Japanese Journal of Ichthyology
Vol. 38, No. 2 1991

魚類学雑誌
38巻2号 1991年

A Unique Adhesion Apparatus on the Eggs of the Catfish *Clarias gariepinus* (Teleostei, Clariidae)

Rüdiger Riehl[1] and Samuel Appelbaum[2]

[1] Institut für Zoologie der Universität Düsseldorf (Morphologie und Zellbiologie), Universitätsstr. 1, D-4000 Düsseldorf, Germany
[2] Ben-Gurion University of the Negev, Jacob Blaustein Institute for Desert Research, 84993 Sede Boqer Campus, Israel

Abstract Eggs of the catfish *Clarias gariepinus* were investigated for the first time using light and electron microscopy (SEM and TEM). In shape, they differ strongly from those of other teleosts, their characteristic profile resembling a fur cap. This shape is unique among the eggs of teleostean fishes. The eggs of *C. gariepinus* are attached to substrata at their animal pole, which shows an annular bulge consisting of numerous tiny attaching-filaments. These filaments seem to be a part of the zona radiata externa. The micropyle, located within the center of the annular bulge, is a straight opening in the zona radiata. Different ways of egg adhesion in teleosts were compared and discussed as well as the position of the micropyle with respect to fertilization.

During spawning demersal eggs of many teleosts adhere automatically to substrata. In most cases, such fastening is related to a part of the egg envelope, the zona radiata externa. In various fish species, the zona radiata externa may be more or less modified. One possible modification is the existence of a multi-layered externa, e.g. in the armed bullhead *Agonus cataphractus* (Götting, 1965) or in the minnow *Phoxinus phoxinus* (Riehl and Schulte, 1977). In the latter, the zona radiata externa is three-layered and consists of a large quantity of mucopolysaccharides. A second modification is the presence of a plug-shaped zona radiata externa in the loach *Nemacheilus barbatulus* and the gudgeon *Gobio gobio* (Riehl, 1978a).

An additional type of modification in many different fish species is the existence of attaching-filaments on the egg surface, as reported for blenniids (Eggert, 1931; Wickler, 1957; Patzner, 1984), cichlids (Wickler, 1956; Busson-Mabillot, 1977), gobiids (Retzius, 1912; Eggert, 1931; Takahashi, 1978; Riehl, 1978b, 1984), cyprinodontids and oryziids (Siegel, 1958; Müller and Sterba, 1963; Wourms, 1976; Dumont and Brummet, 1980; Abraham et al., 1984; Hart et al., 1984; Selman and Wallace, 1989), pseudochromids (Mooi, 1990; Mooi et al., 1990), serrasalmids (Wirz-Hlavacek and Riehl, 1990) and a number of other teleost families (for review see Laale, 1980; Guraya, 1986; Riehl 1991).

In some species, the attaching-filaments are evenly dispersed on the egg surface, e.g. belonids (Russell, 1976) or some pseudomugilids (Howe, 1987), whereas in other species they are found only at specific areas of the egg, such as the vegetal pole, e.g. some cichlids with p-eggs (Wickler, 1956), the animal pole, e.g. blenniids (Eggert, 1931; Patzner, 1984), gobiids (Eggert, 1931; Riehl, 1978b, 1984), serrasalmids (Wirz-Hlavacek and Riehl, 1990) or both poles, e.g. *Pseudomugil gertrudae* (Howe, 1987). In some fish, the attaching-filaments form a regular adhesive disc.

Most catfishes possess demersal eggs, which become sticky after encountering water, and in this way adhere thermselves to substrata. Hitherto, only a few species of catfish have been examined using light and electron microscopy, e.g. silurids (Kobayakawa, 1985; Hilge et al., 1987; Abraham et al., in press) and loricariids (Riehl and Patzner, in press). In both families, the modes of attachment are very different. The eggs of some *Silurus* species adhere with a jelly-layer which covers the zona radiata externa, whereas in the loricariid *Sturisoma aureum* the eggs become attached via numerous attaching-filaments, which are a part of the zona radiata externa at the animal pole (Riehl and Patzner, in press). The eggs of the Japanese *Silurus lithophilus* also have a jelly-layer, but their surface is not adhesive (Kobayakawa, 1985).

During the examination of the eggs of the economically important clariid *Clarias gariepinus*, we found an adhesion apparatus which is apparently unique among catfish, and indeed, even among other teleosts, and was hitherto unreported. Our findings on this unique adhesion apparatus are presented in this paper.

Materials and methods

For the investigation, eggs of the catfish *Clarias gariepinus* (Burchell, 1822), weighting 950 g and originating from the Hulah swamp north of the Sea of Galilee, were obtained by stripping within 12, 24 and 36 hours after receiving a second hypophysis injection (purified carp pituitary extract, product of "Dag-Shan", Israel).

For scanning electron microscopy, the eggs were fixed with 2.5% glutaraldehyde in veronal acetate buffer at pH 7.3 for 24 hours. After several washings in the same buffer, dehydration was carried out in a graded acetone series. The specimens were dried in a Balzers critical point dryer, mounted on brass supports with hotglue and then coated with a 25 nm thick layer of gold in an Emscope SC 500 sputter coater. The specimens were examined with a Leitz AMR 1000 scanning electron microscope.

For transmission electron microscopy the eggs were placed in 3% glutaraldehyde buffered with cacodylate buffer (pH 7.4, 4°C) for 2 to 4 hours. After rinsing with the same buffer specimens were postfixed in 2% osmium tetroxide (2 h) and embedded via propylene oxide in Araldite. A Reichert ultramicrotome OM U 3 was used to cut semi-thin and ultra-thin sections. Semi-thin sections (0.5 μm) were stained with toluidine blue for 30 sec, whereas ultra-thin sections were double stained with uranyl acetate and lead citrate (Reynolds, 1963). Both were examined with a Philips EM 301 at 80 kV.

Results

Breeding. The airbreathing sharptooth catfish, *Clarias gariepinus*, can reproduce in its first year, and has a high fecundity rate resembling that of the carp. According to the literature (for overview, see Britz, 1988) *C. gariepinus* breeds in summer. Spawning usually occurs in areas associated with rain, a rising water level and flood. Courtship, mating and egg deposition take place in shallow water, at night. No parental care of the eggs is known.

Egg morphology. The greenish-yellow eggs are small, measuring 1.2 mm at their shorter diameter and 1.5 mm at the larger diameter. They are protected by a 6 to 10 μm thick zona radiata. The eggs show a spherical or slightly oval shape if they are viewed from the vegetatal pole. Their surface is smooth, exhibiting only some remnants of the follicle epithelium (Fig. 1). We did not find any morphological differences in eggs stripped within 12, 24 and 36 hours.

Compared with this, the egg profile shows a very characteristic form as seen in Fig. 2 (compare also Fig. 5). From the side, the egg resembles a fur cap. The distance from the vegetatal pole to the animal pole is considerably smaller (0.7 mm) than the egg diameter.

The egg adheres at its animal pole, which appears to be flattened (Fig. 2). Examination of the animal pole, however, does not reveal a plain disc, but rather presents an annular bulge measuring 0.5 to 0.7 mm in diameter (Fig. 3). The bulge itself is 250 μm thick. Higher magnification indicated that this annular bulge consists of numerous tiny attaching-filaments (Fig. 4), which are embedded in a certain cementing substance (Figs. 5, 6). The first histochemical tests (PAS-staining) showed that both the attaching-filaments and cementing substance contain an amount of mucopolysaccharides. The attaching-filaments seem to be a part of the zona radiata externa (Fig. 6). Figure 7 shows the zona radiata at the vegetal pole; attaching-filaments are lacking in this region of the egg.

Micropyle. The micropyle of *C. gariepinus* is a small perforation in the zona radiata through which the sperm gains access to the enclosed egg. The micropyle is located at the animal pole in the center of the attaching-filaments arranged in an annular bulge (Fig. 3).

The micropyle is a straight opening with the micropyle canal opening outward like a funnel, though a particular pit is lacking (Fig. 8). The outer diameter of the micropyle canal measures about 5 μm (Fig. 9). The inside of the canal is reinforced by helical thickenings.

Discussion

The eggs of the catfish *Clarias gariepinus* were investigated for the first time by electron microscopy. In shape, they sharply differ from those of other catfish, e.g. silurids such as *Silurus glanis* and other

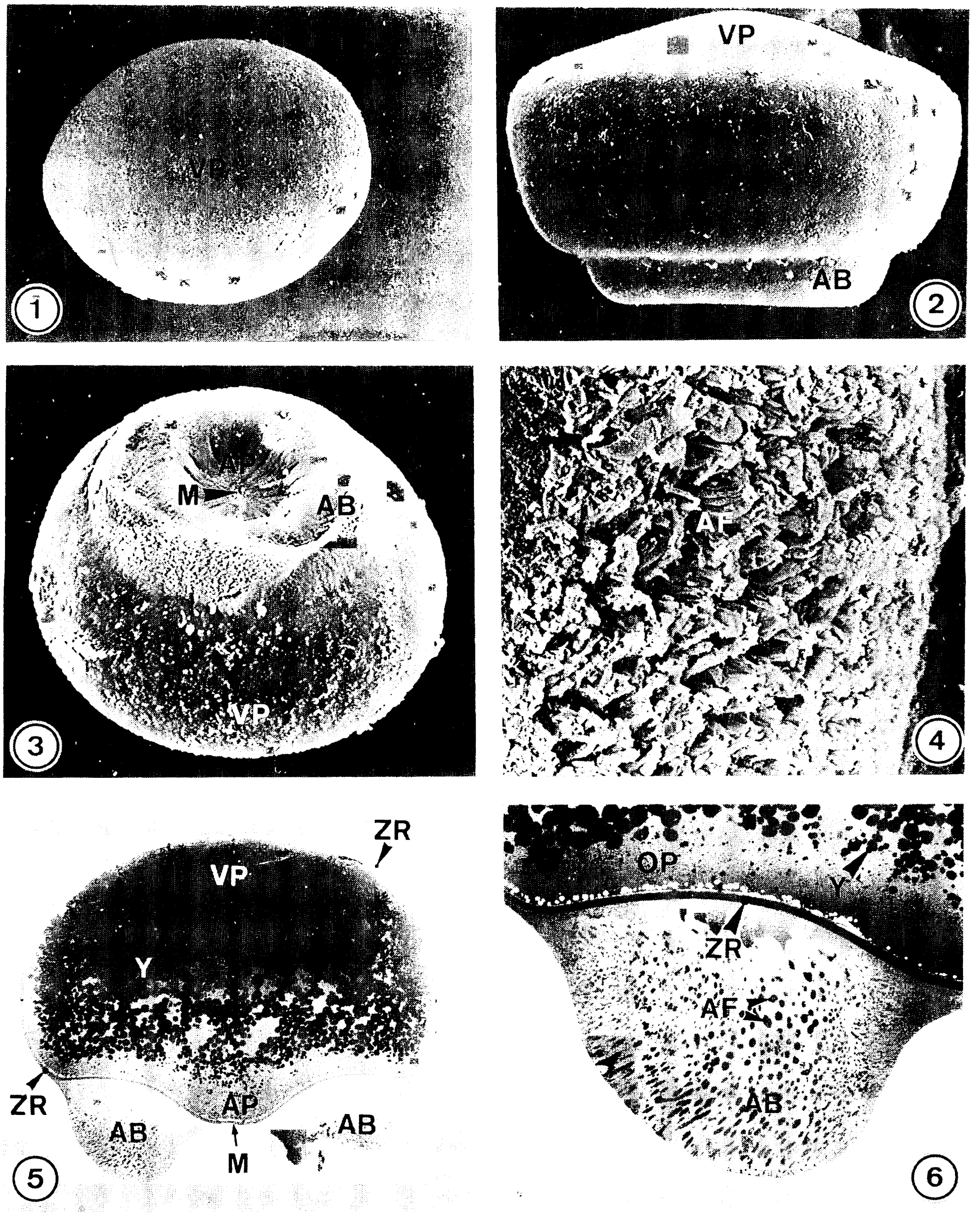

Fig. 1. View on the vegetatal pole (SEM; 40:1). Fig. 2. Side-view of an egg. Note the asymmetric shape (SEM; 61:1). Fig. 3. An egg viewed from the animal pole. Note the annular bulge, in whose centre the micropyle is located (SEM; 57:1). Fig. 4. Higher magnification of the annular bulge showing numerous tiny attaching-filaments (SEM; 515:1). Fig. 5. Semithin section of an egg (61:1). Fig. 6. Higher magnification shows many attaching-filaments embedded in a cementing substance. The attaching-filaments seem to derive from the zona radiata externa. ZR points to the zona radiata interna, the deposited externa is electron-lighter and irregular shaped (semithin section; 170:1). AB=annular bulge, AF=attaching-filaments, AP=animal pole, M=micropyle, OP=oocytoplasm, VP=vegetatal pole, Y=yolk, ZR=zona radiata (interna).

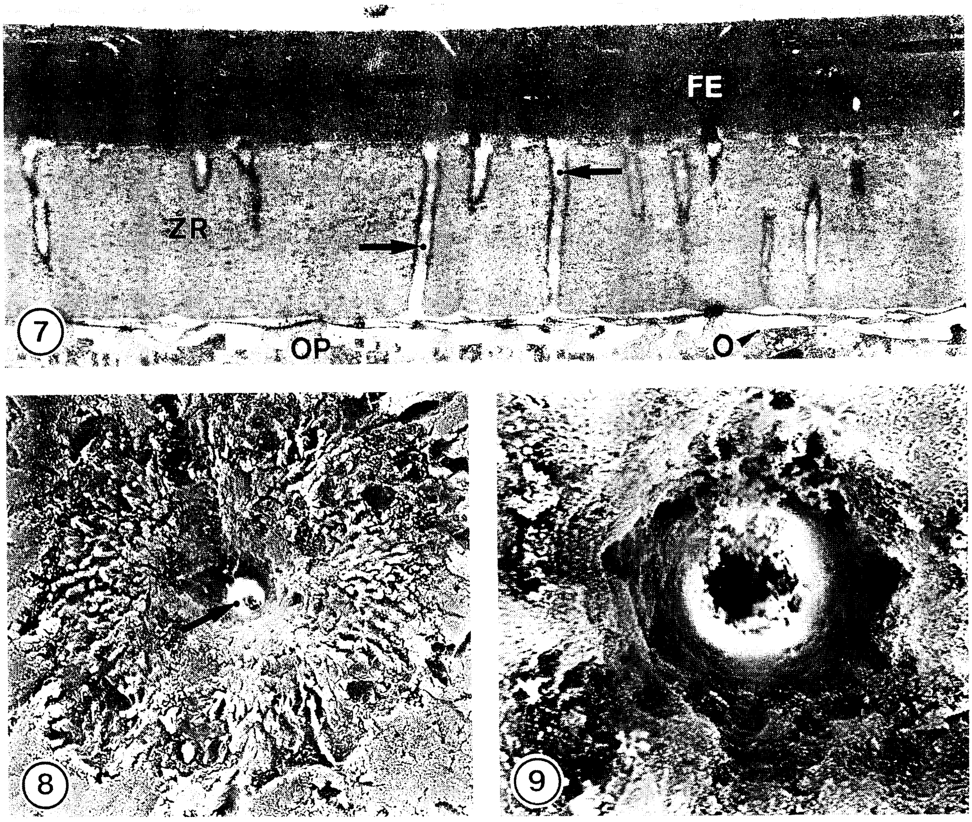

Fig. 7. TEM micrograph of the zona radiata. The arrows point to the radial canals (4725:1). Fig. 8. SEM micrograph of the micropyle, which consists only of a canal (arrow). A micropyle pit is lacking (1080:1). Fig. 9. Higher magnification of the micropyle canal (SEM; 4500:1). FE=follicle epithelium, O=oolemma, OP=oocytoplasm, ZR=zona radiata.

Silurus species (Kobayakawa, 1985; Abraham et al., in press) or the loricariid *Sturisoma aureum* (Riehl and Patzner, in press). The egg profile of *C. gariepinus* has a uniquely characteristic shape among teleosts, resembling a fur cap. In comparison, the eggs of *Sturisoma aureum* are spherical and show a special surface pattern with 22 deep furrows, which run from the vegetatal pole to the animal pole (Riehl and Patzner, in press). *Silurus* species also have spherical eggs, which are surrounded by a voluminous jelly layer (compare Kobayakawa, 1985). Until now, this jelly layer has not been studied using SEM.

Catfish eggs adhere to substrata using several different methods. In *Silurus glanis* and two Japanese *Silurus* species (*S. asotus*, *S. biwaensis*) the eggs adhere via a voluminous jelly layer (Kobayakawa, 1985; Hilge et al., 1987; Abraham et al., in press), whereas the eggs of the Japanese *Silurus lithophilus* are not adhesive. In *Ictalurus nebulosus*, eggs are attached by an adhesion disc (Armstrong and Child, 1962) and in *Sturisoma aureum* the eggs are fastened by numerous, 25 μm long attaching-filaments, which arise at the animal pole (Riehl and Patzner, in press). A special adhesion apparatus is lacking in the latter. On the other hand, in *Clarias gariepinus* a specific adhesion apparatus is present, located at the animal pole. It is formed of a large number of tiny attaching-filaments which are arranged as an annular bulge (compare Figs. 3, 4). Such an adhesion apparatus is unique among teleost fish.

In the blenny *Blennius fluviatilis*, Wickler (1957), having used light microscopy, gave the first indication of the presence of a similar adhesion apparatus. In *Blennius pavo*, Patzner (1984) did indeed show an adhesive disc consisting of numerous clasping fila-

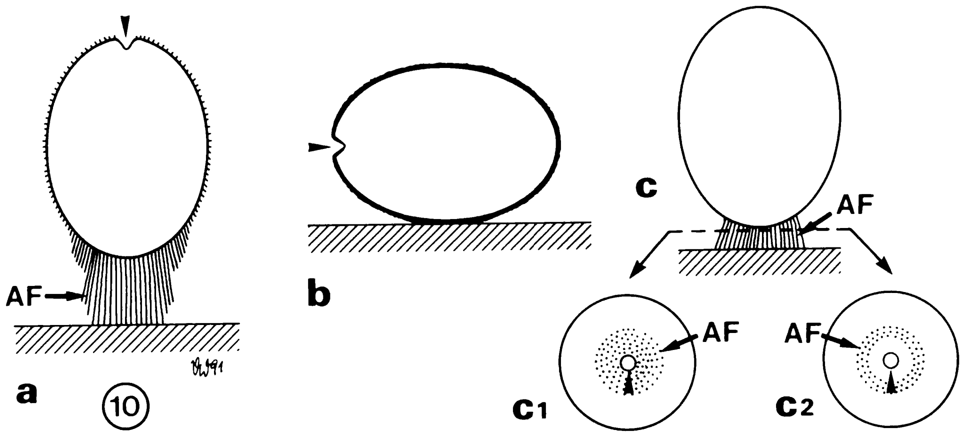

Fig. 10. Schematic drawing of different modes in the attachment of demersal fish eggs to substrata (longitudinal sections): a. at their vegetal pole, b. at their longitudinal side, c. at their animal pole. In most teleosts the attaching-filaments (AF) are arranged as in C1, whereas C2 shows the mode in *Clarias gariepinus.* The arrowhead points to the micropyle. C1 and C2 are cross sections.

ments, but these filaments covered about one-third of the egg surface and never formed an annular bulge as described in *Clarias gariepinus.*

Attaching-filaments are found in a great number of teleost species (see introduction) and also in non-teleost fishes, e.g. cyclostomes (Patzner, 1975). Within the different families of teleost fishes demersal eggs show at least three ways of adhering to substrata:

1. at their vegetatal pole (Fig. 10a),
2. at their longitudinal side (Fig. 10b),
3. at their animal pole (see Fig. 10c).

The position of the micropyle is also of great importance in demersal eggs, as here, too, there are significantly different types.

Some cichlid genera, e.g. *Etroplus*, *Nanochromis* or *Neolamprologus* produce eggs which stick to substrata by attaching-filaments located at the vegetatal pole and are known as "p-eggs" (pole egg, whereas the micropyle opens at the animal pole on the opposite, free side (Wickler, 1956)). In the eggs of other cichlid genera, e.g. *Hemichromis*, *Pterophyllum* or *Symphysodon*, adherence to the substratum is at the longitudinal face, and the eggs are therefore known as "l-eggs" (longitudinal). In both l-eggs and p-eggs the micropyle is located at the animal pole. Wickler (1956) compared l-eggs and p-eggs and found that the latter showed some obvious advantages, such as better guarding and care of the spawn, a better oxygen supply and a high rate of insemination.

In many teleosts, the attaching-filaments of demersal eggs are located at the animal pole, e.g. in blenniids (Eggert, 1931, 1932; Wickler, 1957; Patzner, 1984), gobiids (Eggert, 1931; Riehl, 1978c; Takahashi, 1978), serrasalmids (Wirz-Hlavacek and Riehl, 1990) or loricariids (Riehl and Patzner, in press). In *Clarias gariepinus*, too, the micropyle is situated at the animal pole, which is in the center of the adhesion apparatus! This position raises the question off, how fertilization of these eggs can even take place, as after the attachment to a substratum it would seem that access to the micropyle would be limited or blocked altogether. Spermatozoa would no longer be able to penetrate the egg envelope.

Therefore, in fishes with this unique adhesion mode, synchronization of male and female spawning behaviour is of striking importance. Wickler (1955) did observe such synchronization in the cyprinid *Rasbora heteromorpha.* In the goby *Pomatoschistus minutus* (Riehl, 1978c) and in the piranha *Serrasalmus nattereri* (Wirz-Hlavacek and Riehl, 1990), the eggs are fertilized before they attach to the substratum.

The zona material in teleosts eggs has also been analyzed by histochemical testing. The tests demonstrated that zona material consists of mucopolysaccharides, glycoproteins or protein and polysaccharide combinations (for review see: Guraya, 1986; Riehl, 1991). The biochemical properties of the external and internal layers of the zona radiata in teleost eggs are different. The inner layer of most species consists primarily of proteins (Hagenmaier, 1973; Busson-Mabillot, 1977; Riehl, 1977; Shackley and King, 1977), whereas the zona radiata externa contains a combination of polysaccharides and proteins. In the cyprinid *Gobio gobio* acid mucopolysaccharides predominate in the zona radiata externa (Riehl, 1977).

In adhesive eggs the material of the zona radiata, especially of the externa, undergoes a physicochemical change following deposition (Guraya, 1986; Oppen-Berntsen et al., 1990). The change manifested in an initial acquisition of adhesive properties enables the eggs to become attached to different substrata, such as vegetation, submerged objects (stones, roots etc.), and thus in nature adhesion is a tactic enhancing chances of egg survival.

Acknowledgements

We thank Mrs. Gisela Reuter, Düsseldorf, for technical assistance in preparing the micrographs and Dr. Volker Walldorf, Düsseldorf, for drawing Fig. 10.

Literature cited

Abraham, M., V. Hilge, S. Lison and H. Tibiki. 1984. The cellular envelope of oocytes in teleosts. Cell Tiss. Res., 235: 403–410.

Abraham, M., V. Hilge, R. Riehl and Y. Iger. In press. The muco-follicle cells of the jelly coat in the oocyte envelope of the sheatfish (*Silurus glanis* L.). Cell Tiss. Res.

Armstrong, P. B. and J. S. Child. 1962. Stages in the development of *Ictalurus nebulosus*. Syracuse University Press.

Britz, P. J. 1988. Environmental requirements for the hatchery rearing of African sharptooth catfish *Clarias gariepinus* (Pisces: Clariidae) larvae and juveniles. Thesis for Master Degree, Rhodes University, South Africa.

Busson-Mabillot, S. 1977. Un type particulier de sécrétion exocrine: celui de l'appareil adhésif de l'oeuf d'un poisson téléostéen. Biol. Cell., 30: 233–244.

Dumont, J. N. and A. R. Brummet. 1980. The vitelline envelope, chorion, and micropyle of *Fundulus heteroclitus* eggs. Gamete Res., 3: 25–44.

Eggert, B. 1931. Die Geschlechtsorgane der Gobiiformes und Blenniiformes. Z. wiss. Zool., 139: 249–558.

Eggert, B. 1932. Zur Kenntnis der Biologie, der sekundären Geschlechtsmerkmale und des Eies von *Blennius pavo* Risso. Z. Morphol. Ökol. Tiere, 24: 682–703.

Götting, K.-J. 1965. Die Feinstruktur der Hüllschichten reifender Oocyten von *Agonus cataphractus* L. (Teleostei, Agonidae). Z. Zellforsch., 66: 405–414.

Guraya, S. S. 1986. The cell and molecular biology of fish oogenesis. Monogr. Devel. Biol., 18: 1–223.

Hagenmaier, H. E. 1973. The hatching process in fish embryos. III. Structure, polysaccharide and protein cytochemistry of the chorion of the trout egg, *Salmo gairdneri* (Rich.). Acta histochem., 47: 61–69.

Hart, N. H., R. Pietri and M. Donovan. 1984. The structure of the chorion and associated surface filaments in *Oryzias*—evidence for the presence of extracellular tubules. J. exp. Zool., 230: 273–296.

Hilge, V., M. Abraham and R. Riehl. 1987. The jelly coat of the oocytes in the European catfish. Proc. 3rd Int. Symp. Reproductive Physiology of Fish, St. John's, Newfoundland, Canada: 201.

Howe, E. 1987. Breeding behaviour, egg surface morphology and embryonic development in four Australian species of the genus *Pseudomugil* (Pisces, Melanotaeniidae). Austr. J. Freshwat. Res., 38: 885–895.

Kobayakawa, M. 1985. External characteristics of the eggs of Japanese catfishes (*Silurus*). Japan. J. Ichthyol., 32: 104–106.

Laale, H. W. 1980. The perivitelline space and egg envelopes of bony fishes: a review. Copeia, 1980(2): 210–226.

Mooi, R. D. 1990. Egg surface morphology of Pseudochromoids (Perciformes, Percoidei), with comments on its phylogenetic implications. Copeia, 1990(3): 455–475.

Mooi, R. D., R. Winterbottom and M. Burridge. 1990. Egg surface morphology, development, and evolution in the Congrogadinae (Pisces, Perciformes, Pseudochromidae). Can. J. Zool., 68: 923–934.

Müller, H. and G. Sterba. 1963. Elektronenmikroskopische Untersuchungen über Bildung und Struktur der Eihüllen bei Knochenfischen. II. Die Eihüllen jüngerer und älterer Oocyten von *Cynolebias belotti* Steindachner (Cyprinodontidae). Zool. Jb. Anat., 80: 469–488.

Oppen-Berntsen, D. O., J. V. Helvik and B. T. Walther. 1990. The major structural proteins of cod (*Gadus morhua*) eggshells and protein crosslinking during teleost hardening. Devel. Biol., 137: 258–265.

Patzner, R. A. 1975. Die fortschreitende Entwicklung und Reifung der Eier von *Myxine glutinosa* L. (Cyclostomata). Licht- und elektronenmikroskopische Untersuchungen. Norw. J. Zool., 23: 23–35.

Patzner, R. A. 1984. The reproduction of *Blennius pavo* (Teleostei, Blenniidae). II. Surface structures of the ripe egg. Zool. Anz. (Jena), 213: 44–50.

Retzius, M. G. 1912. Zur Kenntnis der Hüllen und besonders des Follikelepithels an den Eiern der Wirbeltiere. I. Bei den Fischen. Biol. Unters., 17: 2–29.

Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain for electron microscopy. J. Cell Biol., 17: 208–212.

Riehl, R. 1977. Histo- und ultrahistochemische Untersuchungen an den Oocyten von *Noemacheilus barbatulus*

(L.) und *Gobio gobio* (L.) (Pisces, Teleostei). Zool. Anz. (Jena), 198: 328–354.

Riehl, R. 1978a. Licht- und elektronenmikroskopische Untersuchungen an den Oocyten von *Noemacheilus barbatulus* (L.) und *Gobio gobio* (L.) (Pisces, Teleostei). Zool. Anz., 201: 199–219.

Riehl, R. 1978b. Die Oocyten der Grundel *Pomatoschistus minutus.* I. Licht- und elektronenmikroskopische Untersuchungen an Eihülle und Follikel. Helgoländer wiss. Meeresunters., 31: 314–332.

Riehl, R. 1978c. Die Oocyten der Grundel *Pomatoschistus minutus.* II. Lichtmikroskopische Untersuchungen zur Kenntnis der Mikropyle. Microscopica Acta, 80: 287–291.

Riehl, R. 1984. The oocytes of the goby *Pomatoschistus minutus.* IV. Electron microscopic autoradiography of ^{3}H-proline incorporation. Cytologia (Tokyo), 49: 127–142.

Riehl, R. 1991. Structure of oocytes and egg envelopes in oviparous teleosts—an overview. Acta Biol. Benrodis, 3: 27–65.

Riehl, R. and E. Schulte. 1977. Licht- und elektronenmikroskopische Untersuchungen an den Eihüllen der Elritze (*Phoxinus phoxinus* [L.]; Teleostei, Cyprinidae). Protoplasma, 92: 147–162.

Riehl, R. and R. A. Patzner. In press. Breeding, egg structure and larval morphology of the catfish *Sturisoma aureum* (Steindachner, 1910) (Teleostei, Loricariidae). J. Aquaricult. Aquat. Sci.

Selman, K. and R. A. Wallace. 1989. Review: cellular aspects of oocyte growth in teleosts. Zool. Sci., 6: 211–231.

Shackley, S. E. and P. E. King. 1977. Oogenesis in a marine teleost, *Blennius pholis* L. Cell Tiss. Res., 181: 105–128.

Siegel, G. 1958. Zur Morphologie der Eihüllen südamerikanischer bodenlaichender Zahnkarpfen. Wiss. Z. Friedrich-Schiller-Univ. Jena, 7: 229–231.

Takahashi, S. 1978. The adherent filaments of eggs of isaza (*Chaenogobius isaza* Tanaka, Pisces). Zool. Magazine, 87: 216–220.

Wickler, W. 1955. Das Fortpflanzungsverhalten der Keilfleckbarbe, *Rasbora heteromorpha* Duncker. Z. Tierpsychol., 12: 220–228.

Wickler, W. 1956. Der Haftapparat einiger Cichliden-Eier. Z. Zellforsch., 45: 304–327.

Wickler, W. 1957. Das Ei von *Blennius fluviatilis* ASSO (=*Bl. vulgaris* Poll.). Z. Zellforsch., 45: 641–648.

Wirz-Hlavacek, G. and R. Riehl. 1990. Fortpflanzungsverhalten und Eistruktur des Piranhas *Serrasalmus nattereri* (Kner, 1860). Acta Biol. Benrodis, 2: 19–38.

Wourms, J. P. 1976. Annual fish oogenesis. I. Differentiation of the mature oocyte and formation of the primary envelope. Devel. Biol., 50: 338–354.

(Received February 14, 1991; accepted May 17, 1991)

ヒレナマズ科 *Clarias gariepinus* の卵の特異な付着装置

R. Riehl・S. Appelbaum

ヒレナマズ科の一種 *Clarias gariepinus* の卵を，光学及び電子顕微鏡(走査型，透過型)で初めて調べた．その形状は，側面像が毛皮の帽子に似て極めて特異的であることから，他の硬骨魚類の卵とは著しく異なる．この卵の形は硬骨魚類の中でも珍しい．本種の卵は動物極で基質に付着するが，この動物極には非常に多くの微小な付着糸から成る環状の膨隆部が見られる．これらの付着糸は，卵膜外層の一部である．環状膨隆部の内側中央に位置する卵門は，卵膜にまっすぐに開口している．受精にかかわる卵門の位置と併せて，硬骨魚類における卵接着の様々な様式について比較し論じた．