

Supplementary Material

Ontogeny and Dynamics of the Gonadal Development, Embryogenesis and Gestation in Xenotoca eiseni (Cyprinodontiformes, Goodeidae)

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This supplementary material contains:

Table S1: Numbers of fish analysed for the various endpoints on the specified sampling dates.

Page S2

Figure S1: Growth and development of secondary sex characteristics of *Xenotoca eiseni* from birth until the age of six months.

Page S3

Figure S2: Lateral section of an adult (12-week-old) female *Xenotoca eiseni* showing the position of the gonad.

Page S4

Figure S3: Transverse whole body sections of *Xenotoca eiseni* immediately after birth showing the position of the gonads.

Page S5

Figure S4: Sections of *Xenotoca eiseni* at different ages, focused on the gonads.

Page S6

Figure S5: Histological sections of *Xenotoca eiseni* testes.

Page S8

Figure S6: Scheme of a mature, non-gravid ovary.

Page S9

Figure S7: Lateral histological section of an ovary of *Xenotoca eiseni* one week after fertilization.

Page S10

Figure S8: Transverse histological section of an ovary (six weeks into gestation) of *Xenotoca eiseni* with 18 embryos.

Page S11

Figure S9: Female *Xenotoca eiseni* at parturition.

Page S12

Supplementary Material

Table S1: Numbers of fish analyzed for the various endpoints on the specified sampling dates.

Various numbers of randomly sampled fish were sacrificed at each sampling point for histological analysis of gonadal development and gestation. Body weight and total body length were measured for all fish remaining in the study at the respective sampling dates. A total of four fish died during the first experiment: two in the first week, one between week 2 and week 4 and one between week 4 and week 8. **Key:** f: female, m: male.

Study	Sampling Time	Weight and Length	Histology
Ontogeny of gonadal development	Day 1	172 fish	19 fish
	Week 1	151 fish	20 fish
	Week 2	131 fish	20 fish
	Week 4	110 fish	19 fish
	Week 8	90 fish (41 f + 49 m)	16 fish (8 f + 8 m)
	Week 12	74 fish (33 f + 41 m)	19 fish (9 f + 10 m)
	Month 4	55 fish (24 f + 31 m)	20 fish (9 f + 11 m)
	Month 5	35 fish (15 f + 20 m)	16 fish (8 f + 8 m)
	Month 6	19 fish (7 f + 12 m)	19 fish (7 f + 12 m)
Embryogenesis and gestation	Day 2	33 fish	33 fish
	Day 3	33 fish	33 fish
	Day 5	22 fish	22 fish
	Week 1	85 fish	22 fish
	Week 2	63 fish	21 fish
	Week 4	42 fish	21 fish
	Week 6	21 fish	21 fish

Supplementary Material

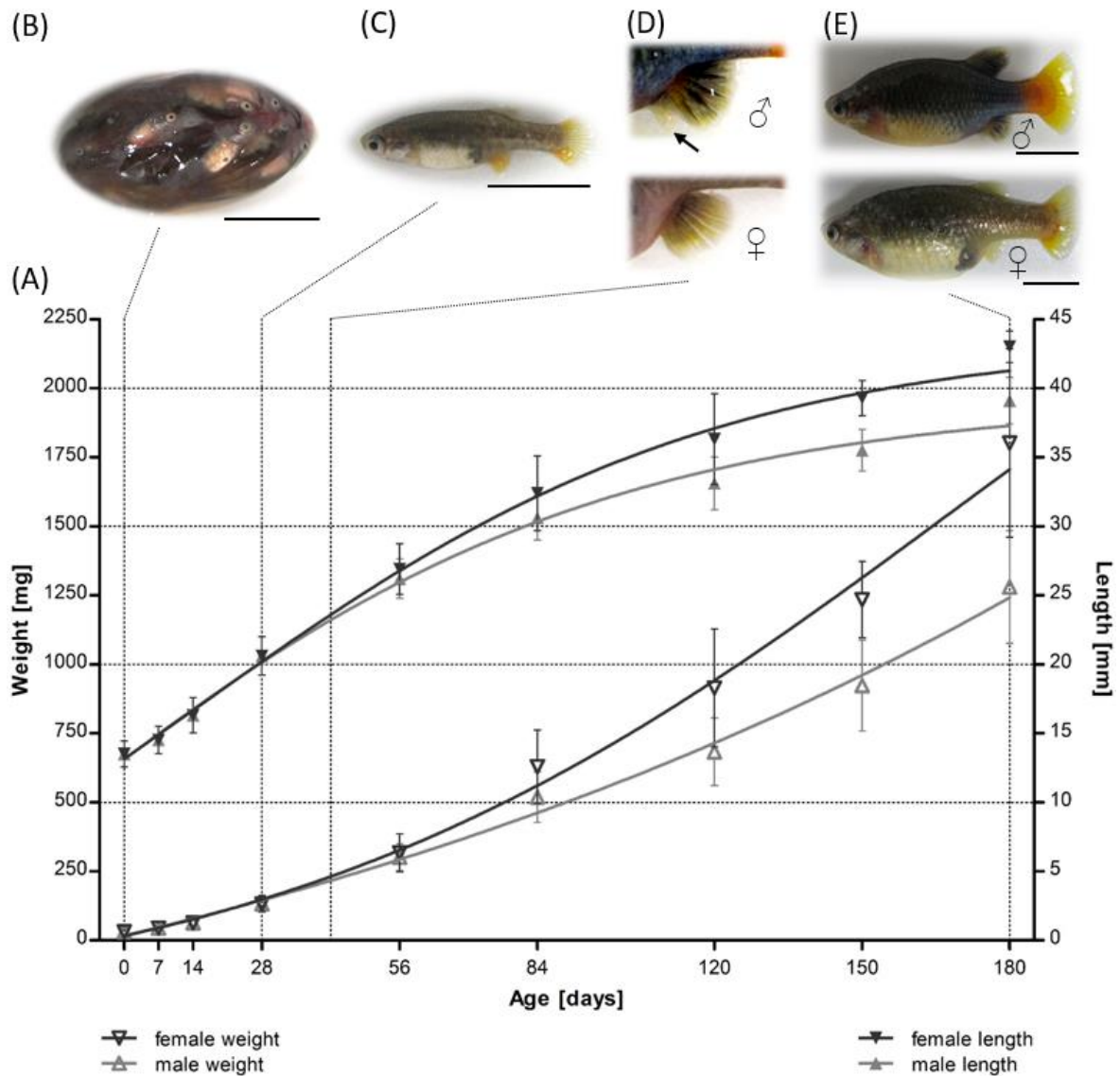


Figure S1: Growth and development of secondary sex characteristics of *Xenotoca eiseni* from birth until the age of six months. **A** Wet weight and total body length of male and female fish. Unpaired t-tests revealed no significant differences between male and female (non-pregnant fish only) growth. **B-E** Illustrations of *X. eiseni* at different ages (at birth (**B**), at four weeks (**C**) and six months of age (**E**)). **D** Anal fins at the age between four and eight weeks (period indicated by the vertical dotted lines) show the notch and the andropodium in the male indicated by the arrow. Bars = 1 cm.

Supplementary Material

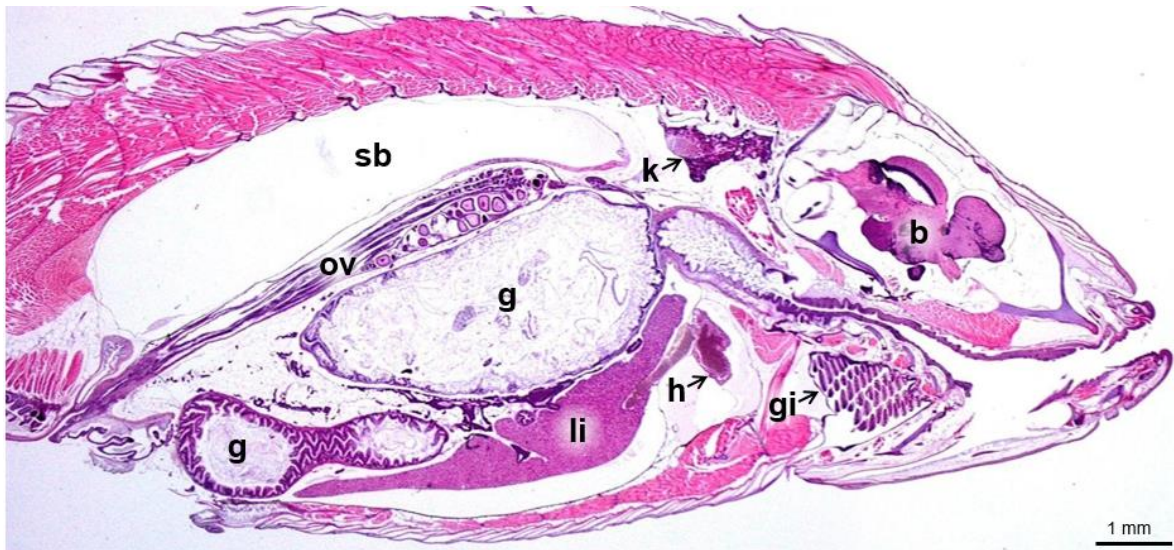


Figure S2: Lateral section of an adult (12 week old) female *Xenotoca eiseni* showing the position of the gonad. **Key:** b: brain, g: gut, gi: gills, h: heart, k: kidney, li: liver, ov: ovary (with oocytes at different developmental stages), sb swim bladder.

Supplementary Material

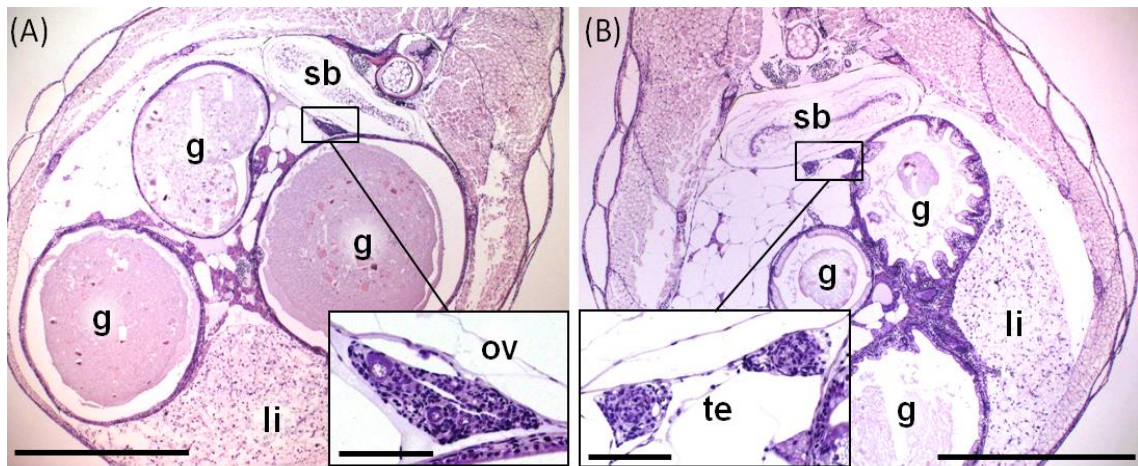
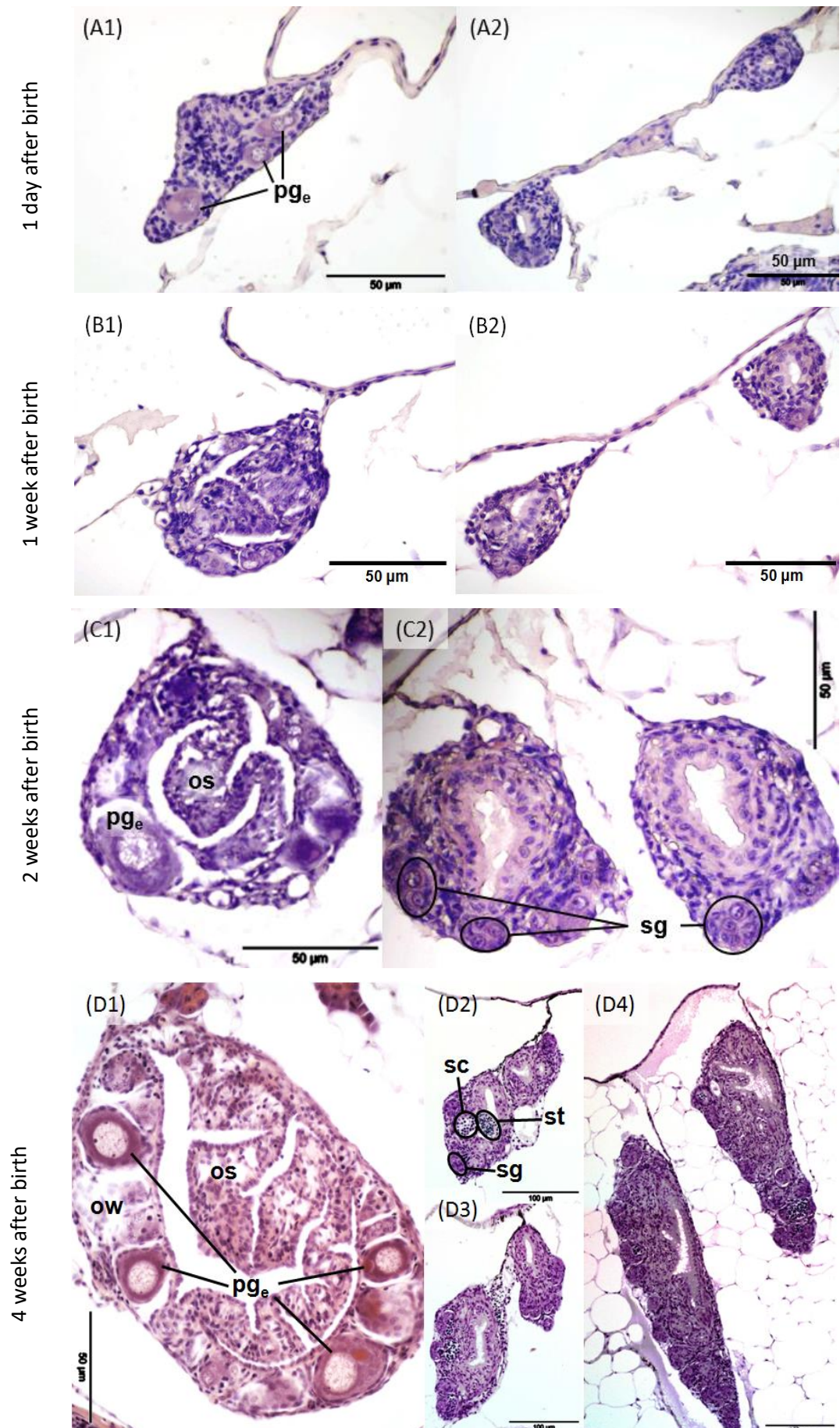


Figure S3: Transverse whole body sections of *Xenotoca eiseni* immediately after birth showing the position of the gonads and a more detailed view of these gonads (inset). **A** Section of a female fish. **B** Section of a male fish. **Key:** g: gut, li: liver, ov: ovary, sb swim bladder (not yet inflated), te: testis. Bars = 500 µm and 50 µm (insets).

Supplementary Material



Supplementary Material

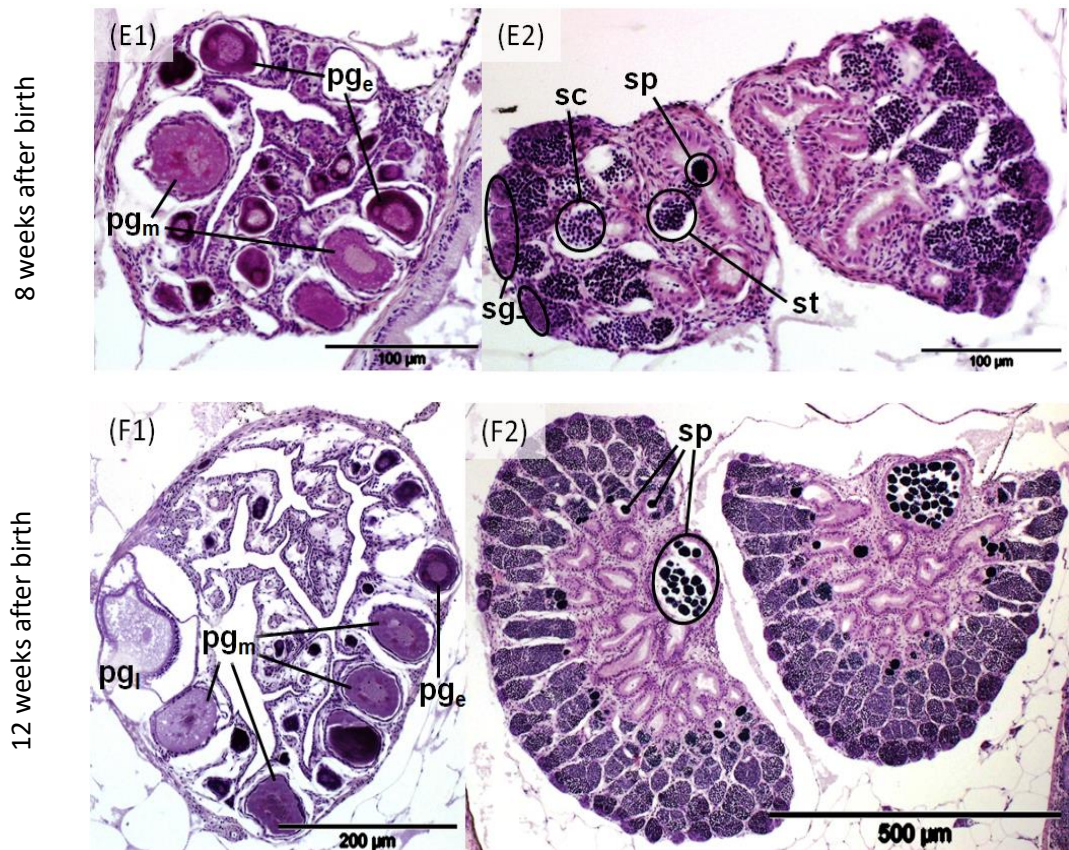


Figure S4: Sections of *Xenotoca eiseni* at different ages, focused on the gonads. **Female** fish: A1, B1, C1, D1, E1, F1. **Male** fish: A2, B2, C2, D2-4, E2, F2. **A1-2:** Transverse sections of gonads of one day-old fish. **B1-2:** Transverse sections of gonads of one week-old fish. **C1-2:** Transverse sections of gonads of two week-old fish. **D1-4:** Transverse sections of gonads of four week-old fish. **E1-2:** Transverse sections of gonads of eight week-old fish. **F1-2:** Transverse sections of gonads of twelve week-old fish. **Key:** os: ovarian septum, ow: ovarian wall, pge: early primary growth oocyte, pgl: late primary growth oocyte, pgm: mid primary growth oocyte, sc: spermatocyte, sg: spermatogonium, sp: sperm package, st: spermatid.

Supplementary Material

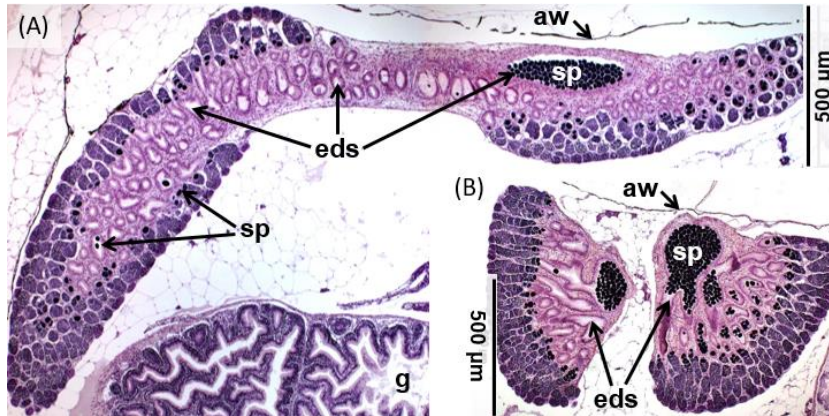


Figure S5: Histological sections of *Xenotoca eiseni* testes. **A** Lateral section of an adult male fish. **B** Transverse section of an adult male fish. **Key:** aw: abdominal wall, eds: efferent duct system, g: gut, sp: sperm package.

Supplementary Material

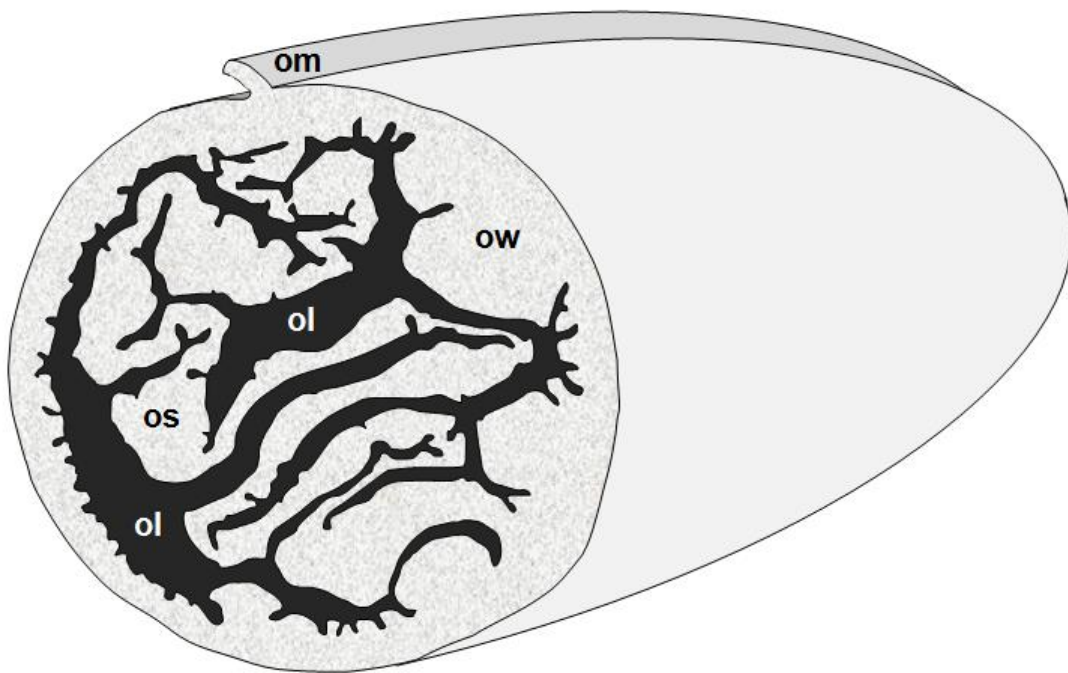


Figure S6: Scheme of a mature, non-gravid ovary; oocytes not shown. **Key:** ol: ovarian lumen, om: ovarian mesentery, os: ovarian septum, ow: ovarian wall.

Supplementary Material



Figure S7: Lateral histological section of an ovary of *Xenotoca eiseni* one week after fertilization. Embryos develop freely in the ovarian lumen but are still within the egg envelope. **Key:** e: embryo, env: egg envelope, os: ovarian septum, ow: ovarian wall, y: yolk.

Supplementary Material

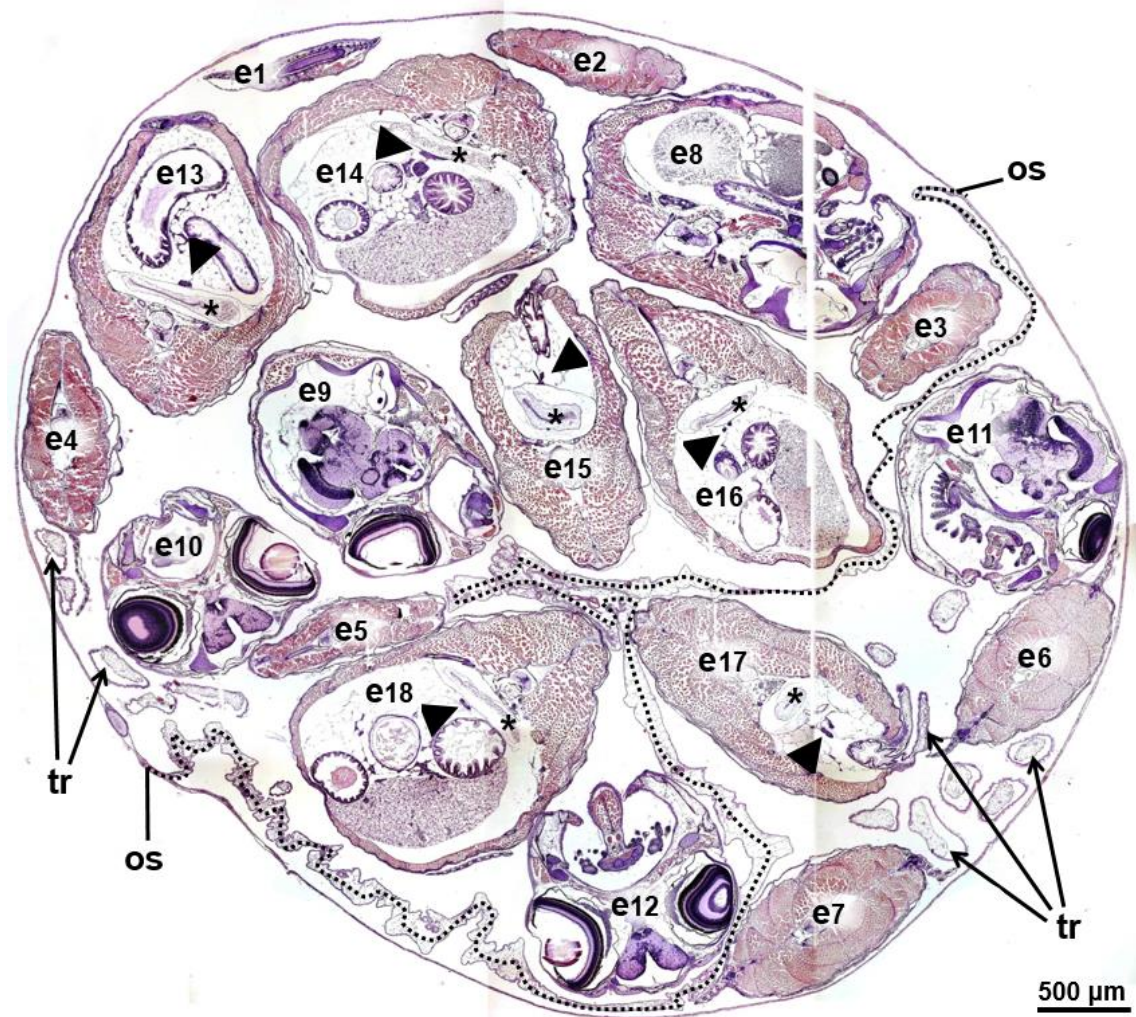


Figure S8: Transverse histological section of an ovary (six weeks into gestation) of *Xenotoca eiseni* with 18 embryos. The image shows seven embryos (e1-7) sectioned through the tail, five embryos (e8-12) sectioned through the head and six embryos (e13-18) sectioned through the abdomen. Of the last six embryos, two (e13-14) have presumptive female gonads, three (e15-17) have presumptive male gonads and one (e18) has a gonad that is not determinable. The dotted line highlights the folds of the ovarian septum. **Key:** e: embryo, os: ovarian septum, tr: trophotaeniae; arrows are pointing to the gonads, asterisks are marking the (not yet inflated) swim bladders.

Supplementary Material



Figure S9: Female *Xenotoca eiseni* at parturition. One embryo (e) is being born while the eye of another one can be seen through the abdominal and the ovarian walls of the mother fish.