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Preliminary Study: Effect of Infrared Soft-Laser Irradiation on Gonadal Maturity Stage of Female Siamese Striped Catfish (*Pangasianodon hypophthalmus*)

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Abstract

Gonadal maturity and spawning of Siamese striped catfish depend on the rainy season, while outside of the season, hormonal treatment is not effective. Alternative technologies should be explored to enhance the gonadal maturity of fish. This study aims to examine the effect of infrared soft-laser irradiation on the gonadal maturity stage (GMS) of female Siamese striped catfish (*Pangasianodon hypophthalmus*). This study was performed through experimental methods using a completely randomized design (CRD) consisting of six treatments with four replications. Treatments were used, namely negative control (K-, no treatment), positive control (K+, injection of human chorionic gonadotropin / hCG and ovaprimTM), soft-laser exposure dose of 0.2 J/cm^2 (T1), 0.4 J/cm^2 (T2), 0.6 J/cm^2 (T3), and 0.8 J/cm^2 (T4). The parameter of GMS was measured. Data was analyzed using ANOVA following Duncan's multiple range test. The result showed that soft-laser irradiation showed significant differences ($P < 0.05$) in GMS of female Siamese striped catfish. The highest GMS was shown through soft-laser exposure dose of 0.4 J/cm2 (T2), with 98.75% of fish reaching GMS IV and 1.25% at GMS III. Infrared soft-laser irradiation dose of 0.4 J/cm2 was the optimal dose to accelerate the gonadal maturity of female Siamese striped catfish.

INTRODUCTION

Siamese striped catfish is one of the cultivated fish with high economic value. However, the spawning of Siamese striped catfish depends on the season, namely during the rainy season. In the spawning process of Siamese striped catfish, the gonadal maturity stage (GMS) plays an important role for both male and female broodstocks. The gonadal maturation process takes a long time and depends on an increase in hormones, so in this case, efforts need to be made to increase the gonadal maturation process in Siamese striped catfish to obtain broodstocks that have the

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optimal GMS to achieve annual production targets (Mukti *et al*., 2020).

In order for the spawning cycle of Siamese striped catfish to be faster, it is necessary to develop technological innovations that are applicable, efficient, and adaptive, such as manipulating external conditions, photoperiod stimuli, which directly influence the neural mechanisms that determine spawning time for fish. Hormonal manipulation is one method to accelerate gonadal maturity (Tahapari and Dewi, 2013). However, it incurs high costs.

Several methods of hormonal research to accelerate the GMS of Siamese striped catfish have been studied, including laserpuncture technology. The use of a helium-neon (He-Ne) soft laser at the reproductive acupoint of female catfish has succeeded in accelerating the GMS; however, the fecundity is low, and the egg diameter is small. He-Ne soft-laser has a power of 4-5 mW and has a light output area of 0.2 cm2 with a wavelength (λ) of 632.8 nm.

Laserpuncture is a type of photobiomodulation therapy or low-level laser therapy (LLLT) which emits light with coherent electromagnetic radiation (Nawawi and Idrus, 2008). One of the laser types that could be used is a red diode soft-laser, which has a λ of 650 nm and produces an optimal output of 20 mW, which falls within the safe range (600-950 nm) (Sulistri and Masturi, 2013). Diode soft-lasers have the following advantages, namely that they are more efficient because they can convert electrical energy into coherent radiation and the light output is directional, which has a small beam, and they have a relatively cheap price compared to other soft-lasers. Meanwhile, the disadvantage of this diode soft-laser is that the wavelength of this laser easily changes due to changes in the environment, and the beam shape is elliptical. Diode soft-lasers could be used at wavelengths ranging from ultraviolet to infrared (Nawawi and Idrus, 2008).

In this study, the soft-laser used was an infrared with λ of 700-800 nm. The price was cheaper compared to other soft-laser types (Rianti, 2013). Infrared soft-laser is one of the sources used by LLLT, because infrared light does not cause pain and does not damage surrounding tissue (Hamblin, 2009). Previous research conducted by Mukti *et al*. (2023) stated that radiation, a soft-laser at the reproductive acupoint, once a week at a dose of 0.4 J/cm^2 could stimulate gonad development. Hardjanto (2001) stated that laserpuncture irradiation has been proven to accelerate the process of growth, development, and maturation of gonads. Laserpuncture exposure can improve the endocrine, vascular, and various other systems of the body. The effectiveness of laserpuncture on gonad development has been studied in several other fish species, such as African catfish (Mantayborbir *et al*., 2013; Hariani and Kusuma, 2019; Kusuma and Hariani, 2019; Kusuma *et al*., 2022), manvis fish (*Pterophyllum scalare*) (Yolanda *et al*., 2016), and mud crab (Kusuma *et al*., 2007). This study aimed to examine the effect of infrared soft-laser irradiation on the GMS of female Siamese striped catfish.

METHODOLOGY

Ethical Approval

In this study, animal treatment and care followed ethical approval by the Scientific Committee, Institute of Research and Community Service, Universitas Airlangga, based on a Decision Letter from the Rector of Universitas Airlangga (Number 546/UN3/2023).

Place and Time

This study was conducted during the rainy season, from January to April at Instalasi Perikanan Budidaya, Mojokerto, East Java, Indonesia.

Research Materials

In this study, the materials used were female Siamese striped catfish of 600-800 g/fish body weight and GMS I. Fish were collected to Instalasi Perikanan Budidaya (IPB) Mojokerto, East Java, Indonesia. Laserpuncture tools used an infrared soft-laser with λ of 954 nm.

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Research Design

The study used a completely randomized design (CRD) and was carried out for four weeks with a frequency of infrared soft-laser irradiation once a week. This study included six treatments with four replications. Treatments of negative control (not any treatment), positive control (injection of hCG and ovaprimTM), and infrared soft-laser irradiation doses of 0.2 J/cm^2 (P1), 0.4 J/cm^2 (P2), 0.6 J/cm^2 (P3), and 0.8 J/cm^2 (P4).

Work Procedure

Selection of Broodstock Candidates

The test animals used were female Siamese striped catfish with uniform size and population, a body weight of 600-800 g/fish and GMS I, consisting of negative controls, positive controls, and four treatments of infrared soft-laser irradiation. Hamid *et al*. (2009) stated that the striped catfish, which has GMS I, had an abdomen that was not soft and slightly hardened, did not look fat or a little big, which indicates that the egg volume was still small. Apart from that, the genitals were not red, so the fish were not ready to spawn.

Laserpuncture Irradiation

Fish were taken from the pond, and the reproductive acupoints were detected using an electroacupuncture device on the ventral 2/3 of the Siamese striped catfish. Once the right acupoint was detected, then laserpuncture exposure was performed using an infrared soft-laser with λ of 954 nm with different treatment doses of 0.2, 0.4, 0.6, and 0.8 J/cm^2.

Injection of Hormones

In week 4 of this study, hormones of human chorionic gonadotropin (hCG) and ovaprimTM were injected intramuscularly into positive control females. After 24 h of hCG treatment, ovaprimTM was injected.

Determination of GMS

GMS of female striped catfish were determined at the end of the study, namely week 5, on positive control (+), negative control (-), and those treated with infrared soft-laser irradiation (doses of 0.2, 0.4, 0.6, and 0.8 J/cm^2). Gonad development was determined morphologically through dissection using surgical tools and removed from the fish's body. GMS was assessed based on the morphological characteristics, such as shape, size, length, weight, color, and development of eggs in the gonads.

Data Analysis

Data were analyzed using analysis of variance (ANOVA) to determine the effect of the treatments. If the results were shown to be significant, the calculation was continued with Duncan's multiple range test to determine the differences between treatments using a confidence level of 0.05.

RESULTS AND DISCUSSIONS

In this study, the GMS of female Siamese striped catfish can be seen in Table 1. Laserpuncture exposure in the ventral 2/3 of the body (governor vessel) influenced the gonad development of female Siamese striped catfish. The results showed a trend in GMS progression with increasing doses of infrared soft-laser irradiation, with the optimal dose being 0.4 J/cm^2, while a dose exceeding 0.4 J/cm^2 causes a slowdown in the gonad development of female Siamese striped catfish.

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Table 1. The GMS of female Siamese striped catfish.

Treatment	Percentages of GMS (%)			
	I	II	III	IV
Negative control	97.50±2.50	2.50±2.50	0.00±0.00	0.00±0.00
Positive control	0.00±0.00	85.00±0.91	15.00±0.91	0.00±0.00
T1 (soft-laser dose of 0.2 J/cm^2)	0.00±0.00	22.50±0.29	77.50±0.29	0.00±0.00
T2 (soft-laser dose of 0.4 J/cm^2)	0.00±0.00	0.00±0.00	1.25±0.25	98.75±0.25
T3 (soft-laser dose of 0.6 J/cm^2)	0.00±0.00	77.50±0.29	22.50±0.29	0.00±0.00
T4 (soft-laser dose of 0.8 J/cm^2)	96.25±0.48	3.75±0.48	0.00±0.00	0.00±0.00

Note: GMS = gonadal maturity stage, GMS I = immature, GMS II = immature, GMS III = late mature, and GMS IV = early mature.

Gonadal maturity is a certain stage of gonad development before and after spawning. During the reproductive process, some energy is allocated for gonad development. The weight of the fish's gonads will reach a maximum just as the fish are about to spawn, then will decrease rapidly as long as the spawning process continues until it is finished. Factors that influence the maturation process of the broodstock's gonads are internal factors (types of fish and hormones) and external factors (temperature, food, and light intensity).

This study shows that the irradiation of infrared soft-laser at the reproductive acupoint could influence the gonad development of female Siamese striped catfish. A similar study on female fish shows that the use of laserpuncture exposure can affect the GMS of female catfish (Mukti *et al*., 2023). Previous studies on other fish species, such as African catfish, irradiation with soft-laser also had a significant effect on the gonadal maturation of fish (Kusuma and Hariani, 2019; Kusuma *et al*., 2022). Usually, in the transition season, the gonadal maturation process of female catfish up to GMS IV is difficult to achieve, and as a result, in that season, female striped catfish usually do not spawn. This proves that the use of laserpuncture exposure is not influence by the season and can be used at any time to accelerate the gonadal maturation of female striped catfish.

This study showed results again that an infrared soft-laser irradiation accelerated the reproductive cycle of female striped catfish compared to control groups (negative and positive controls). This is because a soft-laser exposure at the reproductive acupoint can speed up spawning. At the reproductive acupoint (governor vessel), many active cells are found that have low resistance and high potential. This means that if the cell receives biostimulation from the laser, the cell will experience a process of cellular polarization, ion regulation, and a reaction to form adenosine triphosphate (ATP). ATP will be distributed intracellularly and ultimately causes changes in the potential of other active cells to the target cells (gonads) in the form of energy (Karu, 1988; Alexandratou *et al*., 2002).

Next, ATP stimulates the adenylcyclase enzyme; adenylcyclase catalyzes the formation of the adenosine monophosphate (cAMP) cycle. cAMP will diffuse in the cytoplasm and will combine with intracellular receptors to activate a special enzyme (protein kinase). This enzyme will stimulate mitochondria in the tubular cristae to encourage the conversion of cholesterol into gonadotropin hormone (GtH). This hormone consists of GtH I and GtH II from the anterior pituitary. GtH I stimulate ovarian follicles to produce estradiol-17ß and induce hepatocyte cells to synthesize and secrete vitellogenin for the development of oocytes in the ovaries, so that the gonads develop. This can be followed by an increase in the gonadal maturity index (GMI) value. The GtH II plays a role in stimulating ovarian follicles to produce 17α, 20ß progesterone. This hormone helps oocyte maturation (Kusuma *et al*., 2012; Kusuma and Hariani, 2019).

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CONCLUSION

Infrared soft-laser irradiation at a dose of 0.4 J/cm² was the optimal dose for accelerating the gonadal maturity stage of female Siamese striped catfish.

CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTION

FP collected the materials, performed the experiment, measured parameters, analyzed the data, and wrote the manuscript draft; ATM designed the experiment and analyzed the data, supervised the field treatment, edited and corrected the manuscript; ASM supervised the field experiment and corrected the manuscript draft. All authors read and approved the final manuscript.

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