

**SUPPLEMENTARY MATERIAL**

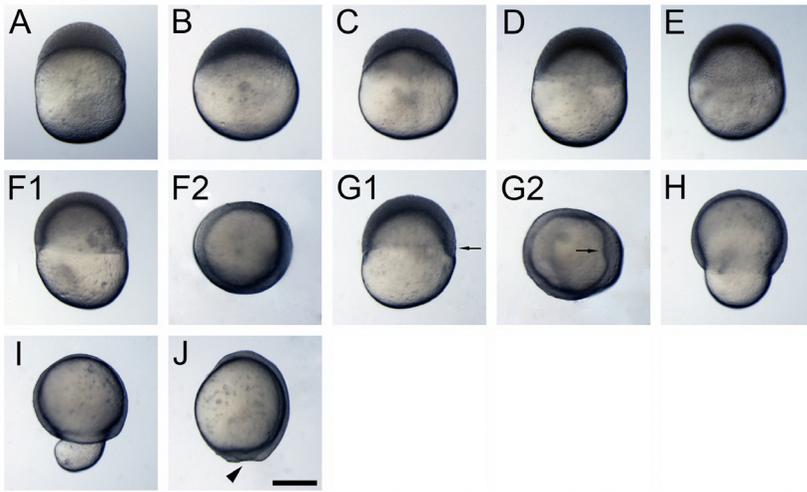
**corresponding to:**

**Morphological differences in embryos of goldfish (*Carassius auratus auratus*) under different incubation temperatures**

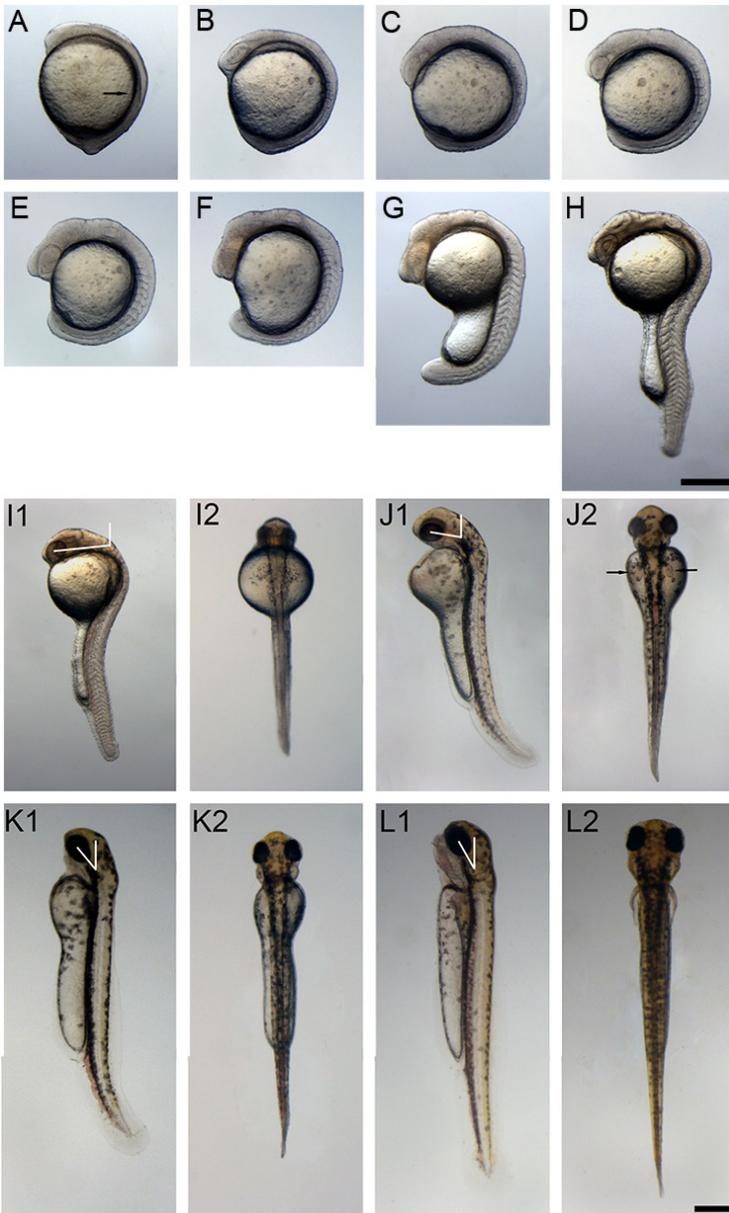
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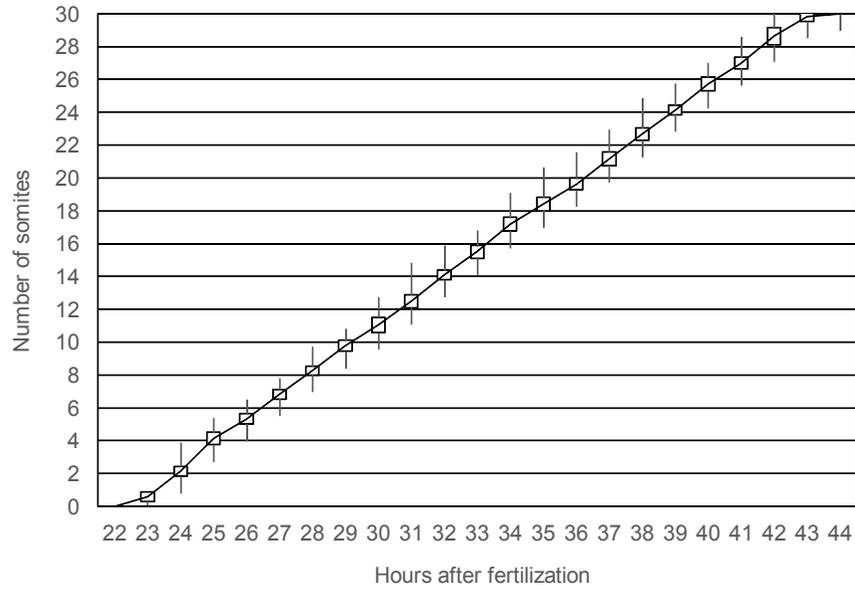
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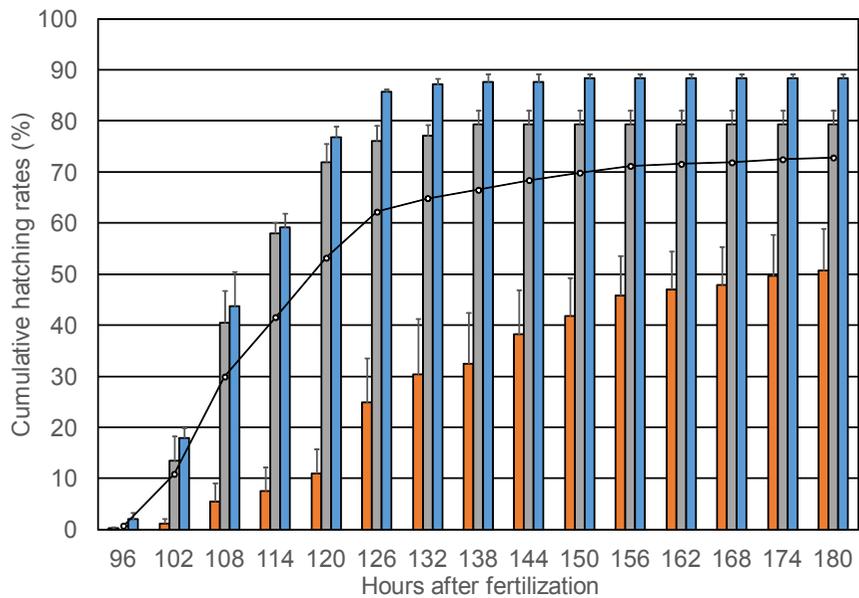
**Supp. Fig. S1. 3D images of embryonic development in goldfish embryos from blastula to gastrula stage, incubated at 20 °C.** **A:** Oblong stage (8 hpf). **B:** Sphere stage (10 hpf). **C:** Dome stage (10.5 hpf). **D:** 30% epiboly stage (11 hpf). **E:** 50% epiboly stage (12 hpf). **F1, F2:** Lateral view (F1) and animal-pole view (F2) of germ-ring stage (13 hpf). **G1, G2:** Lateral view (G1) and animal-pole view (G2) of embryonic shield stage (13.5 hpf). Arrows point to embryonic shield. **H:** 70% epiboly stage (16 hpf). **I:** 90% epiboly stage (18 hpf). **J:** 100% epiboly stage (20 hpf). Arrowhead points to the yolk plug. hpf, hours post-fertilization. Scale bar, 500  $\mu$ m.



**Supp. Fig. S2. (A–H) Embryonic development of goldfish during the segmentation period, incubated at 20 °C.** **A:** 2-somite stage (24 hpf). Arrow indicates the posterior boundary of the second somite. **B:** 5-somite stage (26 hpf). **C:** 8-somite stage (28 hpf). **D:** 11-somite stage (30 hpf). **E:** 14-somite stage (32 hpf). **F:** 17-somite stage (34 hpf). **G:** 20-somite stage (36 hpf). **H:** 30-somite stage (42 hpf). **(I–L) Embryonic development of goldfish during the post-segmentation period, incubated at 20 °C.** **I1, I2:** Lateral (I1) and dorsal (I2) views at 48 hpf. **J1, J2:** Lateral (J1) and dorsal (J2) views at 72 hpf. Arrows point to the pectoral fins. **K1, K2:** Lateral (K1) and dorsal (K2) views at 96 hpf. **L1, L2:** Lateral (L1) and dorsal (L2) views at 120 hpf. Angle between the two lines indicates the head-trunk angle (HTA); HTA decreases between 48 hpf and 120 hpf as a consequence of straightening of the embryo's body. hpf, hours post-fertilization. Scale bar, 500  $\mu$ m.



**Supp. Fig. S3. Graph showing the rate at which somites developed during the segmentation period in goldfish embryos kept at 20 °C.** Box height indicates standard deviation of each development time; bars indicate the range, representing the minimum and maximum number of somites at each time.



**Supp. Fig. S4. Hatching rates 96 hours after fertilization for goldfish embryos incubated at 20 °C.** Columns indicate the average cumulative hatching rate in each of three batches of eggs (orange, grey, and blue bars) at each temperature. Curved line indicates the average hatching rate for all three batches for each developmental time. Bars denote standard error (n = 3).