

# RELEASE OF SEX STEROIDS INTO THE WATER BY ROACH (*RUTILUS RUTILUS*)

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## Introduction

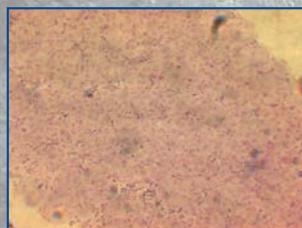
Experiments were carried out to determine the time-course of release of sex steroids by male and female roach (a freshwater cyprinid fish) at various stages of maturity.



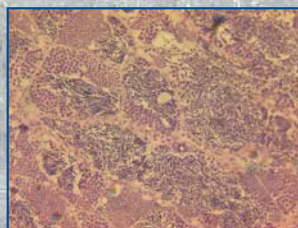
## Method

- Fish were placed in small containers, each filled with 5 litres of tap-water. Water temperature was  $17^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . Photoperiod was 16 hours light: 8 hours dark with lights off at 22:00.
- Fish were either injected ip with 0.5-1mg/100 $\mu\text{l}$  Carp Pituitary Extract (CPE; Argent), or 100 $\mu\text{l}$  saline as a control. All fish were then placed back into individual containers, and for a period of 24 hours, water samples (500 ml) were taken at regular intervals (ranging from 3 to 8 hours). After each sample was collected, fish were transferred to a clean container with 5 litres of fresh water.
- The water samples were pumped through activated C18 Cartridges (Sep-Pak Plus). The extracts were treated to yield free, sulphated and glucuronidated steroids, which were measured by radioimmunoassay.

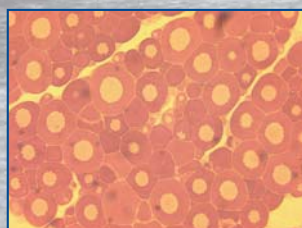
## Results



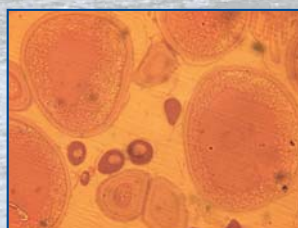
Cross section from immature male roach showing spermatogonia (resting germ cells).



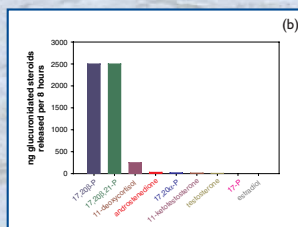
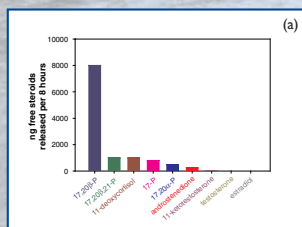
Cross section from mature male roach showing mainly spermatozoa (not yet spermiating). Spermiating males have free running milt upon gentle pressure to the abdomen.



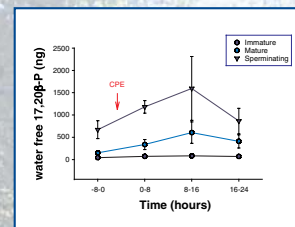
Cross section of immature roach ovary showing peri-nucleolus stage of oogenesis.



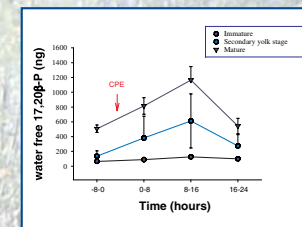
Cross section of roach ovary showing secondary yolk stage of development. Mature females have completed vitellogenesis.



Types of free (a) and glucuronidated (b) steroids (ng/8 hours) released into the water by a mixture of male and female roach in response to CPE-injection (1mg/200 $\mu\text{l}$ ).

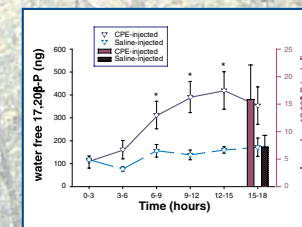


Concentrations ( $\pm$  SEM) of free  $17,20\beta\text{-P}$  released by male roach in response to CPE-injection.



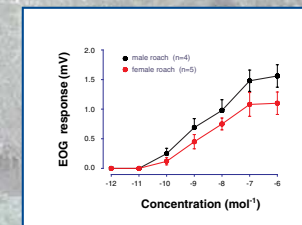
Concentrations ( $\pm$  SEM) of free  $17,20\beta\text{-P}$  released by female roach in response to CPE-injection.

- Substantial amounts of sex steroid were released into the water by sexually mature male and female roach, the most abundant being free  $17,20\beta\text{-P}$  and glucuronidated  $17,20\beta\text{-P}$  and  $17,20\beta,21\text{-P}$ . Levels of sulphated steroids were low.
- Mature females (including those at secondary yolk stage of development) exhibited elevated baseline levels of free  $17,20\beta\text{-P}$  compared to immature females, and showed a greater response to CPE-injection. Spermiating males also released significantly higher concentrations of  $17,20\beta\text{-P}$  at all times compared to immature and mature males.
- Mature males and females both responded to CPE-injection within 8 hours, showing peak concentrations at 16 hours and returning towards baseline levels by 24 hours.
- Immature fish of both sexes also respond to CPE by showing a slight gradual increase in  $17,20\beta\text{-P}$  within 24 hours of injection.
- The olfactory epithelium of male and female roach gives an electro-olfactographic response to  $17,20\beta\text{-P}$ .



Changes in free  $17,20\beta\text{-P}$  ( $\pm$  SEM) released by mature female roach\* represents  $p < 0.05$  compared to the saline-injected (control) group.

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- Immature fish of both sexes also respond to CPE by showing a slight gradual increase in  $17,20\beta\text{-P}$  within 24 hours of injection.
- The olfactory epithelium of male and female roach gives an electro-olfactographic response to  $17,20\beta\text{-P}$ .



## Conclusions

- $17,20\beta\text{-P}$  is released into the water by both male and female roach and is stimulated by carp pituitary extract
- $17,20\beta\text{-P}$  is produced by males and females in similar amounts
- The concentration of sex steroids released is dependent on the maturity of the fish
- Male and female roach both smell  $17,20\beta\text{-P}$
- Roach are opportunistic, single spawners and may use  $17,20\beta\text{-P}$  as a 'bisexual' primer pheromone.

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