

PHEROMONAL EFFICACY ON PITUITARY GLAND RELATIG TO REPRODU CTION THROUGH THE NEUROETHO-HYPOPHYSIO GONADAL PATHWAY IN THE FISH CYPRINIS CARPIO

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ABSTRACT

Conspacifics release chemicals called pheromones, which are known to stimulate spawning behavior such as eliciting courting, sexual attraction, and spawning readiness. (intraspecific communication) In the current investigation, the fish received weekly injections of 1.5 ml/kg body weight of pheromones for a period of six weeks to the fish, *Cyprinus carpio*. It has been observed that pheromone showed stimulatory effect which stimulates the sexual behavior and gonadotrophs were increased in number. Consequently, it was determined that pheromone may be utilized as a stimulant in fish breeding in order to attain early maturity and is associated with induction of spawning reflexes in the fish *Cyprinus carpio*.

INTRODUCTION

Chemical signals that conspecifics release are known as pheromones. Pheromones are known to induce spawning behavior including stimulation of spawning leading into spawning in teleost fishes. Releasing pheromones induce a rapid behavioral. Usually, the central nervous system mediates the recipients' response. Indeed, recent research indicates that olfaction—whether conscious or unconscious—may be important for reproduction. (Karl Grammer *et al.*, 2005). According to Ulrich Boehm *et al.* (2005), several fish species seem to have evolved to be able to discriminate between the odors of mature male and female conspecifics well before they spawn. Many teleost fish react to conspecific odors in the leadup to a spawning during spawning by speeding up gonadal growth and/or changing their hormone levels, which causes ultimate gamete maturation. (Stacey and Sorensen, 2002; Davidson *et al.*, 2008). Recently, Peter Sorenson, 2010 remarked that dwelling in an aquatic habitat that is typically devoid of visual cues but abundant in dissolved substances that encourage synchronization in reproduction. Experimental work on cyprinids, gobies and catfishes provided significant evidence on the role and chemistry of pheromones. In fishes, the male three spines stickle back, *Gasterosteus aculeatus* gets stimulated by smelling the odors of its own nest during the breeding season. Researchers have found that sex pheromone is present in the perspiration of opposite sexes that stimulate the sexual courtship. The ovarian cycle is connected to the thermal effect that male sex pheromone causes in females. (Andrea Mazzatenta *et al.*, 2010)

and the mood of women was surprisingly improved by male sweating. The gonadal, pituitary, and brain axis has been studied by Finn-Arne Weltzien *et al.*, (2004); Adams B.A. *et al.*, (2002); Alok D *et al.*, (2000); Podhorec, P. and Kouril, J. (2009); Arabaci. M. *et al.*, (2004); Chen C.C. and Fernald R.D. (2008); Glasser F. *et al.*, (2004); Kourilet *et al.*, (2006, 2008); Mananoset *et al.*, (2009); Mylonas C. C. and Zohar Y. (2007). Age, composition, growth and reproduction of *Cyprinus carpio* has been studied by Tempero, G.W. *et al.*, (2006); Brown P. *et al.*, (2005); Sivakumaran K.P. *et al.*, (2003). Thus pheromonal efficacy on pituitary gland through neuroetho- hypophysio-gonadal pathway regarding reproduction in the fish, *Cyprinus carpio* (L.) are meager. The current study represents an effort in this regard to evaluate the suitability of substitute inducer as a pheromone for traditional and costly established synthetic hormone products.

MATERIALS AND METHODS

After being gathered, the *Cyprinus carpio* were raised in fiberglass tanks and gradually acclimated to the laboratory environment. The six-week experiment was conducted. Groups for experiments and controls were established. For a period of six weeks, fish in the experimental groups received an intramuscular 1.5 ml pheromone injection every other day, while fish in the control groups received a 1.5 ml injection of distilled water. Fish from the experimental and control groups were sacrificed at the conclusion of the experiment to examine the histomorphological alterations in the gonads and pituitary gland.

OBSERVATIONS /RESULTS

1) CONTROL GROUP

1. **GSI:** The weight of testis and ovary was 80gm and 112 gm respectively. After 6 weeks, GSI of male and female fish was 13.11 and 17.09, respectively.

2. **Pituitary Gland:** Chromophobes, acidophils, and cyanophils made up the pituitary gland. Based on their affinity for AF and PAS-stain, cyanophils were present in the proximal part of the pars distalis. The morphology of these cyanophil cells was spindle-shaped or angular.

3.

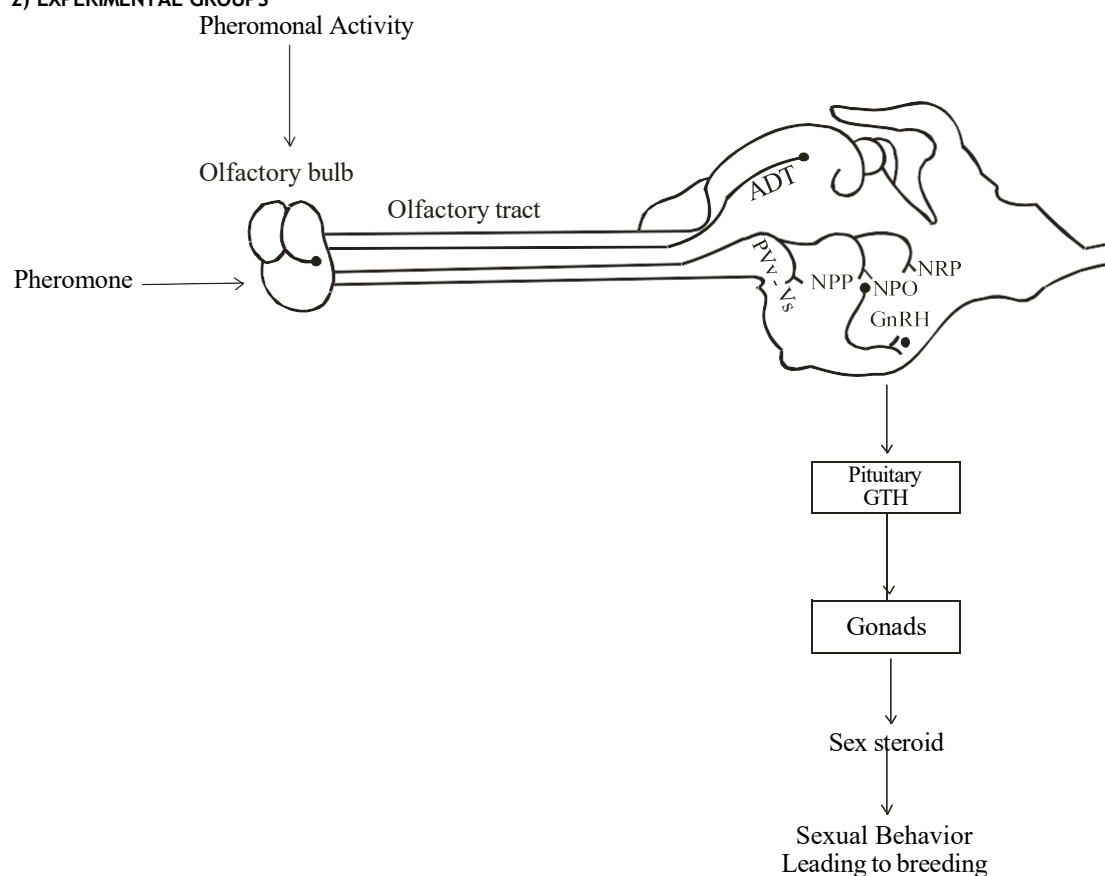
4. 3. Gonads

Ovary: Histologically, the ovary was reorganised into young oocytes, early maturing oocytes (measured), advanced maturing oocytes, and a small number of mature oocytes (prespawning oocyte) when it was in the maturing state. The ovaries had a yellowish hue. Oocyte histological examination revealed nucleus with undulating nuclear membrane and tiny, transparent yolk vesicles.

Testis: The testes were still developing and were opaque. Numerous primary, secondary, and spermatid spermatocytes were seen in one slice.

Courtship: No evidence of courtship was shown even after the control group received an injection of distilled water.

2) EXPERIMENTAL GROUPS



A diagram illustrating the proposed pathway of pheromonal action leading to behavioural and/or ovulation changes in the longitudinal section of the brain (*Cyprinus carpio*) and the pathway from the area dorsalis telencephali (ADT) to the olfactory bulb, which may regulate responsiveness to these stimuli.

1. **GSI:** Weight of testis and ovary was 75gm and 128gm, respectively. After 6 weeks, GSI of male and female was 14.56 and 22.69.

2. **Pituitary Gland:** Pituitary gland showed clear increase of orangeophil. Number of orangeophil was increased. Cyanophils were loaded with secretion.

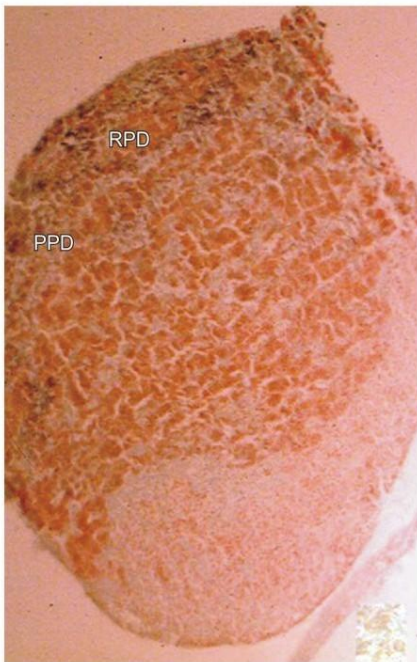
3. **Gonads:**

Ovary: The ovaries were in the prespawning phase at the end of the fifth dose. The turgid, bright yellow-colored ovaries filled the whole chamber of the body. Through the thin ovarian wall, the naked eye could discern the quantity of ova. Gushed out when eggs were pressed. Histologically, a region with transparent yolk vesicles and nucleus was observed to contain a considerable number of oocytes and mature eggs with undulated nuclear membrane.

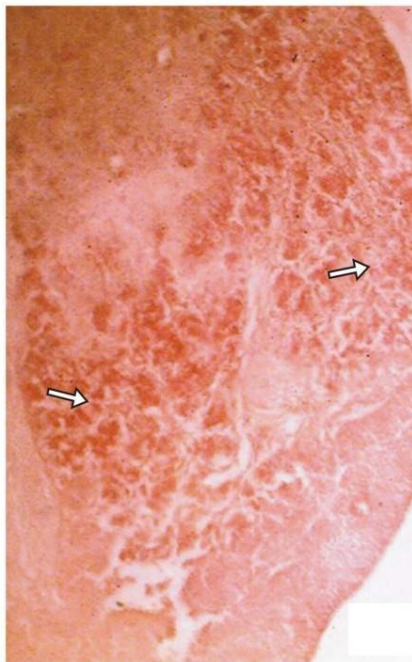
Testis: Testicular weight and volume increased significantly by the end of the fifth dose, becoming turgid. Histologically, spermatids and sperms increased the amount of seminiferous tubules. As soon as I pressed the fish's abdomen, mildew gushed out. After the 5th week, male and female were kept together (2:1).

Courtship: After 3 hrs of 2nd injection, the brooders began swimming vigorously, feeling agitated and euphoric. After four hours of the second injection, the female was pursued by the males and shoved with their snouts, leading to spawning.

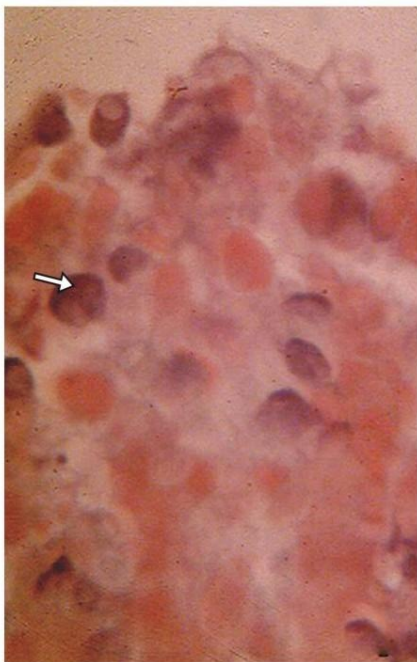
Area ventralis telencephali (PV), nucleus preopticus (NPO), nucleus reaccus posterioris (NRP), nucleus peroximal preopticus (NPP), and gonadotropin-releasing hormone (GnRH) are all present here.



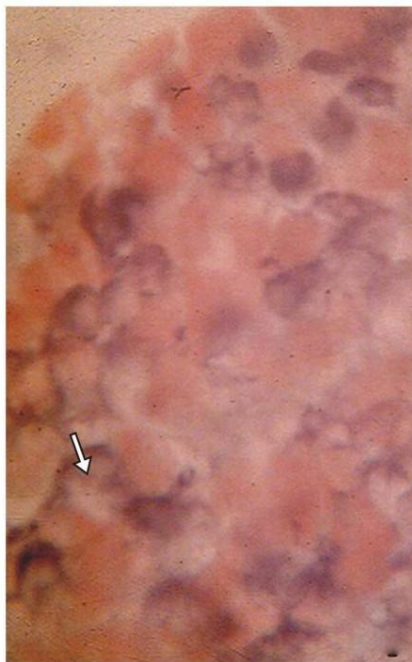
Section of pituitary gland showing rostral pars distalis, proximal pars distalis. AF stain. X10.♂



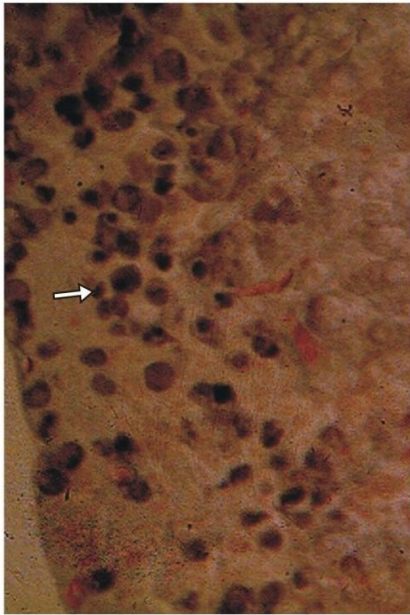
:Section of pituitary gland showing dorsal (→) and ventral proximal pars distalis (→). X 10♀



:Pituitary gland showing turgid cyanophils. (→) AF stain. X100.♂



:Pituitary gland showing completely discharged gonadotrophs (→) AF stain. X 100.♀



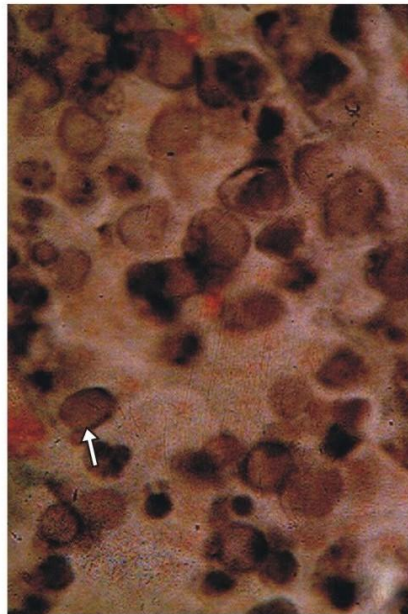
:Section showing turgid cyanophils. (→). X45.♂



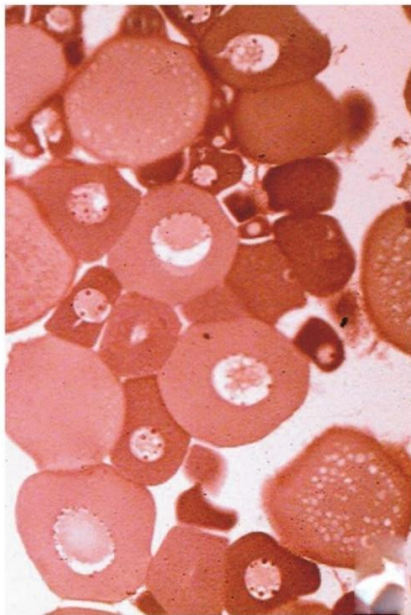
:Pituitary gland showing few turgid cyanophils (→) along with discharged cyanophils. (←). X100.♀



:Pituitary gland showing few turgid (→) and few discharged (←) gonadotrophs. X100.♂



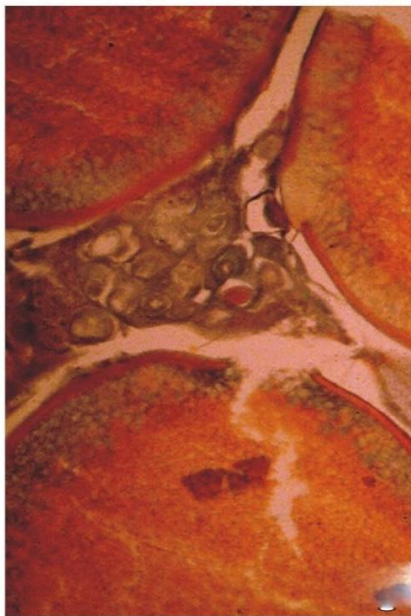
:Pituitary gland showing few discharged gonadotrophs. (→). X100.♀



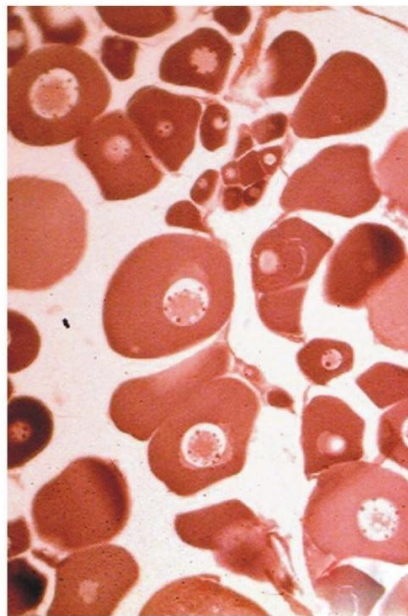
:Section of ovary showing oocytes at different stages of development including developing oocyte, Primary oocyte, Maturing oocyte, yolk vesicles, yolk granules, and atretic oocyte. X10.



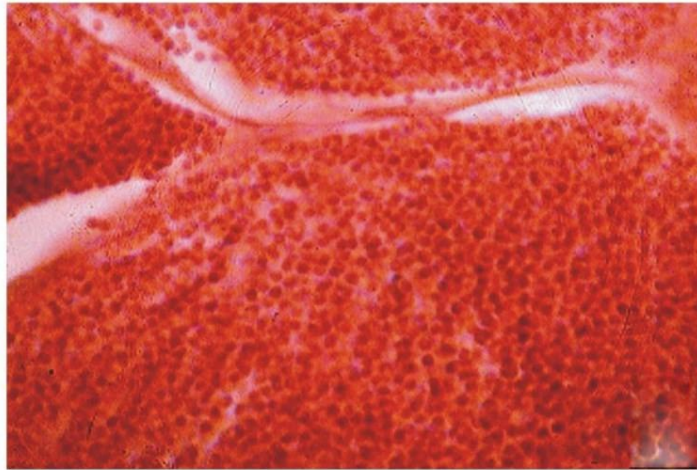
:Maturing oocyte with nucleus showing outer zona granulosa and inner zona radiata. Iron haematoxylin. X10.



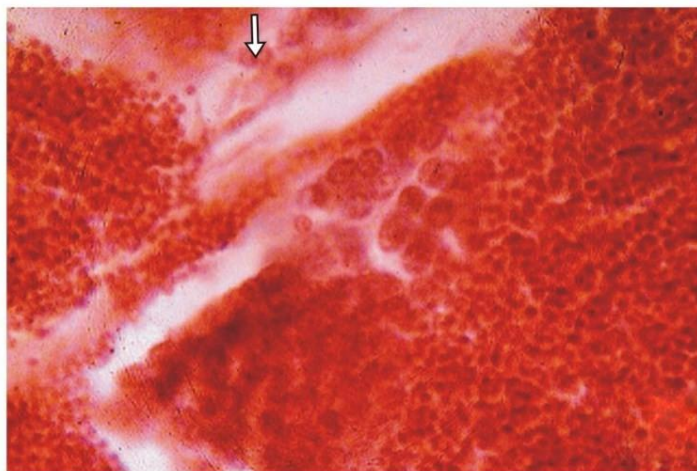
:Mature oocytes showing interstitial cells. Mallory's triple stain. X10.



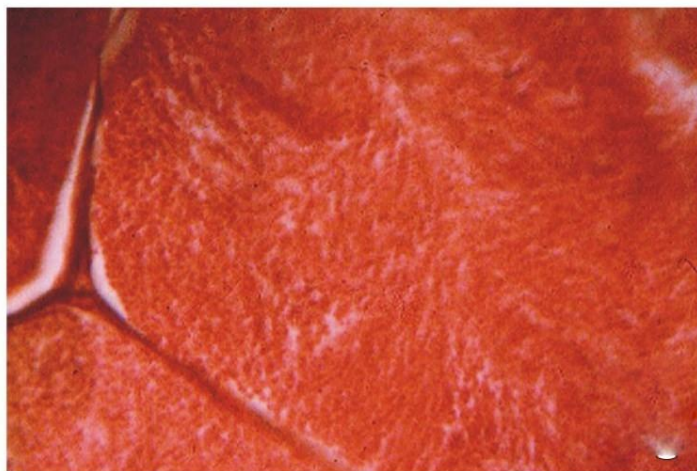
:Section of ovary showing different stages of developing oocytes. Iron haematoxylin.



:Magnified view of testis showing spermatids. Iron haematoxylin stain.



:Section of testis showing primary spermatogonia, secondary spermatogonia and spermatids, interstitial cells (→) (Stages of spermatogenesis). Iron haematoxylin stain.



:Magnified view of seminiferous tubule with intertubular septa of testis filled with spermatids and sperms. Iron haematoxylin stain.

DISCUSSION

In order to elicit the reproductive status, behaviour, and breeding performances of the same or opposite sex within one's own group, sex pheromones are essential. Sex pheromones specifically work by broadcasting reproductive maturity to other spawning individuals, which stimulates ovulation and other converted behaviours and imparts sexual attractiveness. Both internal and external variables, such as hormones, neurotransmitters, and growth factors, as well as environmental signals and pheromones, appear to govern adult neurogenesis in both vertebrates and invertebrates (Scotto Lomassese et al., 2000; Cayre et al., 2002). Numerous teleost species have been found to exhibit phenomenologically mediated spawning synchrony (reviewed by Stacey et al., 1994). Animals frequently communicate using scent, and since humans have been found to have an accessory olfactory system, there has been a significant increase in scientific interest in the possibility of human scent communication. The importance of olfactory signals in human sexual behaviour was examined and evidence of the influence of human pheromone was examined by Karl Grammer et al. in 2005. The apocrine glands in the supplementary and pubic regions are the primary human pheromone makers. Additionally, prior research has shown that female goldfish who are ovulatory produce a range of sex steroids into the water. These steroids act as a pheromonal blend that stimulates the release of male hormones, the creation of sperm, and the behaviour of humans (Stacey and Sorensen, 2002). Very few researchers have studied this line of fish. Based on their cytological characteristics, staining affinities, and distribution, up to eight distinct cell types have been discovered in the adenohypophysis of *Cyprinus carpio*; in *Anguilla*, however, a varied number of cell types, ranging from seven to nine, have been reported. Based on their disposition, staining affinities, and cytological characteristics, up to eight cell types have been identified in the adenohypophysis of *Cyprinus carpio*; in contrast, a variable number of cell types, ranging from seven to nine, were identified in Mexican (Weng et al., 2000) and ploidy level teleost fishes (Long Yu et al., 2006). According to Long Yu et al. (2006), the breeding season is when a higher proportion of gonadotropins (GTH) have been observed. The majority of researchers noted that teleosts lacked a post-ovulatory corpus luteum and that post-ovulatory follicles rapidly disintegrated and vanished (Rastogi, 1969; Lambert, 1970) and a similar condition is noticed in *Cyprinus carpio*.

While gametogenesis is in the secondary spermatocyte stage in *Cyprinus carpio*, the cysts in the majority of the lobules rupture. After that, these cells are freed to complete the remaining phases of spermatogenesis in the testicular lobules (Rai, 1967; Hyder, 1969).

SUMMARY

This study set out to assess the relationship between pituitary glands and pheromones in relation to the breeding effectiveness of *Cyprinus carpio* fish. Gonadosomatic index (GSI) measurements were made in the experimental and control groups in the current investigation. Pheromones have a stimulating impact on the pituitary glands. In particular, early maturity was caused by the gonads being overstimulated. As a result, pheromone, which is a neuroendological mechanism intimately linked to olfactory function, had a stimulatory effect on gonadal secretion in both males and females via the olfacto-hypothalamo-hypophysial route. Therefore, based on the current study's findings, it can be said that pheromone will be a useful replacement in fish breeding technology.

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