

Common Carp Implanted with Prostaglandin F_{2α} Release a Sex Pheromone Complex that Attracts Conspecific Males in Both the Laboratory and Field

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Abstract When ovulated, female fish of many species are known to release a F-prostaglandin-derived sex pheromone that attracts conspecific males. Recently, this pheromone was identified in the common carp as a mixture of prostaglandin F_{2α} (PGF_{2α}) and unidentified body metabolites, which we termed a ‘pheromone complex.’ The present study sought to test the activity of this pheromone complex in the field by developing a system using carps implanted with PGF_{2α} as pheromone donors. An initial experiment determined that osmotic pumps that delivered up to 0.4 mg of PGF_{2α} per hour could be implanted into carp without any apparent effects on their health. A second experiment found that PGF_{2α}-implanted male and female carp released biologically relevant (and equivalent) quantities of PGF_{2α}, along with two of its seemingly inactive metabolites, for up to 2 weeks. Laboratory experiments demonstrated that the odor of PGF_{2α}-implanted carp was highly attractive to male conspecifics, and included necessary body metabolites; it attracted males as strongly as ovulated carp odor, and much better than PGF_{2α} alone. Finally, a field test demonstrated that PGF_{2α}-implanted female carp attracted mature male, but not female carp, from a distance of 20 m. This is the first demonstration of the activity of a PGF_{2α}-based pheromone in a natural environment and confirms the use of a PGF-pheromone complex in the carp. We suggest that the implant technique may be useful in future studies of how PGF pheromones function and could be further developed to attract invasive fish for use in control.

Keywords Hormonal pheromone · Synergism · Pheromone complex · *Cyprinus carpio* · Prostaglandin metabolism · Toxicity · Invasive species · Liquid chromatography-mass spectrometry (LC-MS/MS) · Integrated pest management

Introduction

Female fish of many species release a species-specific F prostaglandin (PGF)-based sex pheromone when ovulated that attracts males and mediates spawning interactions (Stacey and Sorensen, 2009; Lim and Sorensen, 2011). However, with the exception of the common carp, *Cyprinus carpio* (Lim and Sorensen, 2011), studies of this pheromone have been restricted to the laboratory, and have used synthesized PGFs. Our understanding of the identity and biological function of this class of pheromone is incomplete. Recently, we discovered that the pheromone released by ovulated female common carp (hereafter termed ‘carp’) is comprised of a mixture of prostaglandin F_{2α} (PGF_{2α}) and unidentified polar body metabolites, which we termed a ‘pheromone complex’ (Lim and Sorensen, 2011). This discovery suggested that we might be able to induce carp to produce the complete pheromone complex by implanting them with PGF_{2α}, so that they would release various PGFs and body metabolites, thereby permitting larger scale field tests. We were especially interested in these tests because there have been very few tests of fish pheromones in the field (Johnson and Li, 2010), and because the carp is a highly invasive species for which few control options exist (Weber and Brown, 2009; Sorensen and Bajer, 2011).

The possibility that PGF_{2α} implants might elicit normal pheromone release is based on several studies of the closely related goldfish, *Carassius auratus*, which, if injected with PGF_{2α} releases a chemical cue(s) for approximately 2 hrs,

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which mimics both the behavioral activity and olfactory potency of the natural pheromone (Sorensen et al., 1986, 1988; Sorensen and Goetz, 1993). However, little is understood about PGF_{2α} metabolism and clearance in fish. There appears to have been only one study of the metabolism of exogenously administered PGF_{2α} in fish, and it shows (with a single radiochromatogram) that goldfish injected with radio-labeled PGF_{2α} likely release at least five PGF metabolites, in addition to PGF_{2α} (Sorensen and Goetz, 1993). We do not know whether carp would release a similar suite of PGFs and other metabolites. Effects of gender on PGF_{2α} metabolism in fish are also unknown. Additionally, while osmotic pumps have been used to deliver thyroid inhibitors and steroidal hormones in fish (e.g., Comeau and Campana, 2003; Metz et al., 2003), they have not been tested previously for use with PGF_{2α}. There is also the possibility that long-term administration of PGF_{2α} could be toxic to fish, as it is to mammals (Clayman, 1975; Adams, 2001; Lust et al., 2011).

The aim of the present study was to investigate the effects of long-term, constant, inter-peritoneal delivery of PGF_{2α} on pheromone production/release in carp and then, if successful, conduct preliminary field tests. We asked: 1) Is chronically implanted PGF_{2α} toxic to carp? 2) What levels and types of PGFs do implanted male and female carp release, and how do these compare with PGFs released by naturally ovulated carp? 3) Is the odor of PGF_{2α}-implanted carp attractive to carp in the laboratory, and how does it compare to ovulated females and/or PGF_{2α} alone (i.e., is the complex released)? 4) Does the odor of PGF_{2α}-implanted carp attract male and/or female carp in the field?

Methods and Materials

Experimental Fish Juvenile carp were obtained from a fish farm (Osage Catfisheries, Osage Beach, MO, USA) and raised to maturity in 1,000 l, flow-through, circular tanks supplied with 20°C well water at a rate of 10 l/min. Fish were fed a mixture of flake fish food (Aquatic Ecosystems, Apopka, FL, USA) and frozen brine shrimp (San Francisco Bay Brand, Newark, CA, USA) each day, while being maintained on a 16:8 h (Light: Dark) photoperiod. Once mature (3 yr of age, 500–1,000 g weight), they were sorted by sex (fish were stripped to determine whether they were spermiating or rotund with eggs; i.e., females were not ovulatory), and placed in separate flow-through tanks until needed.

All protocols described in this manuscript were approved by the University of Minnesota Institutional Animal Use and Care Committee.

Osmotic Pheromone Pumps Commercially available osmotic pumps (model 2ML1; Alzet, Durect Co., Cupertino, CA, USA) were selected, based on their use in mammals. The

desired quantity of PGF_{2α} (Cayman Chemical, Ann Arbor, MI, USA; see below) was first dissolved in deionized, sterile water, and then injected into the reservoirs of sterile pumps with a syringe. Healthy adult carps were anesthetized by first placing them into an aerated solution of 0.02% MS-222 (Western Chemical Inc., Ferndale, WA, USA), and then into a wet foam cradle. Several scales then were removed from a small area, ventro-posterior to their pelvic fins, and a 4 cm incision made into the body cavity, using a sterile #11 scalpel, through which PGF_{2α}-loaded osmotic pumps were inserted. Four stitches, with absorbable monofilament suture (PDS-II, Ethicon, MI, USA), were used to close the incisions. Finally, fish were removed to a 100 l recovery tank and, once upright and mobile, returned to their holding tanks. This operation took only a few minutes and elicited little bleeding and no infection. Additional technical details on the operation and pumps can be found in a technical manual (Lim and Sorensen, 2010).

Question 1. Is PGF_{2α} Toxic to Carp when Chronically Administered by an Osmotic Pump? Our first experiment sought to determine how much PGF_{2α} we could safely administer to carp. Six doses of PGF_{2α} were selected, based on the quantity of PGF_{2α} we previously injected into goldfish [1 mg·kg⁻¹; Sorensen et al. (1988)] and on published delivery rates of osmotic pumps into the body cavity. These doses were: 0.0 g PGF_{2α}/kg body weight (blank control), 0.04 g/kg (a dose that delivers 0.1 mg/h into the body cavity), 0.2 g/kg (0.5 mg/h), 0.4 g/kg (1.0 mg/h), 1.2 g/kg (3.0 mg/h), and 2.0 g/kg body weight (5.0 mg/h). Three immature female carp [1,020±39 g; gonadosomatic index (GSI: gonad weight/whole body weight × 100%) of 11±2%] were implanted with each dose and then placed into a single large holding tank (1,000 l) that received aerated, 20°C well water. Fish feeding and swimming behavior was observed twice a day, and fish observed not to be feeding or actively moving (i.e., dead or moribund) were removed and euthanized in 0.1% MS-222. The experiment ended after 2 wk when the implants had delivered all of their contents.

Question 2. What is the Fate of the PGF_{2α} Implanted into Male and Female Carp? Having determined the highest dose that a 1 kg carp can tolerate is 0.4 g of PGF_{2α}, we next sought to determine how much PGF_{2α} these fish were releasing into the water, and whether gender had an influence on PGF metabolism and release. All three PGFs [PGF_{2α}, 15-keto-PGF_{2α} (15 K-PGF_{2α}), and 13,14-dihydro-15keto-PGF_{2α} (dh15K-PGF_{2α})] known to be released by naturally ovulated carp were monitored (Lim and Sorensen, 2011). Three males (876±23 g; GSI=8.5±1.1%) and three females (903±20 g; GSI=10.5±2.6%) were implanted with osmotic pumps containing 0.4 g PGF_{2α}, while three others of each sex were implanted with pumps containing

water only, as controls. All fish then were placed into 1,000 l holding tanks supplied with 20°C well water. Implanted fish were fed daily for 18 d while being removed to individual 50 l holding tanks 1, 5, 9, 13, 18 d after implantation, for 1 h, and then returned. One liter water samples were collected from these holding tanks and then either used immediately in behavioral tests (see experiment 3, below) or extracted for analysis, by passing them through activated C18 Sep-Pak cartridges (Waters, MA, USA) at a rate of 1 liter/h and eluted with 5-ml of methanol, following established protocols (Lim and Sorensen, 2011).

For analysis, the eluant from extracted holding tank water was dried under a stream of nitrogen, reconstituted in 1 ml of methanol/distilled water (50:50 v/v), and then analyzed by LC-MS/MS (Liquid chromatography-tandem mass spectrometry) by injection (and standards) onto a LC-MS device equipped with an Eclipse XDB C18-RS column (250×4.6 mm, particle size 5 μm; Agilent Technologies, MA, USA). To separate PGFs, we used a mobile phase of 0.1% formic acid in acetonitrile and 0.1% formic acid in distilled water, at a flow rate of 1 ml/min. The solvent gradient changed from 35% acetonitrile at 0 min to 65% at 12 min (this gradient separated all three PGFs by over 1 min). The LC was interfaced with an ion-trap mass spectrometer (LCQ-1, ThermoScientific, MA, USA) with an electrospray ionization source [ion trap operated in the negative ion mode, with a spray voltage of 5 kV; sheath gas was 99% pure nitrogen at 60 psi; sheath fluid was 50:50 20 mM triethylamine: acetonitrile (v/v)]. We quantified PGF_{2α}, 15K-PGF_{2α}, and dh15K-PGF_{2α} by summing all peaks associated with each compound's daughter ions, and then extrapolating these values to a five-point calibration curve, which we had previously created by adding standards to immature carp holding water, extracting it, and measuring its contents as described above. This technique controls for both extraction efficiencies and matrix effects; both its accuracy and precision have been shown to be high (Fine et al., 2006; Lim and Sorensen, 2011). Our calibration curves were linear (R^2 values of 0.92, 0.96, and 0.96) and had 95% confidence intervals that ranged from 23–28% around the mean. Mean release values of males and females (across days) were compared for each PGF using two-way repeated measure ANOVAs (normality was initially confirmed using a Kolmogorov-Smirnov test) (SigmaStat, Ashburn, VA, USA). If significance ($P < 0.05$) was indicated, daily values were compared to each other using a Holm-Sidek adjustment (Aickin and Gensler, 1996).

Question 3. Is the Odor of PGF_{2α}-implanted Carp Attractive and, if so, How Does it Compare with the Natural Female Pheromone and PGF_{2α} Alone (Is a Complex Released)? Having demonstrated that carp implanted with PGF_{2α} release considerable quantities of PGF_{2α}, our next

step was to determine if the odor of PGF_{2α}-implanted carp was pheromonally active (attractive) and, if so, how it compared with the natural pheromone? Two sets of experiments, using an established 2-choice behavioral assay (Lim and Sorensen, 2011; below), addressed these questions. Our first set of experiments tested the attractiveness of PGF_{2α}-implanted male and PGF_{2α}-implanted female holding water as well as PGF_{2α} against blank water. PGF_{2α}-implanted male and PGF_{2α}-implanted female holding waters were each tested at three concentrations (full strength, diluted 10,000 times, and diluted 100,000 times; dilutions were performed prior to addition to the mazes), using samples collected from female carp (as part of question 2), when PGF_{2α} release rates peaked (days 5–9). Their holding waters contained $0.7 \pm 0.2 \times 10^{-8}$ M PGF_{2α}. Blank water control, the water of blank-implanted carp, and samples of previously collected and thawed, ovulated female carp holding waters (previously estimated to contain $2 \pm 0.3 \times 10^{-10}$ M PGF_{2α}; Lim and Sorensen, 2011), also were tested. Finally, for comparison, a third set of experiments tested the attractiveness of PGF_{2α} alone at concentrations which included those found in PGF_{2α}-implanted fish water (i.e. 10^{-11} M– 10^{-7} M). Responses (relative attraction, see below) within each series were evaluated by one-way ANOVA (Sigmastat, VA, USA) and, if significant ($P < 0.05$), responses to individual odors were compared to each other and blank well water, by pair-wise comparisons using a Holm-Sidek adjustment (Aickin and Gensler, 1996).

The second set of experiments sought to determine the precise potency of PGF_{2α}-implanted carp odor relative to whole pheromone (ovulated female odor), as well as PGF_{2α} alone, using head-to-head tests. It used the same two-choice behavioral assay as the first set of experiments, but substituted a test odor for a control. Holding water from PGF_{2α}-implanted female carp ($0.7 \pm 0.2 \times 10^{-8}$ M PGF_{2α}) was tested directly against ovulated female holding water (2×10^{-10} M PGF_{2α}). Next, we tested PGF_{2α}-implanted female fish holding water against 10^{-7} M PGF_{2α} (the concentration the holding water contained), and then against a concentration of PGF_{2α} (5×10^{-7} M) that was five times greater. As a final follow-up test, we tested 10^{-7} M PGF_{2α} vs. 5×10^{-7} M PGF_{2α}, to determine what role PGF_{2α} concentration alone might have had in the previous experiment. Each experiment was analyzed using a one-sample *t*-test (MiniTab, PA, USA), after confirming that data were normally distributed (Kolmogorov-Smirnov test; see Lim and Sorensen, 2011).

Both sets of experiments used a well-established laboratory assay, which used groups of male carp that had previously been activated ('primed') by exposing them to a pre-ovulatory steroid pheromone 8 h prior to experiments (10^{-10} M 17α,20β-dihydroxy-4-pregnen-3-one; Lim and Sorensen, 2011). Each experiment had 10 trials, each of which used a different group of 5 fish. Briefly, for each

trial, groups of males were placed into circular two-choice (1.5 m diameter) mazes, supplied with well water and equipped with overhead cameras and infrared lighting. The distribution of all carps was noted at 15 sec intervals, during a 15 min pre-test period, after which a test odor was added to one end, and either a control (well water alone) or a matching test odor (for head-to-head tests) to the other, using pumps (10 ml/min). Changes in the percent time spent in the test area were calculated to yield a relative attraction value (%). Fish were re-used after a month-long interval in their holdings tanks if, and as, necessary.

Question 4. Is the Odor of $\text{PGF}_{2\alpha}$ -implanted Carp Attractive to Male and/or Female Carp in the Field? Finally, the attractive properties of implanted-carp water were tested in Lake Keller (Maplewood, MN, USA; 45°00'11.80"N, 93°03'45.48"W). This lake was thought to be representative of many in the region. It is shallow (3–4 m deep), has a surface area of 29 ha, is connected to two other lakes by small streams, and contains a large population of native fish, including bluegill sunfish, *Lepomis macrochirus*, as well as dense beds of submersed vegetation. At the time of the study, Lake Keller was also known to have had approximately 1,500±200 adult carp from mark-recapture studies (Bajer et al., 2011; Chizinski and Sorensen, unpublished results). We used radio-tagged fish to monitor the proximity of male and female carp relative to $\text{PGF}_{2\alpha}$ -implanted females that were placed into one of two screened (darkened) traps. We chose not to use blank-implanted fish as controls, because laboratory experiments showed they have no influence on male behavior (Lim and Sorensen, 2011; this study), and the lake was already filled with spent females.

The experiment started in early May 2009 (prior to carp-spawning season), when we captured 20 adult carp from around the entire perimeter of the lake (12 spermiating males and 8 mature, but non-ovulated, females) using boat-electrofishing, implanted them with radio-tags (F1850, Advanced Telemetry Systems, MN, USA) following established procedures (Bajer and Sorensen, 2010), and then released them where they were caught. We then monitored the lake for spawning activity on a daily basis (carp spawn in floating vegetation and are easy to spot from shore). At the conclusion of the spawning season in mid-June (i.e., when males were likely to be still searching for spawning females, although none would have been present), we captured two spent female carp (2.96 and 2.65 kg) by boat-electrofishing, implanted them with osmotic pumps containing 900 mg of $\text{PGF}_{2\alpha}$ (ca. 0.4 g/kg of fish), and placed them into one of two wood-framed, mesh-covered traps (1.2×1.2×2.4 m in W×H×L; mesh size=5×5 cm; V-shaped funnel opening 50 cm tall and 5 cm wide), which we placed in an area where spawning had been observed (depth=1.2 m), at a distance of 100 m from each other. A battery-powered bilge pump

(4,500 l/h; T1200 Tsunami Bilge Pump, Attwood Marine Products, Lowell, MI, USA) was placed in the back of each trap to create a plume that we estimated, from dye (Bright Dyes, Kingscote Chemicals, Miamisburg, OH, USA) tests, to extend to about 10 m. We then spent 1.5–2.5 h each morning locating and bi-angulating the positions of radio-tagged carp near each trap, using shore-based receivers (estimated precision of 10 m). We focused on regions located 20 m around each trap, as these would have included the pheromone plume. The pair of implanted carp was moved between the two traps every 3 d, so that the position of the control and pheromone traps varied in a way to control for trap location. The experiment continued for 12 d, after which the implants were designed to run out. For analysis, the locations of male and female radio-tagged individuals were determined (LOAS 4.0; Ecological Software Solutions, Sacramento, CA, USA) and analyzed using ArcGIS (ESRI; Redlands, CA, USA). We calculated the number of radio-tagged male and female carp within 20 m of the test and control traps, and compared each by paired *t*-tests (Minitab, PA, USA). Because we were tracking a population of carp that was free to come and go across the lake (and data showed that they did), assumptions of statistical independence were met.

Results

All carp implanted with 0.0, 0.04, 0.1, 0.2, and 0.4 g $\text{PGF}_{2\alpha}$ survived, while all those implanted with either 1.2 or 2.0 g $\text{PGF}_{2\alpha}$ /kg body weight died or were moribund (and sacrificed) within 4 days (Fig. 1). Carp that died fed little, did not move actively, and had skin lesions.

Both implanted male and female carp released very high and similar levels of $\text{PGF}_{2\alpha}$ to the water (about 1 mg/h after day 5), while 15K- $\text{PGF}_{2\alpha}$ and dh15K- $\text{PGF}_{2\alpha}$ were released in far smaller quantities (about 1,000 fold less); the latter did not differ between the sexes (Fig. 2: $P>0.05$). Release rates of all three PGFs peaked at day 5, and declined after 9 days

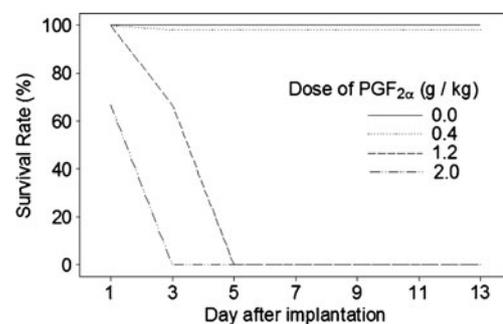


Fig. 1 Survival rates of female common carp implanted with four representative doses of prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$; g $\text{PGF}_{2\alpha}$ /kg body weight). All carp implanted with 0.04, 0.1, and 0.2 g $\text{PGF}_{2\alpha}$ /kg survived, but these data are not shown (to simplify the figure)

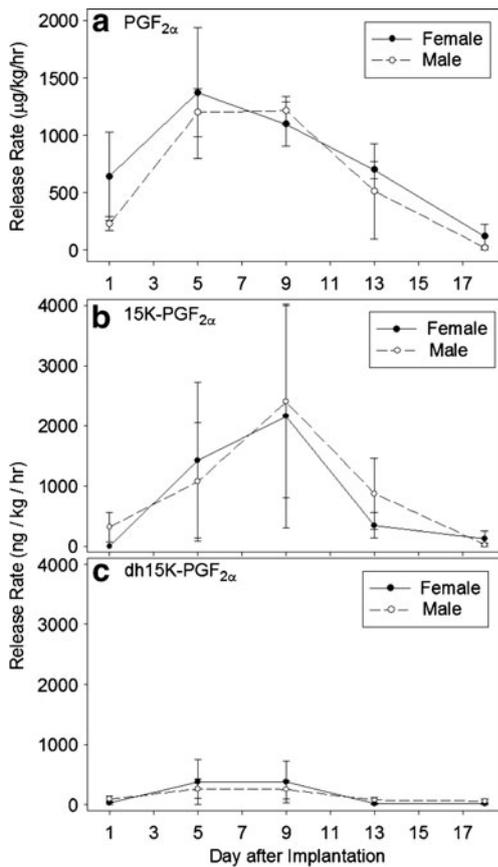


Fig. 2 Release rates (mean ± SEM) of three F prostaglandins a) prostaglandin F_{2α} (PGF_{2α}), b) 15-keto- prostaglandin F_{2α} (15 K-PGF_{2α}), and 13,14-dihydro-15-keto-PGF_{2α} (dh15K-PGF_{2α}), over a 2 wk period, by female (solid circles) and male common carp (empty circles) implanted with 0.4 g/kg body weight of PGF_{2α}

(Fig. 2: $P < 0.05$). The average release rates of all three PGFs could, when summed, account for all of the PGF_{2α} being delivered by the pumps.

While the holding water of blank-implanted female and male carp did not attract males (Fig. 3a, b: $P > 0.05$), both PGF_{2α}-implanted male, and PGF_{2α}-implanted female carp-holding waters attracted male carp in laboratory mazes, even after being diluted 10,000 times (Fig. 3a, b: $P < 0.01$). The concentration of PGF_{2α} in the 10,000x diluted water was estimated to be 10⁻¹¹ M (Fig. 3a, b). The level of relative attraction exhibited to both implanted male and implanted female carp holding water was equivalent in magnitude to that of ovulated female water (Fig. 3a, b: $P > 0.05$). Maze tests of PGF_{2α} alone showed that while it was attractive at 10⁻⁹ M (Fig. 3c: $P < 0.05$), only weak, non-significant attraction was observed at 10⁻¹⁰ M (Fig. 3c). Follow-up, head-to-head tests demonstrated that implanted-carp odor was as potent as ovulated-carp odor, and that implanted odor was far more attractive than PGF_{2α} alone, at both 10⁻⁷ M (equimolar) and 5 × 10⁻⁷ M (Fig. 4). Nevertheless, male carp preferred a

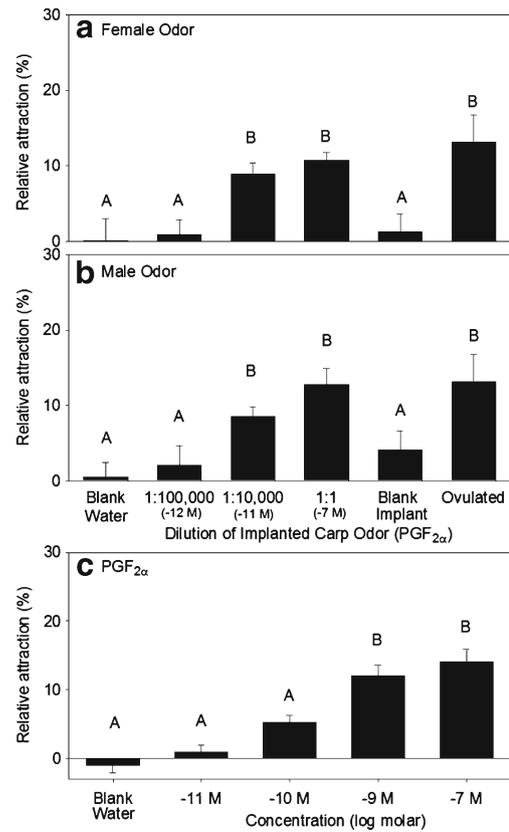


Fig. 3 Attraction (mean relative attraction ± SEM) of primed male common carp to: a) increasing dilutions of prostaglandin F_{2α} (PGF_{2α})-implanted, blank-implanted, and naturally ovulated female carp odor; b) increasing dilutions of PGF_{2α}-implanted, blank-implanted male carp, and naturally ovulated female carp odor; c) different concentrations of PGF_{2α} (log molar). The estimated concentrations of PGF_{2α} in each diluted odor are given in parentheses. Responses were compared by one-way ANOVA. Different letters indicate differences, $P < 0.01$

concentration of 5 × 10⁻⁷ M PGF_{2α} to that of 10⁻⁷ M PGF_{2α} (9.5 ± 1.3% relative attraction; $P < 0.01$).

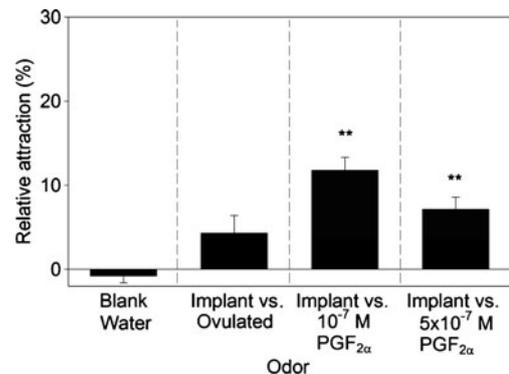


Fig. 4 Attraction (mean relative attraction ± SEM) of primed male common carp to well water vs. well water control, the whole odors from implanted female vs. naturally ovulated carp, the odor of implanted female carp vs. an equimolar concentration of PGF_{2α} (10⁻⁷ M PGF_{2α}), and odor of implanted female carp vs. 5 × 10⁻⁷ M PGF_{2α} (5 times more PGF_{2α} than released by implanted carp). Differences ($P < 0.01$) in attraction between a pair of treatments are denoted by **

In our field test, an average of 0.54 ± 0.2 males was found each morning within 20 m of the trap containing the $\text{PGF}_{2\alpha}$ -implanted carp, and 0.0 ± 0.0 within 20 m of the control trap (Fig. 5a; $P < 0.05$). Eight individual males were found near the traps during the course of this experiment, and two of these were found twice. All eight males came from the southeast area of the lake where the traps were located. In contrast, an average of only 0.11 ± 0.1 females was found within 20 m of the pheromone trap, and 0.0 ± 0.0 within 20 m of the control trap (Fig. 5b; $P > 0.05$). No carp entered the traps, and no mortality was observed. Analysis of the control data suggested there was no effect of trap location ($P > 0.10$).

Discussion

This study showed that $\text{PGF}_{2\alpha}$ -filled osmotic implants placed into common carp stimulate the release of a sex pheromone complex that is attractive to sexually active conspecific males in both the laboratory and field. To our knowledge, this is the first demonstration of the activity of a PGF -based pheromone in the field. The seemingly normal activity of odor of $\text{PGF}_{2\alpha}$ -implanted carp, and the fact that it is more active than the $\text{PGF}_{2\alpha}$ it contained, demonstrates, as previously hypothesized (Lim and Sorensen, 2011), that the natural pheromone of this species is a synergistic mixture of compounds. The technique of implanting pheromones, and/

or their precursors, into fish should be useful in future studies of fish reproductive physiology and pheromone release, as well as in the control of invasive fish species, many of which use known hormonal pheromones whose precursors are commercially available (Sorensen and Stacey, 2004).

This study is an important confirmation of our hypothesis that PGF pheromones function as pheromone complexes that include body metabolites. Previous studies have shown that polar body metabolites are discerned and used by carp and goldfish, and may convey species information (Sisler and Sorensen, 2008; Levesque et al., 2011), as well as demonstrating that chemical fractions containing these metabolites synergize the activity of ovulated fish odor (Lim and Sorensen, 2011). However, this study demonstrated that $\text{PGF}_{2\alpha}$ only has full activity if added to water within the context of whole body odor. Not only was $\text{PGF}_{2\alpha}$ -implanted carp odor much more active than $\text{PGF}_{2\alpha}$ in both head-to-head tests and dose-response relationships, but it was as attractive as ovulated female odor. Even five-fold increases in $\text{PGF}_{2\alpha}$ concentration could not make up for the absence of whole body odor. The PGF metabolite, 15K- $\text{PGF}_{2\alpha}$, also appeared to be relatively unimportant (it was not released in large amounts by fully active implanted fish), even though it is detected by the carp olfactory system (Irvine and Sorensen, 1993) and has pheromonal activity in the goldfish (Sorensen et al., 1988, 1989). Similarly, Lim and Sorensen (2011) found that both dh15K- $\text{PGF}_{2\alpha}$ and 15K- $\text{PGF}_{2\alpha}$ are unattractive to male carp; perhaps these cues are ignored by carp because they strongly characterize female goldfish which, in contrast to carp (Lim and Sorensen, 2011), release them in much greater quantities than they do $\text{PGF}_{2\alpha}$ (Sorensen et al., 1995, unpublished results). Interestingly, the amount of $\text{PGF}_{2\alpha}$ tested seemed to be unimportant in our assay. However, this may make sense because PGFs are likely released as highly concentrated urinary plumes (Appelt and Sorensen, 2007), whose concentrations would change rapidly and, perhaps unpredictably, in the immediate vicinity of female fish and their body odor.

While our study seems to confirm that carp employ a F prostaglandin-based pheromone complex to mediate spawning attraction, and that body metabolites play a key role in this complex, it is premature to conclude that we have characterized all features and components of the complex. In particular, we have yet to test whether $\text{PGF}_{2\alpha}$ -implanted fish odor induces full sexual arousal (chasing and nudging) and reproductive priming (elevated levels of luteinizing hormone), as the odor of naturally ovulated goldfish does (Stacey and Sorensen, 2009). The identity of the body metabolite(s) that synergize the action(s) of $\text{PGF}_{2\alpha}$ also are unknown, although Levesque et al. (2011) provided evidence that they are polar and likely non-hormonal, because all life stages seem to release them.

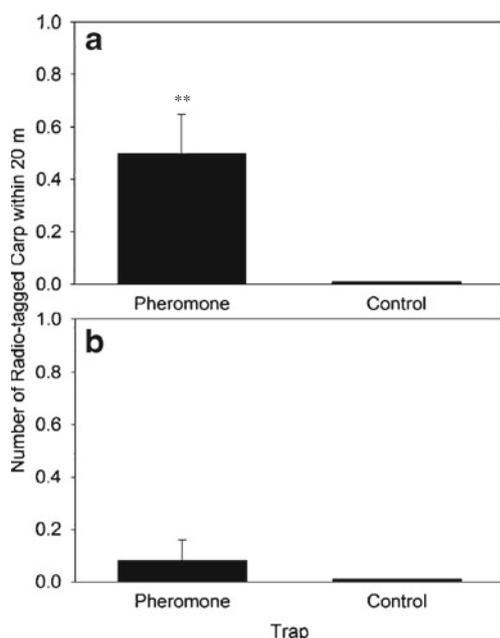


Fig. 5 Daily mean number (\pm SEM) of: a) radio-tagged male, and b) radio-tagged female common carp within 20 m of pheromone-baited and control traps. Because fewer females (8 vs. 12) than males were radio-tagged, their values were expected to be 30% lower. A difference ($P < 0.01$) in mean numbers of fish between a pair of treatments is denoted by **

Although our proof-of-concept field test was only of 12 days duration, it nevertheless confirmed laboratory studies and demonstrated for the first time that a PGF-based pheromone complex functions in the field. It was especially relevant that males, but not females, were attracted to this sex pheromone, because the pheromone attracts only aroused males (Lim and Sorensen, 2011, unpublished results). This observation also suggests that blank-implanted fish odors would have been ineffective in the field, as they were in the laboratory. While the numbers of fish we found near traps may seem low, each radio-tagged male represented about 70 individuals, so, presumably, the numbers of actual fish attracted were much greater. The 20 m range of attraction fits well with our estimates of the size of the plume that these fish release, and which goldfish are known to release in the laboratory (Stacey and Sorensen, 2009); i.e., the active region is relatively small. Adult carp are notoriously loath to enter traps of any kind. So, while our failure to lure carps into traps was disappointing, it was not unexpected. If pheromonal attractants are used, new, more effective types of traps and/or carp capture devices will be needed. We are now planning to conduct studies that include more controls, higher doses of PGF_{2α} in more fish, and different types of plume structures. The present study is one of just a few to demonstrate the actions of a fish sex pheromone in the field (Johnson and Li, 2010).

This study also sheds new light on the metabolism and clearance of PGF_{2α} in fish. It demonstrates that most PGF_{2α} introduced into carp is cleared directly, with lesser amounts being metabolized to 15K-PGF_{2α} and dh15K-PGF_{2α}; there is no evidence of any other biologically active metabolites being produced. This mirrors our earlier results (Lim and Sorensen, 2011) in naturally ovulated carp. In contrast, goldfish injected with PGF_{2α} produce large quantities of 15K-PGF_{2α}, which has pheromonal function and dominates the PGF release mixture (Sorensen et al., 1988, 1995). Injection studies in carp should be conducted, as introduction of a bolus might result in different metabolism, although this seems unlikely. Interestingly, our study did not produce any indication that sex of carp influences the production of the PGF pheromone complex. This is of practical significance, because integrated pest management control programs of invasive species may favor (because escape is a concern) use of males in situations in which females are being targeted for removal (Inland Fisheries Service, 2009). The relatively low toxicity of PGF_{2α} to fish is interesting and fortuitous, as mammals appear much more sensitive (Kennedy and Lukash, 1982; Zelinski-Wooten and Stouffer, 1990); perhaps differences in metabolism related to pheromone signaling are responsible.

The implant technique has potential to be developed to study pheromone function in the field, and as a source of environmentally friendly, species-specific fish attractants to either sample rare fishes and/or remove nuisance fishes.

Notably, many species of fish, including those of the Family Cyprinidae that includes the highly invasive Asian carps (*Hypophthalmichthys* spp.), appear to use PGF-based pheromones (Stacey and Sorensen, 2009; Lim and Sorensen, unpublished results) and are amenable to this technique. The implant technique not only appears to stimulate fish to produce the natural pheromone, but it functions for extended periods of time, while avoiding complications associated with introducing artificial compounds to waterways. Further studies should explore enhancement, including use of higher doses and the possibility that implanted females may also exhibit sexual behaviors that might make them useful as “Judas fish” decoys (Stacey, 1976; Stacey and Sorensen, 2009; Bajer et al., 2011).

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