Functional Sperm of the Yellowtail (Seriola quinqueradiata) Were Produced in the Small-Bodied Surrogate, Jack Mackerel (Trachurus japonicus).

Figure legends

Fig. 1 Section of the donor testis of the yellowtail stained with hematoxylin and eosin. **a** Overview of the testis. Upper and lower rectangles show the area rich in type A spermatogonia and sperm, respectively. **b** and **c** show higher magnification images of upper and lower rectangles in a, respectively. Scale bars: 2 mm (**a**) and 20 μm (**b** and **c**).

Fig. 2 Intraperitoneal transplantation of donor testicular cells into the jack mackerel larvae. **a** Bright-field image. **b** Fluorescent image. **c** Transplanted (TP) and non-transplanted (NTP) individuals. N, transplantation needle. Scale bar = 1 mm.

Fig. 3 Incorporation of transplanted PKH26-labeled cells into recipient genital ridges. Fluorescent (**a**, **c**) and yellowtail *vasa in situ* hybridization (**b**, **d**) images of the excised genital ridges from transplanted (**a**, **b**) and nontransplanted (**c**, **d**) fish at 20 dpt. *Arrowheads* indicate donor-derived germ cells that were labeled with PKH-26 (**a**), and were expressing the yellowtail *vasa* mRNA (**b**). Scale bars = 20 μm.

Fig. 4 Sections of jack mackerel (**a**, **b**) and the yellowtail (**c**, **d**) testis hybridized with antisense jack mackerel (**a**, **c**) and yellowtail *vasa* probes (**b**, **d**). Scale bars = 20 μm.

Fig. 5 RT-PCR amplification of donor-derived yellowtail *vasa* mRNA in recipient gonads.

Electrophoresis patterns of RT-PCR of recipient number 1–14 (**a**) and 15–28 (**b**) using specific primers for the yellowtail *vasa* (upper panels) and jack mackerel *vasa* (lower panels) cDNA sequences. *Lane P* is a positive control obtained using cDNA derived from the yellowtail testis as template. *Lane N* is a negative control containing no cDNA template. *M* represents a molecular weight marker.

Fig. 6 Detection of the colony containing donor-derived germ cells in recipient testis. Serial sections of testis from jack mackerel testis hybridized with yellowtail (**a**) and jack mackerel (**b**) *vasa* probes and were stained with hematoxylin and eosin (**c**). The colony containing donor-derived germ cells is surrounded with a dotted line. Scale bars = 20 µm.

Fig. 7 PCR amplification of donor-derived yellowtail *vasa* genomic DNA fragment in recipient semen. *Lane P* is a positive control obtained using genomic DNA extracted from the yellowtail semen as template. *Lane N* is a negative control containing no DNA template. Electrophoretic mobility of the targeted DNA fragment was indicated by *arrowheads*.

Fig. 8 Embryos at 48 h-post-fertilization obtained from progeny tests. **a** Normal embryos obtained by crossing the yellowtail female and male. **b** Eggs obtained by crossing the yellowtail female and male recipient number 28. *Arrowhead* indicates a normal embryo. **c** Eggs obtained by crossing the yellowtail female and wild-type jack mackerel male. Scale bars = 1 mm.

Fig. 9 Donor-derived offspring obtained from progeny tests. **a** Newly hatched larva obtained by crossing the yellowtail female and male. **b**–**d** Newly hatched larvae obtained by crossing the yellowtail female and male recipient number 28. **b** Normal individual. **c**, **d** Abnormal individuals. Scale bars = 500 µm.

Fig. 10 PCR amplification of *β-actin* and *vasa* genomic DNA fragments in larvae obtained from progeny tests. Electrophoresis patterns of PCR using specific primers for yellowtail *β-actin* (**a**), jack mackerel *β-actin* (**b**), yellowtail *vasa* (**c**), and jack mackerel *vasa* (**d**) DNA sequences. *Lane 1* and *2* are larva samples obtained from the crosses of recipient mackerel number 28 and the yellowtail females. *Lane 3* and *4* are larva samples of wild type yellowtail. *Lane 5* and *6* are wild type jack mackerel. *Lane 7* is a negative control containing no DNA template. *M* represents a molecular weight marker.