SL when the hypural bone started to form and 12 caudal rays were present (Figure 4b).

At 20 days the fish showed rapid growth (for details see Bok 1989). After 22 days the dorsal finfold was in mid-dorsal position and the anal and dorsal fins were forming. The posterior section of the body was still without pigment. Fin rays in the dorsal and anal fins appeared after 24 days at about 6–7 mm SL live, 5–6 mm SL fixed (Figure 4c). At this time the body was covered with a fine pigment and the pigment covered all but the caudal peduncle of the larval fish. At 6,0 mm SL (fixed) the pelvic fin buds were present. The largest specimen sampled after 24 days (7,9 mm SL fixed) had two dorsal fins and the posterior one had nine ray bases. This specimen had 16 caudal rays, 11 anal fin rays and 12 pectoral fin rays.

After approximately four weeks all the fins were formed and silvery scales began to develop below the

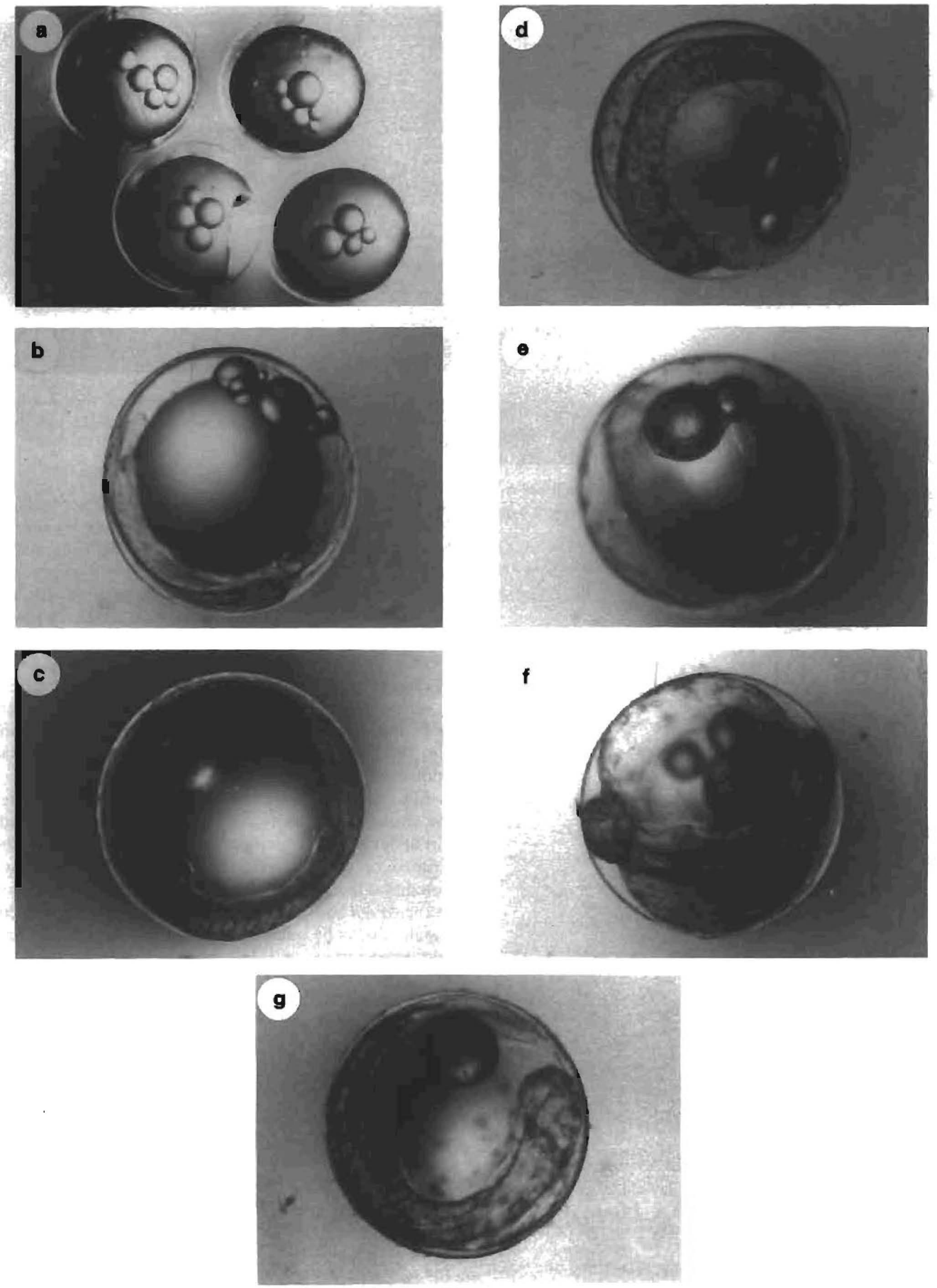

Figure 2 Embryonic development of *L. richardsonii* eggs at 18–23°C, egg diameters in brackets: (a) at fertilization (0,91mm); (b) 13 h after fertilization (0,90); (c) 24 h after fertilization (0,87); (d) 31 h after fertilization (0,87); (e) 34 h after fertilization (0,92); (f) 37 h after fertilization (0,92); (g) 44 h after fertilization (0,95).

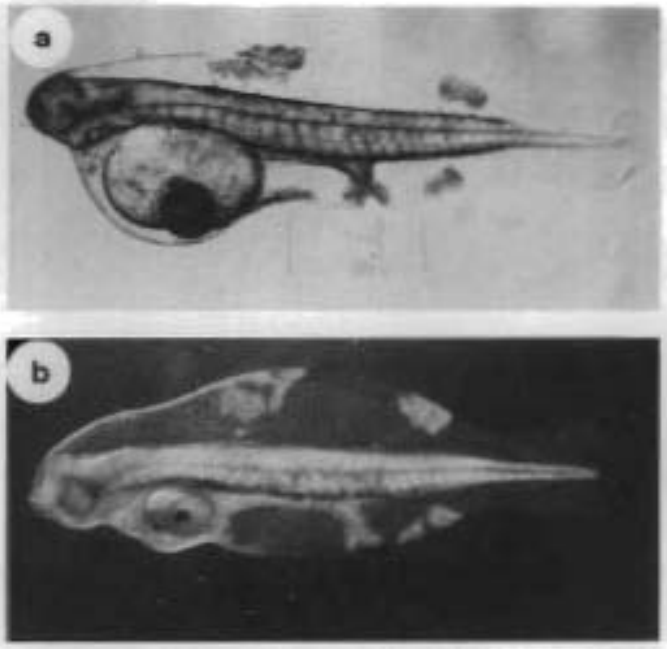

Figure 3 Free embryos of *Liza richardsonii:* (a) 56 h old free embryo, 2,9 mm NL (alive); (b) 73 h old free embryo, 2,9 mm NL (alive).

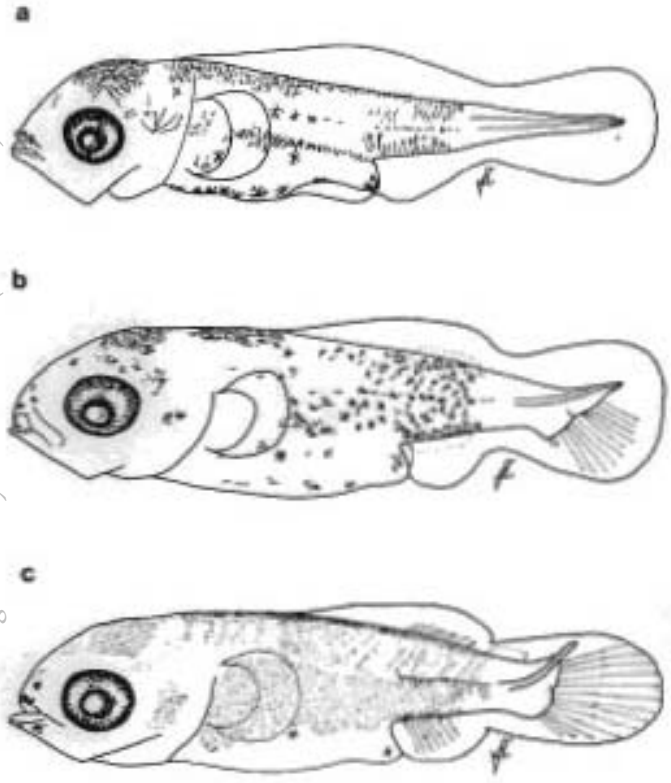

Figure 4 Larval development of *Liza richardsonii:* (a) 15 day old larva, 4,2 m NL (fixed); (b) 19 day old larva, 5,3 mm SL (fixed); (c) 24 day old larva, 5,4 mm SL (fixed).

first dorsal fin. At this stage they resembled the adult fish and began swimming in shoals in rearing tanks.

Discussion

Our results show that the early development of *L. richardsonii* follows the general pattern as described for *Mugil cephalus* (Kuo, Shehadeh & Milisen 1973) and for Mugilidae in general (De Sylva 1984). De Sylva (1984) gave a general overview of each stage. For Mugilidae the eggs are pelagic, spherical and transparent with the surface of the egg being smooth and usually without sculpture. The yolk is unsegmented, the perivitelline space is narrow and there are one or more oil globules (Table 2). De Sylva (1984) also noted that during development several oil globules merge with each other, as is discussed in the present study for *L. richardsonii*, and the oil globule becomes situated on the yolk sac upon hatching.

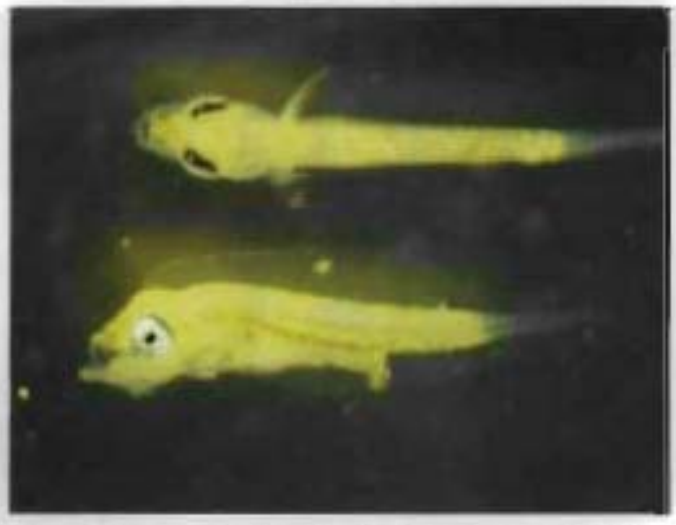

Figure 5 Larval *Liza richardsonii*, four days after hatching showing dorsal (3,0 mm NL) and lateral (3,12 mm NL) pigmentation.

Alhstrom & Moser (1980) noted that in some species the chorion appears to be smooth and unornamented, but under higher magnification the chorion is seen to be striated or wrinkled. Previously various authors have noted that the texture of the chorion is smooth for some of the *Mugil* and *Liza* species (Table 2). This statement should always be qualified as to what magnification and microscope were used. In the present study SEM work revealed an irregular, ragged plate-like ultrastructure of the chorion of *L. richardsonii* (Figure 1). This was quite distinct from the raised patterned surface which occurs on the chorion of *M. cephalus* eggs (Boehlert 1984; Figure 19D).

The egg diameter range found in this study (0,85–1,00 mm) is in agreement with the mean diameter of 0,954 mm reported for ripe, translucent ova from *L. richardsonii*, captured in Algoa Bay on the south-east coast of southern Africa (Lasiak 1983). This is well within the egg diameter range of 0,6–1,3 mm given by De Sylva (1984) for various species of European and African mugilids. The species described as *L. richardsonii* by Brownell (1979), however, had eggs substantially larger (diam. range 1,23–1,35 mm) than those mentioned above for this species (Table 2). The *M. cephalus* eggs and larvae described by Brownell (1979) fit into the size range given here for *L. richardsonii* (Table 2). The

Table 2 Comparison of early developmental characteristics for several mullet species

	Liza richardsonii	*Liza richardsonii*	*Mugil cephalus*	*Mugil cephalus*	*Mugil curema*	General overview
Diameter of egg (mm)	0,85–1,00	1,23–1,35	0,94–1,03	0,89	0,86–0,92	0,6–1,3
Number of oil globules	1–16	1	1 (2–10)	1	1	1 or more
Diameter of oil globules (mm)	0,25–0,40	0,26–0,28	0,30–0,36	0,39	0,27–0,32	–
Perivitelline space	narrow	narrow	narrow	narrow	narrow (0,04–0,12 mm)	narrow
Yolk texture	clear	clear	clear	–	–	–
Chorion texture	smooth*	smooth	smooth	–	etched	smooth
NL (alive) at hatching (mm)	2,20–2,45	3,1–3,4	2,4–3,0	2,68	1,63–1,76(TL)	–
Position of oil globule at hatching	median/ ventral	posterior	posterior	posterior	median (his Figure 3)	–
Reference	This study	Brownell 1979	Brownell 1979	Tung 1973	Anderson 1957	De Sylva 1984

*As seen at ×80 magnification.

eggs Brownell (1979) described as *L. richardsonii* also only had one oil globule of a diameter of 0,26 to 0,28 mm as compared to the multiple oil globules of *L. richardsonii* eggs observed in this study (Figure 2a). In addition, the notochord length range of the newly hatched free embryos in this study (2,20–2,45 mm) were considerably smaller than the equivalent length range (3,1–3,4 mm) given by Brownell (1979). Although differences in egg and larval sizes of the same mullet species collected from different localities have been recorded (Brusle 1981), the above noted differences are substantial.

Another difference between Brownell's (1979) data on *L. richardsonii* and other workers is related to the breeding season. Although the above author sampled extensively throughout the year on both sides of the Cape Peninsula, he only found *L. richardsonii* eggs in winter and spring (July to October). On the south-east coast of southern Africa, however, *L. richardsonii* is considered to be mainly a summer spawner (Marais 1976; Lasiak 1983).

A further important difference is that the larvae described by Brownell (1979) did not have yellow pigment, unlike the larvae found in this study (Figure 5). The presence or absence of yellow pigment was considered an important distinguishing feature by Brownell (1979). Yashouv & Berner-Samsonov (1970) also used the presence or absence of yellow pigment as an important distinguishing characteristic in drawing up a key to the eggs and early larval stages of five mullet species along the Israeli coast.

In addition the shape and pigmentation of the newly hatched free embryos (Figure 3a) are different to that illustrated by Brownell (1979: Figure 126). For example, the yolk sac in the free embryo illustrated by Brownell is relatively larger and extends more anteriorly. Also the pigmentation found on the finfold of the free embryo in this paper is absent from Brownell's drawing. The position of the oil globule in the free embryo is also more anterior in this study than in Brownell's Figure 126. In addition the dorsal section of the finfold has a more distinct raised section than does Brownell's (Figure 126). The overall finfold shape of newly hatched free embryos of *L. richardsonii* in the present study is closer to that of the newly hatched *M. cephalus* illustrated by Brownell (1979: Figure 130). Myomere counts also varied between the two studies. The early free embryos Brownell (1979) studied had either 29 or 30 myomeres. In the present study early free embryos had either 22 or 23 myomeres. De Sylva (1984) noted that the early life history stages of Mugiloidei do not appear to be of any value to phylectic relations with other taxa, except that the Mugiloidei have 23 myomeres during larval development.

The differences between Brownell's data and the findings reported in this paper, therefore suggest that either two genetically distinct populations of *L. richardsonii* exist on the south-east and south-west coasts of southern Africa, or that Brownell misidentified the wild collected material he described as *L. richardsonii*.

Acknowledgements

We thank the Director of the Cape Department of Nature and Environmental Conservation and the Director of the Albany Museum for permission to publish this paper. Mrs E. Betteridge and Mrs P. Stuart are thanked for typing the various drafts.

References

ALHSTROM, E.H. & MOSER, H.G. 1980. Characters useful in identification of pelagic marine fish eggs. *Calif. Coop. Oceanic Fish. Invest. Rep.* 21: 121–131.

ANDERSON, W.W. 1957. Early development, spawning, growth, and occurrence of the silver mullet (*Mugil curema*) along the south Atlantic coast of the United States. *Fish. Bull. U.S.* 57: 397–414.

BOEHLERT, G.W. 1984. Scanning electron microscopy. In: Ontogeny and Systematics of Fishes, (ed.) Moser, H.G. Special Publ. No. 1. A.S.I.H.: 43–48.

BOK, A.H. 1983. The demography, breeding biology and management of two mullet species (Pisces: Mugilidae) in the eastern Cape, South Africa. Ph.D thesis, Rhodes University, Grahamstown, South Africa. 268 pp.

BOK, A.H. 1989. Rearing of artificially spawned southern mullet. *S. Afr. J. Wildl. Res.* 19: 31–34.

BOK, A.H. & JONGBLOED, H. 1987. A preliminary study on the induced spawning of the southern mullet, *Liza richardsonii. S. Afr. J. Wildl. Res.* 17(3): 82–85.

BROWNELL, C.L. 1979. Stages in the early development of 40 marine fish species with pelagic eggs from the Cape of Good Hope. *Ichthyol. Bull. Rhodes Univ.* 40, 84 pp.

BRUSLE, J. 1981. Sexuality and biology of reproduction in grey mullets. In: Aquaculture of grey mullets. International Biological Programme 26, (ed.) Oren, O.H. Cambridge University Press, Cambridge.

DE SYLVA, D.P. 1984. Mugiloidei: Development and relationships. In: Ontogeny and Systematics of Fishes, (ed.) Moser, H.G. Special Publ. No.l. A.S.I.H.: 530–533.

DE VILLIERS, G. 1976. Exploitation of harders along southern African shores. *The South African Shipping News and Fishing Industries Review.* May 1976.

KUO, C.M., SHEHADEH, Z.H. & MILISEN, K.K. 1973. A preliminary report on the development, growth and survival of laboratory reared larvae of the grey mullet, *Mugil cephalus* L. *J. Fish. Biol.* 5: 459–470.

LASIAK, THERESA A. 1983. Aspects of the reproductive biology of the southern mullet, *L. richardsonii,* from Algoa Bay, South Africa. *S. Afr. J. Zool.* 18(2): 89–95.

MARAIS, J.F.K. 1976. Comparative studies on the nutritional ecology of mullet in the Swartkops estuary. Ph.D thesis, University of Port Elizabeth, South Africa, 136 pp.

SMITH, M.M. & HEEMSTRA, P.C. 1986. Smith's Sea Fishes, Macmillan, Johannesburg.

TUNG, I.H. 1973. On the egg and larval stages of the grey mullet, *Mugil cephalus* Linnaeus. *Rep. Inst. Fish. Biol. Taipei* 3(1): 187–210. (In Chinese, English summary).

WALLACE, J.H. & VAN DER ELST, R.P. 1975. The estuarine fishes of the east coast of South Africa. 4. Occurrence of juveniles in estuaries. 5. Ecology, estuarine dependence and status. *Investl Rep. oceanogr. Res. Inst. Durban* (42): 1–63, 26 figures.

YASHOUV, A. & BERNER-SAMSONOV, E. 1970. Contribution to the knowledge of eggs and early larval stages of mullets (Mugilidae) along the Israeli coast. *Bamidgeh* 22(3): 72–89.