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BEHAVIOURAL AND ENDOCRINOLOGICAL RESPONSES OF MATURE MALE GOLDFISH TO THE SEX PHEROMONE 17α,20β-DIHYDROXY-4-PREGNEN-3-ONE IN THE WATER

R. BJERSELIUS[1], K. H. OLSÉN[2] AND W. ZHENG[3]

[1]*Department of Zoophysiology, Uppsala University, Norbyvägen 18 A, S-752 36 Uppsala, Sweden,* [2]*Vertebrate Physiology and Behaviour Unit, Department of Limnology, Uppsala University, Norbyvägen 20, S-752 36 Uppsala, Sweden* and [3]*Department of Zoology, University of Alberta, Edmonton, Alberta, Canada T6G 2E9*

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Summary

The behavioural response of spermiated male goldfish to the sex pheromone 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-P) in the ambient water was measured using a computerized video-image analysis system. The position and spontaneous locomotor activity of single spermiated male goldfish were continuously recorded in an artificial stream. 17α,20β-P (final concentration 10^{-11} mol l^{-1}) was supplied to one half and its ethanol carrier to the other half of the test area. The results showed that the fish spent significantly less time in water scented with 17α,20β-P than in control water. Moreover, both the spontaneous locomotor activity and the gonadotropin II concentration in the plasma increased significantly because of contact with 17α,20β-P in the ambient water. The swimming speed was unchanged, whether the fish resided in the 17α,20β-P section or in the section without 17α,20β-P added. In view of the complex dual hormone–pheromone system in goldfish, the possibility of an additional function of 17α,20β-P is discussed. Because the release of the pheromone 17α,20β-P occurs before ovulation and the level then drops drastically, we suggest that the avoidance reaction observed allows the sexually mature male to avoid misdirected courting of non-ovulated females and thus to continue its search for an ovulated female.

Key words: goldfish, behavioural response, locomotor activity, gonadotropin, pheromone, reproduction, 17α,20β-dihydroxy-4-pregnen-3-one, *Carassius auratus*.

Introduction

In diverse fish species, chemical signals have been shown to participate in the synchronization of spawning between males and females, affecting both behaviour and physiology (Partridge *et al.* 1976; Liley, 1982; Stacey and Sorensen, 1991; Sorensen, 1992; Liley *et al.* 1991; Olsén and Liley, 1993). Olfaction is the sense mediating the pheromonal responses (Stacey and Sorensen, 1986; Hara, 1986; Fujita *et al.* 1991; Sorensen, 1992).

The goldfish (*Carassius auratus*) is the best studied fish species with regard to the role of olfactory signals in reproduction. A dual time-dependent hormone–pheromone system has been proposed for the goldfish, where the sex hormone 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-P) acts as a pre-ovulatory 'priming pheromone' and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and its metabolites as postovulatory 'releaser pheromones'. Both 17α,20β-P and the prostaglandins are secreted into the water by mature females and detected by mature males using their olfactory sense. Female goldfish release 17α,20β-P into the water during the final maturation of their oocytes, which mainly occurs during scotophase (Stacey and Sorensen, 1991). The priming pheromone, 17α,20β-P, is assumed to 'prepare' the male physiologically for the coming spawning, elevating the gonadotropin (GtH) concentration in the blood, which in turn increases milt in the sperm ducts within 3 h (Dulka *et al.* 1987; Stacey *et al.* 1989). The releaser pheromone ($PGF_{2\alpha}$), in contrast, induces active chasing and nudging of the females to complete the spawning act (Sorensen *et al.* 1988, 1989). Electro-olfactogram (EOG) experiments have shown that the olfactory epithelium of goldfish is specifically and extremely sensitive to 17α,20β-P (10^{-12} mol l^{-1}) (Sorensen *et al.* 1987, 1990). It has also been confirmed that 17α,20β-P binds to a steroid receptor in the olfactory epithelium (Rosenblum *et al.* 1991). DeFraipont and Sorensen (1993) recently showed that 17α,20β-P also has a behavioural impact on mature males. Exposure to 17α,20β-P increased the locomotor activity, and the exposed males were also shown to be relatively more aggressive towards other males than were males that had not been exposed to 17α,20β-P. The authors argued that this greater aggressiveness towards other males could favour the exposed males in competitive interactions, leading to greater spawning success. In addition to the endocrinological and behavioural effects, olfactory

signals might have an important function in sex recognition of males and females (Partridge *et al.* 1976; Yamazaki and Watanabe, 1979; Yamazaki, 1990).

The aim of the present study was to determine whether the electrophysiologically (EOG), endocrinologically (GtH II) and behaviourally (locomotor activity, increased aggressiveness) potent steroid 17α,20β-P is also involved in the orientation of mature male goldfish towards the source of the pheromone, the mature female goldfish. This was done by continuously recording the position of one single mature male goldfish in an artificial stream with a supply of 17α,20β-P to one side or the other of the test area. To ensure that the previously documented effects of 17α,20β-P on mature male goldfish did occur under our experimental conditions, spontaneous locomotor activity (swimming distance) and the GtH II concentration in the plasma were measured. The behavioural activity was continuously recorded using a video camera connected to a computerized image-analysis system.

Materials and methods

Fish

Comet goldfish (*Carassius auratus*) were reared in a private hatchery and transported to the Department of Zoophysiology, Uppsala University, 3 months before the start of the study. They were kept in 180 l aquaria at 14–16 °C in a 16 h:8 h L:D schedule. Males were taken out and kept in separate aquaria. At least 48 h before the start of the experiment, a group of six fish was transferred to an aquarium with gravel and artificial floating vegetation. The temperature was 20 °C. Fish were fed Hikari Staple (Kyorin Co. Ltd, Japan) *ad libitum*. The total number of spermiated fish was 37 and their mean mass was 17.7±0.97 g (± S.E.M.; N=37).

Spermiating goldfish males were selected using two criteria: (1) the presence of expressible milt and (2) the presence of pectoral fin tubercles, a sexually dimorphic feature. The mean gonadosomatic index (GSI) of experimental fish was 5.37±0.26 %.

Olfactory stimulant

The steroid, 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-P), was purchased from Sigma Chemical Co. (St Louis, MO, USA). Stock solutions were made up at a concentration of approximately 10^{-5} mol l^{-1} by dissolving 1 mg of 17α,20β-P in 1.5 ml of ethanol and adding 0.5 ml of this solution to 99.5 ml of distilled water. Ethanol control solution was prepared in the same way but without the addition of 17α,20β-P. Stock solutions were stored in the dark at 4 °C and were used within 2 weeks.

The fluviarium

The experiments were conducted in a fluviarium (Höglund, 1961; Olsén and Höglund, 1985), which is an artificial stream with a laminar flow of aerated tapwater (Fig. 1A). The test area, measuring 46 cm×33 cm×11.5 cm (length×width×depth), was limited upstream and downstream by two fine-mesh plastic nets. 17α,20β-P was supplied from a 15 l reservoir (R_1) by means of a peristaltic pump (P_1); 10^{-8} mol l^{-1} 17α,20β-P was delivered at 30 ml min^{-1} to one half or the other of the test area of the fluviarium (Fig. 1A). Ethanol control was delivered simultaneously to the other half of the fluviarium using an identical reservoir (R_2) and pump (P_2). In control experiments, both reservoirs contained ethanol control. To ensure good mixing of 17α,20β-P, a small supply of tapwater (held in a third reservoir, R_3, and delivered at 100 ml min^{-1}) was mixed with the odour before it entered the fluviarium. The flow rate of the water in the fluviarium was 6.6 l min^{-1}. Thus, the final concentration of 17α,20β-P in the test area was approximately 10^{-11} mol l^{-1}. The two water qualities (scented or not scented) were changed between the two halves of the test area at regular 90 min intervals by means of two electromagnetic valves. Water temperature was 20 °C.

The video camera was placed 40 cm above the test area (Fig. 1B). L_1 and L_2 are 20 W halogen lamps. The light from these two lamps was filtered through F, a red glass filter (RG 780, Melles Griot) allowing no transmission of wavelengths shorter than 75 nm, and reflected through the white opalescent glass plate. This arrangement gave a light background, without reflections, against which the fish was readily detected. This infrared light was used as the light source for the video camera during the experimental period (00:00–06:00 h). The daylight was provided by another light source (a 75 W bulb) connected to a timer set to switch the light off at 22:30 h.

The video-computer based image-analysis system

Hardware

The hardware used consisted of an IBM-compatible video digitizer, PC Vision Plus board (Imaging Tech. Inc., Woburn, MA, USA) connected to an IBM-compatible computer (386 DX, 20 MHz). TEA Image Manager TIM 3.30 (Difa Measuring Systems, Breda, The Netherlands) was used as a driver program for the card. Any standard video signal, in our case a camera, can be connected to the digitizer. We used a CCD black-and-white camera (Panasonic WV-BL200, light sensitivity 0.5 lx) from which the infrared filter had been removed.

Software

The program Video Tracking and Motion Analysis System, VTMAS (Noldus Information Technology b.v., The Netherlands), was used for video tracking and motion analysis. An older version of the program, MOTION 0.13β (made by Jacob Rosseu, Institute of Molecular Biology and Medical Biotechnology, University of Utrecht, The Netherlands) has previously been described by Spruijt *et al.* (1992) for behavioural studies on rats and by Winberg *et al.* (1993) for measuring locomotor activity in fish.

For analysis of the experiments, the test area was divided into two halves (A and B) and the computer calculated the time spent and the distance travelled by the fish in each half during

30 min periods (i.e. with 17α,20β-P on one side and its ethanol carrier on the other side). The computer also calculated the distance travelled over 10 min periods.

Gonadotropin II assay

The GtH II levels in male goldfish blood were determined by a validated carp GtH-II-specific radioimmunoassay (RIA) as described previously (Peter *et al.* 1984; Van der Kraak *et al.* 1992).

Experimental procedure

All behavioural tests were conducted on individual fish because of the nature of the image-analysis system. The fish was acclimatized in the test area from 18:00 h (daylight off at 22:30 h) and the measurements started automatically at 00:00 h and stopped at 06:00 h. Substances to be tested were delivered automatically at 00:00 h to one or the other half of the test area, and delivery was then shifted to the other half by means of the electromagnetic valves every 90 min, giving a total of four 90 min periods per night. The supply of tested substance stopped at 06:00 h. Two hours after the delivery of the test substance had stopped (08:00 h), the fish was anaesthetized in 2-phenoxyethanol (0.05 %) and a 200 μl blood sample was obtained from the caudal vasculature. The blood was immediately centrifuged and the plasma collected and stored at −80 °C for further analysis of GtH II concentration. After blood sampling, the fish was decapitated and the testes were weighed to determine GSI (%).

Experiment 1

At least 48 h before the start of the experiment, a group of six fish was transferred from the all-male tank (16 °C) to a 180 l flow-through aquarium (20 °C) with gravel and artificial floating vegetation. The reservoirs containing 10^{-8} mol l^{-1} 17α,20β-P and ethanol control were prepared no earlier than 17:00 h. Individual fish were transferred to the fluviarium 6 h (18:00 h) before the odour supply and behavioural measurements started (00:00 h). The position of the fish was recorded once every 2 s. In this experiment, the fish had a choice to reside either in 10^{-11} mol l^{-1} 17α,20β-P or in its ethanol carrier. A control series of results was obtained by supplying ethanol to both sides of the test area. The concentration used in this experiment (10^{-11} mol l^{-1} 17α,20β-P) was chosen because electrophysiological experiments (EOG) have shown that the detection threshold for 17α,20β-P in goldfish is 10^{-12} mol l^{-1} (Sorensen *et al.* 1987, 1990). Further, since ovulating females during a 2 h period release large quantities of 17α,20β-P (final concentration approximately 10^{-10} mol l^{-1} water) (Dulka *et al.* 1987; Stacey *et al.* 1989), the concentrations in the immediate vicinity of a female would be above threshold (10^{-12} mol l^{-1}). 10^{-11} mol l^{-1} 17α,20β-P should, therefore, not be too high a concentration to risk repellent reaction (Olsén, 1985; Resink *et al.* 1989; Kruzhalov, 1990).

Experiment 2

Water scented by juvenile goldfish was added to the whole width of the fluviarium giving a 'background' of fish odour over the whole test area. 17α,20β-P was supplied to one side or the other of the test area, as described in experiment 1. However, instead of diluting the test substance in plain tapwater, we added 5 l of fish-holding water to each of the two reservoirs and then diluted it to 15 l with tapwater (to which was added 17α,20β-P, to a final concentration of 10^{-8} mol l^{-1}, and corresponding amounts of ethanol). The fish-holding water was prepared by keeping 70 g of juvenile goldfish in an aquarium with 10 l of aerated tapwater for 3 h. The fish were then removed and the water filtered through a fine-mesh net to remove faeces.

Data analysis

Values are presented as means ± S.E.M. Statistical analyses between different experimental groups were compared using the two-tailed Mann–Whitney *U*-test for independent samples (**P*<0.05; ***P*<0.01).

To reduce the likelihood that behavioural analyses would be confounded by data taken during the period when the 17α,20β-P and control sides were being exchanged, only data from the last 60 min of each 90 min delivery period were used to calculate the reaction value (*Rv*), locomotor activity and swimming speed.

Rv was calculated for each experiment based on the four 60 min periods using the following equation:

$$Rv = [(T_1 - T_2)/(T_1 + T_2)] \times 100,$$

where T_1 and T_2 are the times spent on either side of the test area with stimuli added from reservoir R_1 (T_1) or from reservoir R_2 (T_2). R_1 contained a solution of 17α,20β-P or a control solution (ethanol+water). R_2 always contained a control solution. Thus, attraction to 17α,20β-P is indicated by a positive value of *Rv* and avoidance of 17α,20β-P is indicated by a negative value of *Rv*. On the basis of all *Rv* values from repeated tests, the mean *Rv* was calculated (Olsén, 1986). The confidence limits (CL) of mean *Rv* values were also calculated. The reaction was said to deviate from an indifferent reaction, i.e. from mean *Rv*=0, if mean *Rv* ±95 % CL did not include mean *Rv*=0.

Results

Preference/avoidance reaction

In both experiments 1 and 2, the control groups showed indifferent reactions, i.e. the reaction did not deviate from mean *Rv*=0 (−3.0±5.0 and 2.4±7.2, mean *Rv* ±95 % CL, respectively). The reactions in experiments 1 and 2 when 17α,20β-P was added to one or other side of the test area were significantly different, i.e. the reaction deviated from mean *Rv*=0 (−13.6±10.0 and −15.0±11.2, mean *Rv* ±95 % CL, respectively).

When comparing the groups, the spermiated male goldfish in experiment 1 spent significantly (*P*<0.05) less time in the area scented with 10^{-11} mol l^{-1} 17α,20β-P compared with the control area (Fig. 2A). In experiment 2, where fish odour was continuously added to the whole test area and the choice situation was 10^{-11} mol l^{-1} 17α,20β-P or ethanol control, the

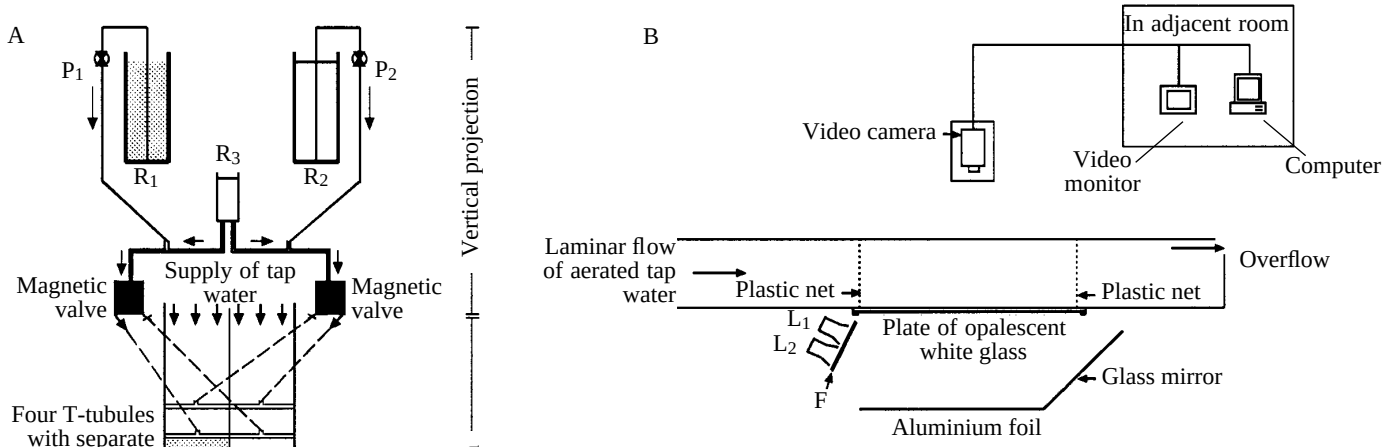

Fig. 1. (A) The fluviarium used for studying preference/avoidance, locomotor activity and the response to GtH II of spermiated male goldfish in response to 10^{-11} mol l^{-1} 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-P) in the ambient water. P, peristaltic pump; R, reservoir. (B) The video camera arrangement. L, 20 W halogen lamp; F, glass filter. The daylight was provided by a 75 W bulb connected to a timer (not shown in figure). See Materials and methods for further details.

males also spent significantly (P<0.05) less time in the area scented with 10^{-11} mol l^{-1} 17α,20β-P compared with the control area (Fig. 2B).

Locomotor activity

In experiment 1, the experimental fish (17α,20β-P/ethanol control) showed a progressively increasing locomotor activity (distance moved per 10 min period) compared with the control fish (ethanol control/ethanol control). The locomotor activity reached a plateau after 210 min of 17α,20β-P supply and was then maintained throughout the rest of the experiment (Fig. 3A). Thus, the locomotor activity measured over 1 h periods was significantly higher (P<0.05) in fish with a supply of 17α,20β-P during test periods 3 and 4 compared with the control periods (Fig. 3B). In experiment 2, the fish showed a significantly higher locomotor activity (P<0.05) throughout the experiment compared with the control fish (Fig. 4A,B). There were no significant differences in locomotor activity between the control fish in experiments 1 and 2.

Analysis of the swimming speed (m min^{-1}) in each section (with 17α,20β-P and its ethanol carrier added or ethanol carrier in both sections) of the fluviarium showed that the fish maintained its swimming speed irrespective of its position in the test area, i.e. in the 17α,20β-P section or in the section with only ethanol carrier added (Fig. 5A,B).

Endocrine gonadotropin II response

In experiment 1, experimental males exposed to 17α,20β-P had significantly higher GtH II levels than control males

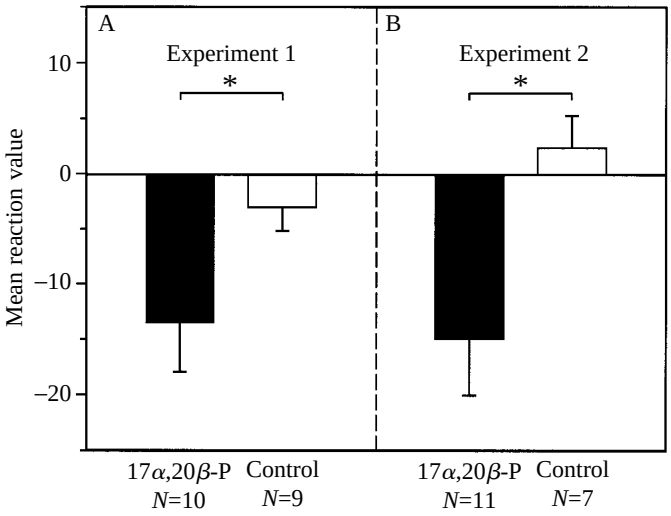

Fig. 2. Mean reaction value (see text) of spermiating male goldfish during a simultaneous choice between 10^{-11} mol l^{-1} 17α,20β-P and its ethanol carrier in the fluviarium. (A) Experiment 1. In B (experiment 2), juvenile goldfish odour was administered over the whole test area during the entire experiment (see Materials and methods for further details). Choice between 17α,20β-P and water (filled bar) and between water and water (control) experiment (open bar). Values are given as means + S.E.M. A significant difference from the control is denoted by an asterisk. N, number of fish. *P<0.05 Mann–Whitney U-test, two-tailed.

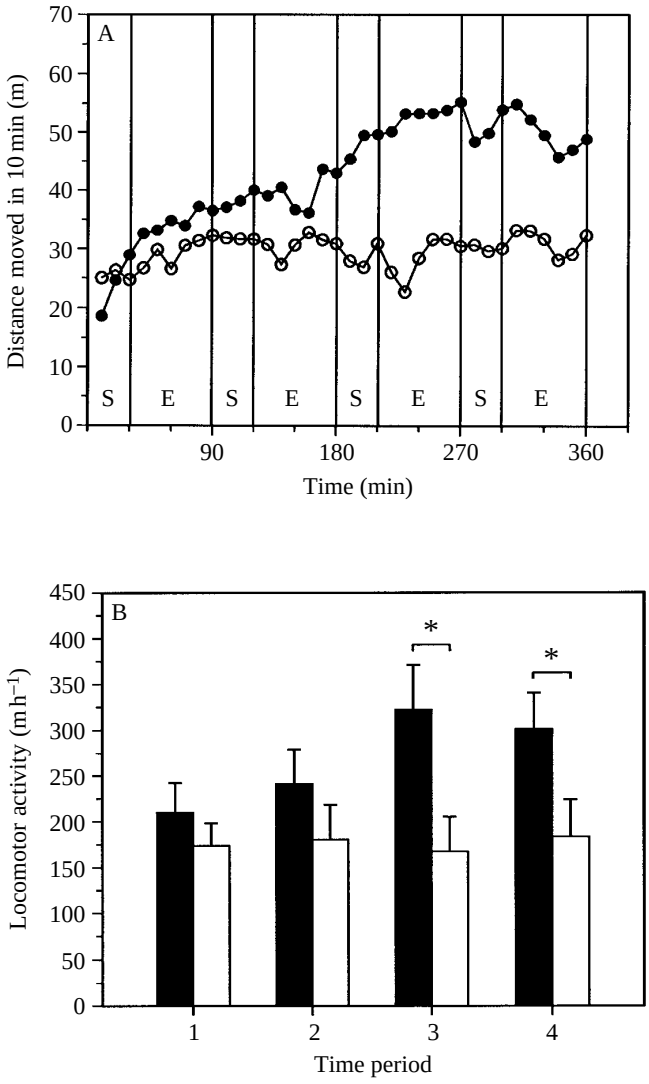

Fig. 3. (A) Locomotor activity (measured every 10 min) of spermiating male goldfish in the fluviarium in experiment 1 during a simultaneous choice between 10^{-11} mol l^{-1} 17α,20β-P and its ethanol carrier in the fluviarium (see Materials and methods for further details). S, change of side for the test substance; E, established odour conditions in the test area. Values are means. (B) Locomotor activity during 1 h periods (E in Fig. 3A). Period 1, 00:30 h to 01.30 h; period 2, 02:00 h to 03:00 h; period 3, 03:30 h to 04:30 h; period 4, 05:00 h to 06:00 h. Filled symbols (*N*=10 fish) represent the locomotor activity of fish that had a choice between 17α,20β-P and water, and open symbols (*N*=9 fish) represent the locomotor activity of fish that had a choice between water and water (control). Values are means + S.E.M. **P*<0.05 Mann–Whitney *U*-test, two-tailed.

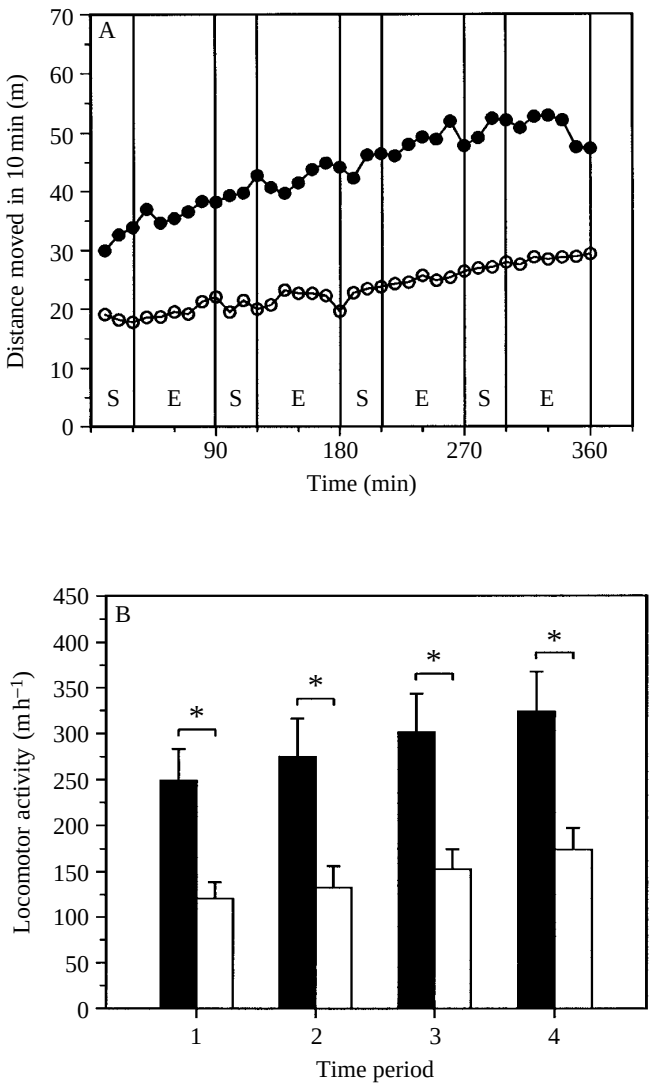

Fig. 4. (A,B) Locomotor activity of spermiating male goldfish in experiment 2. Juvenile goldfish odour was administered over the whole test area during the entire experiment; otherwise conditions were identical with those for Fig. 3. Filled symbols (*N*=11 fish) and open symbols (*N*=7 fish) represent the locomotor activity of fish that had a choice between 17α,20β-P and water and between water and water (control), respectively. Values are means + S.E.M. **P*<0.05 Mann–Whitney *U*-test, two-tailed.

exposed to ethanol (*P*<0.01; Fig. 6A). A similar trend was seen in experiment 2, although the difference was not significant (Fig. 6B). In experiment 1, the GtH II concentration was doubled in those fish that had a supply of 17α,20β-P (34.3±2.94 ng ml^{-1}) to either side of the test area compared with the controls (16.9±4.33 ng ml^{-1}, *P*<0.01) (Fig. 6A).

Discussion

The results of this study confirm previous reports (Sorensen *et al.* 1989; DeFraipont and Sorensen, 1993) that exposure to 17α,20β-P induces a rapid increase in locomotor behaviour in mature male goldfish. However, this study is the first to examine the behavioural reaction of male goldfish given a choice between water containing 17α,20β-P and water containing its ethanol carrier.

Both experiments 1 and 2 showed that the goldfish spend significantly less time in the area scented with 17α,20β-P. Two separate sets of experiments were carried out, one without (experiment 1) and one with (experiment 2) a background odour of fish-holding water, because some authors have

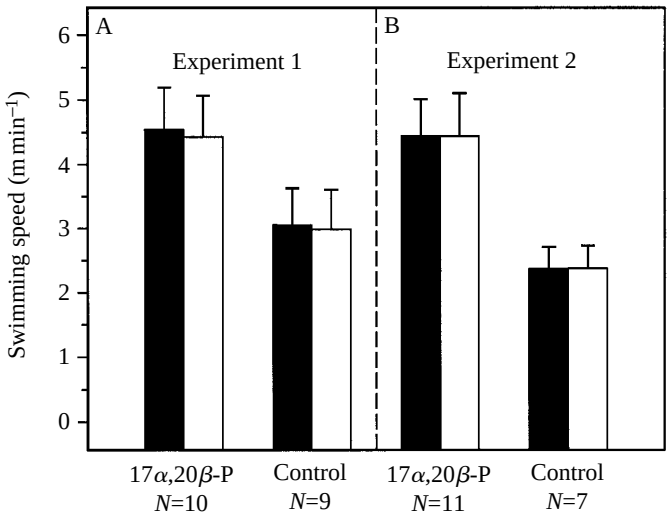

Fig. 5. (A,B) Swimming speed ($m\,min^{-1}$) of spermiating male goldfish during simultaneous choice between $10^{-11}\,mol\,l^{-1}$ 17α,20β-P and its ethanol carrier in the fluviarium. (A) Experiment 1. In B (experiment 2), juvenile goldfish odour was administered over the whole test area during the entire experiment. Filled bars represent the swimming speed in the section of the fluviarium with 17α,20β-P added and the corresponding section (i.e. in the control experiment) of the fluviarium with ethanol carrier added. Open bars represent the swimming speed in the section of the fluviarium with ethanol carrier added (in the 17α,20β-P experiment) and the corresponding section of the fluviarium with ethanol carrier added (in the control experiment). Values are given as means + S.E.M. *N*, number of fish tested.

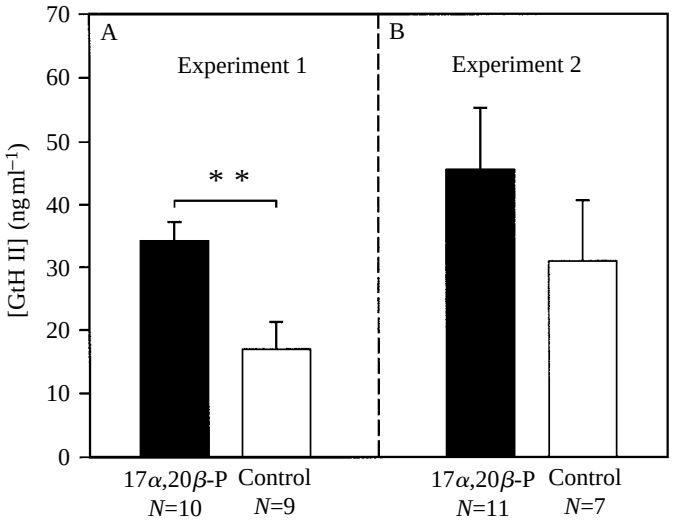

Fig. 6. (A,B) Average gonadotropin (GtH II) levels in plasma of spermiating male goldfish 2 h after (08:00 h) the supply of test solutions to the fluviarium had stopped. (A) Experiment 1. In B (experiment 2), juvenile goldfish odour was administered over the whole test area during the entire experiment. Filled and open bars represent the GtH II levels in fish that had a choice between 17α,20β-P and water and between water and water (control), respectively. Values are given as means + S.E.M. *N*, number of fish tested. ***P*<0.01 Mann–Whitney *U*-test, two-tailed.

suggested that intraspecific odours, secreted from the skin mucus, are important in cyprinids during schooling and other pheromone-mediated behaviour (Keenleyside, 1955; Hemmings, 1966; Saglio and Fauconneau, 1985; Saglio and Blanc, 1989). The goldfish has been shown to be strongly attracted to water scented by conspecifics (Le Martret and Saglio, 1982; Saglio and Blanc, 1989), and results from our laboratory have shown that the closely related crucian carp (*Carassius carassius*) is significantly attracted to the scent of conspecifics under experimental conditions similar to those described in this study (R. Bjerselius, unpublished data). Indeed, in African catfish (*Clarias gariepinus*), the importance of 'body odour' in chemical communication was demonstrated in an attraction experiment in which the odour of a male without seminal vesicles was necessary to stimulate mobility of individual females (Resink *et al.* 1987, 1989). Nevertheless, the observed avoidance of the 17α,20β-P in the present study was apparently independent of goldfish odour, because a similar avoidance of the 17α,20β-P was observed both in the absence and in the presence of a background odour of juvenile goldfish. We used juvenile fish as odour donors to be sure that no additional sex pheromones were added from the donors themselves.

Still, we cannot argue that this second experiment gave the correct pheromone information to the fish. The present study examined male behavioural responses to a single pheromonal compound (17α,20β-P). It therefore remains to be determined whether similar avoidance behaviours would be observed if males were exposed to the mixture of steroidal olfactory stimulants (17α-hydroxyprogesterone, androstenedione, glucuronidated and sulphated 17α,20β-P) reported to be released by peri-ovulatory goldfish (Sorensen *et al.* 1991, 1992; Van der Kraak *et al.* 1989). This possibility is suggested by studies with zebrafish, *Brachydanio rerio* (Van den Hurk and Lambert, 1983), which showed that males are attracted to a mixture of oestradiol and testosterone glucuronides, but not to the individual components of the mixture.

In both experiments 1 and 2, a significant increase in the locomotor activity was shown in the 17α,20β-P groups compared with the respective control group. This is in accordance with earlier studies that have shown an increase in locomotor activity due to 17α,20β-P exposure (addition of 17α,20β-P to an aquarium) for male goldfish (Sorensen *et al.* 1989; DeFraipont and Sorensen, 1993). It is demonstrated in the present study that the swimming speed is increased both in the 17α,20β-P section and in the section without 17α,20β-P added. Thus, the male's increase in locomotor activity is not dependent on continuous exposure to 17α,20β-P, nor is the locomotor activity decreased when the fish is outside the 17α,20β-P plume.

The significant increase in plasma GtH II in experiment 1 in response to $10^{-11}\,mol\,l^{-1}$ 17α,20β-P in the ambient water is in agreement with earlier 17α,20β-P exposure studies on male goldfish (Dulka *et al.* 1987; Sorensen *et al.* 1989). Since the blood sample was taken 2 h after the supply of 17α,20β-P had stopped, the contact with 17α,20β-P induced an increase in GtH II level that lasted for up to 2 h. It has been shown for

male goldfish that the GtH II levels return to basal levels within 4 h after removing the 17α,20β-P stimulus (Stacey *et al.* 1991). This time-dependent decline in GtH II level after removal of the 17α,20β-P stimulus probably accounts for the lack of significance in experiment 2, although the results indicate higher GtH II concentrations in the 17α,20β-P group.

Some authors have reported avoidance reactions to proffered pheromones. The seminal vesicles in male African catfish (*Clarias gariepinus*) secrete an odour that makes them less attractive to unovulated females (Resink *et al.* 1987). In catfish (*Ictalurus melas*), extracts of the seminal vesicles have been shown to be involved in both avoidance and attraction responses (Rubec and Thomas, 1979). The observed avoidance reaction to 17α,20β-P reported here could be related to the ability of mature male goldfish to detect the degree of sexual maturity of the female. As soon as the female goldfish ovulates, there is a dramatic decrease in the level of 17α,20β-P in the blood and in the rate of its release to the water (Kobayashi *et al.* 1987; Stacey *et al.* 1989). In a shoal of spawning goldfish, the females may co-exist at two different stages of sexual maturity: females that are commencing the final maturation of their oocytes (and are releasing 17α,20β-P) and females that have ovulated (and ceased releasing 17α,20β-P but are releasing $PGF_{2\alpha}$ and its metabolites). In the female zebrafish, the production of ovarian steroid glucuronides (male attractants) takes place shortly after ovulation (Van den Hurk and Lambert, 1983; Van den Hurk *et al.* 1987). It has also been suggested that the postovulatory pheromones in the zebrafish inform the males about the female's readiness to spawn (Van den Hurk and Lambert, 1983). Partridge *et al.* (1976) have demonstrated that male goldfish can discriminate between ovulated and unovulated females, spending significantly more time following the ovulated female. This behaviour was, according to the authors, mediated almost entirely by olfaction, with gustatory or visual signals playing a relatively minor role. It would probably increase the male goldfish's reproductive success if he were able to identify and court only those females that had ovulated and avoid misdirected courting of non-ovulated females (i.e. those still releasing 17α,20β-P). In a recent study (Bjerselius *et al.* 1995), we have shown that immature males of the crucian carp (*Carassius carassius*), a close relative of the goldfish and with a similar sensitivity to 17α,20β-P (Bjerselius and Olsén, 1993), are indifferent to 17α,20β-P, while mature males avoided the steroid in the same way as has been demonstrated for the mature male goldfish in the present paper.

In conclusion, this study shows that mature male goldfish spend significantly less time in water scented with the pheromone 17α,20β-P than in water scented with its ethanol carrier. The male showed significantly increased locomotor activity and plasma GtH II concentration as a result of contact with 10^{-11} mol l^{-1} 17α,20β-P. We suggest that 17α,20β-P might be a signal to the sexually mature male not to court a non-ovulated female but to continue its search for an ovulated female. In view of the extremely intense competition among males in a natural group of spawning goldfish (DeFraipont and Sorensen, 1993), this seems to be an adequate explanation, providing the male with a higher chance of finding, courting and spawning with as many females as possible.

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