

# Atlas of Medaka Gonadal Histology

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Medaka (*Oryzias latipes*) has been widely used as laboratory animal in various fields in biology. Its relatively short life cycle, capacity to reproduce, and ease of breeding are chiefly responsible for its popularity. In addition, medaka has been commonly used as test species for ecotoxicity tests. Standardized acute (OECD, 1992), prolonged (OECD, 1984) and early-life stage (OECD, 1992) toxicity have been developed for assessment, Medaka also is a suitable test organism for investigations of sex reversal and intersex in fish because this species is characterized as a differentiated gonochorist.

We provide here a basic data of normal development of testis and ovary in development of medaka as well as growth, secondary sexual characteristics and vitellogenin synthesis. In addition, we show several images of gonadal tissues in the fish exposed to model endocrine disrupting chemicals. We hope that these data will be used for the further study of ecotoxicity and endocrine disruptors testing in medaka.

## About Medaka (*Oryzias latipes*)

### Commonly used test species

### Biologically detailed research

- differentiated gonochorist
- early maturation (6 to 8 weeks)



### Sensitive to endocrine-disrupting chemicals

- intersex condition (testis-ova)



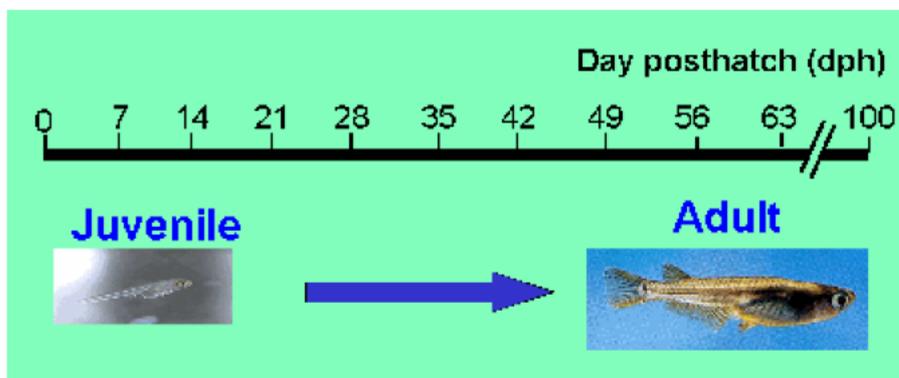
Medaka is a suitable test organism for investigations of gonadal histology as a tool for endocrine disruption.

## Normal Development and Gonadal Histology in Medaka

### Presentation

We present basic data on normal development of testis and ovary as well as growth, secondary sexual characteristics and vitellogenin synthesis

### Measurement



- Gonadal histology
- Growth (total length and body weight)
- Secondary sexual characteristics (after 35 dph)
- Hepatic vitellogenin concentration (VTG) (after 35 dph)
- Gonadosomatic index (GSI) (after 35 dph)
- Hepatosomatic index (HIS) (after 35 dph)

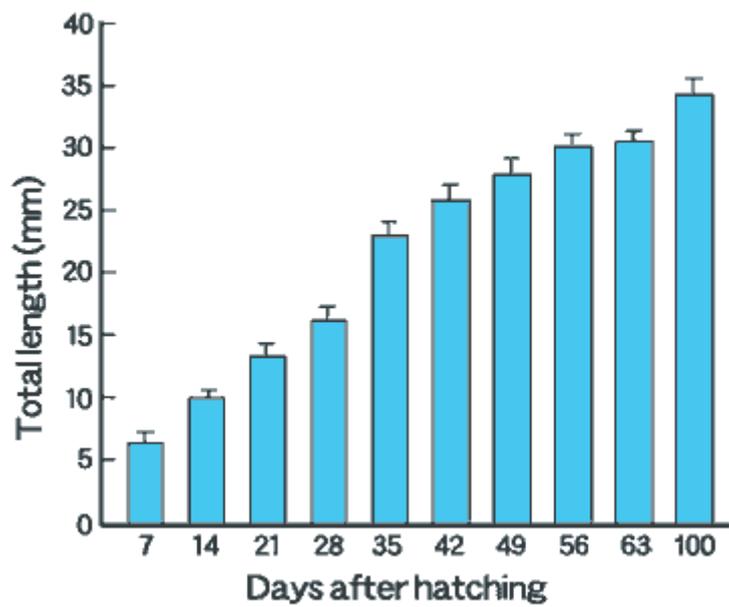
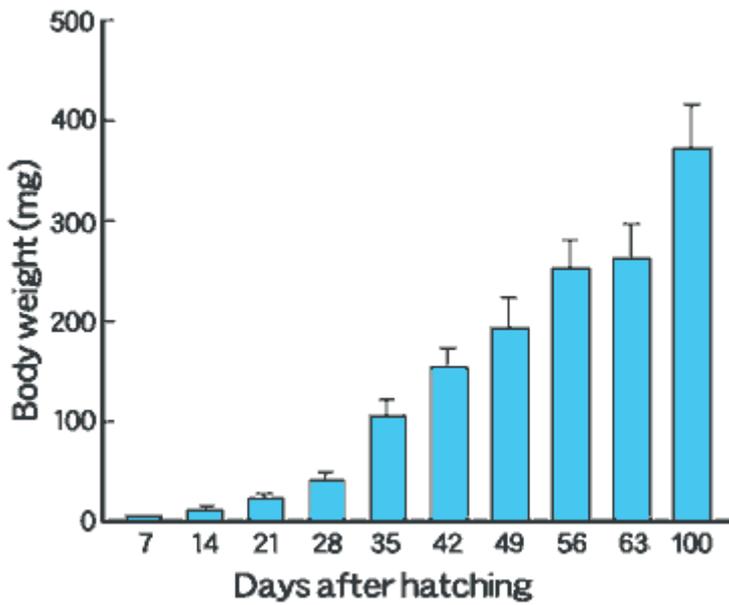
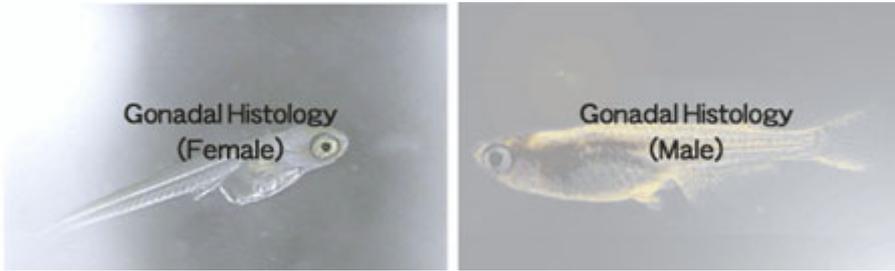
### Outline of the study

#### Breeding conditions

Species	Medaka ( <i>Oryzias latipes</i> )
Breeding system	Flow-through
Water temperature	24 ± 1°C
Breeding water	dechlorinated tap water
Photoperiod	16h light, 8h dark
Test chamber size	2.5L
Water volume	1.8L
Volume exchange of water	12 times a day
Number of fish per test vessel	15(15 fish × 9 vessels)
Food	newly hatched <i>Artemia</i> nauplii(<24h)

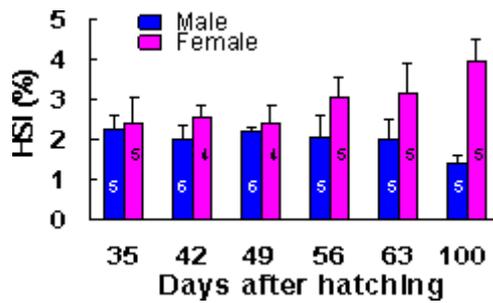
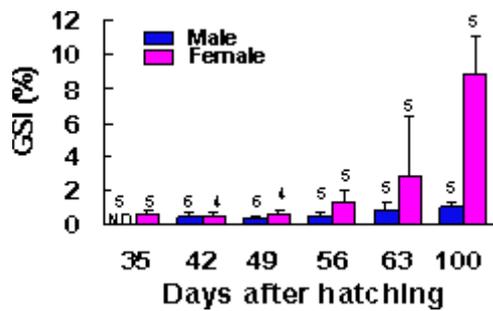
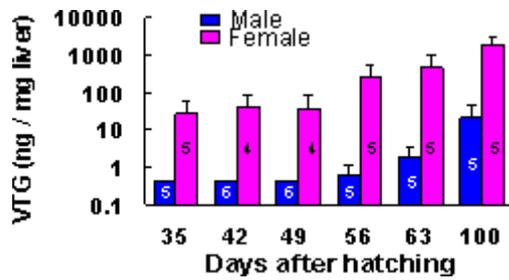
## Normal Development and Gonadal Histology in Medaka

### Normal development of the gonad



## Normal Development and Gonadal Histology in Medaka

### GSI, HSI and VTG levels from 35 to 100-dph



$$\text{GSI} = \frac{\text{Gonad weight (mg)}}{\text{Body weight (mg)}} \times 100$$

$$\text{HSI} = \frac{\text{Liver weight (mg)}}{\text{Body weight (mg)}} \times 100$$

## Atlas of Gonadal Histopathology in Medaka Exposed to Endocrine Disruptors

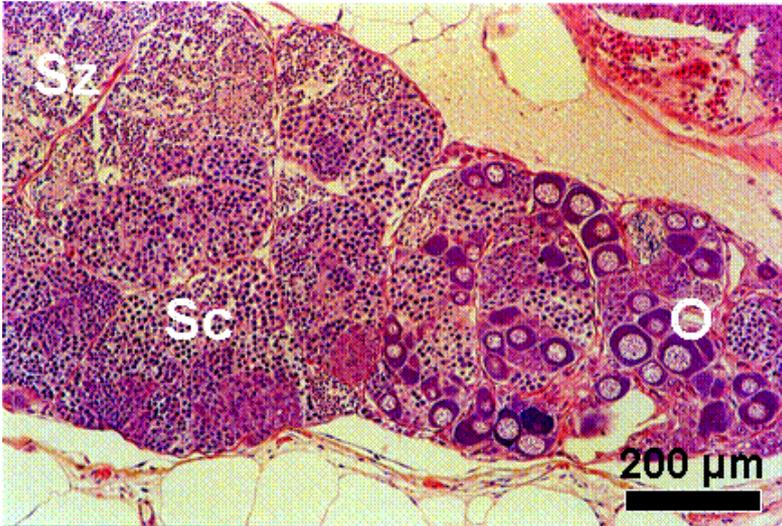
### Effect of estrogen exposure

#### Testis-ova

Testis-ova from a fish exposed to 224  $\mu\text{g/L}$  of 4-tert-pentylphenol from fertilized egg to 61-d posthatch (Bouin, H&E).

O: oocyte, Sc: spermatocyte, Sz: spermatozoa.

Oocytes appear in clusters within the testicular tissue. Numerous spermatocytes and spermatozoa are still present in a compacted mass in this section.

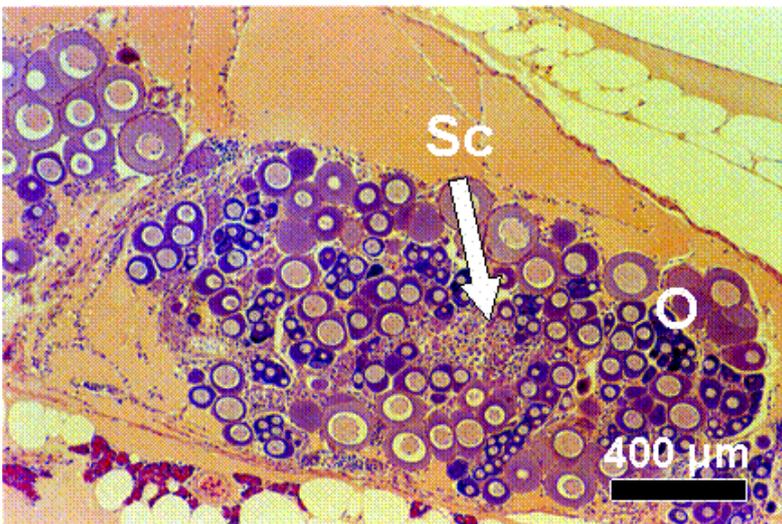


#### Progressed testis-ova

Testis-ova from a fish exposed to 931  $\mu\text{g/L}$  of 4-tert-pentylphenol from fertilized egg to 61-d posthatch (Bouin, H&E).

O: oocyte, Sc: spermatocyte, Sz: spermatozoa.

A more progressed testis-ova. Almost the entire area is composed of oocytes, accompanying small testicular tissues interspersed with a few spermatocytes.

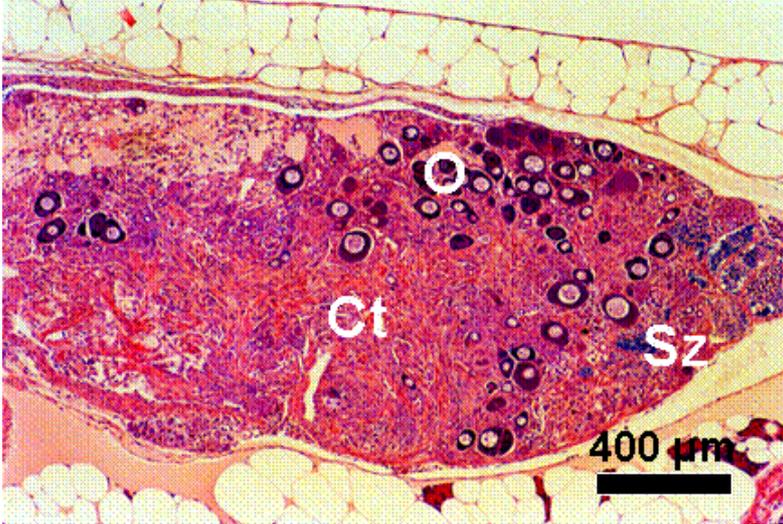


### Testis-ova & connective tissue

Testis-ova from a fish exposed to 931  $\mu\text{g/L}$  of 4-tert-pentylphenol from fertilized egg to 61-d posthatch (Bouin, H&E).

O: oocyte, Ct: connective tissues, Sz: spermatozoa.

Connective tissues develop well in a testis-ova specimen with a few spermatozoa.

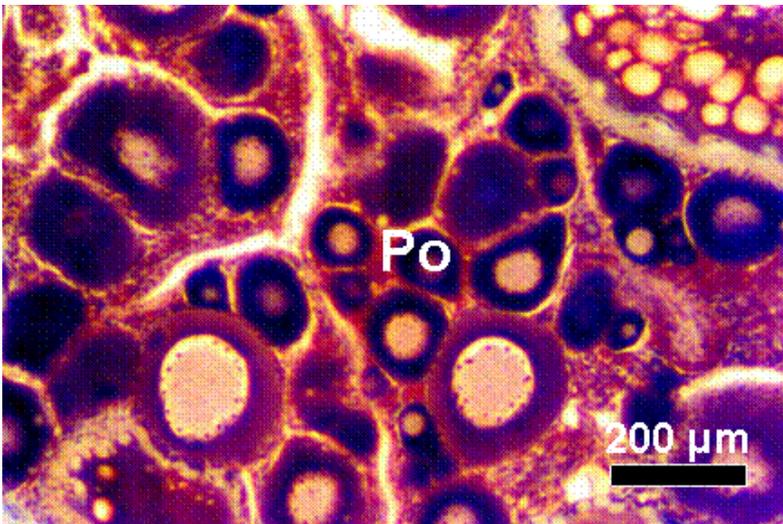


### Regressed ovary

Ovary from an adult female fish exposed to 488  $\text{ng/L}$  of ethinylestradiol for 3 weeks (Bouin, H&E).

Po: previtellogenic oocyte

Many previtellogenic oocytes exist in this specimen, suggesting regressed ovary.



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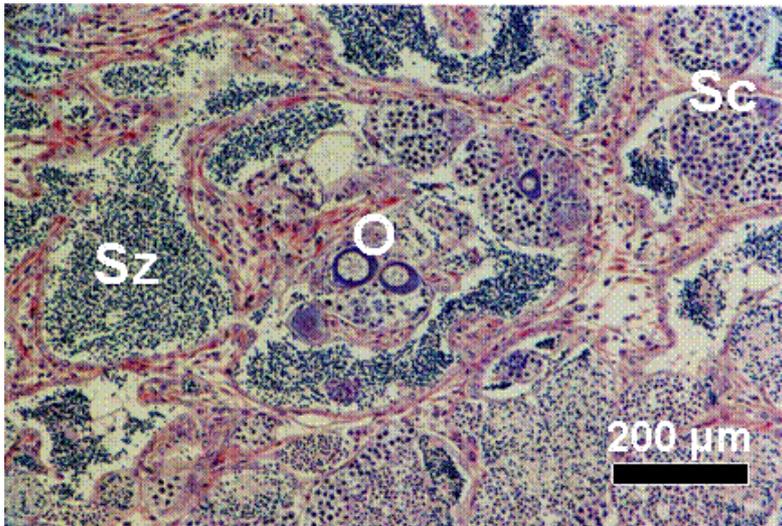
### Effect of androgen exposure

#### Testis-ova

Testis-ova from a fish exposed to 27.7 ng/L of methyltestosterone from fertilized egg to 61-d posthatch (Bouin, H&E).

O: oocyte, Sz: spermatocyte, Sc: spermatozoa.

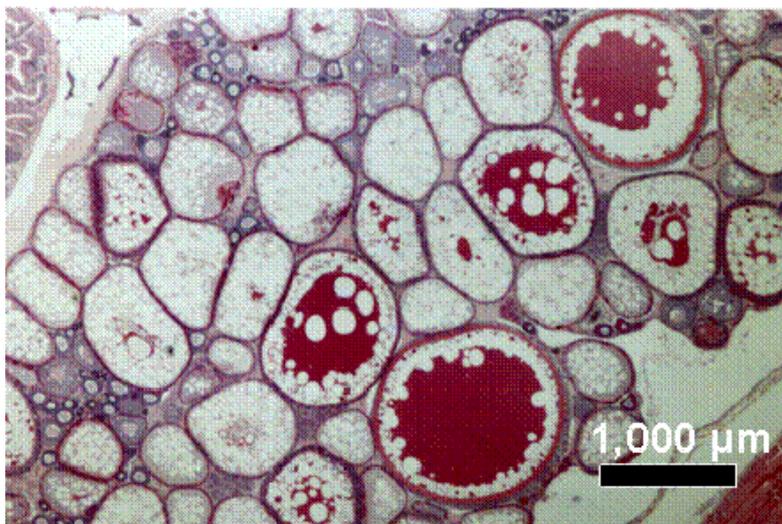
Three oocytes exist within the testicular tissue. This fish probably have been on the process of sex reversal from female to male.



#### Ovary

Ovary from a fish exposed to 9.98 ng/L of methyltestosterone from fertilized egg to 61-d posthatch (Bouin, H&E).

No effects are observed.



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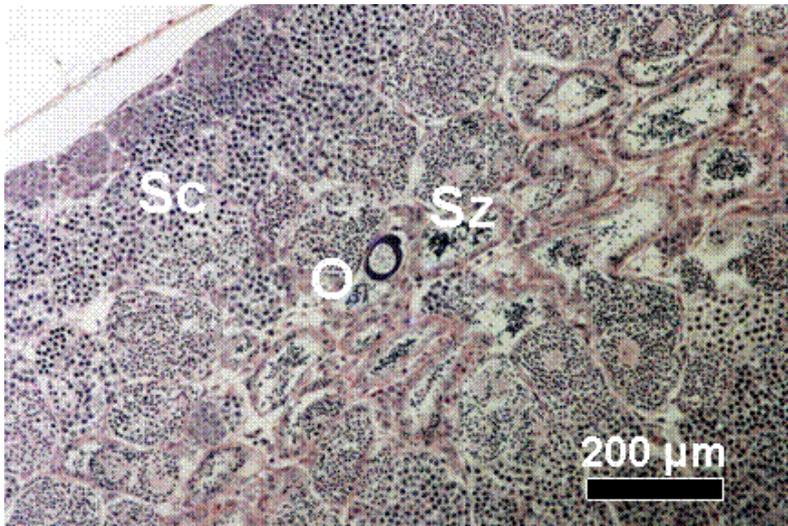
### Effect of estrogen exposure

#### Testis-ova

Testis-ova from a fish exposed to 958  $\mu\text{g/L}$  of flutamide from fertilized egg to 60-d posthatch (Bouin, H&E).

O: oocyte, Sc: spermatocyte, Sz: spermatozoa.

Single oocyte exists within the testicular tissue. It is not clear that what mechanisms have been involved in the induction of testis-ova when exposed to anti-androgen.



#### Ovary

Ovary from a fish exposed to 958  $\mu\text{g/L}$  of flutamide from fertilized egg to 60-d (Bouin, H&E).

No effects are observed.

