**A Novel Strategy of Reproduction in *Oryzias eversi* (Adrianichthidae, Teleostei)**

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**ABSTRACT**

Reproductive features in an oviparous teleost, *Oryzias eversi* known as a pelvic brooder were examined. Eggs possess two distinct types of filaments on the surface of the chorion which are formed during the oogenesis: long attachment filaments at the restricted area around the vegetal pole, and exiguous, poor short filaments only around the animal pole (micropyle). After spawning, females carry a fertilized egg cluster hanging from the genital pore by a bundle of the long attachment filaments on the chorion around the vegetal pole. By mating with male, females spawn eggs through the genital pore and fertilized eggs were covered and protected with a pair of their long pelvic fins in the abdominal concavity behind the pelvic fins in the belly, until they hatch after about 12 days post-oviposition. While a female carried the egg cluster in her belly, the oocyte growth proceeded slowly in her ovary. Furthermore, ovulation never took place in the ovaries of the embryos-hanging females. Some of the female occasionally lost their hanging eggs accidentally and the next spawning came earlier. Thus, the regulation of the reproductive cycles in the females of *O. eversi* appear to entirely depend upon the presence of the egg-cluster hanging from the genital pore.

**Key words**: *Oryzias eversi*, attachment filament, pelvic brooder, ovulation, oviposition cycle

**INTRODUCTION**

The genus *Oryzias* (Family, Adrianichthyidae) comprises 24 species, or more which ranges broadly throughout fresh and brackish waters of Central, Nouth and Southeast Asia and Indro-Malay-Philippines Archipelago as far east as Timor (Parenti, 2008). Oviparous mode of reproduction in *Oryzias* is categorized as lecithotroph according to *in vitro* development. Most of these oviparous species exhibit photo-periodically synchronized ovulation. Under the natural daylight conditions in the breeding season, mature females of *Oryzias latipes* lay eggs in the early morning every day. When males engaged in the mating behavior, females lay eggs almost immediately after ovulation (Robinson and Rugh, 1943; Egami, 1954). According to Egami (1954) and Yoshioka (1964), the time of oviposition can be, however, modified whenever if female fish are artificially exposed to an appropriate photoperiod.

Eggs of Adrianichthidae, as well as Scomberesocidae, Hemiramphidae and Exocoetidae belonging to Beloniformes, are spherical and have filament-like appendages on the chorion (egg envelope, *zona radiata*) (Rosen and Parenti, 1981; Collette et al., 1984). In the eggs of *Oryzias* species, there are two distinct types of filaments, viz. long attachment filaments (AFs) on the restricted surface of the chorion in its vegetal pole area (VPA), and short villi on the whole surface of the chorion except for the VPA. Spawned eggs are entangled into objects floating in the water by the long AFs (Yamamoto, 1975). In *O. latipes,* females frequently hang spawned eggs with AFs in their belly until the subsequent spawning. Most *Oyzias* species including *O. latipes* are called “transfer brooders” (Spanke, et al., 2021) and their females deposit spawned eggs in any water plants or solids. In contrast, in some of oviparous *Oryzias* and *Xenopoecilus* species the AFs appear to play an important role not only for keeping embryos in the belly of the female until embryos hatch, but also for a physiological function to regulate the reproductive cycle. These species are generally called “pelvic brooders” (Kottelat, 1990).

Fishes are the most diversified vertebrates with respect to their morphology and ecology (Wourms, 1981). Particularly, the ovulation of viviparity establishes the specialized endocrinological maternal-fetal relationships. In the diversity of reproductive strategies, it is not surprising to find that fishes exhibit nearly all the reproductive modes found in the other vertebrates. Even in the oviparous fishes, reproductive cycle is regulated by embryos as reported in the mouth-brooders (Specker and Kishida, 2000). So far, pelvic brooders have also been reported in two monophyletic genera, *Adrianichthys* and *Oryzias* (Parenti, 2008); *Oryzias sarasinorum* (Pota, 1905; Iwamatsu and Sato, 2004; Iwamatsu et al., 2007), *O. eversi* (Herder et al., 2012; Mokodongan and Yamahira, 2015; Schüller et al., 2022) and *O. kapimpaaensis* (Gani et al., 2022) as well as *Adrianichthys poptae* (Weber and Beautfort, 1922), and *A. oophorus* (Kottelat, 1990). These females carry a cluster of developing embryos in the posterior region of the genital papilla (protuberance) in the abdominal concavity by AFs on the chorion until embryos hatch. In these pelvic brooders, females do not ovulate while they carry fertilized eggs and developing embryos (Iwamatsu et al., 2007, 2008). This reproductive event of the pelvic brooders completely differs from that of the transfer brooders in which spawning takes place photo-periodically every day. In the present study, we focused on the morphological and reproductive features of a pelvic brooder *O. eversi* of which so excellent histological investigations has been reported by Schüller et al. (2022). In order to understand the regulation of reproductive cycle, we also examined the composition of various-sized follicles (oocytes) and post-ovulatory follicles present in one ovary. The observational results imply that in *O. eversi* females carrying embryos in their belly, oocytes develop slowly in the vitellogenic phase of their oogenesis. Consequently, oocyte maturation and ovulation do not take place during the pelvic brooding.

**MATERIALS AND METHODS**

Animals

In the present study, 3 females (43.7+3.5 mm in total length, TL) and 5 males (43.9±2.6 mm TL) of *Oryzias eversi* kept in the World’s Medaka Aquarium, Higashiyama Zoo, Nagoya-city, Japan were examined. Adult females were kept under reproductive conditions (13 hr light, 6:00-19:00, 27 ℃), and fed on live foods (*Halla parthenopeia*) and dry powder diet (Otohime, Marubeni Nisshin Feed Co., Ltd, Tokyo, Japan). Females carrying a cluster of spawned eggs were deeply anesthetized in a mixture of water-saturated phenylurethane (7 parts) and ethanol (3 parts) and examined under a binocular dissecting microscope (SZX12, Olympus, Tokyo, Japan). The anesthetized females were then euthanized by pithing with a sharp tip of ophthalmic scissors. After the abdominal cavity was opened, the ovary was isolated and quickly fixed in cold 0.05% glutaraldehyde in saline (4 ℃). This study was conducted following the Guidance for the use of fishes, in Research of the American fisheries Society.

Observations of oocytes, eggs and pelvic girdles

Females carrying a cluster of spawned eggs by a bundle of attachment filaments were deeply anesthetized in saline containing the mixture of water-saturated phenylurethane and ethanol and euthanized as described above. Then, they were laparotomized and the ovaries were isolated with fine forceps and a scalpel in saline under a binocular stereomicroscope (SZX12, Olympus). Pelvic girdles were also isolated with pelvic fins from the belly, stained with 0.001 alizarin red S, followed by incubation in 0.5 % KOH. These samples were cleared in 50% glycerol before observation. Stages of oogenesis are represented in accordance with stages in *O. latipes* (Iwamatsu et al., 1988), although the size of oocytes is a little different.

Histological examination and 3D reconstruction of female genital organ

The fish were anaesthetized and fixed in Davidson’s fixative solution (22% of a 37% solution of formaldehyde, 33% ethanol, 11.5% glacial acetic acid, and 33.5% distilled H2O) at room temperature and stored util further processed. The fixed samples were dehydrated with ethanol, embedded in paraffin (Parabett, Muto Pure Chemicals Co., Ltd., Tokyo, Japan) and longitudinal-sectioned (5 μm) throughout the whole body using a sliding microtome (REM-700, Yamato Koki Industrial Co., Ltd., Saitama, Japan). The sections were stained with haematoxylin and eosin (HE) for light microscopy observation. 3D image of the genital organs were reconstructed from the cross sections using ReconstructTM 1.1.10 (Lu et al., 2009).

**RESULTS**

**Traits of sexual dimorphism**

Adult males of *O. eversi* exhibit 6-9 blackish spots on their lateral body surface (Fig. 1A), as previously described by Herder et al. (2012). They have the elongated anal fins and dorsal fins with long fin-rays, and have small urogenital papilla (UGP) although some males lack this. In contrast, adult females are characterized by having less developed anal fins and dorsal fins with short fin-rays, along with long pelvic fins and a well developed single-lobed UGP (Figs. 1B, 2A and 2B). In the females, the elongated pelvic fins help to hold and to protect a cluster of spawned eggs in the inconspicuous belly concavity (Figs. 1A and 2B). Distal tips of long pelvic fins are overhanging the anterior part of the anal fin (Fig. 1B, Figs. 2A and 2B). Anatomical observations also showed that the female has a pair of the well-developed pelvic girdles (Fig. 2C), which play a role in moving the long pelvic fins for protecting embryos hanging, connected with the muscle which moves the pelvic fins.

The length of anal fin (mean 29.1±2.1% SL) in males are significantly longer than those in females (mean 18.2±0.6% SL, Fig. 3C). In sexually matured males, the tip of anal fins has long fin rays without the fin fold (membrane) at the distal region and the fin-rays in the posterior region of the male anal fin possess small papilla processes, which females anal fin-rays do not (Fig.1A). However, the notch that the last fin ray displays by separating from the rest is not seen in the anal and dorsal fins. The dorsal fin has 9-12 fin-rays and the length of dorsal fin with 11 fin-ray was also larger in males (mean 24.7±1.9% SL, n=8) than in females (mean 16.4±1.0% SL, n=3) (Fig. 3C). *O. eversi* males use their dorsal and anal fins for grasping gently but firmly on the tail of the female at the time of mating in the same way as *O. latipes* males do.

In contrast to the anal and dorsal fins, *O. eversi* females have longer pelvic fins (11.3±0.7% SL) than those of males (5.6 ±1.3% SL) (Fig. 3C). The female pelvic fins have 6 fin rays and the total number of ray nodes was 111.0±10.7 (range 93-130) and the total number of ray ramification was 6.3±0.9 (range 5-8). When compared with those (ray node, 84.0±4.1; ray ramification, 5.2±1.6) in the male pelvic fins, these values in the female pelvic fins were significantly larger than those in the male pelvic fins.

**Ovary and genital organ**

The ovary (2.9-3.4 mm in width, 3.4 mm in length) of mature females shortly after oviposition consists of the stromal compartment and the dorsal ovarian cavity, which is surrounded by ovarian wall with the intricate folds. The dorsal surface of the ovary connects to the median surface with the mesovarium that attaches to the dorsal peritoneum. The width of the ovary that is divided by the mesovarium is smaller in the left side than the right side. In the stromal compartment, oocytes in various stages of oogenesis (I-VIII) within all the various-sized developing follicles and the post-ovulatory follicle (POF) are observed and these follicles and POFs connect with the follicular stalks. Follicular stalks are located on the opposite side of the follicle attaching to the ovarian wall (oe) and anchored respective follicles to the abdominal reticular connective tissue (rt) (Figs. 4A and 4B).

In the belly of the adult females there is an anus at the anterior region of UGP with the intricate folds on their surface (Fig. 2B). The orifices of oviduct and urinary duct open at the posterior end of the UGP. The ovarian cavity connects to the short oviduct lumen being surrounded by the markedly modified inner wall of the oviduct.

3D results will be added

**Reproductive cycle**

Females carry a cluster of spawned eggs in the belly by long AFs which located on the outermost layer of the chorion in the vegetal pole area of the egg. After the egg spawning, these females continue to carry the developing embryos for more 10-13 days (mode 12 days) until the embryos hatch (Fig. 5). During pelvic breeding, female often shakes a body by moving the pelvic fins as if to confirm the presence of hanging embryos, providing fresh water to the embryos at the same time.

**Morphological features of growing oocytes and eggs**

The ooplasmic volume gradually increase as oocytes grow. All oocytes are anchored to the abdominal ovarian rete by thread-like follicular stalks (Fig. 4). In the same as described previously in *O. latipes* (Iwamatsu et al., 2020), a line connecting the follicular stalks and the center of the follicle area attaching to the ovarian wall might be called follicle axis. The large spherical egg nucleus (germinal vesicle, about 48 µm in diameter) contains many chromatin–threads and various sized nucleoli, distributed in the peripheral region of the nucleus (the perinucleolus stage). On the surface of the chorion (egg envelope) of oocytes more than 135 µm in diameter, the peculiar primordia of attachment filaments (AFs) can be observed as minute bumps (verrucose rudiments) for the first time in the presumptive vegetal pole area (VPA) of the oocyte. The inner layers of the chorion are not yet formed in the young oocyte at this developmental stage. In the cytoplasm of such oocytes, there is a single yolk nucleus (Balbiani body) which is different from the ubiquitously formed some nuages in the juxtanuclear cytoplasm (Hamaguchi, 1987). In oocytes of around 250 µm in diameter, oil droplets appeared in the cortical cytoplasm adjacent to the nucleus (oil droplet stage VI). When oocytes grow with its rotation ascertainable by state of FAs in the oocyte more than 300 µm in diameter, about a third part of the oocyte surface are covered by winding of elongated AFs. In the oocyte at this stage, the tips of all AFs on the chorion uniformly bend at right angles to the animal-vegetal (A-V) axis, or the hypothetical line which connects a micropyle and a center of the distribution area of AFs (Fig. 7A). As vitellogenesis progresses, small yolk globules fused with each other to form a single spherical mass (yolk sphere), of which the size enlarges in proportion to the growth of oocytes (Fig. 6). The layer of granulosa cells surrounding the VPA became thicker as compared to that of the animal hemisphere.

**Changes in AFs and the oocyte composition in an ovary following oviposition**

On the following day after the oviposition, females (43 mm TL) were carrying a batch of eggs (1,497.4±23.4 µm in diameter, n=63) at the embryonic shield stage 17. The distal parts of AFs formed a tangled mass and adhered on the inner surface of the ovarian cavity. Micropyle is detectable on the just opposite side of VPA in the chorion (Fig. 7A) and the number of poor villi (mean 95.4±0.4 µm in length, range 10-450 µm, n = 40) averaged 16.0±0.7 (n =20) on the chorion of each egg. These short villi are distributed only in the restricted area (about 450 µm x 180 µm in diameter) around the micropyle at the animal pole (Figs. 7A and 8A).

Total number of oocytes in an ovary was 2,440. The largest population of stage I oocytes occupied 60.3% of the oocytes in an ovary (n = 4), and nearly full-grown oocytes of vitellogenetic stage IX (more than 800 µm in diameter; range 846-1,026 µm) were only 1.2% of the total oocytes (Fig. 9). Mean size of post-ovulatory follicles was 741.7±47.6 µm in length, 214.2±26.8 µm in width and 67.1±18.6 µm in thickness (n=15).

In a female (45 mm TL) on 5 days post–oviposition, the tangled AF mass contains the two types of interstitial (or epithelial) cells within the inner wall of the ovarian cavity (not shown). The AF mass became hardened as a compact plug (1.0–1.5 mm in diameter) of the genital pore. In its ovary, the total number of oocytes was counted to be 1,820. The cluster of fertilized eggs (stage 34) (mean diameter 1,562+13.2 µm; range 1,525-1,630 µm, n=14) was kept at the anterior region of the anal fin by a cluster of long AFs (13.6 + 0.4 in number, n = 67, Fig. 1B), which were located on the restricted area (about 216 x 189 µm) of the chorion surface in VPA (Figs. 8A, 8B and 8C). In this female, the ovary exhibited a significant decrease of the smallest oocytes in stage I (33.4%), while the oocytes in stage II increased definitely (31.5%) and nearly full-grown oocytes remained unchanged (0.1%) in number (Fig. 9). Mean size of POFs shrank to 486.0±41.2 µm in length and 261.0±44.4 µm in width. In contrast, full-grown vitellogenetic oocytes larger than 800 μm in diameter were hardly seen. Just after hatching, AFs remain attaching on the very thin residue (outermost layer) of the chorion which was not dissolved by hatching enzymes. The rotatory direction of oocytes is implied by left- or right-handed bending of their AFs on the slightly thin chorion (Fig. 8D). Thus, oocyte composition in an ovary exhibited the vitellogenetic pattern without full-grown oocytes.

**DISCUSSION**

The egg is a crucial ingredient in studying evolutional diversity of the reproductive strategy. Morphology of developing oocytes in O. eversi is quite similar to that of O. latipes oocytes (Iwamatsu et al., 1988; Iwamatsu and Nakashima, 1996).

Females of O. latipes spawn at dawn (shortly before the onset of the light phase) and carry spawned eggs in the belly by AFs for about ten hours or more, since AFs are pushed out with subsequently spawned eggs if it were not for something to adhere eggs. In these female, AFs do not, however, transform into the plug-like complex structure as a plug of the oviduct, so the AFs are lost with eggs from the belly at least until next oviposition. Unlike these AFs, a loosely tangled mass of the tips of AFs of O. eversi (Herder et al., 2012; Hilgers et al., 2022; Shüller et al., 2022; the present study) and O. kalimpaaensis (Gani et al., 2022) as well as that of O. sarasinorum (Iwamatsu et al., 2008), remains in the ovarian cavity, due to their adhesion to the inner wall of the ovarian cavity with the mucus secreted just after oviposition. Hilgers et al. (2012) and Shüller et al. (2022) hypothesized that an inflammetory reaction by the retention of attachment filaments gives rise to the pathways forming the oviduct plug. The epithelial (interstitial) cells lining the inner wall of the oviduct appear to migrate among the tangled tips of AFs probably due to allergic inflammation (Hilgers et al., 2022; Shüller et al., 2022) to constrict a short oviduct. Consequently, female O. eversi carry fertilized eggs until the embryos hatch.

In oviparous O. eversi, ovulation never occurs while females carry fertilized eggs in the belly for about 12 days, in a way mimicking the pregnant period in viviparous animals. At present, although we have no knowledge as to whether the function of the plug–structure in this fish may correspond to the placenta during the pregnancy, a close relationship between the ovarian activity and the presence of the plug–structure (or developing embryos in the belly) can be inferred from the experimental observation that the artificial removal of embryos (AFs) from the belly shortens the intervals of the reproductive cycle (Iwamatsu et al., 2007). Therefore, the presence of the plug–structure fused with the maternal tissue may be constrained from progressing oogenesis and so the next oviposition is retarded during carrying embryos. This situation resembles the relationship between the retardation of oogenesis and the parturitions in the viviparous fishes. The retardation effect of the presence of embryos on the development of oocytes has been reported in viviparous fishes (Turner, 1937). In Gambusia affinis, young oocytes grow slowly up to the next gestation and reach the maturation stage within about 5 days after parturition (Koya et al., 2000). This retardation effect of the ovarian gestation on oocyte maturation seems to resemble the effect of cross-talk with the plug–structure in O. sarasinorum and O. eversi, as estimated in oocyte composition in an ovary in the presence of the plug structure. Thus, the oviduct plug may be specialized to accomplish the two functions of carrying the egg cluster and regulating the maturation of oocytes.

If once O. latipes females are artificially inseminated in the ovarian cavity by introducing a sperm suspension shortly after ovulation, fertilized eggs with a hardened chorion (egg envelope) fail to be squeezed out of the ovarian cavity through a narrow genital pore (Iwamatsu et al., 2022). Therefore, fertilized eggs begin to develop within the ovarian cavity. However, those pregnant females never stop ovulating every morning, even if developing embryos exist in the ovarian cavity, and consequently eggs are in turn accumulated in the ovarian lumen by every ovulation. Namely, the transfer brooder O. latipes depends on the environmental factors such as light, temperature and so on, in spite of pregnancy, whereas the reproductive cycle of such oviparous pelvic brooders as O. eversi and O. sarasinorum is influenced by embryos to be carrying in the belly until hatching, similarly to that of viviparous fishes. The queer reproduction mode of these oviparous fishes still remains little known.

Our study will provide the aquarists as well as biologists with much unexpected but interesting knowledges that AFs form and elongate on the chorion at the vegetal pole region (VPA) of young oocytes, winding around the vegetal hemisphere in fully grown oocytes as oocytes grow (Iwamatsu et al., 1988). The tuft of long AFs on the chorion indicates the vegetal pole of the egg and is a phylogenetic trait of Oryzias species belonging to the family Oryziatidae, although its number is different among them (Iwamatsu, 2018). As shown in the present observation, O. eversi eggs also indicate a left- or a right-handed rotation on the animal-vegetal (A-V) axis that isascertainable with a visual evidence that the tips of AFs indicate the bending to a uniform direction. In these eggs, the A-V axis is recognized as a line that connects the vegetal pole (AFs) with the opposite animal pole (micropyle). It implies that the egg polarity of O. eversi oocytes is determined in the same manner as seen experimentally in O. latipes oocytes which rotate on the A-V axis of the oocyte during oogenesis (Iwamatsu, 1994).

Further, the other similarity of the genus Oryzias is in short villi (non-attaching filaments) distributed on the surface of the chorion except for the VPA (see Iwamatsu, 2018). Eggs of O. eversi possess the very short villi poor in size and number, locating in only restricted area around the micropyle. As seen in another pelvic brooder O. sarasinorum, eggs of this pelvic brooder also are morphologically similar with respect to the context of villi which might be the result of phylogenetic loss. In Xenopoecilus oophorus, there also are no short villi on the chorion (Kottelat, 1990). Pelvic brooders display a similarity in developmental patterns of the villi on the chorion of eggs.

Interestingly, pelvic brooders such as O. eversi (1.4 mm in Herder et al., 2012; 1.46±0.02 mm in the present data), O. kalimpaaensis (2.19±0.10 mm in Gani et al., 2022), O. sarasinorum (2.09±0.01 mm in Iwamatsu et al., 2008) and Xenopoecilus oophorus (2.0-2.1 mm in Kottelat, 1990) spawn in common very large eggs, in comparison with transfer brooders Oryzias species. These features on the egg seem to support a phylogenetic relationship reported by Rosen (1964) and Rosen and Parenti (1981). The developmental period of 12 days in O. evesi of which the egg size was about 1. 5 mm as observed at the present time is longer than that of 9 days (26℃ in the transfer brooder O. latipes with small size (1.2 mm in diameter). In O. sarasinorum with more large eggs (2.1 mm in diameter), the developmental period is too longer (15 days). Thus, the developmental period of eggs may have a close relation to the size of (egg yolk) volume. That is, in pelvic brooders, the period that female pelvic brooders carry fertilized eggs in the belly appears to be dependent on the egg size.

In common with many transfer brooders, O. curvinotus, O. latipes, O. luzonensis, O. mekongensis of the genus Oryzias (Iwamatsu, 2018), the male possesses longer in the anal and dorsal fins longer in comparison with the female. Moreover, numerous small papillar processes on the internodes in the posterior region of the long fin rays of the male anal fin are well known (Oka, 1931; Yamamoto and Egami, 1974, cf. Yamamoto, 1975). The presence of the papillar processes in long fin rays of the anal fin also has been reported in some transfer brooders as a sexual character. To contrary, such a sexual character is absent in males of pelvic brooders, O. sarasinorum, O. eversai, Xenopoecilus oophorus and X. poptae (unpublished) in many species of the endemic Oryzias species in Sulawesi including O. eversi as well as O. melastigma in India. Therefore, the papillar processes in the anal fin is not always a significant sexual characteristics in males. In addition to this fin trait, a sexual dimorphism that the length of pelvic fins is shorter in the male in comparison to that of the female is commonly comfirmed in most Oryias species such transfer brooders as O. celebensis, O. javanicus and O. latipes, and pelvic brooders O. eversi and O. sarasimorum. In the pelvic brooders of Oryzias species (Iwamatsu, 1986) including O. sarasinorum (Iwamatsu and Sato, 2004; Iwamatsu et al., 2007), O. eversi (Shüller et al., 2022) and O. kalimpaaensis (Gani et al., 2022) as well as Xenopoecilus poptae (Weber and Beautfort, 1922) and X. oophorus (Kottelat, 1990), females can use the elongated pelvic fins of which distal tips meet beyond the anal fin origin. The female traits of pelvic brooders are likely beneficial and gainful in the context of the common reproductive strategy. Especially, the long pelvic fins of these pelvic brooder females protect a cluster of embryos from egg predators for a long developmental period. Therefore, in pelvic brooders such as O. eversi (the present data) and O. sarasinorum (Iwamatsu et al., 2008) pelvic girdles of females functionally supporting the long pelvic fins develop greater than those of males. Furthermore, detailed observations with the utmost care and attention on sexually dimorphic traits such as pleural ribs (Spanke et al., 2021) will be required for understanding the diversity in evolution of medaka-fish. The present results will provide aquarists as well as biologist with much unexpected but interesting knowledges.

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**FIGURE LEGENDS**

Fig. 1. Living Oryzias eversi and a cluster of developing embryos being hung in a

female abdomen by a tuft of attachment filaments (AFs).

A. A sexual pair of O. eversi exhibiting traits of sexual dimorphism. Bar=10mm

B. The pelvic region during pelvic brooding. Developing embryos (about 6 days

after spawning) being hung at the posterior end of urogenital papilla (u) by long

attachment filaments (arrowheads). Pelvic fins (pf) overhanging embryos. af, anal

fin. Bar = 1mm

Fig. 2. Abdominal region of O. eversi female.

A. Side view of abdominal region: long pelvic fins (pf) beyond the origin of the

anal fin (af) and attachment filaments (α) which are seen at the end of a single urogenital papilla (ugp). af, anal fin; pf, pelvic fins. Bar = 1mm

B. A single-lobed urogenital papilla of the female. Arrowhead indicates an anus

(an) in the anterior region of a urogenital papilla (UGP) with a complicated pattern.

Bar = 0.1mm

A pair of left (L) and right (R) elements of pelvic girdles (pg) with pelvic fins (pf).

C. Female pelvic girdles (pg) exhibiting more specialized parts facing each other in in comparison with male ones (D). Bar=1mm

Fig. 3 Sexual dimorphism of fins in O. eversi.

A. Total number of ray ramification. B. Total number of ray node

C. Length /SL (%)

Fig. 4 Oocytes and post-ovulatory follicle in the ovary of O. eversi.

Oocytes (A) and post-ovulatory follicle (pof, B) are anchored by follicular stalks (fs) to reticular connective tissue (rt). Follicular stalks attach the opposite side of ovarian epithelium (oe). ap, animal pole; vp, vegetal pole.

Fig. 5. Frequency distribution of the day from spawning to hatching in O. eversi.

Fig. 6 Changes in spherical yolk mass within vitellogenetic oocytes of O. eversi.

Fig. 7 Attachment filaments and villi on the chorion of O. eversi.

A. The animal pole area showing a micropyle (m), villi (v) and oil droplet (o).

B. Attachment filaments (α) in the vegetal pole area.

Fig. 8 Developing embryos being carried by the female.

A. The following day after oviposition, the egg envelope (chorion) indicate the animal-vegetal (A-V) axis which connects a micropyle (arrowhead) at a thick chorion and a center of the distributed area of attachment filaments (α). Small arrows point out poor villi.

B. Five days after oviposition, the chorion displays a micropyle (arrowhead) and attachment filaments (α). cd, Cuvier duct; ha, atrium of heart; hv, ventricle of heart; sv, sinus venosus; vc, median yolk vein.

C. Attachment filaments on slightly thick region (arrowhead) of the chorion just before hatching,

D. After hatching attachment filaments remain unchanged on the thin outermost layer of the chorion and exhibit a right-handed arrow or a left-handed arrow (rotation of the egg).

Fig. 9 Oocyte composition in an ovary of O. eversi females carrying developing embryos.

A. The following day after oviposition (oocyte rotation).

B. Five days after oviposition.