Physiological response of the puerulus of scalloped spiny lobster, *Panulirus homarus*, raised at different tank color

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Abstract. By influencing crustacea's behavior, habitat preferences, and metabolic rates, tank color can act as a stressor. The study's objective was to assess how scalloped spiny lobsters, *Panulirus homarus*, raised in various colored containers-transparency (K), red (A), black (B), and blue-reacted physiologically and biochemically (C). An experimental model with a fully randomized design, comprising 4 treatments and 3 replications, was employed in this work as the methodology. The treatment feed was administered for 30 days. The findings demonstrated a survival of 81.33 (an increase of at least 2.31%, compared to the other treatments) when red containers were used. Black tanks, on the other hand, were able to enhance the growth performance ($P<0.05$) by lowering the stress levels, as demonstrated by lower SGPT, SGOT, ALP, total protein, and glucose levels, in comparison to controls ($P<0.05$). The abdomen of *P. homarus* raised in the red tank tended to be brighter than those raised in the other environments. The *P. homarus* tails raised in the blue tank typically had a brighter appearance than those raised in the other environments. The *P. homarus* carapaces raised in the red tank tended to be brighter than those raised in other environments.
Key Words: biochemical, environment, growth performance, stressor, survival.

Introduction. In Indonesia, a nation with high potential for lobster fishing, *Panulirus homarus* is the variety that is third most abundant in nature, behind ornate lobster and rock lobster (Wahyudin 2018). Excessive natural lobster catching operations will reduce the availability of lobster, hence culture activities must be carried out. Although there have been attempts to grow lobster in Indonesia, little has changed. Low survival and growth brought on by high levels of stress are the key factors preventing Indonesia from developing its lobster cultivation. High levels of stress are hypothesized to be caused by unfavorable environmental variables, such as the tank's color not matching, which will therefore hinder the *P. homarus*'s ability to thrive. According to Sykes et al (2011), tank color can act as a stressor by influencing fish and crustacean behavior, habitat preferences, and metabolic rates (Maciel & Valenti 2014; Jensen & Egnotovich 2015). There appears to be a species-specific response to background color (Sykes et al 2011). The specific growth rate and survival of *P. homarus* juveniles were impacted by the color of the tank (Saktya 2020). Numerous studies have shown that the background colors of culture units have a significant impact on the growth and survival of cultured animals, including freshwater prawns (*Macrobrachium rosenbergii*), snakeskin gourami (*Trichogaster pectoralis*), and barramundi (*Lates calcarifer*) (Ninwichin et al 2018). Other species, including spotted sand bass (*Paralabrax maculatofasciatus*) (Pena et al 2010) did not experience any differences in growth, feed efficiency, or survival as a result of their exposure to the color of the tank. The color of the tank had an impact on the crabs as well, the mud crab, *Scylla serrata*, performed substantially better with a black background of the tank (Rabbani & Zeng 2005). To maximize *Pornutus trituberculatus*'s performance as a larva, background color must be optimized (Shi et al 2019). The animals would choose a background that would decrease the energy cost of body coloring, while also lowering the risk of predation (Rodgers et al 2013). The selected habitats may indicate the ideal background color for physiological and growth conditions. Consequently, a color preference study could be a useful method of determining the animals' ideal tank color (Li et al 2016). In order to determine the ideal tank color, the current study was conducted to examine *P. homarus* larvae's preference for colors, as well as their survival, growth, and oxygen consumption rates in various backgrounds. The metabolic and biochemical physiology of developing *P. homarus* is poorly understood. The current study brings a better understanding of *P. homarus*' physiological reactions to various environmental and culture circumstances.

Material and Method

Containers and media research. The experiment was conducted in an indoor laboratory using 12 aquariums (for 4 treatments in 3 replications) of 100 x 50 x 50 cm. The treatment consisted of three aquariums with transparent backgrounds, three aquariums with red backgrounds, three aquariums with black backgrounds and three aquariums with blue backgrounds. A recirculating system with physical, chemical, and biological filters was used in this study. Feces, sediments, and leftover lobster feed were removed daily to maintain water quality.

Lobster seed. Specimens of the puerulus of *P. homarus* (Linnaeus, 1758), with an average length of 2,210.01 cm ind^{-1} and an average body weight of 0.28 g ind^{-1}, served as experimental animal. *P. homarus* seeds were obtained from wild catches in West Java Province, Sukabumi District, and Ujung Genteng Beach, Indonesia.

Experimental design. A completely randomized experimental model with four treatments and three repetitions was employed in this investigation as the methodology, which is detailed in Table 1.

Table 1

Research layout

Code	*Treatment*
K	Transparent background (Control)
A	Red background
B	Black background
C	Blue background

Tank preparation and acclimatization. The 12 aquariums with filtration tanks utilized in this study were cleaned and sanitized before being dosed with 5 mg L^{-1} of chlorine. Following a thorough cleaning, the tanks were drained and filled with 200 L of water. The procedure of acclimatizing lobsters took place in the acclimatization tank for 7 days.

Feed management. Green mussels *Perna viridis* from the Anyer region were used as feed. There were two feedings every day at 7:00 am and 17:00 evening.

Water quality parameters. According to APHA (2012), there were performed daily measurements of the water's pH, salinity, temperature, and dissolved oxygen (DO). Ammonia, nitrite, and nitrate levels in the water were measured on day 0 and then every 10 days until the study's conclusion.

Phisiologycal and biochemical response parameters. According to Blaxhall & Daisley (1973), the measurement of total hemocyte count (THC) of the hemolymphwas carried out. The blood biochemical analysis consisted of total protein (TP), blood glucose, triglycerides (Trigly), cholesterol (CH), glutamic pyruvic transaminase (SGPT), and glutamic oxaloacetic transaminase (SGOT) (GH). An ARKRAY blood chemistry analyzer

(SPOTCHEM-EZ sp 4430) with a paper test indicator for each parameter was used to conduct the analysis. On a panel inside the tool, paper test indicators are produced and set up. The blood chemical analyzer machine is configured and coded in accordance with the course of therapy; after a 10-minute analysis, the machine automatically prints out the results.

Pigmentation (brightness relatives and total carotenoid). A brightness relative study of *P. homarus* was performed using ImageJ software and normalized to the control. Calculating the total area tested is comparable to measuring the area in line with the area to be tested. There are 3 different examples in each of the 5 sections. The statistical analysis employed the ANOVA test with a 95% confidence level. Additional tests were performed on homogenous, normally distributed data. The process used to extract from fish samples the total carotenoids per replication was developed by Schiedt & Liaaen-Jensen (1995), and the results were assessed using a spectrophotometer (Thermo Scientific Genesis 10S UV-Vis).

Production performance parameters. Weight was addressed by biometric measurements taken every ten days on *P. homarus*. Every ten days during the maintenance, the specific growth rate was observed. At the conclusion of the research, the lobster survival rate was assessed.

Data analysis. This study employed a totally random design (CRD). Analysis of variance (ANOVA) F-test with a 95% confidence interval was used to statistically analyze the data of THC, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), triglycerides (Trigly), cholesterol (CH), total protein (TP), blood glucose (GH), survival rate, and absolute weight and if there was a significant difference, a Tukey test was also performed to look at the variations between treatments. Data on water quality were descriptively examined.

Results and Discussions

Physiologycal and biochemical response. At the end of the trial, the THC, SGPT, cholesterol, and total protein levels in lobsters were substantially different between treatments, according to the physiological and biochemical analysis ($P<0.05$). The usage of the black tank had no impact on the lobsters' SGOT, ALP, Triglyserida, or glucose levels, according to this study. Table 2 displays the physiological and biochemical responses of *P. homarus* to various colored tanks.

Table 2

Physiological and biochemical response of *Panulirus homarus* to different color tank

Parameter	*Treatment*			
	K	*A*	*B*	*C*
THC (x 10^3 cell mm^{-3})	6.15 ± 0.79^{ab}	7.05 ± 0.68^{a}	5.47 ± 0.23^{b}	6.23 ± 0.34^{ab}
SGPT (UL^{-1})	30.73 ± 0.54^{a}	4.10 ± 0.66^{b}	3.21 ± 0.46^{b}	3.20 ± 1.12^{b}
SGOT (UL^{-1})	49.03 ± 0.77^{a}	4.46 ± 1.55^{b}	7.51 ± 2.43^{b}	25.10 ± 3.46^{ab}
ALP (UL^{-1})	263.40 ± 1.30^{b}	192.60 ± 1.75^{b}	508.50 ± 2.67^{a}	231.6 ± 3.56^{b}
Trigly (mg dL^{-1})	292.9 ± 2.49^{a}	324.3 ± 160.3^{a}	377.7 ± 4.89^{a}	262.1 ± 3.22^{a}
CH (mg dL^{-1})**	125.04 ± 0.24^{b}	207.2 ± 3.36^{ab}	289.9 ± 1.44^{a}	225.7 ± 2.53^{ab}
TP (g dL^{-1})	7.72 ± 1.57^{a}	4.39 ± 1.04^{b}	6.02 ± 0.32^{ab}	7.19 ± 1.09^{ab}
GH ($mgdL^{-1}$)	105.4 ± 1.69^{a}	94.5 ± 2.05^{ab}	50.9 ± 1.29^{ab}	27.04 ± 0.61^{b}

Values with different superscripts are significantly different ($P<0.05$); ** ($P<0.1$)

In general, the term "stress" refers to a change in the physiological state, determined by internal or external stimuli (stressors) that are outside of the normal physiological range (Barton 2002). Reversible stressors produce physiological reactions that endanger or disturb the homeostatic balance and give rise to a series of behavioral and physiological reactions, thought to be compensatory or adaptive, enabling the animal to get through the stressful situation (Iversen & Eliassen 2009). The proportion of lobsters' hemocyte in red-colored tanks is larger than in other treatments and is statistically different ($P<0.05$). Increased bodily resistance (immunity) is indicated by an increase in the number of hemocytes (Hartinah et al 2014). In another study, the high THC value under a glucan treatment caused a very high level of hemocyte mobilization in the shrimp body, increasing the immunity and improving the identification of pathogens or foreign bodies entering the body (Sahoo et al 2008).

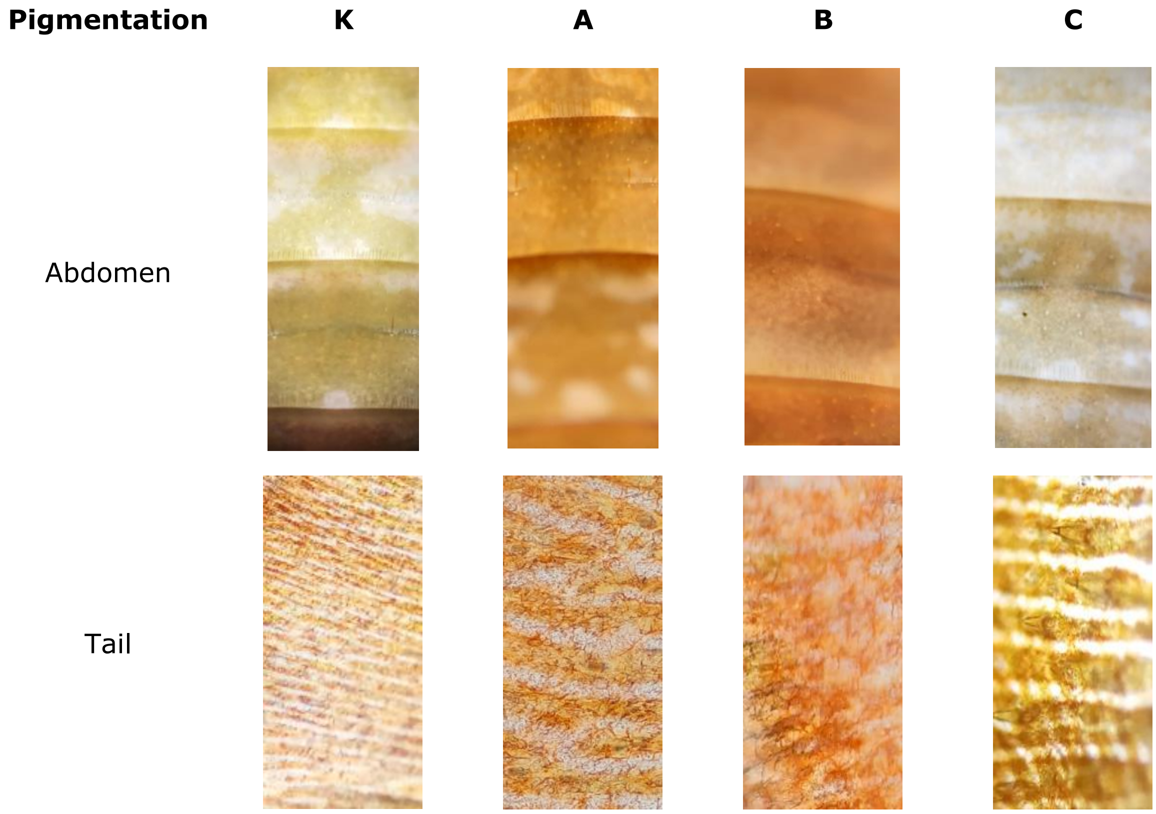
The comparative physiologic research on the hematology and blood biochemistry of several lobster species is of a great interest. A number of cultivable crustacean species' hematological traits have been examined in order to define normal value ranges, any deviation from which may point to a disturbance in the physiological processes. The liver parenchyma cells that produce the enzymes serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). The liver is where the SGPT transaminase enzyme is primarily located, with smaller amounts also being present in the kidneys, heart, and skeletal muscle. The SGOT, which may also be raised in disorders affecting other organs, such as the heart or muscles in myocardial infarction, as well as in acute pancreatitis, acute hemolytic anemia, and acute renal disease, is therefore a more general predictor of organ inflammation than the SGPT (Kulkarni & Pruthviraj 2016). In this study, control lobsters had significantly lower SGPT levels than those under treatment ($P<0.05$). The lobsters that are raised in transparent environments are likely to be under stress. The SGOT measurement in control lobsters (transparency background) also revealed lower values compared to the treatments ($P<0.05$). While the SGPT activity is higher in the fish *N. notopterus*, the SGOT and ALP activities were found to be higher than those reported in *Acipenser stellatus*. This pattern has also been noted in channel catfish, *Ictalurus punctatus*, common carp, and goldfish. Variations of the values of different enzymes in fish species may be due to the method of sampling, the method of analysis, the age of the fish, the habitat, and the diet. Important factors that can lead to intra-species variances in hematological parameters include the stress factors resulting from the manner of capture, handling, and sampling (Kulkarni & Pruthviraj 2016).

The triglyceride levels of the lobsters kept in the blue tank were higher than those of the other treatments ($P<0.05$). In the hemolymph of crustaceans, triglycerides and cholesterol are the two circulating lipid groups (Musgrove & Babidge 2003). Triglycerides are essential for the production of energy and are often regarded as the principal metabolic reserve in an organism (Gurr et al 2002). In contrast, because it is a crucial part of cellular membranes, cholesterol has a greater structural function (Gurr et al 2002). In addition to being a steroid, cholesterol is also employed in the creation of certain hormones (Kanazawa & Koshio 1993) that regulate many aspects of crustacean physiology, particularly during the moult cycle (Chang & Mykles 2011). Therefore, it was not surprising to find strong connections between these two hemolymph lipid measures and tissue lipid. Other crustaceans' growth and health have been evaluated by looking at the hemolymph's lipid contents (Musgrove & Babidge 2003).

P. homarus kept in the black tank had higher cholesterol levels than the controls and other treatments ($P<0.05$). The steroid hormones necessary for moulting and reproduction are precursors of cholesterol (Kumar et al 2018). Additionally, cholesterol gives cell membranes more structural integrity against environmental aberrations like heat or salt stress (Yang et al 2016). Because the same hormones control both moulting and reproduction in crustaceans, they are closely related (Nagaraju 2011). Sesquiterpenoids, which include methyl farnesoate (MF) and juvenile hormones (JH), or ecdysteroids, are two classes of such hormones. It has been hypothesized that distinct receptor isoforms for these hormones exist in crustaceans in order to control more effectively the moulting and reproduction (Nagaraju 2011). The total protein levels of control *P. homarus* were higher than those of the treatments ($P<0.05$). Blood protein levels change in response to changes in physiological and environmental factors, and they serve as crucial functions in the physiology of crustaceans, including O_2 transport, reproduction, and stress reactions (Lorenzon et al 2011). The primary factors altering the relative proportions and total amounts of hemolymph proteins include the moulting, reproduction, nutritional status, infection, hypoxia, and salinity fluctuations. Total protein concentrations in hemolymph vary depending on the species, with penaeid showing the greatest quantities and freshwater crayfish showing the lowest. Because *P. homarus* have a high total protein content compared to other seafood, it is thought that they are more able to adapt to their environment. The amount of proteins in an animal expresses its capacity for adaptation and its acclimatization tactics (Lorenzon et al 2011).

The glucose values of lobsters kept in a tank with a transparent background were greater than those receiving a treatment ($P<0.05$). It is hypothesized that these lobsters experience stress. The backdrop color of the culture system is very important, especially in the larval stages of fish and crustaceans, as the stress of aquatic animals can negatively affect their health state and growth (El Sayed & El Ghobashy 2011). Additionally, glucose is a crucial preventative measure in dealing with stresses like hypoxia and emersion (Lorenzon et al 2007). The hemolymph glucose concentration may experience short-term changes as a result of such stressors, which could reduce the overall correlation due to a greater variability. The largest increase in blood glucose levels in crustaceans during stressful situations were of 20.80 mg dL^{-1} in *P. homarus* (Adiyana et al 2014), 14.41 mg dL^{-1} in *Helix pomatia,* 13.20 mg dL^{-1} in *Astacus leptodactylus* and 80 mg dL^{-1} in *P. monodon* (Rustam et al 2013).

Pigmentation (brightness relatives and total carotenoid). Figure 1 shows the alterations in the *P. homarus* body coloration. The abdomen, tail, carapace, and eye stalk of *P. homarus* were the areas with the highest brightness level, as seen in the image. The abdomen of lobsters raised on a red background tended to be brighter than those raised with other backgrounds. *P. homarus* tails raised in the blue tank typically had a brighter appearance than those raised in the other environments. The carapace of *P. homarus* raised on the red backdrop tended to be brighter than those raised on other backgrounds. Their eyestalks also tend to be brighter than in the other treatments (Figure 2).



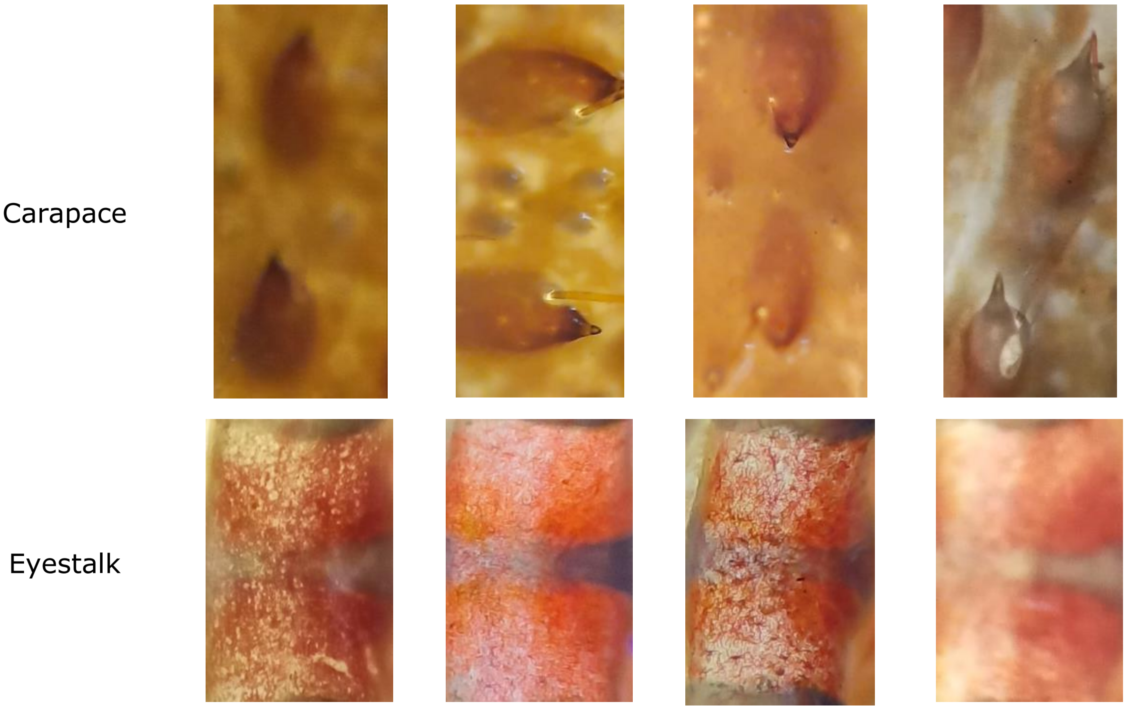


Figure 1. Body coloration of the *Panulirus homarus* puerulus in different color tanks.

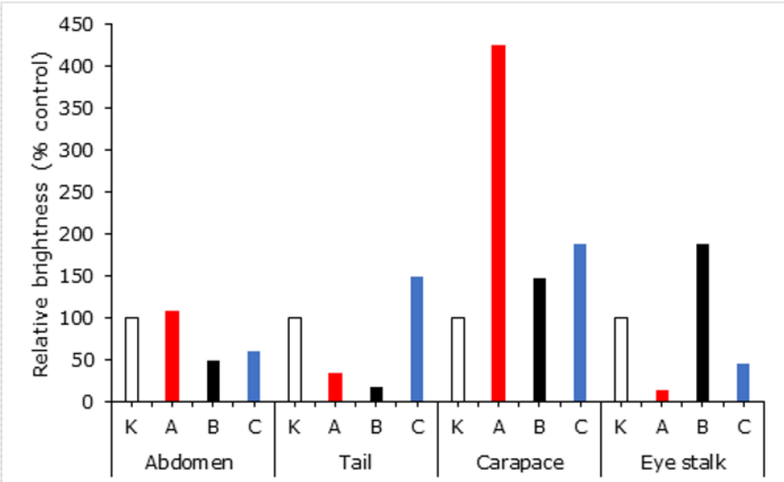


Figure 2. Relative brightness of the *Panulirus homarus* puerulus body pigmentation.

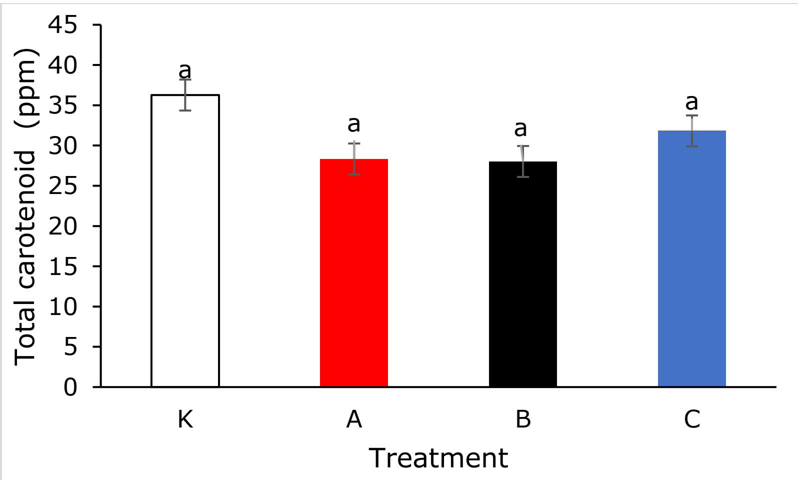


Figure 3. Total carotenoid of *Panulirus homarus.*

In *P. homarus* exposed to either a black or a transparent background, the total amount of carotenoids extracted from the whole tissue was identical, but the fraction of astaxanthin esters in lobsters exposed to a transparent background was higher (Figure 3). Despite the fact that this effect was not substantial, the trend was consistent with significant effects seen in earlier investigations (Tume et al 2009). The information provided here shows that the adaptive response to the background color and the coloration of the control crustaceans are based on more than just the expansion or contraction of the pigment structures. This research demonstrated a significant interaction between the physiological and morphological causes of lobster coloration. *P. homarus* with dilated chromatophores similarly accumulates CRCN protein bound with free astaxanthin within hypodermal tissues when adapted to a black backdrop. In contrast, the CRCN protein is depleted in control (transparent background) adapted animals with restricted chromatophores, while astaxanthin is preserved in the hypodermal tissue in an esterified form. This adaptation to backdrop color has a significant influence on the intensity of the lobster's outward appearance (Wade et al 2012).

Physiological or morphological reasons may be to blame for changes in the coloration of crustaceans (Wade et al 2012). The vibrant carotenoid pigments are recognized as biologically active substances that have favorable impacts on both animal and human metabolism. Carotenoids have crucial functions in vision, operate as precursors of transcription regulators, and defend against a variety of stressors such as UV radiation, reactive oxygen species, and free radicals. (Calvalho & Caramujo 2017).

Production performance. For all treatments, the starting mean weight of individuals in the various lobster groups is approximately 0.28 g. *P. homarus* raised in black tanks had the highest absolute weight and specific growth rates after 30 days of feeding (P>0.05). The maximum survival rate was, however, seen in red tank-raised *P. homarus* (P<0.05). Figures 4 through Figure 6 show the growth and survival rates of lobsters raised in various colored tanks for 30 days.

The *P. homarus* puerulus survival rates (%) varied according to the treatments, indicating that changing the background color has an impact on them. It was noted during the investigation that the lobster in the treatments with black backgrounds grew more than the control lobster (transparent background), which might be related to an improved camouflage and consequently to less stress with a dark background. In comparison to the treatments with a white background, which are more exposed, the shrimp in the treatments with a black background (darker environment) grew more, for the same reasons (El-Sayed & El-Ghobashy 2011). The adaptation to the backdrop color (camouflage) in crustaceans develops primarily as a strategy for lowering photoreception and, in turn, avoiding predator assaults (Shiraki et al 2010).

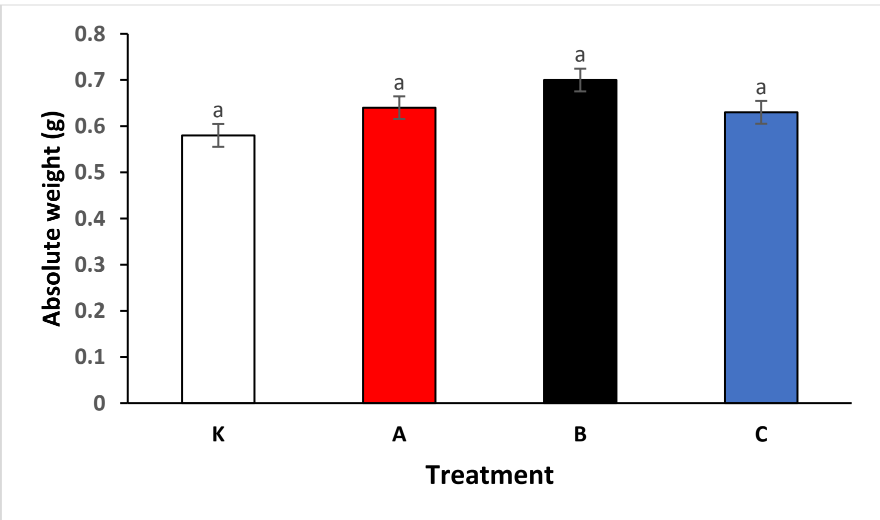


Figure 4. Absolute weight of *Panulirus homarus* puerulus.

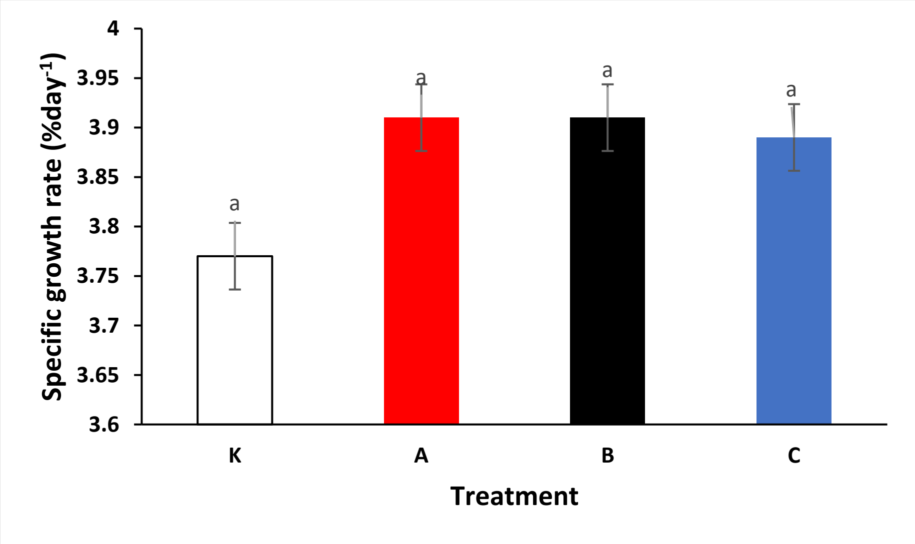


Figure 5. Specific growth rate of *Panulirus homarus* puerulus.

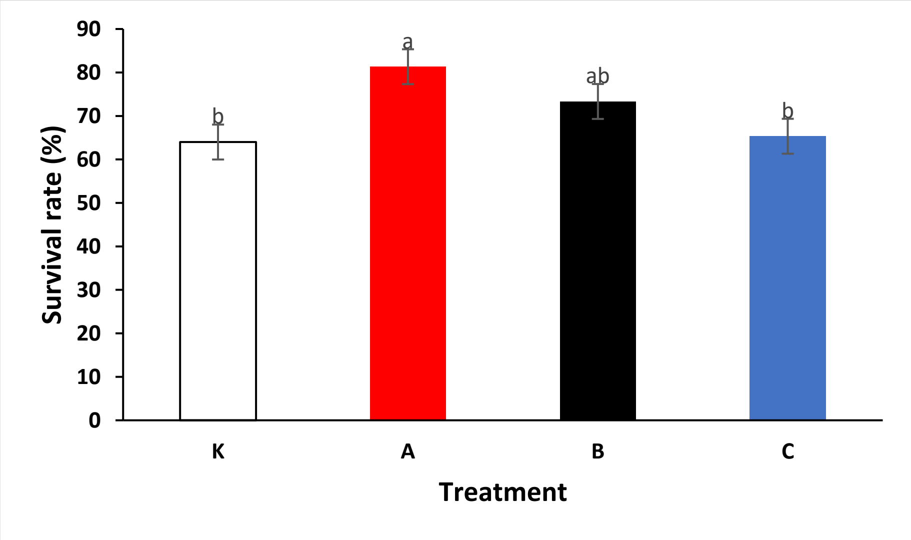


Figure 6. Suvirval rate of *Panulirus homarus* puerulus.

Growth statistics demonstrate that a black background provides a high value. It is believed that because of their gloomy environment, which affects their feeding, since *P. homarus* are nocturnal and actively engaged in foraging during the night period. *P. homarus* tends to grow slowly in a control (transparent) environment, and it is thought that this is because the lobster's eyes are prone to refraction, which makes it difficult for it to see the food that is being provided. Compared to a white background, a black background might produce more natural lighting conditions, whereas artificial lighting situations might be distressing (Shi el al 2019). The light reflection lured the larvae on the white backdrop to the bottom of the tank, which caused them to become disoriented. This reduced their ability to successfully eat and increased their susceptibility to diseases (Rabbani & Zeng 2005). Because Jundia fish, *Rhamdia quelen*, prefer to stay in the dark, the white tank's brightness and clarity make it difficult for the fish to live in (Barcellos et al 2009). In contrast to those housed in containers with lighter backgrounds, the *Scylla serrata* crab larvae exhibited a higher survival rate when kept in containers with dark backgrounds (black, dark green, and brown), according to the research performed by Rabbani & Zeng (2005). Furthermore, it was found that the black backgrounds encouraged faster development rates, which were linked to a more effective eating as a result of the background's contrast with the prey's color and the larvae's decreased stress. Because many larvae are positively phototactic, which induces them to gravitate toward rejective surfaces, several studies advise using black color tanks for larval rearing. Larval striped bass (*Morone saxatilis*) have been observed to suffer from stress, poor feeding, and bodily injury as a result of the larvae adhering to the walls of light-colored tanks (Martin-Robichaud & Peterson 1998).

Water quality. One of the environmental factors that affects lobster survival is water quality. Table 3 lists the water quality metrics that were logged during this study.

Table 3

Average values for water quality parameters during *Panulirus homarus* peurulus' maintenance period

Parameters	*Treatment*			
	K	*A*	*B*	*C*
pH	7.75-8.34	7.72-8.36	7.72-8.46	7.73-8.35
Temperature (^{0}C)	25.00-30.00	25.33-29.00	25.67-28.33	25.65-28.67
Salinity (g L^{-1})	31.00-36.00	30.67-36.00	30.33-36.00	31.00-36.00
DO (mg L^{-1})	7.57-8.83	7.60-8.88	7.36-8.80	7.47-8.63
Nitrite (mg L^{-1})	0.13-1.02	0.13-1.28	0.13-1.17	0.13-0.96
Nitrate (mg L^{-1})	0.16-3.66	0.16-3.37	0.16-3.63	0.16-3.84
Total amonia (mg L^{-1})	0.02-0.77	0.02-0.72	0.02-0.76	0.02-0.81

Animal health and productivity can be dramatically impacted by environmental stressors, particularly temperature. For poikilothermic lobsters, ambient temperatures have an immediate impact on their metabolism, growth, and molting, and high temperatures can have a significant negative impact on their immune systems (Ooi et al 2019). The water quality conditions during the study were generally favorable. The pH range used for the study was from 7.72 to 8.46, fairly steady. This figure falls within the ideal range according to the study of Vijayakumaran et al (2010), which found that the best pH range for lobster rearing is between 7.8 to 8.59. The study's temperature range, 25 to 30˚C, was quite steady and remains within the ideal range (Vijayakumaran et al 2010). The fastest growth in juvenile *P. homarus* can be accomplished at a temperature of 28˚C, with a carapace length of 60 mm obtained in 18 months, according to Phillips & Kittaka (2000). The lobster species grow best at different temperatures: *Panulirus argus* (between 25 and 27˚C), the *P. homarus* (30˚C), the *Panulirus cygnus* (25 to 26˚C), and the *Interrupt panulus* (28˚C), all show different temperature tolerances.

Salinity levels throughout the investigation were quite constant and fell between 30.33 and 36.00 ppt. According to studies by Verghese et al (2007), such values are within the ideal range for lobster rearing, which is between 20 and 40 ppt. The conditions for dissolved oxygen throughout the investigation were quite constant and fell between 7.36 and 8.88 mg L^{-1}. According to Boyd's study's (1990) findings, the ideal range for dissolved oxygen for lobster rearing is >5 mg L^{-1}. Total ammonia levels were rather steady throughout the trial, ranging from 0.02 to 0.81 mg L^{-1}. According to the study of Vijayakumaran et al (2014), the ideal range of TAN indicated for lobster rearing is <0.1 mg L^{-1}. Nitrite levels throughout the trial were rather constant and ranged from 0.13 to 1.28 mg L^{-1}. According to Drengstig & Bergheim's (2013) research, the ideal range for nitrite for *P. homarus* rearing is <5 mg L^{-1}. Nitrate was in the range of 0.16-3.84 mg L^{-1} during the study, which was consistent with the mentioned nitrate threshold. According to a study of by Philips & Kitaka (2000), the recommended ideal range of nitrate for lobster raising is in the region of 100 mg L^{-1}.

Conclusions. The survival rate of *P. homarus* puerulus varied significantly (P<0.05) depending on color in the maintenance tank. Analysis of physiological and biochemical parameters revealed significant differences (P<0.05) in the following biomarkers: total hemoglobin count, SGPT, SGOT, ALP, cholesterol, total protein, and glucose. *P. homarus* puerulus housed in a transparent background (control) are typically under stressful circumstances.

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Conflict of interest. The authors declare no conflict of interest.

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