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Light colour affect the survival rate, growth performance, cortisol level, body composition, and digestive enzymes activities of different Snubnose pompano (*Trachinotus blochii* (Lacépède, 1801) larval stages

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ABSTRACT

Snubnose pompano (*Trachinotus blochii*) are widely cultured in the world. Light colours are among factors affecting fish performance in captivity. Snubnose pompano larvae often reared under white light. However, no study supports its choice or informs the extent of its effect or its constituent colour. The study investigated the influence of green, blue, yellow, purple, and white lights on growth, survival, cortisol, body composition, and digestive enzymes activities of one-day post-hatch Snubnose pompano larvae reared in light magenta tanks under a photoperiod of 24 Light:0 Dark hours for 25 days. Overall, the best growth performance of snubnose pompano larvae was in purple and white, and green lights during early and late larval stages, respectively. White light promoted survival rate while that of green light reduced. Larvae were less stressed and had enhanced body contents under white and purple light environments. Purple and yellow lights influenced low trypsin and pepsin activities. The study reports for the first time the effect of the light colour environment on the digestive enzyme activities and body composition of the finfish larvae. Generally, the study provides novel insights on optimal light colour in the larval rearing protocol of this species. Through this study, the use of white light during the first feeding (zero to four days post-hatch) and purple light in later stages (five days post-hatch until metamorphosis) is encouraged while discourages green and yellow lights in the larval rearing of Snubnose pompano.

1. Introduction

Snubnose pompano (*Trachinotus blochii* (Lacépède, 1801) is among promising aquaculture species although its exceptionally high larvae mortality presents a significant bottleneck for increasing its global output. Factors influencing successes of larval transition to adulthood include light, with different fish species and age groups perform optimally under specific light wavelengths (light colour) (Ruchin, 2020a). The light colour influence survival rate (Yan et al., 2019), growth performance (Villamizar et al., 2009; Wu et al., 2020), stress response (Song et al., 2016; Wu et al., 2020), body composition (Aly, 2017), and digestive enzyme activities (Hou et al., 2019) of various fish species in captivity. However, the effects are species-specific, as low survival rates in Turbot, *Scophthalmus maximus* larvae (Wu et al., 2019) and Atlantic cod, *Gadus morhua* (Sierra-Flores et al., 2016), for example, have been reported under green light. Contrary, the green light was among light colour that increased the survival rate of shrimp, *Penaeus vannamei* (Fei et al., 2020). On growth, blue light improved the performance of Senegal sole larvae, *Solea senegalensis* (Villamizar et al., 2010), European sea bass, *Dicentrarchus labrax* larvae (Villamizar et al., 2009), Guppy fish, *Poecilia reticulata* (Ruchin, 2004), and turbot larvae (Wu et al., 2020), but reduced performance of Rainbow trout, *Oncorhynchus mykiss* (Karakatsouli et al., 2007). However, light colour does not affect the growth of Haddock, *Melanogrammus aeglefinus* larvae (Downing, 2002).

Light colour has also been associated with fish body composition, behaviours, hormone and enzyme response. For example, green, blue, and red lights have been shown to induce stress response in turbot larvae (Wu et al., 2019) and red light in Goldfish, *Carassius auratus* (Song et al.,

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2016). Green and blue, and white lights decrease cortisol levels in goldfish (Noureldin et al., 2021; Song et al., 2016) and Atlantic salmon, *Salmo salar* (Migaud et al., 2007), respectively. Aly (2017) reported that light colour affected body composition of hybrid red tilapia, *Oreochromis mosambicus* × *O. hornorum* fingerling, and Karakatsouli et al. (2007) showed no effect in gilthead seabream. The effect of light is stage-specific in some species such as turbot larvae (Wu et al., 2020).

Important enzymes found in early fish larval stages include trypsin, pepsin, and amylase with the proteolytic pancreatic trypsin and gastric pepsin being important mobilization of a protein crucial for high larval growth rate. Pancreatic amylase digests complex carbohydrates (Rønnestad et al., 2013; Suzer et al., 2007). However, their activities before or after the onset of exogenous feeding vary with species (Rønnestad et al., 2013; Yúfera, 2018). Activation of the pancreatic trypsin often comes earlier than gastric pepsin (Rønnestad et al., 2013). In red drum larvae, the pepsin assumes an important proteolytic role toward the end of the larval period at 10–14DPH (Lazo et al., 2007). Coordination of these enzymes is crucial for promoting digestion of ingested feed and larval nutrient influx for successful larval survival, growth and development (Rønnestad et al., 2013; Rungruangsak-Torrissen et al., 2006; Suzer et al., 2007). However, studies on the influence of light colour on digestive enzyme activities are limited. The lone study on juvenile spotted sea bass, *Lateolabrax maculatus* (Hou et al., 2019) shows blue light offering best while red light disrupting digestive enzyme activities.

Wrong light colour can affect the production and quality of various fish age groups under captivity and hence the returns of farmers. Establishing the best light colour for each development stage of specific fish of interest, including snubnose pompano, is crucial. In Snubnose pompano, white light is often used in rearing them. However, no studies support its choice, and its effects on performance are unknown. The present study investigated the effect of various light colour on growth, survival, cortisol, body composition, and digestive enzymes activities of the Snubnose pompano larvae in the hatchery. It is an effort to establish optimum light colour requirements to improve the hatchery outputs of this species.

2. Materials and methods

2.1. Experimental setup and management

One-day post-hatch (1dph) Snubnose pompano larvae (2.9 ± 0.17 mm, 1.4 mg) from Duong De Marine Fish Hatchery (Nha Trang, Vietnam) were stocked to all fifteen (15) light colour treatments tanks (light magenta in colour, fibre-glass and conical in shape, 0.2 m^3 each) in triplicate at a stocking density of 5 larvae/litre (1000 fish larvae per tank). Light-emitting diodes (LEDs) (15 W, Waterproof; supplied by Roxin Company) were used to emit five different lights: green (495–530 nm, blue (450–480 nm), yellow (580–595 nm), purple (460−465 nm), and white. The LED lights were set on the water surface at the center of each tank (one colour per tank). The photoperiod was 24 Light: 0 Dark as recommended by Alejos and Serrano (2018) for Snubnose pompano larvae. All tanks were covered by black screen covers to prevent light pollution. The light intensity was on average of 1252 lx. Green water was maintained by adding live microalgae *Nannochloropsisoculata,* 3 × 10^5 cells/mL when larvae were aged between 1dph and fifteen days post-hatch (15dph). Larvae were fed with enriched rotifers *Brachionus plicatilis*, at a density of 1 individual/mLfor larvae aged two to four days post-hatch (2−4dph) and later 3–10 individuals/mL for five to fifteen days post-hatch (5−15dph). Finally, mixed-size enriched Artemia (Gold Artemia Brand Brine Shrimp Eggs, Vietnam), 0.5 individual/mL were fed twice per day for larvae with an age of eleven to twenty-days post-hatch (11– 20dph). Liquid enrichment diets-DHA Protein Selco and DHA Selco (INVE aquaculture, Belgium) were used for the rotifer and Artemia, respectively. The availabilities of rotifers and artemia in the rearing tanks were checked around 1 h before each feeding time to ensure the presence of feed in the tanks is not a limiting factor for the study. Weaning diet, ROYAL CAVIAR microencapsulated feed (BERNAQUA) was provided to larvae from sixteen to twenty five-days post-hatch (16 to 25dph) (4–5 times per day) at a rate recommended by the supplier. Throughout the experiment, average water quality parameters in the tanks were as follows: salinity (35‰), temperature (31.0–31.4 °C), pH (7.56–7.70), total ammonia nitrogen (<0.2 mg/L) and dissolved oxygen (>5 mg/L). Leftover feed and dead larvae were siphoned daily. Water exchanges were performed before evening feed from 6 to 25dph at a rate of 30–50 %.

2.2. Sampling and analytical methods

2.2.1. Length and weight

Larvae between 2.90 mm and 6 mm were measured to the nearest 0.01 mm by stereomicroscope equipped with a micrometer and weighed using a sensitive balance (Sartorius CP224S, Data Weighing System Inc., USA) to the nearest 0.0001 g. Large larvae (from 6.8 mm) were measured using a plastic ruler and weighed using TL-series digital scale (50g × 0.001g). Water temperature and pH were recorded by pH meter (HANNA, HI98115, USA). Light intensity recorded by a light meter (Fuyi FY836, China) and dissolved oxygen (DO) by multi-parameter DO meter (HANNA HI9147-04, USA). Salinity and ammonia were recorded using a salinity refractometer (ATC Extech RF20, China) and an ammonia kit (API AMMONIA 130, Germany), respectively. Initial body length and weight were estimated from 18 larvae fish sampled from the hatching tanks at 1dph. Randomly sampled larvae (n = 20–21, 7 per replicate) were taken at 4dph, 12dph, nineteen days post-hatch (19dph), and (n = 45–50, 16–17 per replicate) at 25dph and immediately immobilized in ice, blotted, and measured for total length and weight. Larvae were counted manually to estimate the survival rate at the end of the experiment. Survival and growth parameters were estimated using the formulas

$$\text{Survival } (\%) = [(N_0 - N_t)/N_0] \times 100$$

$$SGR_{TL}\ (\%day^{-1}) = [(\ln TL_f - \ln TL_i) \times t^{-1}] \times 100;\ DBWG = (F_{BW} - I_{BW}) \times t^{-1};$$
$$\text{and } SGR_{BW}\ (\%day^{-1}) = (\ln F_{BW} - \ln I_{BW}) \times 100.$$

Where N_0: Initial number of larvae; N_t: Final number of larvae; TL: Total length; TL_i: initial total length; TL_f: final total length; SGR_{TL}: specific growth rate in total length; BW: body weight; I_{BW}: initial body weight; F_{BW}: final body weight; DBWG: daily body weight gain; SGR_{BW}: specific growth rate in body weight; t: 25 days of the experiment; ln: natural logarithm.

2.2.2. Cortisol extraction and analysis

Batches of 45 larvae (15 larvae per treatment tank) were collected during the morning before feeding at 4dph, 12dph, 17dph, and 25dph for cortisol determination. Sampled larvae were immediately immobilized in ice-cold water, transferred to pre-chilled 1.5 ml cryotube vials with excess water removed and frozen in ethanol at -80 °C until further analysis. Analysis of whole-body cortisol used methods described for Zebrafish (Yeh et al., 2013). Briefly, samples were thawed on ice, homogenized with a pellet mixer (VWR International LLC, Radnor, PA, USA) for 20 s and vortexed for 30 s at maximum speed. Extraction of supernatant done after mixing 1000 μl ethyl acetate with homogenate at 4 °C. The mixture was allowed to evaporate for 30 min at 30 °C in a speed-vacuum concentrator. Cortisol was dissolved in 0.2 % bovine serum albumin (BSA; A7030, Sigma) in phosphate-buffered saline (PBS) and frozen at -20 °C. Cortisol antibody solution (P01-92-94M-P, East Coast Bio 1.6 g/mL in PBS) used to coat plates for 16 h at the temperature of 4 °C. Then, washed and blocked with 0.1 % BSA in PBS. Later, incubation of cortisol samples and cortisol conjugate (HRP: P91-92-91H, East Coast Bio) set at room temperature for two hours and washed three times with PBS containing 0.05 % Tween-20 (Roth). Colour reactions developed using tetramethyl benzidine (TMB: 22166-1, Biomol) and

tetrabutylammonium borohydride (TBABH: 230170-10 G, Sigma). Sulphuric acid (1 M H_2SO_4) stopped the colour reaction. Enzyme-linked immunosorbent assay (ELISA) plate reader (Multiskan Ascent Microplate Photometer, Thermo Scientific) read absorbance at 450 nm. Cortisol levels were normalized to total protein and denoted as cortisol concentration (pg/μg protein).

2.2.3. Body composition

Determination of protein, lipid, moisture, and ash followed the standard methods of the Association of Official Analytical Chemists (AOAC). Protein was measured by determining total nitrogen using the Kjeldahl device, while lipid was extracted by dissolving samples in petroleum ether and quantified by the Soxhlet's method. Samples of 105 larvae (35 per tank colour) were collected randomly at the end of the experiment and stored at −80 °C for whole-body composition analysis. Moisture content was determined by drying the larvae at 105 °C to a constant weight and ash by combustion of larvae at 550 °C for 24 h.

2.2.4. Enzymatic activities

A total of 45 larvae (15 per replicate) were sampled at 12, 19, and 25dph before morning feeding and frozen for analyzing digestive enzyme activities. Sampled larvae were thawed on ice and homogenized potassium dihydrogen phosphate (20 mM KH_2PO_4) and sodium chloride (6 mM NaCl) buffer maintained at pH 6.9. The mixture was centrifuged for 30 min at 4,200 rpm 4 °C. Supernatants were collected and stored at -80 °C until further analysis. Trypsin, amylase, and pepsin activities were measured based on methods by Bernfeld (1951); Tseng et al. (1982), and Worthington (1982), respectively. Determination of total protein concentration done by Biorad protein assay based on the Bradford dye-binding method (Bradford, 1976). Enzyme activities are expressed as U/mg protein.

2.3. Data analysis

All results presented as mean ± standard error of the mean (SD). Data were tested for the normality (Shapiro's test) and homogeneity of variance (Levene's test). All data except growth parameters were analyzed by One Way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test to determine the significant differences between the means. Growth parameter data were analyzed by Kruskal Wallis's test followed by Dunn's Multiple Comparison Post-hoc test. The P-value of < 0.05 represented the significance level between treatment groups. Program R (R Foundation for Statistical Computing, Vienna, Austria) version 4.0.4 was used for all data analysis.

3. Results

3.1. Survival rate

The survival rates of Snubnose pompano larvae were significantly affected by the different light colour ($P < 0.05$, Fig. 1). At the end of the experiment (25dph), larvae had a significantly higher survival rate when reared under white light than under green, purple, yellow, and blue lights ($P < 0.05$). Snubnose pompano larvae reared under the purple, blue, and yellow lights had no significant differences in the survival rates ($p > 0.05$).

3.2. Growth performance

The growth performance of Snubnose pompano larvae was significantly affected by light colour ($P < 0.05$, Fig. 2, Table 1). At the age of 4dph, larvae had significant higher total lengths when grown under white and purple lights than under green, yellow and blue lights ($P < 0.05$, Fig. 2). At the age of 12dph, the body length of the larvae under green, yellow, and blue lights were significantly higher than those under white and purple lights ($P < 0.05$). At the end of the experiment

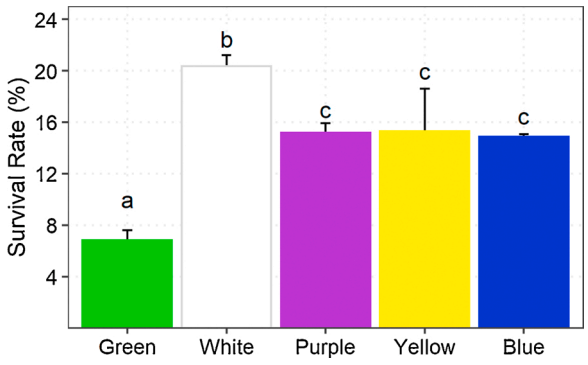

Fig. 1. Survival rate (%) of the Snubnose pompano larvae reared under green, white, purple, yellow, and blue light environments for 25 days and sampled at 25 days post-hatch. Different letters in the bar diagram show groups that differ significantly ($P < 0.05$).

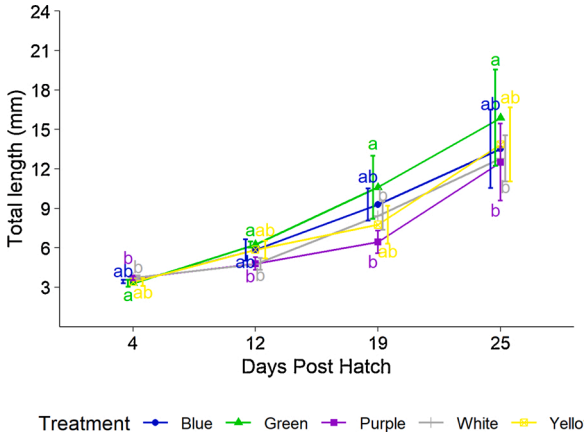

Fig. 2. Weakly growth in total length (mm) of Snubnose pompano larvae cultured in green, white, purple, yellow, and blue light environments for 25 days. The results are expressed as Mean ± Standard error of three replicates. Different superscripts indicate significant differences ($P < 0.05$).

(25dph), larvae under white, purple, yellow, and blue lights attained significantly lower final body weight, specific growth rate in body weight (SGR_{BW}), and daily body gain in weight (DBWG) than those under green light ($P < 0.05$, Fig. 2, Table 1). Besides, larvae under green light had significantly higher final total length, length gain rate, and specific growth rate in body length (SGRTL) than those under blue, purple and white light ($P < 0.05$). Although, green lights attained a higher final total length and length gain rate than yellow light, but were statistically the same (Fig. 2, Table 1).

3.3. Body composition

Crude protein, lipid, ash, and moisture contents in Snubnose pompano larvae were significantly affected by different light colour at 25dph ($P < 0.05$, Fig. 3A–D). White light significantly increased lipid and moisture contents while reducing crude protein contents compared to green, purple, yellow, and blue lights ($P < 0.05$). Purple light increased larval crude protein and decreased ash contents than green, white, yellow, and blue lights. Green light significantly influenced the lowest larvae lipid and moisture contents and highest ash contents compared to white, purple, yellow, and blue lights ($P < 0.05$).

3.4. Digestive enzymes activities and cortisol levels

3.4.1. Trypsin activities

Significantly highest trypsin activities were measured in Snubnose

Table 1
Initial total length (mm), Final total length (mm), Length gain rate (%), the specific growth rate in length (SGR_{TL}, % total body length per day), Initial body weight (mg), final body weight (mg), the specific growth rate in body weight (SGR_{BW}, % body weight/day), and daily body weight gain (DBWG, mg/day) of Snubnose pompano larvae reared in a different light colour for 25 days.

Growth results	Treatment (light colour)				
Growth parameters	Green	Blue	Yellow	Purple	White light
Initial total length	2.9 ± 0.17^{a}	2.9 ± 0.17^{a}	2.9 ± 0.17^{a}	2.9 ± 0.17^{a}	2.9 ± 0.17^{a}
Final total length	15.9 ± 3.65^{b}	13.5 ± 2.98^{a}	13.8 ± 2.82^{ab}	12.5 ± 2.93^{a}	12.8 ± 1.76^{a}
Length gain rate	448 ± 1.26^{b}	366 ± 1.03^{a}	378 ± 0.97^{ab}	331 ± 1.01^{a}	341 ± 0.67^{a}
SGRTL	6.69 ± 0.98^{c}	6.06 ± 0.90^{ab}	6.18 ± 0.80^{b}	5.75 ± 0.88^{a}	5.90 ± 0.57^{ab}
Initial body weight	1.4^{a}	1.4^{a}	1.4^{a}	1.4^{a}	1.4^{a}
Final body weight	102 ± 0.06^{b}	63 ± 0.04^{a}	61 ± 0.03^{a}	45 ± 0.03^{a}	50 ± 0.020^{a}
SGR_{BW}	16.2 ± 2.98^{b}	14.4 ± 2.78^{a}	14.5 ± 2.18^{a}	13.0 ± 2.87^{a}	14.0 ± 1.58^{a}
DBWG	4.0 ± 0.003^{b}	2.5 ± 0.001^{a}	2.4 ± 0.001^{a}	1.7 ± 0.001^{a}	1.9 ± 0.0007^{a}

Data are presented as Mean ± Standard error of three replicates. Different superscripts in the same row were significantly different ($P < 0.05$).

pompano larvae reared under green light compared to those under white, purple, yellow, and blue lights at 12dph ($P < 0.05$, Fig. 4A). Larvae subjected to white light had significantly the highest trypsin activities at 19dph, followed by blue light, while other remaining groups were statistically the same ($P < 0.05$). At the end of the experiment (25dph), larvae of pompano had significantly lower trypsin activities when subjected to purple light relative to green, white, yellow, and blue lights ($P < 0.05$). Trypsin activities of each treatment group decreased at 19dph but increased again by 100 % at 25dph (Fig. 4A).

3.4.2. *Pepsin activities*

At 12 dph and 25dph, Snubnose pompano larvae had significantly higher pepsin activities when reared under green lights than in white, purple, yellow and blue lights ($P < 0.05$, Fig. 4B). At 25dph, both larvae under green and blue lights had significantly higher pepsin activities than when grown under white, purple, and yellow lights ($P < 0.05$). At 19dph, elevated pepsin activities were observed in all treatments, followed by decreased activities at the end of the experiment (25dph) (Fig. 4B).

3.4.3. *Amylase activities*

Amylase activities were significantly highest in the Snubnose pompano larvae under yellow light at 12dph and 25dph than the green, white, purple, and blue lights. Green and purple lights influenced significantly higher larvae amylase activities at 19dph than in white, yellow and blue lights ($P < 0.05$, Fig. 4C).

3.4.4. *Cortisol levels*

Significant effects of the different light colour on Snubnose pompano cortisol levels were in their different larval stages ($P < 0.05$, Fig. 4D), with larvae at 4dph having the highest cortisol levels than at 12−25dph in all light regimes. In larvae exposed to the green light, cortisol level was significantly higher at 4 and 12dph than in white, purple, blue and

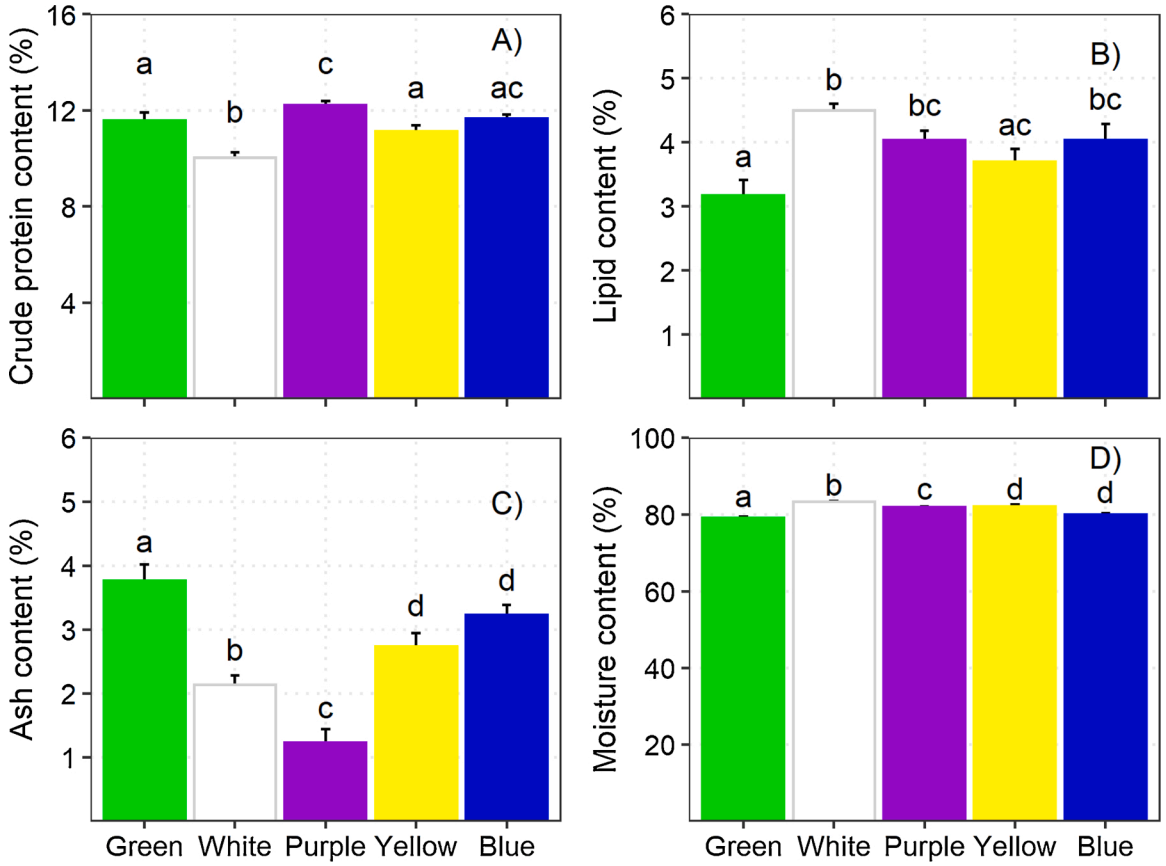

Fig. 3. Mean ± Standard error crude proteins (A), Lipid content (B), and Ash content (C) of Snubnose pompano larvae cultured in triplicate in green, white, purple, yellow, and blue light environments for 25 days and sampled at 25 days post hatch. Significant differences ($P < 0.05$) among treatments at the same sampling point are indicated by different letters above error bars.

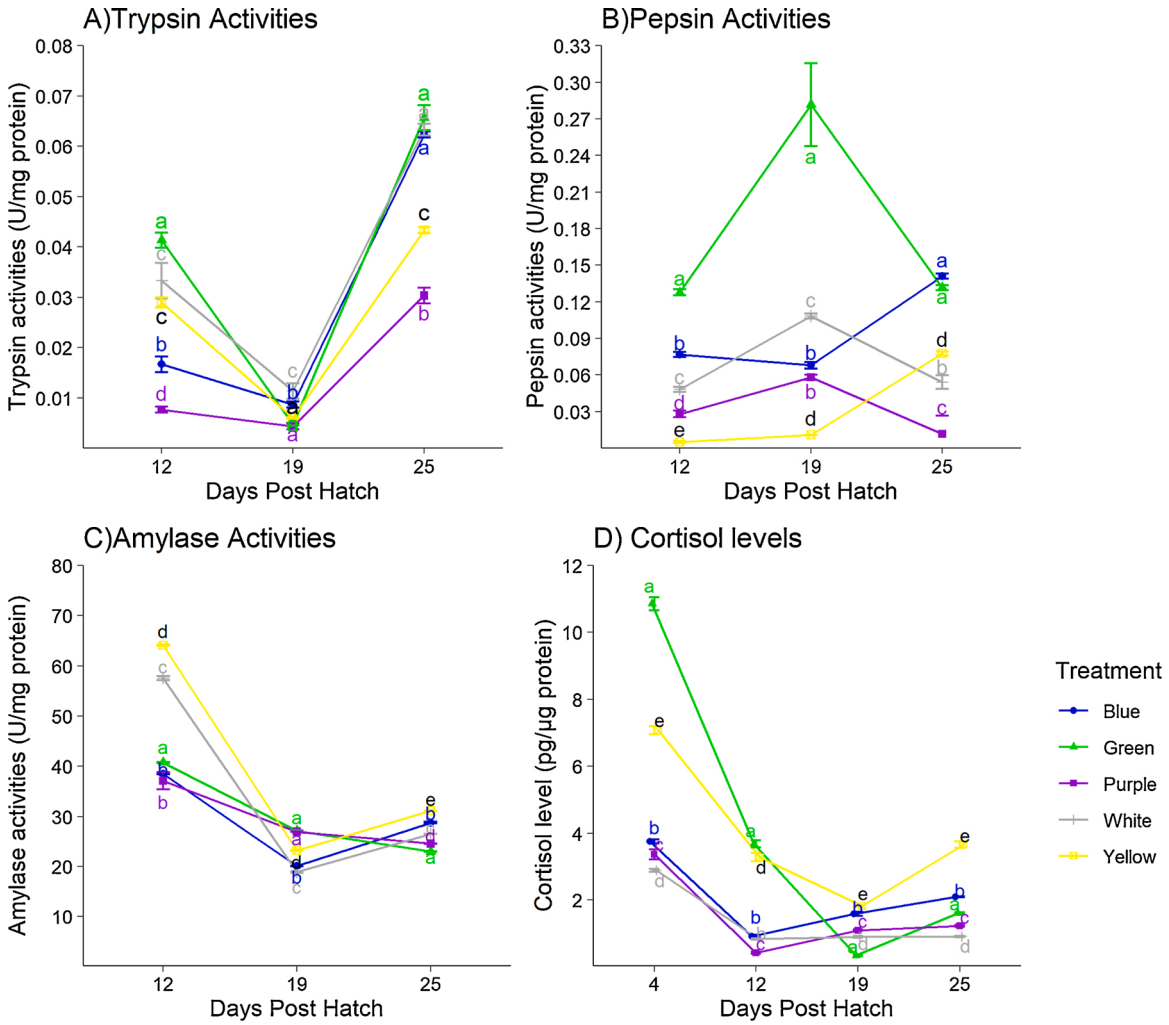

Fig. 4. Mean ± Standard error trypsin activities (A), pepsin (B), amylase (C) and cortisol concentrations (D) of Snubnose pompano larvae cultured in triplicate in green, white, purple, yellow, and blue light environments and sampled at 12, 19, and 25 days post hatch. Significant differences ($P < 0.05$) among treatments at the same sampling point are indicated by different letters above or below error bars.

yellow lights ($P < 0.05$). During the age of 19 and 25dph, larvae under yellow light had significantly higher cortisol levels than larvae reared under green, white, purple, and blue lights ($P < 0.05$). Significantly lower cortisol levels were measured in larvae under white light at 4dph and 25dph; purple light at 12dph; and green light at 19dph ($P < 0.05$). Larvae reared in green and yellow lights, had their cortisol levels decreased as the larvae approached 19dph and raised again at 25dph. In contrast, larvae reared under blue and purple lights had their cortisol levels decreased until 12dph and started to rise at 19dph until 25dph. Larvae under white light showed a sharp decrease of cortisol at 12dph, which remained stable until metamorphosis at 25dph (Fig. 4D).

4. Discussion

This study focused on revealing the effect of light colour environments on the performance and physiology of different larval stages of Snubnose pompano. Their survival and growth rates suggest some of the light colour environments caused elevated stress and reduced digestive capacity resulting in the poor larval performance of the Snubnose pompano. The results provide novel insights and crucial optimal light colour environments for the marine hatchery industry of this species. These results may be improving larval rearing protocols for this species. The results are crucial to the scientific community because this is the first study to report the effect of light colour environment on the digestive enzyme activities and body composition of the finfish larvae. Thus, it provides novel information on the digestive physiology and biochemistry crucial in the aquaculture nutrition industry. Besides, cortisol results provide information on the welfare and tolerance level of stress of this species when cultured under different light environments.

Ambient light assists fish to form visual images crucial for their ability to detect, distinguish, select and capture prey (Rønnestad et al., 2013; Ruchin, 2020b). Light environment, therefore, affects the first feeding, growth and survival of marine fish larvae (Alejos and Serrano, 2018; Jayakumar et al., 2018). In the present study, larvae subjected to green light had a significantly lower survival rate while growth in white light enhanced it. Over 80 % mortality of the larvae were observed around the time of first feeding in 3 to 5dph larvae grown in green light. As growth in green light also increased significantly cortisol level at 4dph, the poor survival in the green light environment during 3–5dph may be associated with high stress, starvation due to limited reserves of endogenous energy and essential fatty acids needed for normal survival (Adams, 1999) and hence failure to maintain homeostasis. Larvae under green light were also shown to have the lowest lipid content whereas those under white light had the highest lipid content and highest survival rate. The effect of green light in the present study is not unique as the light environment has also been shown to affect the survival rate of Turbot larvae at 2dph (Wu et al., 2019) and 60dph (Sierra-Flores et al., 2016), European sea bass larvae (Yan et al., 2019), and Atlantic cod

(Sierra-Flores et al., 2016). Contrary to the present study, a higher survival rate has been shown in Barfin flounder (Takahashi et al., 2018, 2016) and Crucian carp (Ruchin, 2004) grown in the green light, Haddock larvae subjected to blue and green lights (Downing, 2002), juveniles of Rainbow trout under green and blue lights (Timucin et al., 2016), and *Penaeus vannamei* under green, blue, yellow, and dark lights as compared to red and white lights (Fei et al., 2020). Although poor early larval survival in the present study has been associated with stress in green light, the possible effect of green water in further reducing incident irradiance and thus creating a darker light environment and/or changing larval behavior cannot be overruled (Cobcroft et al., 2001).

Unlike in early larval stages, green light in the present study influenced better growth performance in larvae of 12dph until metamorphosis (25dph). At such larvae ages, cortisol levels were also lowered, with 19dph having the lowest level. Perhaps, the changes in growth performance at older larval age under green light may be related to the ability of larvae to either adapt to lowered stress or decrease in density of competing larvae as there was high mortality at 3–5dph. The green light has been reported to enhance the growth performance of Crucian carp and Rotan fish (Ruchin, 2004). Growing Haddock larvae in blue and green lights enhanced growth performance (Downing, 2002). Green or red light has also been stated to improve the growth performance of European sea bass larvae (Yan et al., 2019). These findings suggest that optimal lighting spectrums may differ widely between species.

Cortisol is a stress response to glucocorticoid steroid hormone released by activating the hypothalamic-pituitary axis when the animals experience stressful environments (Aluru and Vijayan, 2009). The underwater light environment is one of the critical parameters that affect physiological processes and reactions at various development stages of the fish (Ruchin, 2020a). Following their lower survival and growth performance, we found that larvae under green light had significantly higher cortisol levels at 4dph and 12dph than the other light colour, with cortisol level at 12dph being three times lower than at 4dph. Yellow light influenced the second-highest cortisol level at 4dph and nearly half of that at 12dph and 25dph. Suggesting green light (at 4dph and 12dph) and yellow lights (at 4dph, 12dph and 25dph) may induce stress to snubnose pompano larvae if their respective levels have surpassed threshold levels for causing stress. Studies using turbot larvae have shown green, blue, and red lights inducing stress response at the early (2dph) larvae stage (Wu et al., 2019). However, studies using Goldfish (Song et al., 2016) and Gold-stripped amberjack (Choi et al., 2016) have shown the green light to decrease cortisol and stress levels. Also, yellow light reduces the stress-induced cortisol response of Pearl gourami fish, *Trichopodus leerii*(Heydarnejad et al., 2017). Once again showing to differential light preferences in different species. In the present study, Snubnose pompano larvae grown under white light had the lowest cortisol levels at 4dph. Its levels at 12dph, 19dph and 25dph, were nearly half of the 4dph level. Other light regimes with generally low cortisol levels were purple (violet) and blue. Cortisol levels under white and blue lights seem low as in the Atlantic salmon (Migaud et al., 2007) and Goldfish (Noureldin et al., 2021), respectively. In the present research, the cortisol level decreased below 2.5 pg/μg protein (50–125 % decrease) across the ages 12dph, 19dph and 25dph in all light colour groups except yellow. The decrease-increase trend of cortisol levels indicates cortisol levels are higher during first feeding (many larvae are starving) and metamorphosis. Contrary, stress in Turbot larvae was induced before and after metamorphosis (Wu et al., 2020). The present result shows that Snubnose pompano larvae stress is generally low in white, purple and blue light (short wavelengths below 480 nm) during the early to late larvae stage. Cortisol levels in green light lowered with age. As well, cortisol levels rise during the first feeding and metamorphosis stages.

Fish body composition is of paramount interest in aquaculture partly is an indicator of energy allocation among various compartments and affects growth, the efficiency of food utilization and survival (Breck, 2014). Previous studies have shown light colour's effect on body composition in juvenile and adult fish (Aly, 2017; Karakatsouli et al., 2007). For example, light colour did not affect the proximate body composition of the Gilthead sea bream and Rainbow trout (Karakatsouli et al., 2007). Our study focused on the larvae stage and showed the body composition of the Snubnose pompano larvae being significantly affected by the light colour. The high lipid and lowest crude protein content of the larvae were under white light. Larvae under white light had low cortisol levels throughout the development. Suggesting larvae utilized protein and stored lipid to maintain body homeostasis compared to the other groups. The lower lipid level of the larvae under green light shows that energy source was used to cope with a stressful environment, as indicated by their high cortisol levels. Larvae in green light hardly captured prey at early ages (4 to 12dph), affecting their lipid content. Our results with crude protein, lipid, moisture, and ash contents indicate that larvae under purple light captured prey sufficiently. There are no reports on the effect of light colour on the body composition of fish larvae.

Light aids fish to visualize prey. In turn "promotes their digestion process and prepare the digestive tract for the expected arrival of feed by stimulating secretions including digestive enzymes and their activities (Trypsin, Pepsin, Amylase, Lipase)" (Rønnestad et al., 2013). As digestive enzymes break down food, their presence and activities are good indicators for assessing the maturation and function of the digestive system (Rønnestad et al., 2013; Zambonino Infante and Cahu, 2001). The impact of light colour on the activities of these enzymes has been reported only in juvenile Spotted sea bass (Hou et al., 2019). Blue light with 18 h of light increased trypsin and lipase activities, while white light with 12 h of light and red lights with 12 h, 18 h and 24 h of light decreased trypsin activities. However, there was no effect of the light spectrum on amylase activities (Hou et al., 2019). Hou et al. (2019) indicate that photoperiod under different light colour affects the activities of the digestive enzymes of the juvenile fish. There is no information in the literature about the influence of light colour on the activities of digestive enzymes on the fish larvae. In this study, larvae were reared under continuous light (24 h light) because Snubnose pompano larvae perform better under this photoperiod (Alejos and Serrano, 2018). The digestive enzyme activities effect was growth stage/age-specific with pancreatic proteolytic trypsin and amylase being active in early larval days and stomach proteolytic pepsin assuming an important role toward the end of the larval period at 12–25DPH. However, the different light colour influenced the extent of the three enzyme activities, with green light promoting trypsin (at 12dph and 25dph) and pepsin (at 12dph and 19dph), white for trypsin (at 19dph) and blue pepsin (at 25dph), and yellow for amylase (at 12dph and 25dph) activities.

In the present study larvae with high protein contents were observed to have low tryptic levels across the age and low pepsin activities at 25dph. Suggesting a high amount of food is not the only factor for the high digestive enzymes activities. Light colour research involving measuring digestive enzymes activities before and after a feed is needed. The decrease-increase trend of amylase activities was in all light colour groups. Trypsin and pepsin activities showed decrease-increase and increase-decrease trends, respectively. A similar tryptic trend is also reported in other marine fish larvae (Rønnestad et al., 2013). A peptic trend observed in our study differs from what has been discussed by Rønnestad et al. (2013). In the present study, we did not manage to show enzymes activities at first feeding because of insufficiency. Since our study indicated that food is not the only factor for enzymatic activities, the observed trends can also be genetic controlled (Lovett and Felder, 1990). The present study suggests that the effect of light colour on digestive enzyme activities is stage-specific.

In the present study, shorter wavelengths below 480 nm have been shown to favor the performance of the snubnose pompano larvae. Whilst, few studies reported that shorter wavelengths are more prominent with increased intensity. Besides, the present study observed that along with the light colour environments, the density of the larvae could

affect the growth of the Snubnose pompano larvae. Furthermore, the present study analyzed the digestive enzymes activities and body composition as the indicators of the food acceptability and nutrition of snubnose pompano larvae. However, we find it is crucial to study the light colour effects along with feeding activities, particularly capturing and ingestion rate of the prey. These areas provide an opportunity for snubnose pompano larvae future researches.

5. Conclusion

Light colour significantly affects growth performance, survival rate, cortisol level, body composition and digestive enzymes activities of Snubnose pompano larvae at different larval ages. Although light colour induced stress response of Snubnose pompano larvae at the early life stage, they were able to/adapt to the stressful environment as they grew. White and purple lights form the least stressful environment across a period of snubnose pompano larval rearing. At the same time, green and yellow proved to be the worst. Lipid was a source of energy used by larvae to cope with the stressful environment. Therefore, the difference in survival rate probably may be associated with the lipid contents of the larvae. The present study encourages white light during the first feeding (0dph to 5dph) and purple light in later stages while discourages green and yellow lights in the larval rearing of Snubnose pompano. Besides, the present study implies that Snubnose pompano larvae respond well in white, purple and blue light (short wavelengths below 480 nm) during the early to late larvae stage.

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Data availability

Data are available upon request from the Authors.

Author contributions

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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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