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Photobiomodulation effect of infra-red laser on the level of gonad maturity in the Simese Catfish (*Pangasianodon hypophthalmus*)

Suryani Dyah Astuti [a,*], Amalia Rizky Febriastri [b], Akhmad Taufiq Mukti [c], Ahmad Khalil Yaqubi [d], Yunus Susilo [e], Ardiansyah Syahrom [f]

[a] *Department of Physics, Faculty of Sciences and Technology, Airlangga University, 60115, Surabaya, Indonesia*
[b] *Faculty of Sciences and Technology, Airlangga University, 60115, Surabaya, Indonesia*
[c] *Department of Fish Health and Aquaculture Management, Faculty of Fisheries and Marine Affairs, Airlangga University, 60115, Surabaya, Indonesia*
[d] *Doctoral Program Faculty of Science and Technology, Airlangga University, 60115, Surabaya, Indonesia*
[e] *Faculty of Engineering, Dr Soetomo University, 60118, Surabaya, Indonesia*
[f] *Department of Applied Mechanics and Design, Faculty of Mechanical Engineering, Universiti Teknologi Malaysia, 81310, Johor Bahru, Malaysia*

A R T I C L E I N F O

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Diode laser
Therspeutic laser
Gonads maturation

A B S T R A C T

The purpose of this study is to determine how photo biomodulation therapy utilizing infrared diode laser irradiation (975.2 nm) affects the gonadal maturity level (GML) of male Siamese catfish (Pan-gasianodon hypothalamus). The interest in applying laser therapy in medicine and dentistry has remarkably increased in the last decade. Different types of lasers are available, and their usage is well-defined by different parameters, such as wavelength, energy density, power output, duration of radiation, power density and radiation mode. Infrared diode laser irradiation is used at the reproductive point (governor's vessel), situated 2/3 of the way between the anus and the pectoral fin. This study examined the metrics GML, gonads somatic index, and hep-atosomatic index. The treatments were Control+ (ovaprim), Control- (without the treatment), P1 (0.2 J/cm^2), P2 (0.4 J/cm^2), P3 (0.6 J/cm^2), and P4 (0.8 J/cm^2). Therapy with infrared diode laser irradiation can modify gonad maturity (GML), gonadosomal index, and hepatosomatic index in male Siamese catfish. The photobiomodulation effect of an infrared laser stimulated the gonadal maturation of Siamese catfish. This is based on the values of wavelength (nm), power (mW), beam area (cm^2), time (s), radiation mode (rad) and energy dose (J/cm^2) in Control- (no treatment), control+ (ovaprim), P1, P2, P3, and P4. The increase in the observed parameter values is due to the vitellogenesis process. The fish gonads at the GML IV had the highest GML at P2 (dose 0.4 J/cm^2), with a GSI value of 1.02% and an HSI value of 1.46%. According to the study's findings, photo biomodulation therapy with infrared diode laser exposure at a dose of 0.4 J/cm^2 is the best way to increase the gonad maturity of male Siamese catfish.

1. Introduction

The Siamese catfish is one of the freshwater fish that can reproduce. Siamese catfish is very well-liked by consumers since it is inexpensive, has a good flavour, has a high protein content, and has low cholesterol [1]. The benefits of this Siamese catfish have led to

* Corresponding author.
E-mail address: suryanidyah@fst.unair.ac.id (S.D. Astuti).

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increased production to fulfil consumer demand. Quality seeds are obtained through brood-stock management to satisfy the rising demand for catfish. Because it is challenging to find broodstock in hatcheries that are ready to spawn, customer demand for catfish still needs to be met [2]. Seasonal factors affect when Siamese catfish spawn, with the rainy season being when catfish naturally reproduce. For both male and female catfish brood stock, the gonad maturity stage is crucial to the Siamese catfish spawning process. It is necessary to make efforts to speed up the gonadal maturation process in Siamese catfish in order to obtain broodstock with the ideal level of gonadal maturity to meet the annual production target because the catfish gonad maturation process takes a long time and is dependent on increased hormones. Siamese catfish can have their spawning accelerated by hormones. However, using hormones is expensive, and using them in fish results in pollution since they leave residues in the water and at the bottom of the pond. This makes using hormones unfavourable to the ecosystem [3].

Based on this, technology is necessary to meet the demand for Siamese catfish with faster growth and more mature gonads. The Siamese catfish's development may be sped up using photobiomodulation therapy, often known as low-level laser therapy (LLLT). A laser is a light source that produces an electromagnetic beam with a narrow focus. One of the various types of lasers is the diode laser. A semiconductor element is a coherent, monochromatic laser diode. Due to their smaller beam size, diode lasers have the benefit of being more effective at converting electrical energy into coherent radiation. They also produce collimated light [4]. Due to its advantages, diode lasers are the most popular laser type and are employed in a wide range of applications, including photodynamic treatment (PDT) in the medical field [5] and photobiomodulation therapy (PBMT) [6]. PBM or LLLT is the application of a low-level laser (class IV) with an output power of less than 500 mW to the biological systems of people and animals to promote tissue healing, reduce inflammation, and relieve pain [7]. One advantage of tissue repair is the biological process that can boost the synthesis of hormones and enzymes [8]. Both human and animal reproductive systems are biologically affected by LLLT, including those of aquatic animals like fish [9]. LLLT employs low-power lasers in the visible (400–700 nm) and infrared (700–1000 nm) wavelength ranges [10]. The source employed in this study was infrared light with a wavelength of 975.2 nm. Infrared light is one of the sources utilized in low-level laser therapy since it is painless and does not harm nearby tissue [11].

The red and infrared wavelength ranges have the most significant penetration depth in the network at the optical window [12]. Photons emitted into the tissue by infrared diode lasers cause two interactions, namely absorption, and scattering interactions. The chromophore absorbs the absorbed photons; the main tissue chromophores are water, oxyhemoglobin (HbO_2), deoxyhemoglobin, melanin, and cytochromes. Endogenous photosensitizers include the chromophore oxyhemoglobin (HbO_2) and water [13]. Infrared light penetrates the tissue to a depth of 5–7 mm, allowing it to reach the subcutaneous tissue (hypodermis), where it can directly affect capillary blood vessels, lymph vessels, nerve endings, and other tissues beneath the skin [14].

The reproductive point, 2/3 of the distance between the anus and the pectoral fin, was exposed to an infrared diode laser. This exposure demonstrates that it can stimulate active cells during reproduction. These active cells will go through a process of cellular polarization, ion regulation, and a reaction for converting adenosine triphosphate (ATP) from the mitochondria into electrical energy in the form of a flow of electrons that are then distributed intracellularly and will then experience changes in the potential of other active cells in the form of energy [15]. After photobiomodulation therapy at the reproductive point, energy formation is linked to specific proteins in cells and has a direct impact on increasing metabolic processes for target cell development. Depending on the therapist's diagnosis, the dose of photobiomodulation therapy using the given infrared light varies. In addition to distance, the intensity of infrared rays influences environmental temperature. The faster the heat is produced, the higher the intensity of the irradiation, and vice versa [16].

Previous research has shown that laser induction at the reproductive point in the ventral 2/3 of the body once a week with a dose of 0.4 J/cm^2 or 0.44 p. J/cm^2 and 0.1 E has been shown to stimulate gonadal development. The optimal dose for increasing the gonads of male Siamese catfish to TKG IV with red light and output power of 20 mW at the reproductive point with a dose of 0.5 J/cm^2 or 0.55 p. J/cm^2 and 0.12 E [6]. This study aims to determine the effect and optimal dose of photobiomodulation therapy by infrared diode laser (975.2 nm) exposure on gonad maturity level in male Siamese catfish (Pangasianodon hypophthalmus). Infrared diode laser irradiation is used at the reproductive point (governor's vessel), 2/3 of the distance between the anus and the pectoral fin. TKG, gonadosomatic index, and hepatosomatic index were the parameters studied in this study.

2. Method

2.1. Time and place of research

This research was done in several places, including the Aquaculture Installation (IPB) in Mojokerto for photobiomodulation therapy in experimental animals, the Pathology Laboratory, Faculty of Veterinary Medicine, Airlangga University for the preparation of gonadal organs, and the Pathology Laboratory, Faculty of Veterinary Medicine, Airlangga University to characterize the infrared diode laser light source. The study took place over around 5 months.

2.2. Test animals and materials

Infrared diode laser light sources with the following specifications were used in this study: 975.2 nm wavelength, 4.36 mW power, 0.19 cm^2 laser beam area, and 28.8 °C laser output temperature a series of instrument performance tests for lasers, surgical scissors, digital scales, hanging scales, rags, sample bottles, and cameras; additionally, experimental Siamese catfish (*Pangasianodon hypophthalmus*) with up to 24 tails of male sex, ovaprim hormone 0.2 ml/kg, and buffer neutral formalin (BNF) with 9, 18, 26 and 36 s time were used.

2.3. Treatments

Male catfish were exposed to infrared diode lasers over four weeks, one laser every week, to induce ovaprim hormone induction as a positive and negative control. The method employed is a completely randomized experimental model (CRD) with two treatment groups: laser-exposed and untreated samples. Catfish Siamese males are exposed to infrared diode lasers at the site of reproduction, which is positioned at 2/3 of the ventral body and may be determined using a ruler from the anus up to the pectoral fins. For each male Siamese catfish exposed to the laser, dosage values of 0.2 J/cm^2, 0.4 J/cm^2, 0.6 J/cm^2, and 0.8 J/cm^2 were employed. The infrared diode lasers object exposure time was calculated based on laser power and laser beam area. After laser exposure, Mojokerto performs catfish dissection utilizing surgical techniques once weekly for four weeks. The parameters assessed involved evaluating gonad maturity levels in both the control and treatment groups through macroscopic observations, which included factors such as coloration, body shape, body length, and body weight.

Fig. 1 details the laser exposure scheme applied to an object.

2.4. Morphology and histology of gonadal maturity, GSI, and HSI 132

Following the raising process, fish were sedated with MS222 at a dosage of 100 ppm for 10 min. The 134 fish had their abdomens dissected from anal to ventral. One hundred thirty-five mature specimens undergoing histological and morphological investigation were all observed in shape, length, weight, color, and gonadal development. Gonad sample 137 was produced following Junqueira and Carneiro's guidelines to test the GSI and HSI.

2.5. Data analysis

The data analyzed were from SPSS version 23.0 for Windows and the Kruskal-Wallis to determine whether there was a difference between treatments for gonadal maturity level parameters; if there were significant results, further tests were performed using the Mann-Whitney test. The parameters of the gonadosomal index and hepatosomal hepatic index are compared using the one-way ANOVA test, and the data is said to differ if the significance value is greater than $p < 0.05$. Using the Tukey method post hoc test; we can determine which treatment has the greatest effect on infrared laser exposure in mice.

3. Result

3.1. Diode laser beam characterization

A continuous output infrared diode laser with a wavelength of 975.2 nm and a power of 4.36 mW was used in this study. An infrared diode laser is the light source for photobiomodulation therapy in the Siamese catfish's reproductive organs. Prior to perborate treatment, an infrared diode laser characterization test is necessary. Measurements made during the laser characterization tests included wavelength, laser power vs time and distance, temperature, and the infrared diode laser's output beam.

The results of laser beam characterization show that at a distance of 1 cm the laser has a value of $dx_0 = 0.72 \pm 0.01$ cm and $dy_0 = 0.34 \pm 0.01$ cm, resulting in a beam area of $A = 0.19 \pm 0.01$ cm^2. Table 1 shows the infrared diode laser characterization.

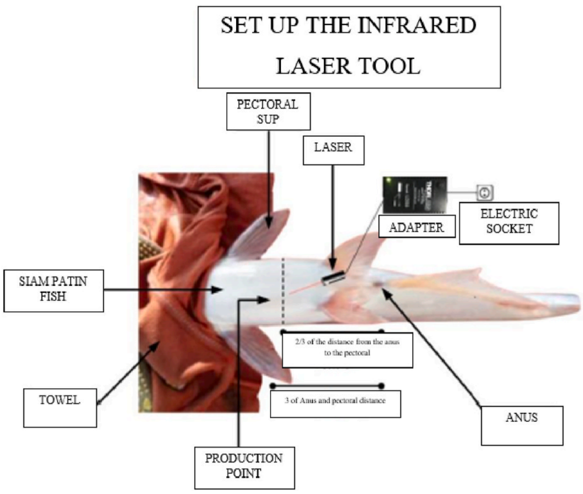

Fig. 1. Laser exposure scheme on an object.

3.2. Gonad maturity level

Infrared exposure for photobiomodulation therapy in Siamese catfish at the reproductive point on the ventral 2/3 of the body affects gonadal development. Statistical analysis was conducted to determine the effect of exposure on gonadal maturity level and the optimal dose of exposure. Kruskal Wallis test showed a significant difference in the gonad maturity level ($P = 0.01$) between treatments based on the gonadal maturity level of male Siamese catfish. Male Siamese catfish exposed to an infrared diode laser at a dose of 0.41 J/cm^2 showed a good effect, allowing the gonads to reach GML IV, in contrast, the lowest gonad maturity level was without diode laser exposure in the control (−) treatment.

Infrared hormone induction with or without ovaprim had no significant effect, and the gonads only reached GML I. The results of gonadal morphology in positive controls, namely treatment with ovaprim hormone induction, resulted in GML II. Infrared diode laser exposure resulted in GML III at an energy dose of 0.20 J/cm^2 (P1), GML IV at an energy dose of 0.41 J/cm^2 (P2) and 0.61 J/cm^2 (P3), and GML II at doses of 0.82 J/cm^2 (P4). Table 2 shows the results of the gonad maturity of male Siamese catfish.

The Lwanga and Lemeshow formula in the equation may be used to calculate the number of sample replications:

$$n = \frac{2\sigma^2 \left(Z_{1-\frac{\alpha}{2}+} + Z_{1-\beta} \right)^2}{(\mu_1 - \mu_2)}$$

The Mann-Whitney test revealed that the negative control treatment did not differ from the positive control statistically significantly ($P > 0.05$). The infrared diode laser exposure treatments P1, P2, P3, and P4 substantially differed from the negative control treatment ($P > 0.05$). The P4 treatment and the P1, P2, and P3 infrared diode laser treatments did not differ from the positive control treatment considerably ($P > 0.05$) or significantly ($P > 0.05$), respectively. Fig. 2 visually represents the male Siamese catfish's gonad shape under different treatment scenarios. Treatment-free Control Group (Control -): As a point of comparison, this panel displays the standard gonad morphology of male Siamese catfish without special treatment. Treatment with Ovaprim Induction (Control +): The gonad morphology is shown after ovaprim induction is applied. Ovaprim induction is a frequently used technique in aquaculture to induce fish spawning. Exposure to Infrared Diode Laser with a particular Energy dosage of 0.20 J/cm^2 (P1): This panel shows how the Siamese Catfish's gonad morphology is affected by an infrared diode laser with a particular energy dosage of 0.20 J/cm^2. This therapy condition investigates the possible consequences of low-intensity laser exposure. 0.41 J/cm^2 Laser Energy dosage (P2): This second laser treatment condition uses a greater energy dosage of 0.41 J/cm^2, which sheds light on how a higher energy level can affect the male Siamese catfish's gonad structure. 0.61 J/cm^2 Laser Energy dosage (P3): This panel displays the gonad morphology after exposure to a 0.61 J/cm^2 laser, a greater energy dosage. This condition investigates the Siamese Catfish gonads' possible dose-dependent reactions to laser therapy. 0.82 J/cm^2 Laser Energy dosage (P4): The last panel shows the gonad morphology when subjected to an even more outstanding 0.82 J/cm^2 laser energy dosage. This condition examines how more muscular laser therapy affects the gonads of male Siamese catfish.

The Kruskal-Wall test revealed significant differences between treatments in the male Siamese catfish's gonad maturity level ($P < 0.01$). Table 3 shows the histological observations of the gonad maturity level of male Siamese catfish and the average score of gonadal histology.

A control group (control -), an ovaprim induction treatment group (control +), and exposure to an infrared diode laser with varied energy doses (P1, P2, P3, and P4) are shown together with the gonad histology of male catfish under various treatment circumstances in Fig. 3 group under Control (Control -): no treatment. With no further processing, this panel depicts the typical gonad histology of a male catfish. Induction Therapy with Ovaprim (Control +): Here, ovaprim induction revealed the gonad histology. To facilitate comparison, this acts as a positive control. The gonad histopathology after exposure to an infrared diode laser with a particular energy dosage of 0.20 J/cm^2 is shown in this panel, "Exposure to Infrared Diode Laser with an Energy Dose of 0.20 J/cm^2" A greater laser energy dosage of 0.41 J/cm^2 is shown in this panel (P2), which displays the histopathology of the gonads after exposure. 3. Laser Energy Dose of 0.61 J/cm^2: In this case, the histology of the gonads is shown after exposure to an additional 0.61 J/cm^2 increase in laser energy. The histopathology of the gonads exposed to the maximum laser energy dosage of 0.82 J/cm^2 is shown in the last panel (P4).

To ascertain which treatment groups contributed to the various interpretations, the Mann-Whitney test was utilized. Based on the test results, there was no significant difference between negative control and positive control therapy ($P > 0.05$). Based on the test results, there was a significant difference in the treatment of infrared diode laser exposure P1, P2, P3, and P4 in the negative control treatment ($P < 0.05$). While the treatment of P4 with positive control was not significantly different ($P > 0.05$). P1, P2, and P3 treatments with positive controls showed significant differences ($P < 0.05$). Spermatids were formed in negative and positive controls,

Table 1
Infrared diode laser characterization test results.

Wavelength (nm)	Power (mW)	Beam area (cm^2)	Time (s)	Energy Dose (J/cm^2)
975 ± 0.05	4.36 ± 0.01	0.190 ± 0.01	9	0.20
			18	0.41
			26	0.61
			36	0.82

Table 2
Results of summary of GML statistical test for control group treatment and infrared laser diode exposure treatment group of Simese Catfish (*Pangasianodon hypophthalmus*).

Treatment	N	GML		Kruskal Wallis Test	
		Mean	SD	Significance	Result
Control (−)[a]	4	1.00	0.00	0.001	There is a significant difference
Control (+) [ab]	4	1.50	0.57		
P1 (0.20 J/cm^2)[c]	4	2.75	0.50		
P2 (0.41 J/cm^2)[d]	4	4.00	0.00		
P3 (0.61 J/cm^2)[c]	4	3.50	0.57		
P4 (0.82 J/cm^2)[b]	4	2.25	0.50		

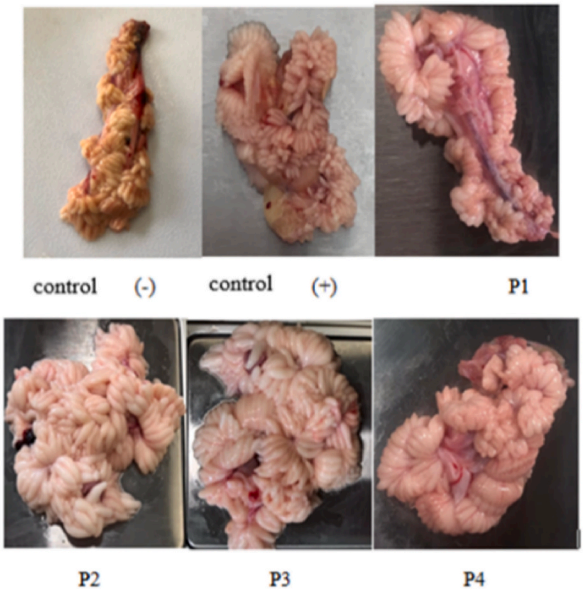

Fig. 2. Gonad morphology of male siamese catfish.

Table 3
Results of Gonadal histology statistical test for the negative, positive control group and the infrared diode laser exposure treatment group of Simese Catfish (*Pangasianodon hypophthalmus*).

Treatment	N	GML		Kruskal Wallis Test	
		Mean	SD	Significance	Result
Control (−)[a]	5	3.40	0.89	0.000	There is a significant difference
Control (+) [ab]	5	3.80	1.09		
P1 (0.20 J/cm^2)[c]	5	5.80	0.84		
P2 (0.41 J/cm^2)[d]	5	7.00	0.00		
P3 (0.61 J/cm^2)[c]	5	6.80	0.45		
P4 (0.82 J/com^2)[b]	5	4.60	0.55		

scoring 3. Infrared laser treatment P2 with an energy dose of 0.41 J/cm^2 showed a score of 7, which produced 75–100 % spermatozoa.

3.3. Gonado somatic index

Based on the calculation of the gonadosomatic index (GSI) of male Siamese catfish, it showed that photobiomodulation therapy using infrared diode lasers at various energy doses was significantly different ($p < 0.05$) from the control group. The analysis's means range between 0.34 and 1.02%. The greatest GSI value was in treating infrared laser exposure with an energy dose of 0.41 J/cm^2. Table 4 shows the information on male Siamese catfish's mean gonado somatic index (GSI).

Description: Different superscriptions in the same column show a noticeable difference (p < 0to 0.05). Fig. 4 compares the gonadosomatic index (GSI) with the histology score of Siamese Catfish (Pangasianodon hypophthalmus).

Gonad maturity is determined by the somatic index of the gonads, which is a measure of the amount of sperm in the fish's gonads.

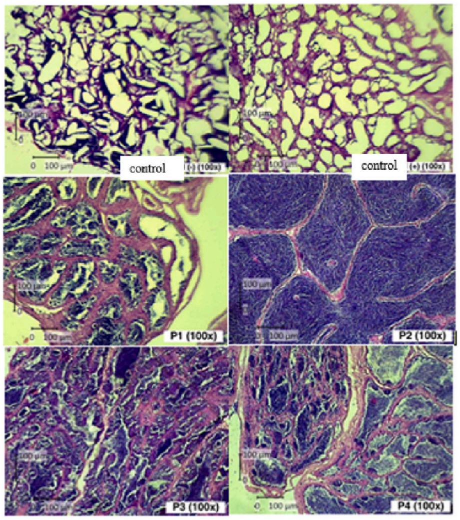

Fig. 3. Comparative gonad histology of male catfish under varied treatment conditions.

Table 4
The average value of gonadosomatic index (GSI) of Male Simese Catfish (*Pangasianodon hypophthalmus*).

Treatment	N	Gonadosomatic Index (%)		One-Way ANOVA Test	
		Mean	SD	Significance	Result
Control (−)[a]	4	0.34	0.04	0.000	There is a significant difference
Control (+)[b]	4	0.54	0.02		
P1 (0.20 J/cm^2)[c]	4	0.71	0.03		
P2 (0.41 J/cm^2)[e]	4	1.02	0.03		
P3 (0.61 J/cm^2)[e]	4	0.94	0.01		
P4 (0.82 J/cm^2)[d]	4	0.67	0.04		

The effective dose of infrared diode laser exposure and the impact of laser exposure on gonadal maturity level were determined using statistical analysis.

Based on the results of the one-way ANOVA test, it is known that there is a significant difference between each treatment group, with a significance result of $p = 0.000$. Tukey's test determined which treatment group produced a significant difference. The test findings showed that when compared with the other irradiation groups, an irradiation dose of 0.41 J/cm^2 yielded substantial results. Gonado somatic index increased by 1.02% with an infrared diode laser exposure time of 17.63 s and a dose of 0.41 J/cm^2.

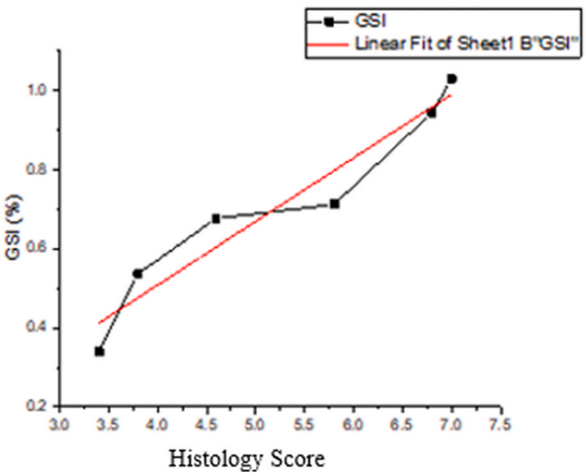

Fig. 4. Comparison of gonadosomatic index (GSI) with histology score of simese catfish (*Pangasianodon hypophthalmus*).

3.4. Hepato somatic index

The photobiomodulation therapy treatment utilizing an infrared diode laser was significantly different ($p < 0.05$), according to the examination of the hepatosomatic index (HSI) of male Siamese catfish. The range of the analysis's average value was between 1.13% and 1.46%. Table 5 provides information on the average hepatosomatic index (HSI) of male Siamese catfish.

Description: Different superscripts in the same column show a noticeable difference ($p < 0.05$). Fig. 5 illustrates the comparison between hepatosomatic index (HSI) and histological score.

Increased liver activity in the generation of vitellogenin results in an increase in the quantity of sperm in fish gonads. The liver's vitellogenin synthesis is normally linked to the female fish's reproductive cycle and is not directly tied to the male fish's generation of sperm. However, the general function and condition of the liver might indirectly affect fish reproduction. Infrared diode laser exposure affects the gonad maturity level. A one-way ANOVA test was used for the hepatosomatic index, assuming that the data were normally distributed.

Based on the results of the one-way ANOVA test, it is known that there is a significant difference between each treatment group ($p = 0.000$). Tukey's test determined which treatment group produced a significant difference. The test findings showed that when compared with the other irradiation groups, an irradiation dose of 0.41 J/cm^2 yielded substantial results. The hepatosomatic index value increased by 1.46% with an infrared diode laser exposure time of 17.63 s and a dose of 0.41 J/cm^2.

4. Discussion

The study was carried out to determine the effect of infrared diode laser exposure on gonadal maturity level in male Siamese catfish and the optimal radiation dose for photobiomodulation therapy. Because of its advantages, such as being monochromatic, coherent, and having a directional beam, the diode laser is one of the light sources used in the health sector. The infrared diode laser wavelength is 975 nm, with power stability over time after 250 s at a distance of 1 cm, a power of 4.36 mW, and a laser beam area of 0.19 cm^2 based on the characterization test results. To get accurate findings from research requiring laser exposure, it is crucial to maintain stable settings. It is possible to guarantee that the laser energy hitting the target is constant across all experimental participants by establishing a fixed distance of 1 cm between the laser and the target. Contrarily, the distance in contact mode may not be constant, resulting in fluctuations in the laser energy used. The laser output beam has a temperature stability of 28.8 °C, close to room temperature. A temperature of 28.8 °C generated by exposure to an infrared diode laser has no biological effects. As a result, there is no photothermal interaction in this photobiomodulation therapy because photobiomodulation therapy is a therapy that employs a low-power diode laser [17].

One of the determining stages in a male's development is the level of gonadal maturity, which determines whether or not the egg will be fertilized [18]. Photobiomodulation therapy with diode laser exposure stimulates tissues for growth, hormones, proliferation, and differentiation. PBMT is a therapy that uses a low-power laser to avoid damaging the affected tissue. Photobiomodulation therapy can stimulate tissue growth and development at the appropriate energy dose [19].

When fired, A photon collides with a tissue, causing absorption, reflection, and scattering. To maximize the absorption effect on the tissue, the laser diode is perpendicular to the reproduction point to avoid reflection and scattering of laser diode light [17]. A chromophore is a part of the tissue that can absorb laser light energy. Melanin, hemoglobin, and water are the primary chromophores in the skin. The absorbed wavelength is primarily between 600 and 1200 nm, referred to as the optical window [20]. The infrared diode laser used for photobiomodulation therapy has a penetration depth of 5–7 mm, allowing it to penetrate the hypodermis layer and affect capillaries, lymph vessels, nerve endings, and other tissues under the skin, causing a stimulating effect [21,22].

Light exposure increased ATP levels in Cox10 KO cells but not in the control cells. ATP is the substrate for adenyl cyclase, which regulates cAMP levels. Ca^{2+} and cAMP are important hormones and regulate almost every process in the body [23,24].

Adenosine exposure causes energy production, which is then used to promote the development of target cells. It stimulates the enzyme adenyl cyclase, which functions as the formation of cyclic adenosine monophosphate (cAMP) and stimulates GABAergic neurons to synthesize GABA [25].

After acclimatization, catfish brood stock was taken, and laser puncture was induced at the reproductive point for 15 s for 4 weeks. This helium-neon (He–Ne) soft laser has a safe wavelength range for the biostimulation of biological organs [26]. For 9 weeks, dumbo catfish were taken and laser punctured at the reproductive point, precisely in the 2/3 ventral body for 15 s. $GSI = 0.465 \pm 0.0004$ [27]. According to this research, there were significant treatment differences for GSI and HSI ($p < 0.05$). Although the HSI is comparatively

Table 5
Average hepatosomatic index (HSI) of Male Simese Catfish (*Pangasianodon hypophthalmus*).

Treatment	N	Hepatosomatic Index (%)		One-Way Anova Test	
		Mean	SD	Significance	Result
Control (−)[a]	4	1.13	0.01	0.000	There is a significant difference
Control (+)[b]	4	1.25	0.03		
P1 (0.20 J/cm^2)[c]	4	1.31	0.02		
P2 (0.41 J/cm^2)[e]	4	1.47	0.02		
P3 (0.61 J/cm^2)[d]	4	1.41	0.01		
P4 (0.82 J/cm^2)[c]	4	1.31	0.01		

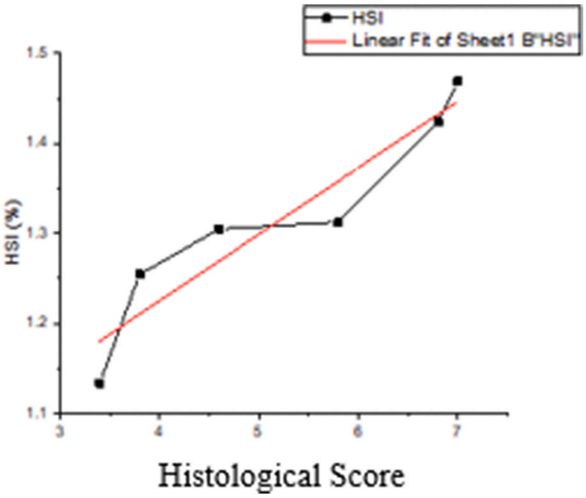

Fig. 5. Comparison of hepatosomatic index (HSI) with histological score.

not substantially different across treatments, 189, except when compared to the negative control, the laserpuncture therapy of 0.5-J had the greatest GSI and HSI compared to the 188 other treatments.

As a form of biostimulation, laser puncture is used. Siamese catfish were laser punctured in the 2/3 ventral body for 20 s four times (1 month). A control group of fish had their ventral bodies laser-punctured for 14 s twice (14 days) [6]. Laser puncture is used as a form of biostimulation in this study. It uses a He–Ne soft laser with a wavelength of 632.8 nm, an output area of 0.2 cm^2, and a laser beam output power of 4.36 mW/cm^2.

The study was conducted by using the infrared diode laser exposure method to induce ova prim hormone induction in mice. The control group was divided into 2, namely the control group (−) with untreated samples and a control group (+) with samples given ovulation-promoting hormone induction. The treatment was carried out on male Siamese catfish samples with the same origin and body weight between 550 g and 650 g. Infrared diode laser exposure is carried out at the reproductive point (governor vessel) which is 2/3 of the distance from the anus.

Ripe fish gonads can be marked by the release of sperm when stripping the fish's genital organs. This research was carried out with two times of exposure to infrared diode lasers within two weeks and 7 days of exposure. The quantity of sperm would grow as the fish's gonads matured to a larger degree, impacting both the fish's body weight and gonad weight. The pituitary produces 260 follicle-

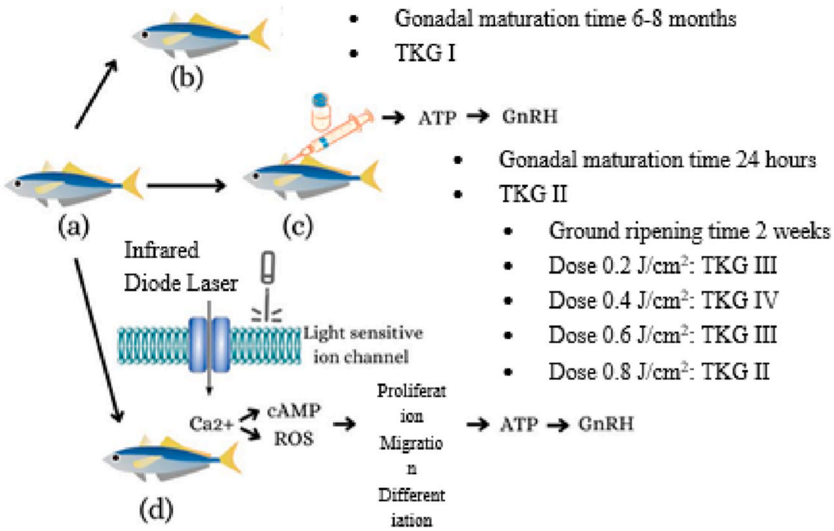

Fig. 6. Illustration of Photobiomodulation therapy Mechanism with Exposure of Infrared Diode Laser to Siamese Catfish (Pangansianodon hypophthalmus). (a) The sample divided into two groups: the control group and the treatment group. (b) The group was not given any treatment or negative control and (c) the group was given an induction treatment of the hormone ovaprim or positive control. (d) Exposure of infrared diode lasers with dose variations that provide photochemical interactions with exposed tissues and increase the production of ATP and the hormone Gonadotropin (GnRH) to accelerate gonad maturity levels.

stimulating hormone (FSH) and LH when the 259 hypothalamus is stimulated by laser acupuncture induction at the reproductive acupoint. The FSH will activate Leydig cells, which will generate testosterone. The testosterone hormone helps to promote spermatogonia's 262 division into spermatocytes.

Photobiomodulation therapy with infrared diode laser exposure at the reproductive point had different effects on the gonad development of male Siamese catfish in the treatment group. The dose that gave the best effect for increasing gonadal maturation in four doses was 0.41 J/cm^2.

When pituitary gonadotroph cells are stimulated to produce gonadotropin hormone (GtH), which is involved in spermatogenesis for the growth of spermatozoa and steroidogenesis for the production of testosterone, the hypothalamus releases gonadotropin-releasing hormone (GnRH). To regulate spermatogenesis and the production of sperm, testosterone in these granulosa cells will bind to androgen binding protein (ABP) [26]. The mechanism of photobiomodulation treatment is shown in Fig. 6.

The fertility rate that is "PBM-assisted" refers to the rate at which PBM is used as a method to improve Siamese catfish reproduction. As part of this investigation, infrared laser light was used to promote and enhance the fish's gonads' (reproductive organs) maturation. Through the stimulation of hormone synthesis, gamete formation, and general reproductive preparation, this method can improve the fish's reproductive efficiency.

The importance of this comparison lies in evaluating the efficacy of PBM in enhancing the reproductive capacity of Siamese catfish, and it includes elements like accelerated rate of gonad maturation, enhanced egg and sperm production, enhanced fertilization rates, accelerated hatching, accelerated survival rates of offspring.

Photobiomodulation therapy with infrared diode laser exposure can increase gonad development in male Siamese catfish. This occurs because male fish experience a 5–10 % increase in gonad weight during the reproductive process. The gonads will be more mature, and the sperm count will be higher [26]. It is stated that the synthesis of vitellogenin in the fish body occurs in the liver, thus increasing the value of the hepatosomatic index. The activity of this vitellogenin can cause the hepatosomatic index to rise, but it remains lower than the gonadosomatic index.

This is because high doses of exposure lead to decreased production of gonadotropin hormone production, causing the gonads to fail in the maturation process.

In this study, the optimum dose was 0.48 J/cm^2. In determining the optimum dose, that is through the equation on the polynomialgonadosomatic index regression graph, because at the optimum dose it represents mature gonads and at the optimum dose it will affect the development and weight of the gonads and the whole body of the fish and will affect the calculation analysis of the gonadosomatic index. The photobiomodulation therapy using infrared diode lasers can affect the development of gonad maturity in male Siamese catfish (*Pangasianodon hypophthtalmus*). The process of stimulating the reproductive organs can effectively increase the gonad maturity of these fish.

Previous studies have used light at various wavelengths to provide antimicrobial [28] and photobiomodulation [29] effects. With wavelength as its primary variable, photobiomodulation treatment is utilized to modify neuronal oscillations non-invasively. In the output findings of power spectrum analysis, this may enhance the intensity of the higher oscillation frequency bands, such as alpha, beta, and gamma, while decreasing the strength of the lower frequency bands, such as delta and theta. In order to stimulate or inhibit cells and biological tissue to engage in photochemical interactions, a light source in the red and infrared spectrum is utilized in photobiomodulation therapy (PBMT) or low-level laser therapy (LLLT) [30]. The light source utilized in this procedure can effectively penetrate the skin, soft tissue, and hard tissue to perform as it should without endangering the nearby tissue to which it is exposed. Suppose a low-power laser of 5 mW induces an induction dose of 0.5–1.0 J/cm^2 with a wavelength of 632.8 nm. In that case, it will improve the ability of peripheral and central nerves to regenerate, causing an increase in cellular activity and the production of hormones and enzymes. It showed that using laserpuncture might enhance the action of a hormone in regulating reproduction, resulting in a faster supply of catfish growth, development, and gonad maturity [31]. The combination of antimicrobial laser and photobiomoulation will have a cell regeneration effect and accelerate wound healing. Generally, the spectrum of light used is red and infrared. The advantage of infrared light is that it penetrates deeper into the skin, with a lower energy density, but for far infrared, the light beam is invisible. Meanwhile, the red spectrum has a shorter penetration and visible light beam, making it easier to use. In this study, laser photobiomodulation with an infrared spectrum was used to accelerate gonadal maturation in fish. This treatment aims to optimize fish farming which in turn helps the economy of fish farmers.

Based on research, photobiomodulation therapy using infrared diode laser irradiation may be a potential strategy to accelerate the maturity of the gonad organs of male Siamese catfish. Induced hormones, which may have adverse environmental impacts due to residues left in the air, can be replaced by this non-invasive technique. The results of this study need to be confirmed and improved through additional research and experiments, and this is important to mention. Further research will be carried out to determine the effect of PBM on the quality of fertility (fertilization), hatching rates, abnormalities and survival rates of the larvae produced. In conclusion, this study offers in-depth information on the possible use of PBM therapy using infrared diode lasers in the maintenance of fish reproduction.

5. Conclusion

Photobiomodulation therapy using infrared diode laser irradiation (975.2 nm) on the reproductive point of male Siamese catfish *(Pangasianodon hypophthalmus*) was shown to increase gonadal maturity. Photobiomodulation therapy with infrared diode laser exposure can affect the level of gonad maturity (GML), gonadosomatic index, and hepatosomatic index in male Siamese catfish (*Pangasianodon hypopthalmus*). The fish gonads at the GML IV stage had the highest GML at P2 (dose 0.4 J/cm^2), with a GSI value of 1.02% and an HSI value of 1.46%. Infrared diode laser exposure at an energy dose of 0.41 J/cm^2 is the optimal energy dose for photobiomodulation therapy to increase the gonad maturity of male Siamese catfish (*Pangasianodon hypophthalmus*).

Ethics statement

This study was conducted at Instalasi Perikanan Budidaya Mojokerto, Jawa Timur, Indonesia. Histological analysis of fish gonads was held at the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, EastJava, Indonesia. In this study, the experimental protocols were approved by the Scientific Committee, Institute of Research and Innovation, Universitas Airlangga (Protocol Number 251/UN3/2022).

Data availability statement

The data that supports the findings of this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Suryani Dyah Astuti: Writing – review & editing, Visualization, Validation, Supervision, Funding acquisition, Conceptualization. **Amalia Rizky Febriastri:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Akhmad Taufiq Mukti:** Software, Resources, Methodology, Data curation, Conceptualization. **Ahmad Khalil Yaqubi:** Writing – review & editing, Visualization, Validation, Software, Resources, Formal analysis, Conceptualization. **Yunus Susilo:** Visualization, Validation, Methodology, Data curation, Conceptualization. **Ardiansyah Syahrom:** Visualization, Validation, Resources, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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