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Enhancement of Gonadal Maturity in Female Sand Lobster *Panulirus homarus* Using Shortwave Radiation Beams (Laser Puncture)

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ABSTRACT
Panulirus homarus, an important export commodity, faces challenges in mastering artificial breeding techniques, with limited practical applications despite advancements in seed production. This study investigated the effects of laser exposure on gonadal maturation, gene expression (Molting-Inhibiting Hormone, MIH), and hormone levels (17β-estradiol) in *P. homarus*. A total of 50 female broodstock were acclimated for 7 days and exposed to a soft He-Ne laser for 4, 6, or 8 seconds. Gonadal maturity was assessed using the gonadal maturity level (GML) and gonadal maturity index (GMI), while MIH gene expression was quantified by quantitative PCR, and 17β-estradiol levels were measured using ELISA. Result revealed that laser exposure significantly influenced gonadal maturation and gene expression, with the 4-second exposure resulting in a 2.68-fold increase in MIH expression, and the 8-second exposure showing the highest increase at 4.21-fold. Both exposure groups exhibited advanced gonadal stages, with the 8-second exposure achieving the most mature gonads (GML 3). Serum 17β-estradiol levels were significantly higher in both the 4-second (2763.36 pg/mL) and 8-second (130.64 pg/mL) groups compared to controls. These findings suggest that laser exposure has a dose-dependent effect on gonadal maturation, MIH expression, and 17β-estradiol production. The results highlight the potential of laser technology as a biostimulant to enhance reproductive processes and optimize breeding practices in aquaculture.

INTRODUCTION

Panulirus homarus, commonly found in the coastal waters of Banyuwangi, Indonesia, is a prominent export commodity with significant economic value in the global

seafood market. However, overexploitation, driven by the collection of larvae from the wild, has led to a considerable decline in the population of this species, raising concerns about its sustainability and conservation **(Pratiwi, 2018)**. To address this, the development of effective aquaculture practices, particularly for the controlled breeding of lobster seeds, has become increasingly urgent. Ensuring the sustainability of the species requires reliable methods for preparing broodstock capable of spawning and producing mature eggs **(Petersen & Phuong, 2010)**.

Despite its importance, Indonesia faces significant challenges in mastering artificial breeding techniques for lobsters. While progress has been made in commercial seed production technologies, practical applications remain limited. Female lobsters are capable of producing large quantities of eggs, ranging from 32,269 to 142,971, with an incubation period of 3-4 weeks **(Kuslani & Sumindar, 2017)**. However, the success of hatching is highly dependent on the selection and conditioning of broodstock, as healthy and well-managed individuals are crucial for successful reproduction **(Hall *et al*., 2013)**. These broodstock are typically sourced from fishermen or live seafood markets, but their use requires careful management to ensure optimal maturity and health before breeding **(Adiputra *et al*., 2018)**.

Current approaches to enhancing gonadal maturation in lobsters have primarily involved dietary interventions, hormone treatments, and invasive techniques such as eye-stalk ablation **(Phillips & Matsuda, 2011)**. While eye-stalk ablation has proven effective in stimulating gonad development, it carries limitations, including the high cost of hormone treatments and the potential for permanent eye damage **(Mantayborbir *et al*., 2013)**. These challenges highlight the need for more efficient and non-invasive methods of gonadal maturation that do not compromise the health or well-being of the broodstock.

Recent advancements in soft laser beam technology, specifically laser puncture, have emerged as a promising non-invasive alternative to traditional methods of gonadal maturation in crustaceans. Unlike invasive techniques like eye-stalk ablation, laser puncture uses low-energy laser beams to stimulate hormonal changes and promote gonadal development without damaging the animal's physical integrity. This technology has already shown success in stimulating gonadal maturation in various species, including crabs, giant tiger prawns, vannamei shrimp, and freshwater lobsters, demonstrating its potential in aquaculture applications. The novelty of laser puncture lies in its ability to provide a cost-effective and non-invasive solution to enhance reproductive processes, offering a more sustainable approach to breeding practices in *Panulirus homarus*.

The primary objective of this study was to assess the effectiveness of soft laser beam technology (laser puncture) in inducing gonadal maturation in female *Panulirus homarus* within a short time frame. By achieving efficient gonadal maturity, this technology could facilitate sustainable lobster seed production in aquaculture, reducing reliance on wild populations and contributing to the conservation of this valuable species.

MATERIALS AND METHODS

Ethical statement

All procedures and applications conducted in this study were approved by the Ethical Commission of Universitas Brawijaya. The study adhered to the ethical guidelines established by the Animal Behavior Society.

Time and location

This study was conducted at the "Mina Bahari" Fish Farming Group, Grand Watu Dodol, Bangsring District, Banyuwangi, and at the Fish Reproduction Laboratory, Faculty of Fisheries and Marine Science, Brawijaya University, Malang, over a period of 3 months.

Experimental design

The research employed an experimental design with a completely randomized design (CRD), consisting of three treatments: laser exposure durations of 4, 6, and 8 seconds, administered once every 7 days for a period of 2 months. A control group was included, which received no soft laser beam (laser puncture) treatment. Each treatment was replicated three times.

The preparation of female sand lobster broodstock

It must be ensured that the gonadal maturity level (GML) has reached at least stage 1. The sample in this study consisted of 50 female sand lobster broodstock, with a total body length ranging from 16.2 to 23.3cm, body weight ranging from 154 to 333g, and carapace length between 7.5 and 10.1cm. These lobsters were caught in the waters of Banyuwangi and the Bali Strait. The sand lobsters were maintained for 7 days in tanks to acclimate to the breeding environment, with a diet consisting of squid or fresh fish, chopped into small pieces, comprising 3-5% of their body weight. The feed was provided once daily.

Preparation of soft laser beam (Laser puncture) equipment

The laser puncture used as a biostimulant in this study was a soft He-Ne laser, with a wavelength of 632.8 nm, a light output area of 0.2 cm^2, and a laser power output of 5 mW/cm^2, equivalent to 0.375 $Joule/cm^2$.

Soft laser beam exposure (Laser puncture)

- The laser puncture target points were located at the left eye stalk and the reproductive point (Fig. 1).

- The distance between the laser probe tip and the target surface was maintained such that the laser probe tip was perpendicular to the surface of the target area.
- The duration of laser puncture exposure was set to 4, 6, and 8 seconds by adjusting the timer until it automatically counted down to zero.

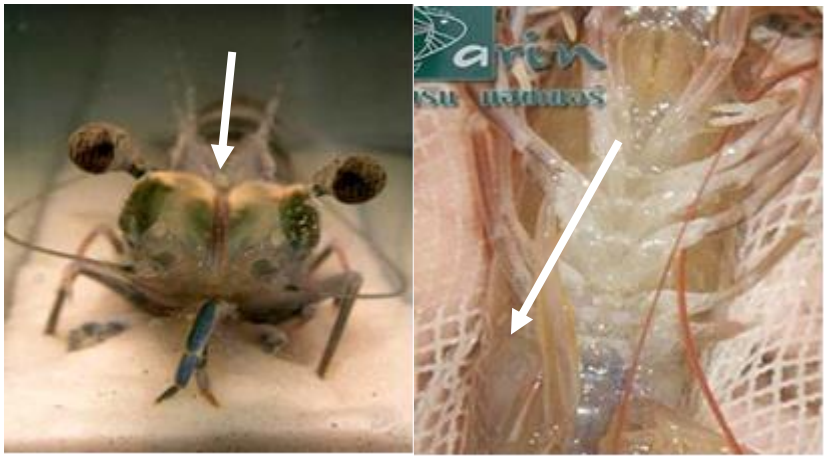

Fig. 1. (a). Eye stalk (b) Thoracic ganglion

Gonadal maturity level (GML)

The GML of female lobsters was determined following the classification in reference **(Silva & landim, 2006)**:

- **GML I**: Immature ovaries, white in color, and difficult to distinguish from the surrounding circular muscles.
- **GML II**: Premature stage, where the ovaries grow in volume and expand with a pink or light yellow color.
- **GML III**: Mature stage, where the gonads are fully developed, filling all available space in the body cavity. The gonads become more convoluted and can extend to the second segment of the abdomen, with an orange or reddish color.
- **GML IV**: Spawning or resorption stage, where the ovaries may reabsorb ovulated oocytes and other cells.

Duration to achieve GML 3

The duration to reach GML 3 refers to the time (in days) required for female sand lobsters to progress from eggless ovaries to the production of eggs with a dark orange color (GML 3) after laser puncture exposure.

Gonadal maturity index (GMI)

The GMI and egg diameter were calculated using the following formula **(Tarsim *et al.*, 2007)**:

$$GMI = \frac{Gonad\ weight}{Body\ weight}\ x\ 100\%$$

MIH gene expression

MIH gene analysis was obtained from the lobster's eye stalk, which was placed in a microtube and labeled accordingly. The eye stalk was weighed using an analytical scale until a weight of 0.05g was achieved. The following steps involved DNA extraction, using the GeneAll Kit (GeneAll Biotechnology Co., Ltd, South Korea), DNA quantification, amplification, electrophoresis, sequencing, and BLAST analysis to determine the potential effects of laser puncture on female lobsters. The primers used were Primer F MIH (5'– ATT ATA CAC TCA TGT ATC GGC TGG – 3') and Primer R MIH (5'-AGA GGC TTG TCC CAA CAA CTA CAA T -3').

17B-Estradiol Hormone

Hemolymph from the lobster was collected from the base of the pleopods near the genital opening using a 1ml syringe pre-wetted with an anticoagulant solution (EDTA 10%) in a 1:1 ratio. The Hemolymph was then tested for 17B-Estradiol hormone levels using 17B-Estradiol ELISA Kits.

Water quality

Observation of water quality parameters was conducted throughout the study period, as detailed in Table (1)

Table 1. Water quality parameters evaluated during precise period

No	Parameter	Sampling Time
1	Temperature	Daily (07:00 WIB & 16:00 WIB)
2	Salinity	Daily (07:00 WIB & 16:00 WIB)
3	Dissolved Oxygen (DO)	Daily (07:00 WIB & 16:00 WIB)
4	pH Level	Daily (07:00 WIB & 16:00 WIB)
5	Ammonia	Weekly (07:00 WIB)
6	Nitrite/Nitrate	Weekly (07:00 WIB)

Data analysis

Quantitative data for GML and GMI were analyzed using ANOVA in SPSS 26. Descriptive analysis was performed on the MIH gene expression and the anatomical changes in the ovaries, which were captured using a digital camera and processed using Adobe Photoshop CS. Water quality parameters were compared descriptively with reference data on lobster aquaculture. The research flowchart is shown in Fig. (2).

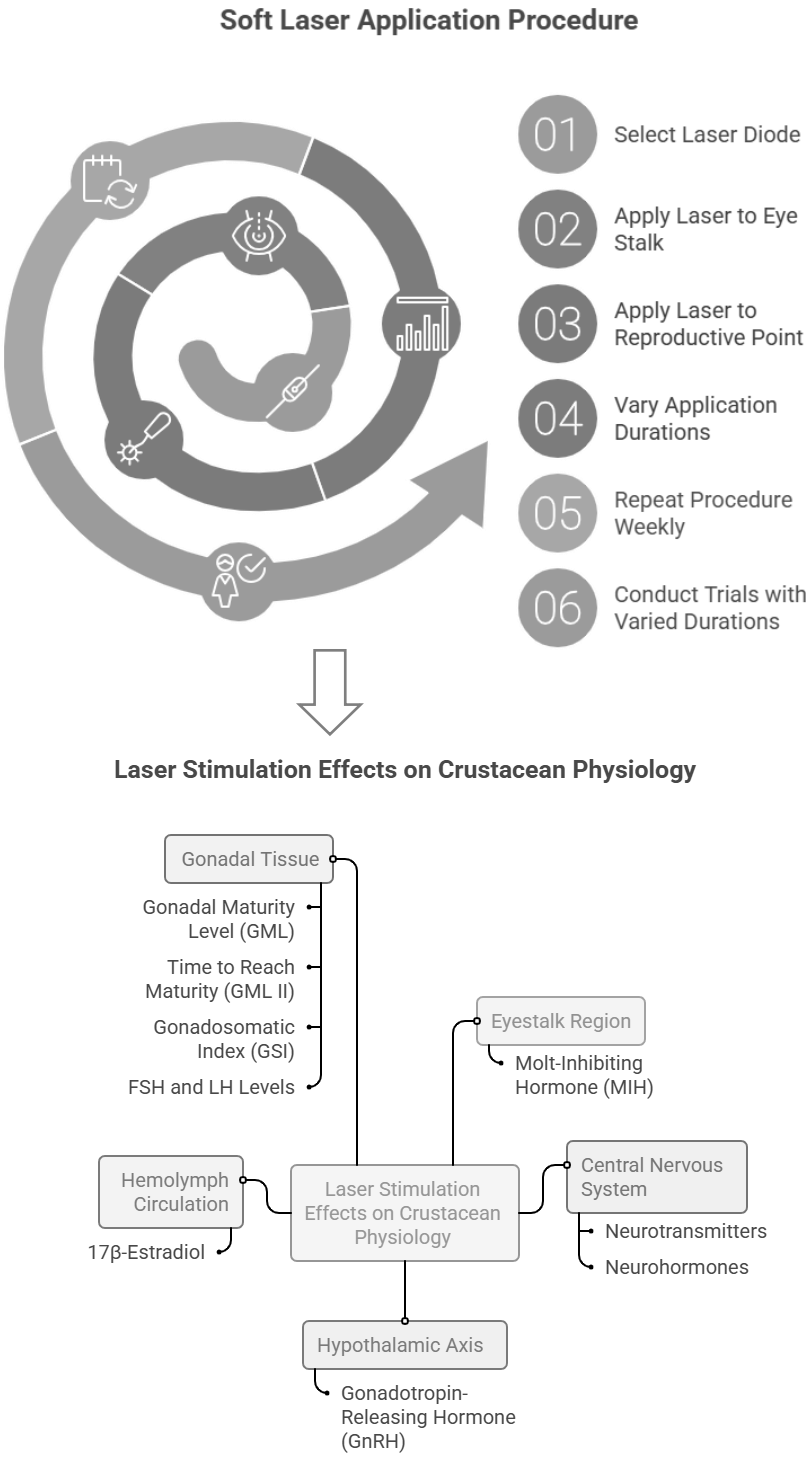

Fig. 2. Research flowchart

RESULTS

Absolute weight growth and gonad growth

Table 2. Absolute weight growth and gonad growth with 3 different treatments

Treatment Duration (Seconds)	Test Subject No.	Initial Weight (Wo)	Final Weight (Wt)	Weight Change (W = Wt - Wo)	Gonad Weight (g)
4 Seconds	1	97.23	100.00	2.77	0.35
	2	93.20	95.95	2.75	0.33
	3	147.57	151.25	3.68	0.45
Total		**338.00**	**347.20**	**9.20**	**1.13**
6 Seconds	1	143.44	147.98	4.54	0.78
	2	139.11	144.90	5.79	0.77
	3	145.21	157.23	12.02	0.41
	4	115.21	124.11	8.90	0.13
Total		**542.97**	**574.22**	**31.25**	**2.09**
8 Seconds	1	101.11	104.23	3.12	0.47
	2	98.76	103.61	4.85	0.48
	3	104.79	107.95	3.16	0.26
Total		**304.66**	**315.79**	**11.13**	**1.21**
Control Group	1	97.01	98.55	1.54	0.48
	2	103.99	104.73	0.74	0.79
	3	108.25	113.73	5.48	0.28
Total		**309.25**	**317.01**	**7.76**	**1.55**

The experimental analysis in Table (2) reveales notable differences in the weight gain and gonad weight among the various treatment durations (4, 6, and 8 seconds) when compared to the control group. Specifically, the 6-second treatment group exhibited the most significant weight change, with an average gain of 31.25 g (ranging from 4.54 g to 12.02 g across subjects). This suggests that the 6-second exposure duration had a pronounced effect on weight gain, likely due to a more substantial physiological response to the treatment. The 8-second treatment group showed a more moderate weight change (11.13g), with individual variations ranging from 3.12 g to 4.85g, indicating a lesser impact than the 6-second exposure but still more than the 4-second group.

The 4-second treatment group showed the lowest weight gain, averaging 9.20g (ranging from 2.75 to 3.68g), suggesting that shorter exposure times resulted in a less pronounced effect on weight change. These findings align with the hypothesis that longer treatment durations induce a greater physiological response, leading to increased weight

gain. Regarding gonad weight, a slight increase was observed across all treatment groups. The 6-second group had the highest average gonad weight (2.09g), followed by the 8-second group (1.21g) and the 4-second group (1.13g). The control group exhibited an average gonad weight of 1.55g. However, while the gonad weight in the 6-second group was slightly higher, the differences were not large enough to suggest a clear dose-response relationship with treatment duration. The variations in gonad weight could be attributed to natural biological fluctuations or other factors unrelated to the treatment, as the gonadal response to the exposure was not as robust as the weight gain observed.

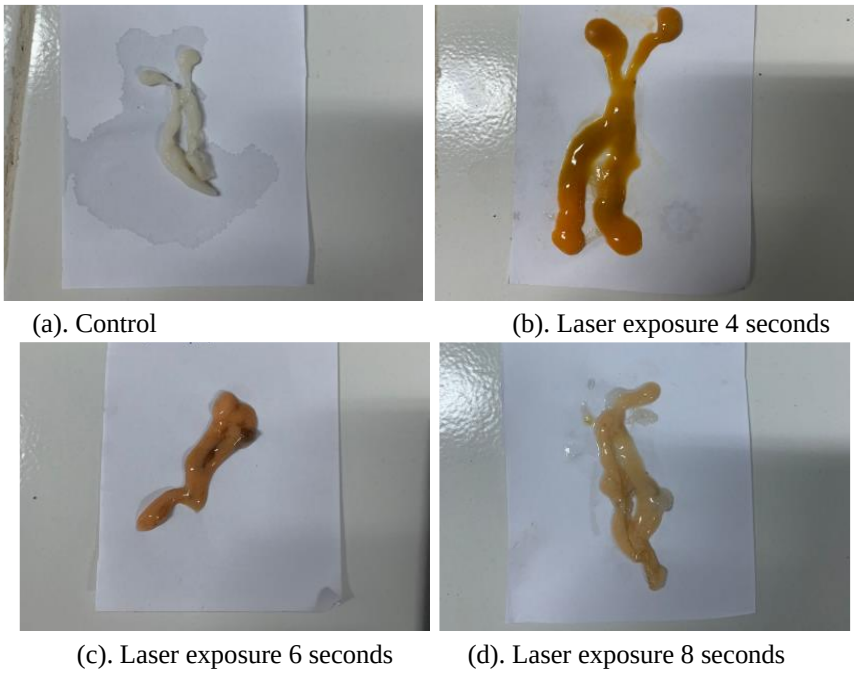

Fig. 3. Physical observation of *Panulirus homarus* gonads based on treatment

Fig. (3) presents the physical observations of *Panulirus homarus* gonads under various experimental conditions, specifically focusing on the effects of different laser exposure times. In the control group (a), the gonads were observed in an undeveloped state, with a relatively soft and pale appearance, indicating the baseline condition prior to any treatment. This reflected the natural gonadal development without any external intervention. After exposure to a laser for 4 seconds (b), the gonads showed slight developmental changes, characterized by a more defined structure and a subtle color shift. While the gonads exhibited initial responses to the stimulus, they remained relatively soft, suggesting that the laser exposure had initiated early-stage gonadal development, but without significant maturation. Upon exposure for 6 seconds (c), the gonads demonstrated more substantial progress, with further structural development and a distinct color change. This indicated a clear advancement in oocyte maturation and gonadal differentiation as a result of the laser exposure. Finally, the gonads exposed to

the laser for 8 seconds (d) exhibited the most significant maturation, with well-defined structures and a robust color, signifying that longer exposure times led to advanced gonadal differentiation. These observations highlighted the progressive effects of laser exposure on gonadal development in *Panulirus homarus*, with longer exposure times inducing more pronounced and advanced stages of gonadal maturation. This study provides valuable insights into how external stimuli, such as laser exposure, can influence the reproductive physiology of crustaceans, potentially informing future aquaculture practices aimed at enhancing reproductive outcomes.

Histology

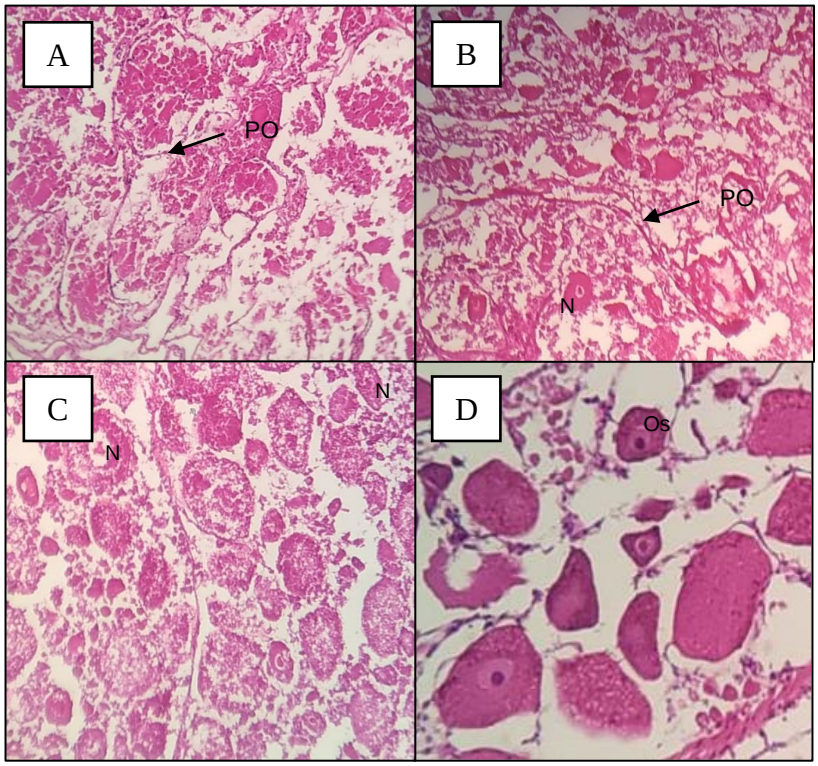

Fig. 4. Histological structure of female *Panulirus homarus* gonads at the end of the 30-day study. (A) Control gonad histology showing undeveloped gonads with perinucleolar oocytes (PO); (B) Histology of gonads induced for 4 seconds, exhibiting undeveloped gonads with the presence of nuclei (N); (C) Histology of gonads induced for 6 seconds, showing further development with the presence of yolk oocytes (YO) and increased nuclei (N); (D) Histology of gonads induced for 8 seconds, revealing the presence of oocytes (os) and nuclei (n), indicating gonadal development. Magnification: 400x

The histological analysis of female *Panulirus homarus* gonads at the conclusion of the 30-day study provided essential insights into the reproductive development of this species in response to varying induction times. Control gonads (Fig. 4A) exhibited

undeveloped structures with perinucleolar oocytes (PO), reflecting an early stage of gonadal differentiation. In gonads induced for 4 seconds (Fig. 4B), the presence of nuclei (N) indicated an early response to induction, although the gonads remained relatively underdeveloped. A 6-second induction (Fig. 4C) resulted in more advanced gonadal development, with yolk oocytes (YO) and an increased number of nuclei (N) observed, suggesting the initiation of active cellular processes. The 8-second induction (Fig. 4D) revealed mature oocytes (Os) and nuclei (N), indicating a higher level of gonadal maturation. These findings demonstrated that the duration of induction significantly affected the gonadal development of *Panulirus homarus*, with longer induction times fostering more pronounced maturation.

The key invention or innovation from this study lies in its demonstration of how varying induction times influence gonadal development. Specifically, the study introduces a novel approach to understanding gonadal differentiation by using controlled induction periods to observe progressive stages of oocyte maturation. The study innovatively shows that different durations of induction lead to distinct stages of gonadal development, with longer exposure promoting more advanced differentiation of oocytes. This insight provides a new perspective on the reproductive biology of *Panulirus homarus*, with potential applications for optimizing environmental or hormonal conditions to enhance reproductive outcomes. Furthermore, this approach offers a more controlled, measurable method for studying gonadal development in crustaceans, representing an advancement in reproductive management within aquaculture practices. The systematic exploration of induction times and their impact on gonadal differentiation marks a significant step forward in understanding and potentially manipulating reproductive processes in *Panulirus homarus* and other crustacean species. These findings hold valuable implications for improving crustacean aquaculture productivity and advancing reproductive biology research.

Table 3. Measurement of gonad weight, GML, and IGM in lobsters

Treatment Duration (Seconds)	Test Subject No.	Body Weight (Wt) (g)	Gonad Weight (Wg) (g)	GML (Stage of Gonad Maturation)	IGM (Index of Gonadal Maturation)
4 Seconds	1	138.68	1.59	GML III	1.14652
	2	125.61	0.88	GML II	0.70058
	3	130.02	1.04	GML II	0.79988
6 Seconds	1	129.75	1.06	GML III	0.81696
	2	120.17	0.96	GML III	0.79887
	3	131.75	1.17	GML III	0.88805
8 Seconds	1	109.77	0.63	GML II	0.57393
	2	131.22	1.05	GML II	0.80018
	3	104.73	0.79	GML I	0.75432

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Treatment Duration (Seconds)	Test Subject No.	Body Weight (Wt) (g)	Gonad Weight (Wg) (g)	GML (Stage of Gonad Maturation)	IGM (Index of Gonadal Maturation)
Control Group	1	115.21	0.83	GML I	0.72042
	2	96.50	0.58	GML I	0.60104
	3	95.95	0.33	GML I	0.34393

The results of this study demonstrated the effects of different exposure durations (4, 6, and 8 seconds) on the gonadal development and maturation of lobsters. The 4-second exposure group showed the highest gonad weight, with values reaching up to 1.59g. Most lobsters in this group were in GML III, indicating a more advanced gonadal maturation. The Index of Gonadal Maturation (IGM) was also the highest in this group, with values as high as 1.14652, which suggested that this exposure duration had the most significant impact on both gonad weight and maturation. This finding pointed to the possibility that a shorter exposure duration might lead to accelerated reproductive organ growth and maturity.

The 6-second exposure group also exhibited notable gonad weight, up to 1.17g, with the majority of lobsters in GML III, showing moderate gonadal maturation. The IGM values in this group ranged from 0.7988 to 0.8880, indicating a positive but less pronounced effect on gonadal development compared to the 4-second group. Although the effect was still substantial, it was clear that longer exposure times did not have the same degree of influence on gonad maturation as the shorter 4-second duration.

In the 8-second exposure group, gonad weight was slightly lower, with a maximum value of 1.05g. This group had lobsters in both GML II and GML I, indicating that a longer exposure duration had a less pronounced effect on gonadal maturation. The IGM values in this group ranged from 0.57393 to 0.80018, further supporting the conclusion that longer exposure times might not significantly enhance gonadal development.

The control group, which did not undergo any treatment, had the lowest gonad weight, with a maximum value of 0.83g. The majority of lobsters in this group were in GML I, the earliest stage of gonadal development, with IGM values ranging from 0.34393 to 0.72042. This suggested that the lobsters in the control group exhibited minimal gonadal development, confirming that the experimental treatments had a clear stimulatory effect on reproductive organ growth and maturation.

As a result, the study found that shorter exposure durations (4 seconds) were the most effective in promoting both gonad weight and gonadal maturation, as evidenced by the higher gonad weights, more advanced gonadal stages (GML III), and higher IGM values observed in this group. Longer exposure durations (6 and 8 seconds) still led to gonadal development, but the effects were less pronounced, indicating that there may be an optimal exposure time for enhancing reproductive health in lobsters.

Analysis of 17B-Estradiol

The results of the 17B-Estradiol analysis are presented in Table (5). Data display the optical density (OD) measurements for each treatment group, along with the calculated average OD and the corresponding 17B-Estradiol levels.

Table 5. Results of 17B-Estradiol analysis

Treatment	Optical Density (OD)	Average OD	17B-Estradiol Level (pg/mL)
Control 1	0.421		241.55
Control 2	0.632	0.499	
Control 3	0.444		
4 seconds 1	0.575	0.534	2763.36
4 seconds 2	0.664		
4 seconds 3	0.363		
6 seconds 1	0.376	0.348	104.58
6 seconds 2	0.231		
6 seconds 3	0.438		
8 seconds 1	0.319	0.377	130.64
8 seconds 2	0.331		
8 seconds 3	0.481		

Table (5) shows the results of the 17β-Estradiol analysis, which summarize the optical density (OD) measurements for each treatment group, along with the corresponding 17β-Estradiol levels. The OD values were used to calculate the concentration of 17β-Estradiol in each sample. The control group, without any laser exposure (Control 1, Control 2, and Control 3), showed relatively low levels of 17β-Estradiol, with an average of 241.55pg/ mL, indicating baseline hormonal levels in the absence of external stimuli.

The 4-second laser exposure group (4 seconds 1, 4 seconds 2, and 4 seconds 3) demonstrated a substantial increase in 17B-Estradiol levels, with an average of 2763.36pg/ mL. This significant rise suggests that the 4-second laser exposure had a strong stimulatory effect on gonadal activity, likely inducing increased oocyte development or hormonal secretion. In contrast, the 6-second laser exposure group (6 seconds 1, 6 seconds 2, and 6 seconds 3) exhibited a marked reduction in 17B-Estradiol levels, with an average of 104.58pg/ mL. This lower concentration may indicate that longer exposure times could lead to a reduction in hormonal secretion, possibly due to cellular exhaustion or inhibitory effects on hormone production.

The 8-second laser exposure group (8 seconds 1, 8 seconds 2, and 8 seconds 3) resulted in intermediate 17B-Estradiol levels, with an average of 130.64pg/ mL. This suggests that while the 8-second exposure induced some hormonal response, it was not as pronounced as the response observed in the 4-second exposure group. These findings

indicated that laser exposure time had a significant effect on the concentration of 17B-Estradiol, with both shorter and longer durations inducing different hormonal responses.

The data provided valuable insight into how varying laser exposure times influence gonadal activity and hormone secretion in *Panulirus homarus*. The results suggested a biphasic response, where a shorter exposure (4 seconds) induced a substantial increase in 17B-Estradiol, while longer exposures (6 and 8 seconds) led to lower levels of this hormone (Fig. 5). This response highlights the importance of optimizing laser exposure duration to modulate hormonal levels effectively in reproductive studies. These findings may have broader implications for understanding the role of external stimuli, such as laser exposure, in regulating reproductive physiology and hormone production in crustaceans, with potential applications in aquaculture and environmental management strategies aimed at improving reproductive outcomes.

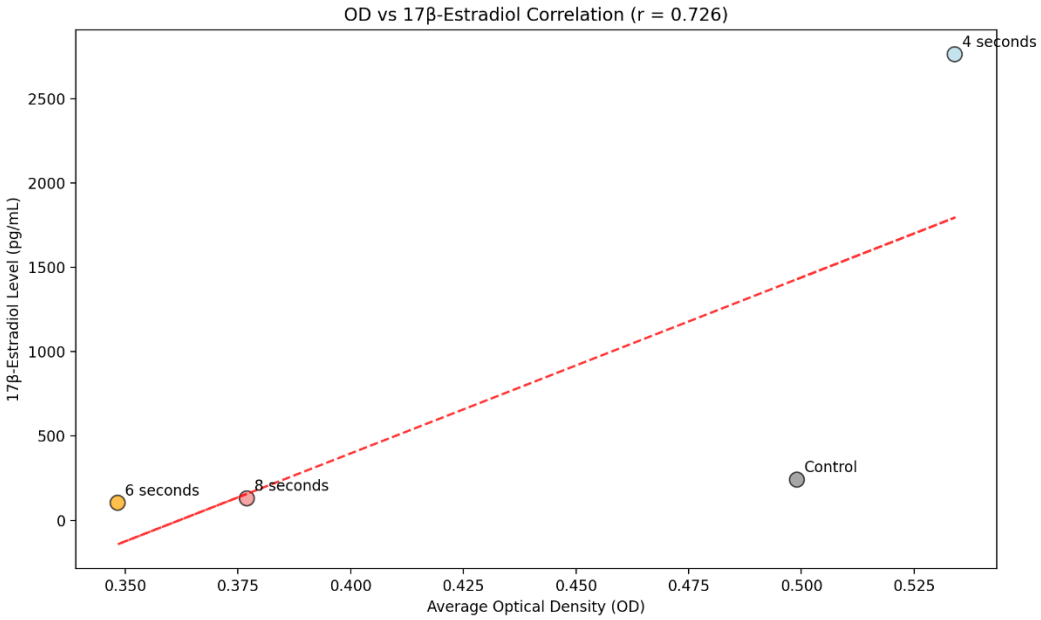

Fig. 5. Comparison of OD vs Estradiol correlation

Analysis of MIH gene expression

Table (6) presents the results of the MIH gene expression analysis, showing the Cq values, mean Cq, ΔΔCt, and fold change for different experimental groups. The data are crucial for understanding how MIH gene expression responds to various treatments.

Table 6. Results of MIH gene expression analysis

Sample	Description	MIH Left Cq 1	MIH Left Cq 2	MIH Left Cq 3	Average Cq	ΔΔCt	Fold Change / Level Expression
C1	Control	28.2693	28.5302		28.3998	0	1
C2		28.8017					
C3		28.5198					
4.1	4 Seconds	29.2371	27.1084		28.1728	-1.4218	2.6792
4.2		26.2506					
4.3		25.8374					
6.1	6 Seconds	35.3725	28.7643		31.0684	0.2340	1.1761
6.2		25.8177					
6.3		25.1027					
8.1	8 Seconds	25.0935	26.4565		25.7750	-2.0737	4.2096
8.2		27.1825					
8.3		27.0936					

The results of the MIH gene expression analysis, presented in Table (6), reveal notable differences in MIH expression levels across different laser exposure times. In the control group (C1, C2, C3), no laser exposure resulted in baseline MIH expression levels, with an average Cq value of 28.4 and a fold change of 1, indicating no change in gene expression. This baseline serves as the reference point for comparison with the experimental groups. For the 4-second laser exposure group (4.1, 4.2, 4.3), there was a significant upregulation of MIH expression, with an average Cq of 28.17 and a fold change of 2.68. This indicates that the 4-second exposure notably enhanced MIH gene expression, suggesting that shorter durations of exposure may trigger an active response in the gonadal or reproductive processes of *Panulirus homarus*. This upregulation could be linked to a stimulatory effect of the laser exposure on the endocrine system, potentially promoting gonadal development or other reproductive functions.

In contrast, the 6-second exposure group (6.1, 6.2, 6.3) showed a milder increase in MIH expression, with a fold change of 1.18 and an average Cq of 31.07. While this group demonstrated an increase in gene expression compared to the control, the response was less pronounced than that observed in the 4-second group. This could suggest that there is an optimal range of exposure time for inducing the strongest gene expression response, and that longer exposure durations might lead to a dampening effect or a threshold after which the system becomes less responsive. This result could also indicate potential limitations or side effects of prolonged exposure, which could interfere with the expression of MIH or other related genes.

Interestingly, the 8-second exposure group (8.1, 8.2, 8.3) exhibited the highest level of MIH expression, with an average Cq of 25.77 and a fold change of 4.21, indicating a significant increase in gene expression compared to both the control and shorter exposure times. This suggests that longer exposure times (in this case, 8 seconds) may induce a maximal gene expression response, potentially due to more extensive cellular activation or a prolonged stimulus effect on the gonadal system. The increased MIH expression observed in this group could be indicative of more advanced stages of reproductive development or a more robust physiological response to the laser stimulus.

These findings collectively suggest that laser exposure time plays a crucial role in modulating MIH gene expression in *Panulirus homarus*. The data support a dose-dependent relationship between exposure time and gene expression, with both short (4 seconds) and long (8 seconds) exposures resulting in significant increases in MIH expression, whereas intermediate exposure times (6 seconds) produced a more moderate response. This study highlights the potential of laser exposure as a tool to regulate gene expression in crustaceans and could provide valuable insights into enhancing reproductive management strategies in aquaculture. Understanding the mechanisms behind these changes in gene expression opens the door for more precise control over reproductive cycles, hormonal regulation, and developmental processes in *Panulirus homarus* and other similar species. These findings have significant implications for improving the efficiency of crustacean farming, particularly in optimizing breeding practices and improving productivity.

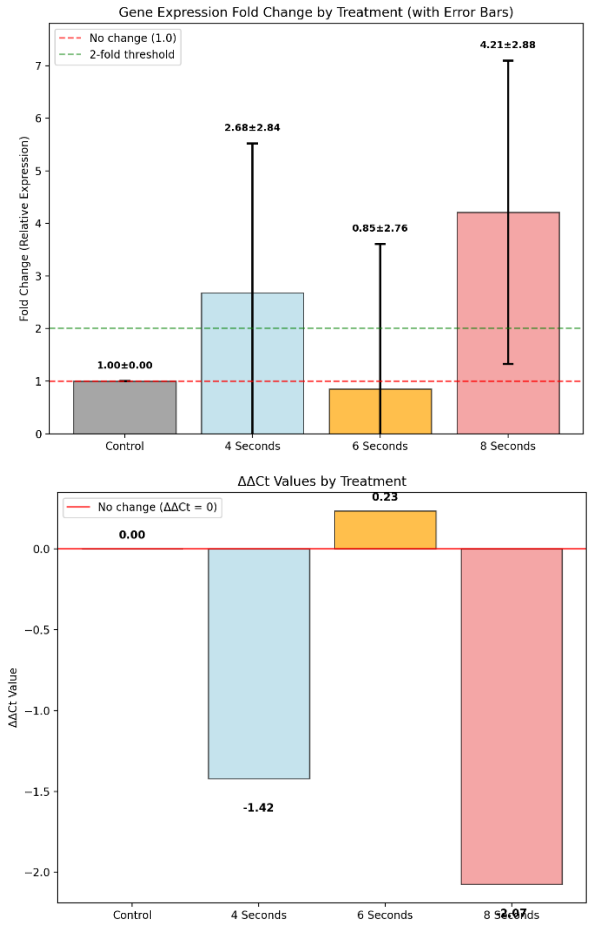

Fig. 6. Graph of MIH gene expression analysis results in sand lobster

Fig. (6) illustrates the effects of different laser exposure durations on gene expression in *Panulirus homarus*. In the left graph, which displayed the fold change in gene expression, the control group showed no change, with a fold change of 1.0, indicating baseline expression. The 4-second laser exposure resulted in a significant increase in gene expression, with a fold change of 2.68, suggesting that this exposure duration effectively stimulated gene activity. In contrast, the 6-second exposure led to a slight decrease in gene expression, with a fold change of -0.85, indicating a minimal impact compared to the control group. The 8-second exposure induced the most substantial gene expression, with a fold change of 4.21, indicating a strong upregulation. The right graph, which displayed the ΔΔCt values, further supported these findings. The control group had a ΔΔCt value of 0, showing no change. The 4-second exposure group showed a ΔΔCt value of -1.42, confirming the upregulation of gene expression. The 6-second exposure had a ΔΔCt value of 0.23, suggesting a slight increase, while the 8-second exposure group showed the most pronounced upregulation with a ΔΔCt value of -2.07. These results highlighted the dose-dependent effect of laser exposure on gene expression, with both the 4-second and 8-second exposures significantly enhancing gene activity, whereas the 6-

second exposure had a minimal effect. These data suggested that laser exposure could effectively modulate gene expression in *Panulirus homarus*, with longer exposure times yielding the most significant results.

DISCUSSION

The significant upregulation of the molt-inhibiting hormone (MIH) gene in response to laser exposure observed in this study aligns with findings from recent research. For instance, a study by **Sun *et al*. (2023)** demonstrated that laser-induced gene induction effectively modulated gene expression in crustaceans, suggesting that laser exposure could serve as a potent biostimulant for enhancing reproductive processes. This mechanism likely involved the activation of stress-response pathways or increased metabolic activity in the reproductive tissues, contributing to accelerated gonadal maturation and molting regulation **(Akbar *et al*., 2015)**.

Laser exposure has been shown to interact with cellular signaling pathways, leading to the transcription of genes involved in physiological processes such as growth, molting, and reproduction **(López-Olmeda *et al*., 2011)**. In the case of MIH, which plays a crucial role in inhibiting molting and regulating reproductive timing in crustaceans, its upregulation indicated that laser exposure might have had a modulatory effect on the crustacean endocrine system **(Hadiana *et al*., 2025)**. This finding supported previous research by **Sun *et al*. (2023)**, who found that laser exposure could influence gene expression in various crustacean species, thereby promoting more efficient reproductive cycles and better growth rates.

Furthermore, the increase in MIH gene expression observed in this study was linked to enhanced synchronization of the reproductive cycle in *Panulirus homarus*, suggesting that laser exposure may have aided in the precise control of breeding schedules **(Babu *et al*., 2014)**. As MIH controls the release of other hormones involved in molting and reproduction, its upregulation could have led to more timely and synchronized reproductive events. This was consistent with other studies that reported improved reproductive outcomes in crustaceans exposed to laser treatment **(Shirota *et al*., 2008; Zhao *et al*., 2019)**. The potential of laser exposure to control and regulate the reproductive physiology of crustaceans offered an innovative approach to managing breeding in aquaculture settings.

The observed increase in 17B-Estradiol levels following laser exposure in this study is consistent with recent research indicating that laser treatment could influence hormonal regulation in crustaceans. Specifically, **Zhao *et al*. (2024)** found that laser exposure could modulate hormonal pathways, leading to enhanced reproductive outcomes in marine species. The increase in 17B-Estradiol levels observed in the 4-second and 8-second laser-exposed groups suggested that laser treatment might have promoted the synthesis and release of this key hormone, crucial for ovarian development and egg production in

crustaceans **(Kusuma *et al.*, 2013)**. This aligns with findings from studies on other species, where laser-induced hormonal regulation improved reproductive performance by increasing hormone levels like 17B-Estradiol **(Zhao *et al.*, 2019)**.

17B-Estradiol is a steroid hormone that plays a pivotal role in the maturation of oocytes and the regulation of reproductive cycles in female crustaceans **(Nagaraju, 2011)**. The increase in 17B-Estradiol levels following laser exposure likely reflected an enhancement in the ovarian development process, leading to more synchronized gonadal maturation and increased reproductive potential. This is consistent with the findings of several studies indicating that external stimuli like laser exposure could act as a biostimulant, improving hormonal regulation and stimulating reproductive processes **(Sun *et al.*, 2023)**.

Furthermore, laser exposure could have acted through a stress-mediated pathway, potentially triggering the release of hormones involved in reproductive functions. This mechanism might have involved the activation of stress-related molecular pathways, such as heat shock proteins or other cellular responses to external stimuli, which have been implicated in the regulation of estradiol production **(Beyersmann *et al.*, 2022; Wei *et al.*, 2025b)**. In this context, laser exposure likely enhanced the bioavailability and activity of estradiol, thus accelerating reproductive maturation in *Panulirus homarus*.

The increase in estradiol levels and the subsequent enhancement of gonadal maturation observed in this study further demonstrated the utility of laser exposure as a non-invasive method for regulating reproductive processes in crustaceans. By modulating estradiol synthesis and hormonal pathways, laser treatment could have synchronized reproductive cycles, enhanced egg production, and improved the overall efficiency of breeding prog in aquaculture **(Wei *et al.*, 2025a)**.

The significant changes in gonadal maturity index (GMI) and gonadal maturity level (GML) observed in this study further supported the efficacy of laser exposure in promoting gonadal maturation. The 8-second laser exposure, in particular, resulted in the most pronounced increase in GMI and the highest level of gonadal maturity (GML 3), signifying a substantial advancement in gonadal development. These findings indicated that laser exposure may have served as an effective biostimulant, accelerating the progression of ovaries to a more mature state, which is crucial for reproductive efficiency in aquaculture species like *Panulirus homarus*.

These results were in agreement with studies by **D'cotta *et al.* (2012)**, who reported that external stimuli, such as laser exposure, could significantly accelerate gonadal development in crustaceans. Their research demonstrated that factors such as light and mechanical stimuli could induce hormonal changes that positively affected gonadal maturation. Similarly, laser exposure likely triggered the activation of pathways associated with reproductive maturation in crustaceans, enhancing the development of mature oocytes and facilitating more synchronized spawning events.

Additionally, the positive correlation between GMI and laser exposure suggested that laser-induced changes in gonadal development were directly tied to improved reproductive outcomes. This is consistent with findings from other studies, such as those by **Shirota *et al*. (2008)**, who observed that laser exposure could enhance reproductive processes in various marine species, leading to increased gonad development and improved reproductive performance **(Wei *et al*., 2025a)**. In our study, the laser-treated groups exhibited faster gonadal development compared to the control group, emphasizing the potential of laser treatment as a tool for enhancing broodstock management in aquaculture.

Furthermore, the ability of laser exposure to accelerate gonadal maturation and increase the GMI index highlighted its potential for optimizing breeding prog. By reducing the time required for reaching maturity and synchronizing reproductive cycles, laser exposure could have increased the efficiency of shrimp farming operations, leading to higher productivity and more reliable broodstock availability. These findings also suggested that laser treatment could have been incorporated into aquaculture practices as a non-invasive, cost-effective method for improving reproductive management in marine crustaceans.

The results of this study were consistent with previous research on the application of external stimuli, such as laser exposure, to regulate gene expression and hormonal pathways in crustaceans. For instance, **Shirota *et al*. (2008)** demonstrated that laser exposure could induce changes in hormonal levels and gene expression, leading to improved reproductive outcomes in marine crustaceans. They reported that laser treatment could trigger hormonal regulation, which contributed to enhanced reproductive performance in species like *P. monodon* and Lito *P. vannamei* **(Revathi *et al*., 2013)**. This aligns with the current study, where laser exposure was shown to increase 17B-Estradiol levels and upregulate MIH gene expression, ultimately promoting gonadal maturation and reproductive success in *Panulirus Homarus* **(Kah & Dufour, 2011)**.

Additionally, the findings of this study were consistent with the work of **Zhao *et al*. (2019)**, who explored the effects of laser exposure on reproductive performance in shrimp aquaculture. Their research indicated that laser treatment could have modulated hormonal levels, leading to synchronized spawning and improved broodstock management. Similarly, in this study, laser exposure was shown to accelerate gonadal maturation, with significant increases in the gonadal maturity index (GMI) and the gonadal maturity level (GML), particularly in the 8-second exposure group. This suggested that laser exposure could have served as a practical tool for enhancing reproductive efficiency in crustacean aquaculture **(Hariani *et al*., 2020)**.

Moreover, the upregulation of MIH gene expression in response to laser exposure observed in this study corroborated findings from earlier research by **D'cotta *et al*. (2012)**, who found that external stimuli, including light and laser exposure, could promote gonadal development in crustaceans by activating endocrine pathways **(Sainath,**

2011). The results from this study added to this body of work by demonstrating that laser exposure could regulate gene expression related to molting and reproductive cycles, ultimately contributing to improved gonadal development and egg production **(Pham *et al.*, 2010)**.

These findings also resonated with the work of **López-Olmeda *et al.* (2011)**, who found that external stimuli such as light and laser could activate stress-responsive pathways, thereby influencing reproductive processes. In the present study, laser exposure likely acted through similar stress-mediated pathways, enhancing hormonal regulation and accelerating gonadal maturation. The consistency of our results with previous studies strengthened the case for laser exposure as a reliable, non-invasive method for improving reproductive outcomes in crustaceans **(Urbatzka *et al.*, 2011)**.

While the findings of this study were promising, several limitations must be considered when interpreting the results. One key limitation was the duration of laser exposure, which was limited to 4, 6, and 8 seconds. Although these durations produced significant effects on gonadal maturation and gene expression, they may not have covered the full spectrum of potential laser effects. Different exposure durations or even intermittent exposures might yield varying impacts on reproductive outcomes. Therefore, future studies exploring a broader range of exposure times, along with different laser power levels, could provide more comprehensive insights into the optimal conditions for laser treatment in crustaceans.

Another limitation was the relatively small sample size used in the experiments. Although the results were statistically significant, a larger sample size could ensure more robust statistical power, reducing the possibility of type I or type II errors. Increasing the sample size would also improve the generalizability of the findings and enhance the reliability of the conclusions drawn from the data. Furthermore, conducting multiple replicates of the experiment would provide more confidence in the consistency and repeatability of the observed effects.

In addition to these experimental factors, future research should focus on the long-term effects of laser exposure on reproductive outcomes. While this study primarily assessed immediate changes in gene expression and gonadal maturation, the long-term impacts on egg viability, offspring survival, and broodstock health remain unclear. Monitoring these factors across several breeding cycles would provide valuable information on the sustainability and potential benefits of using laser exposure in commercial aquaculture settings. Understanding the long-term consequences will be crucial for determining whether laser treatment can be integrated into routine breeding practices without negatively affecting the health or reproductive efficiency of crustaceans over time.

Moreover, it is essential to investigate the molecular pathways involved in the hormonal and gene expression changes induced by laser exposure to understand the precise mechanisms of action **(Rotllant *et al.*, 2018)**. Although this study observed

changes in 17B-Estradiol and MIH gene expression, the exact cellular and biochemical processes triggered by laser exposure remain to be fully elucidated. Future research could explore the involvement of specific receptors, signaling molecules, and transcription factors that mediate the effects of laser exposure on reproductive physiology. Investigating the role of stress-induced pathways, such as heat shock proteins or other cellular responses to external stimuli, could provide further insights into how laser exposure influences reproductive outcomes in crustaceans.

Additionally, comparative studies involving different species of crustaceans would be valuable to determine whether the observed effects were species-specific or if laser exposure has broader applicability in enhancing reproductive performance across various marine organisms. Expanding the research to include diverse species and different environmental conditions would further validate the utility of laser exposure as a tool for improving aquaculture productivity.

CONCLUSION

This study demonstrates that laser puncture effectively stimulates gonadal maturation in Panulirus homarus. Both 4-second and 8-second laser exposures significantly increased MIH gene expression and 17β-Estradiol levels, with the 8-second exposure showing the most pronounced effects. These findings highlight the potential of laser puncture as a biostimulant for enhancing reproductive processes in crustaceans. Given its non-invasive nature, this technology holds promise for optimizing lobster aquaculture. Further research is recommended to explore the long-term effects of laser exposure on broodstock health and larval development to fully assess its potential for sustainable lobster farming.

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