because, sometimes, the secondary constriction of pair 4 cannot be well observed and the chromosomes may be considered as metacentrics.

The different patterns of C-bands associated with the morphological differences of the chromosomes allowed a precise identification of all the chromosome pairs. The distribution of the constitutive heterochromatin associated with the NOR observed in the long arm of pair 4 is different from that described for another fish, *Umbra limi*, where sequential banding analysis showed that the silver-stained region was also C-band positive[3]. In *A. albifrons*, however, the NOR is not C-band positive, as shown in figure 2d. This situation is very similar to mammalian species where NORs have never been found to be C-band positive[15].

Most of the available data in the literature[3,7,9] and unpublished data of the present authors, involving 3 more species of Gymnotiformes, show that in many fishes a NOR seems to exist in only 1 pair of homologues; in some cases there is a suggestion of the involvement of more than 1 chromosome pair in the nucleolar organization, either by the presence of tiny dark areas over another chromosome pair[2], or by the indication that the silver-stained chromosomes were not apparently homologues[8]. These facts lead us to speculate that in fishes the usual condition could be the presence of only one or a few chromosome pairs involved in the nucleolar organization. Inversion and translocation mechanisms could explain the different morphology of the NOR bearing pair even in closely related species, as is the case in the Anostomidae[9].

The identification of all the chromosome complement of *A. albifrons* by banding procedures may help in the studies of the cytotaxonomy and evolution of the order Gymnotiformes, and also in experimental studies involving fish chromosomes.

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## Effect of progesterone on the formation of the ovipositor in female bitterlings (*Rhodeus sericeus amarus* BLOCH, 1782) (Teleostei, Cyprinidae)[1]

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*Summary.* Female bitterlings *(Rhodeus sericeus amarus)* were treated with the sexual hormone progesterone at a dose of 170 µg/l for 2 weeks. Ovipositors from 1–4 centimetres in length grew out in all females within 24 h. The ovipositors were studied by electron microscopy (SEM, TEM).

The bitterling *Rhodeus sericeus amarus* is a small European fresh-water fish with remarkable breeding habits. During the spawning period the female developes an ovipositor from which eggs are injected into the inhalent siphon of a mussel of the genera *Anodonta* or *Unio*.

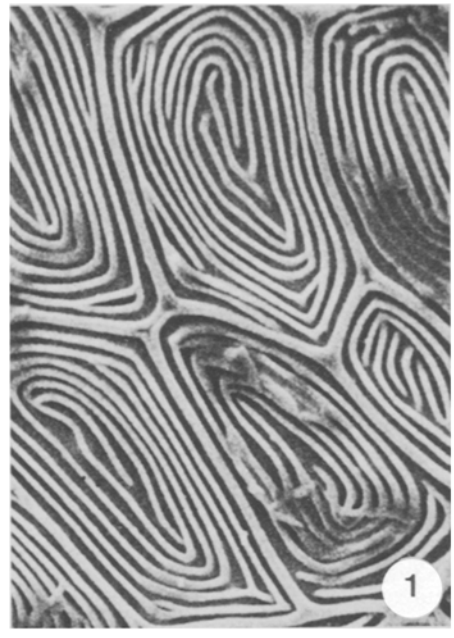
The purpose of this paper is to clarify whether or not progesterone can induce the development of an ovipositor and/or control its appearance. Female bitterlings lacking ovipositors were treated with the female sex hormone progesterone and then checked for the occurrence of egg depositors. The induced ovipositors were examined ultrastructurally.

24 female *Rhodeus sericeus amarus* were placed in 3 60-l aquaria (8 per tank). 1 60-l tank was used as a control aquarium containing 6 bitterlings. The hormone progesterone was dissolved in 96% ethyl alcohol at a concentration of 10 mg/ml. This solution was added to the 3 aquaria at a dose of 1 ml/60 l of aquarium water, creating a concentration of 170 µg of progesterone/l[2]. The control aquarium was given a dose of 1 ml of 96% ethyl alcohol/60 l of water. The fish were treated for 2 weeks.

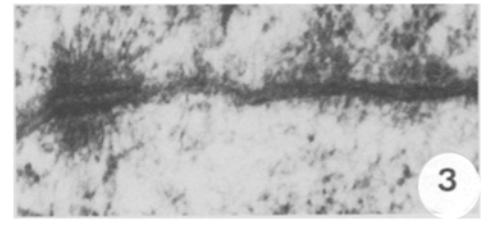
The treated females were anesthetized with 0.5–1% Anestesin (=4-aminobenzoic-acid-ethylester, Serva). The ovipositors were then cut off with fine scissors and placed in 5% glutaraldehyde buffered with veronal acetate and phosphate buffers pH 7.4, 4 °C for 2–10 h. After washing with adaequate buffers (3 times) ovipositors were postfixed in 2% osmium tetroxide, dehydrated in a series of ascending alcohols and embedded via propylene oxide in Vestopal W and Araldite. Ultrathin sections were cut with a Reichert Om U 3, double-stained in uranyl acetate and phosphotungstic acid en bloc[3] or in uranyl acetate and lead citrate[4]. They were then viewed in a Zeiss EM 9S and a Philips EM 400.

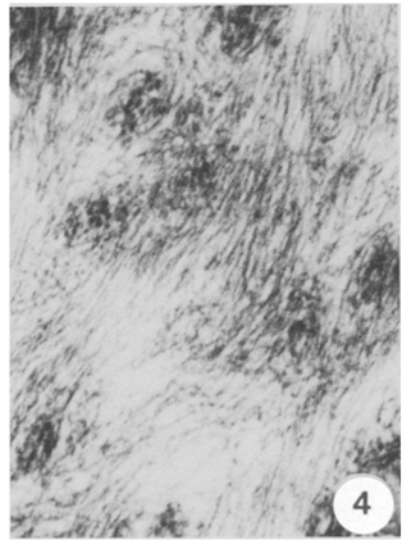
1 of 30 females treated with 170 µg of progesterone/l aquarium water died during the 1st week of testing and 2 died during the 2nd week. In all females ovipositors were observed within 24 h, the length ranging from 1 to 4 cm. The 6 control females showed no sign of an ovipositor during the 2-week testing period.

Ovipositors can also be induced by addition of 1–2 ml urine of gravid women/60 l aquarium water (Riehl, unpublished). After this treatment only 80% of the female bitterlings show ovipositors, which also grow out within 24 h. The induction of ovipositors by treatment with the urine of gravid women was formerly employed in diagnosis of







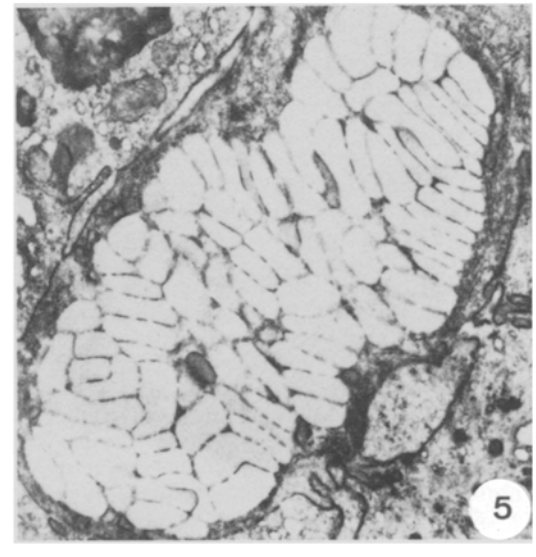




Figure 1. Pattern of meandrian microridges on the surface of the ovipositor. SEM; × 6300.

Figure 2. Interdigitations and gap junctions which link the ovipositor cells. Note the annular gap junction. TEM; × 40,000.

Figure 3. Desmosome between 2 ovipositor cells. TEM; × 42,000.

Figure 4. Network of cytofilaments in most of the cells. TEM; × 40,000.

Figure 5. Goblet cell from the tip of the ovipositor. TEM; × 11,700.

pregnancy. If the addition of progesterone or urine of gravid women is stopped, the ovipositors disappear within 2 or 3 days. The observation that the administration of female sexual hormones causes the formation of ovipositors in all females agrees well with what has been observed in nature; all females have ovipositors during the spawning period.

It is known that factors such as temperature-changes, bright light or administration of alcohol can influence the appearance of certain secondary sex characters. Subjecting female bitterlings *(Rhodeus sericeus amarus)* to these conditions causes the artificial lengthening of the ovipositors[5]. The treatment of female *Cobitis taenia bilineata* with methyltestosterone (100 µg/l of aquarium water) induces the formation of Canestrini's organ, which is a male secondary sex character[6,7].

The surface of the ovipositor is covered by a pattern of meandrian microridges as shown by scanning electron microscopy (fig. 1). The transmission electron microscopic examination of the induced ovipositor reveals polygonal cells with large nuclei and some organelles. The ovipositor contains a system of longitudinal muscles, which enable it to contract or to extend during egg deposition. Furthermore nerves and blood vessels occur in the ovipositor. All cells are linked by desmosomes and/or interdigitations and gap junctions (figs. 2 and 3). Most of the ovipositor cells contain a dense network of cytofilaments (fig. 4). The occurrence of a great number of goblet cells is striking. These are chiefly found at the tip of the ovipositor (fig. 5). Mechanoreceptors were not found up to this time.

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