

# Effect of Blue Light on the Growth of the Red Swamp Crayfish *Procambarus clarkii* Larvae - Seasonal and Sexual Differences

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Organisms have the ability to adapt their behavior and physiology in response to seasonal changes in their habitat's environments. Although it is known that a specific light wavelength affects growth and reproduction in various animal taxa, its effect on sexual and seasonal differences in year-round breeding animals remains unclear. Here, we demonstrate that a blue light stimulus promotes or suppresses larval growth in the red swamp crayfish *Procambarus clarkia* depending on the season. During the spawning season (natural growing period), blue light irradiation accelerates female growth faster than in males, but suppresses growth in both females and males in the overwintering season. Moreover, these seasonal plastic effects of blue light show apparent sexual differences, with female juveniles exhibiting the greatest sensitivity. Our findings provide an opportunity to research how the red swamp crayfish can adapt to various habitable niches from the point of view of light color perception, and can be applied for the development of a more effective aquaculture system, not only for crayfish, but also for other commercially available decapod crustaceans using a specific light environment.

**Key words:** Crayfish, Sexual difference, Seasonal difference, Larval growth, Light effect.

## BACKGROUND

Light stimulus is an important environmental cue that allows living animals to recognize day and night alterations and seasonal changes. In aquatic environments such as rivers and ponds, light signals are attenuated as they pass through water, and blue light with a wavelength of about 470 nm can penetrate to an appreciable depth, even in clear water (Wurts and Stickney 1984). Although the physiological effects of light stimuli such as light intensity and photoperiod in growth and reproduction have been investigated

in various animals such as mammals (Dardente et al. 2010), birds (Yoshimura et al. 2003), fishes (Nakane et al. 2013; Takahashi et al. 2016 2018; Yamanome et al. 2009), and insects (Saunders 2020), the effects of light color and wavelength are still poorly understood. In the aquaculture field, it has been widely believed the significant differences of light intensity and spectrum between the organically rich waters including a lot of nutrients and outbreak of phytoplankton and the organically poor waters (McFarland 1986). The high density of plankton from eutrophication results in attenuation of light transmittance, especially in blue

light. Therefore, decapod crustaceans living in natural sea or freshwater experience different light colors environments. Indeed, previous studies found that light spectrum triggers significant differences, for example, in the molting, ovarian maturation, reproduction, and growth of some crustaceans (Emmerson et al. 1983; Guo et al. 2011; Primavera and Caballero 1992; Wang et al. 2003). In the Chinese shrimp *Fenneropenaeus chinensis*, the growth of juveniles was suppressed by a blue light stimulus (Wang et al. 2003). Although the growth of the penaeid prawn *Penaeus indicus* was reduced in response to blue light because they were more active and therefore more energy was allocated to respiration, spawning capacity was apparently higher in blue or green light than that in natural light (Emmerson et al. 1983). Likewise, blue or green light induced ovarian maturation and increased the number of spawns in the white shrimp *Penaeus setiferus* (Wurts and Stickney 1984). Taken together, blue light has the potential to influence the growth and reproduction of decapod crustaceans, although not much attention has been paid to the effects of light color on growth from the point of view of sexual differences and seasonal changes. Moreover, current studies on the impacts of light spectrum on growth and other life traits have tended to focus on marine decapod crustaceans, particularly to important fishery species such as *F. chinensis* and *Litopenaeus vannamei*. Fewer studies have addressed how light affects the growth of freshwater crustaceans.

The red swamp crayfish *Procambarus clarkii* is a well-known invasive species that has successfully spread widely into various freshwater environments such as rivers, ponds, swamps, and paddy fields. They show high tolerance to poor environmental conditions (Cruz and Rebelo 2007) and cold water habitats (Chucholl 2011), and have the ability to spread rapidly to new niches causing a devastating impact on the biodiversity of plankton, invertebrates, and small vertebrates such as fishes and tadpoles (Jin et al. 2019a). *P. clarkii* exhibits sexual dimorphism and several reproductive morphotypes (Hamasaki et al. 2020). This species was originally distributed in northeastern Mexico and the south-central United States and has since been introduced into many other countries in Asia, Africa, and Europe as a commercially farmed species (Hobbs et al. 1989). The aquaculture industry around this species is growing in the United States and China, and *P. clarkii* is the second most produced crustacean species in terms of aquaculture production (Jin et al. 2019b). Despite accumulating knowledge about rearing methods for red swamp crayfish, less is known about the effects of specific light wavelength on larval growth.

In this study, we hypothesized that specific light

wavelength can affect growth and/or sexual maturation in crayfishes. The present study, therefore, aimed to demonstrate the following effects on larval growth of representative freshwater species, red swamp crayfish: (1) of specific light color, (2) sexual differences, and (3) seasonal differences (tested periods in February–April, May–July, and December–January). Rearing experiments were employed for 8 weeks. Moreover, it was recently demonstrated that green light irradiation of teleost larval fish of medaka *Oryzias latipes* reversed the female-to-male sex ratio (Hayasaka et al. 2019). Based on this finding, the sex ratio of surviving juveniles after 8 weeks of a rearing experiment was verified by morphological sexual characteristics.

## MATERIALS AND METHODS

### Animals

Red swamp crayfishes were captured by hand between June and September of 2018–2019 in the paddy field in Hiratsuka city (35°21'N, 139°17'E), Kanagawa prefecture, Japan. They were transferred to the laboratory at Kanagawa University, where the air temperature was maintained at 24–27°C. They were kept in 50 L volume plastic containers and fed daily with an artificial prawn diet (size 9; Vitalprawn: Higashimaru Co., Ltd., Kagoshima, Japan). After rearing for over a month, only mated females were transferred to a 2 L volume plastic container covered with a shading net and reared until the offspring were released.

### Rearing experiments of juvenile crayfish

This series of experiments was conducted using light-emitting diode (LED) lights of either blue (475 nm), green (525 nm), red (640 nm), or white colors (made by mixing blue, green, and red LED) on the 5 L volume plastic container covered with a shading net (Fig. 1). LED light units were FLAT BEAM AURORA (GEX, Osaka, Japan) and were connected to an external power supply with a time switch that set the light:dark cycle to 14:10 h. The light intensities of each LED bulb were adjusted precisely to a photon flux density of 4.0–4.2  $\mu\text{mol photons s}^{-1} \text{m}^{-2}$ .

In this study, we set three experiments that were conducted in different seasons with duplicate experimental design (round 1 and round 2). The periods of rearing experiments were as follows: experiment 1 (round 1: 1st February–28th March 2019; round 2: 3rd March–28th April 2019), experiment 2 (round 1: 21st May–16th July 2019; round 2: 22nd May–17th July 2019), and experiment 3 (round 1: 2nd December

2019–27th January 2020; round 2: 6th December 2019–31st January 2020) (Fig. 4). Each experiment used juveniles obtained from different single mothers to remove any genetic influence. Juveniles were removed from individual mothers immediately after hatching and transferred to each well in a plastic six-well plate with a plastic lid containing an air vent (~5 mm diameter) (Asahi Glass Co., Ltd., Tokyo, Japan) (Fig. 1). They were kept in 9 mL of water and fed daily with an artificial prawn diet (size 7–8). Rearing water was changed daily and total body length was measured once a week by ImageJ software (Schneider et al. 2012). Each experimental period was 56 days (8 weeks). The sex of individuals was judged by the existence of abdominal gonopores (female-specific) using a stereomicroscope at the end of the experiment (day 56).

### Data analysis

Statistical analyses were performed using R statistical software (R3.5.3; R Core Team 2019) at a 5% level of significance. On day 56, the females and males were separated and differences in total body length in each LED light color condition were evaluated by one-way ANOVA with the post hoc Tukey-Kramer test. Likewise, the proportion of female and male individuals were evaluated by a Chi-square independent test to assess whether specific light wavelengths affected the sexual characteristics of juvenile red swamp crayfish.

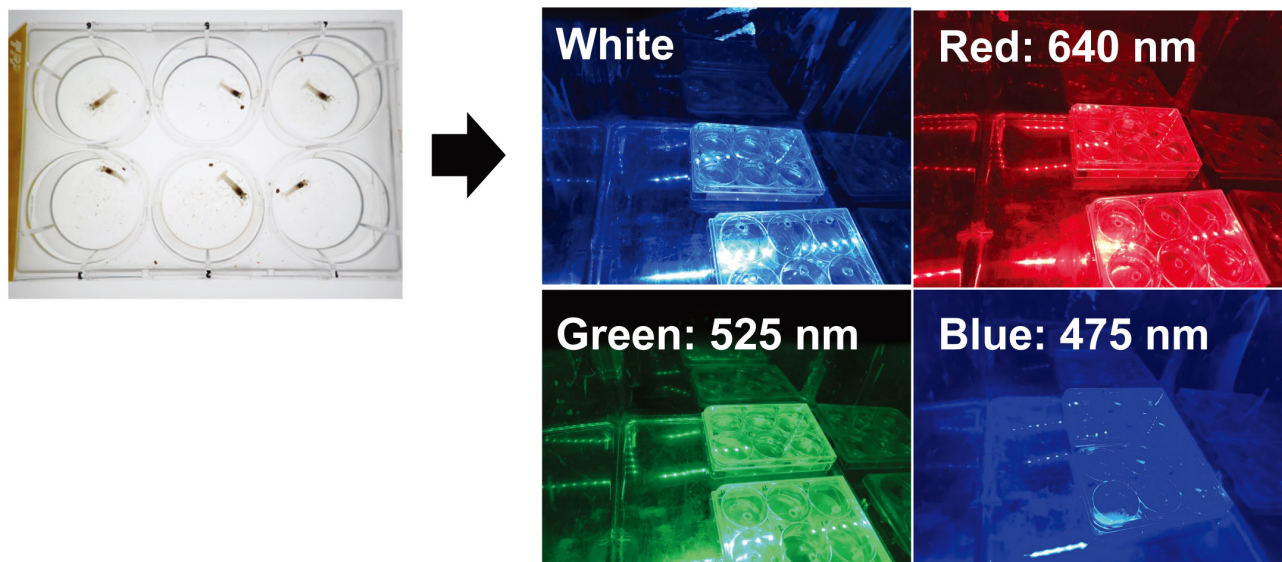
## RESULTS

### Effect of blue light on growth of *P. clarkii* in early spring (Experiment 1, February to April)

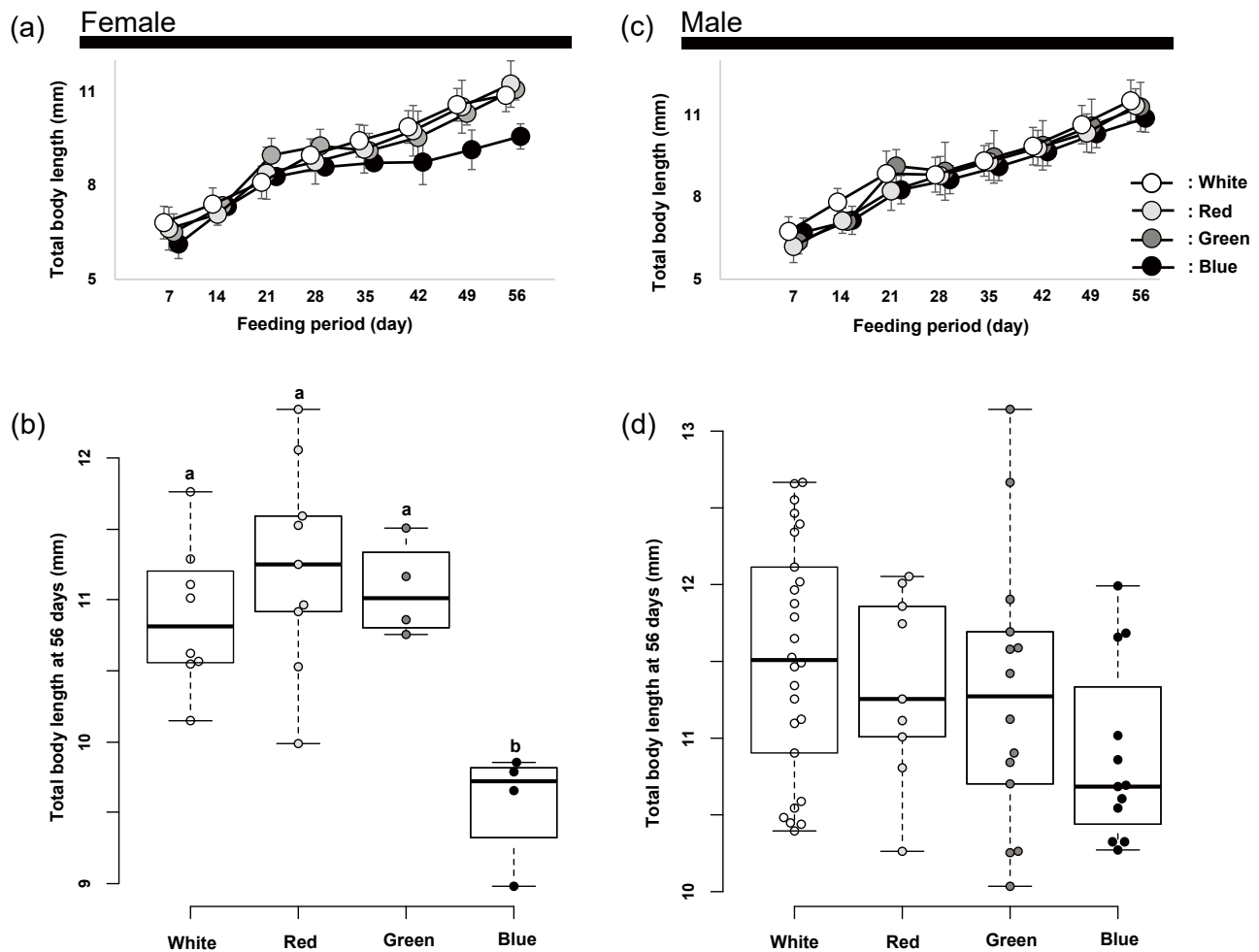
All experimental schedules are described in figure 4. At the beginning of the experiment, the number of individuals in each LED light condition was as follows (rounds 1 and 2): white (36, 24), red (18, 12), green (18, 12), and blue (18, 12). There was no difference in the ability to survive in each LED light condition, and 0–2 individuals died. Among females, growth, as assessed by total body length, appeared to be suppressed by blue light from 35–42 days after initial exposure (round 1 shown in Fig. 2a and round 2 in Fig. S1). On day 56, this suppressive phenomenon was clearer (round 1 shown in Fig. 2b and round 2 in Fig. S2). Although there were no statistical differences among light conditions in males (Fig. 2d), blue light tended to suppress larval growth as much as in females (white-blue comparison,  $p < 0.079$ ).

### Effect of blue light on the growth of *P. clarkii* in summer (Experiment 2, May to July)

The number of individuals at the beginning of each LED light condition was 18 and 12 in rounds 1 and 2, respectively, and survival in each condition ranged between 56 and 89%. Although males showed no differences among LED conditions, the growth of females was promoted in response to blue light (round 1 shown in Fig. 3 and round 2 in Figs. S1 and S2).



**Fig. 1.** Rearing equipment. Each juvenile crayfish was placed in one well of a plastic 6-well plate (individual/well). Each 6-well plate was moved to each light condition (white, red, green, and blue) and maintained for 8 weeks.



**Fig. 2.** Results of early spring (experiment 1, round 1). Left (a, c) and right (b, d) panels are females (a, b) and males (c, d), respectively. Upper panels show the time-course growing patterns with error bars showing standard deviation. Each point was measured once a week for eight weeks. Black, dark grey, light grey, and white indicate blue, green, red, and white LED lights, respectively. Lower panels show a comparison of total body length among the four LED light conditions at the end of the experiment (day 56). Different lowercase letters denote significant differences (one-way ANOVA post hoc Tukey-Kramer test,  $p < 0.05$ ). The number of individuals is shown in table 1.

**Table 1.** Comparison of sex ratio after 8-week rearing experiment under different LED light conditions

Exp	Sex	White	Red	Green	Blue	<i>p</i> value
1_1	male	26	9	14	12	0.181
	female	8	9	4	4	
1_2	male	12	7	5	5	0.850
	female	10	4	5	6	
2_1	male	7	7	5	9	0.614
	female	3	9	5	7	
2_2	male	4	4	2	4	0.750
	female	4	7	6	5	
3_1	male	17	20	18	17	0.800
	female	19	15	18	13	
3_2	male	11	16	14	7	0.456
	female	16	13	13	13	

## Sex ratio

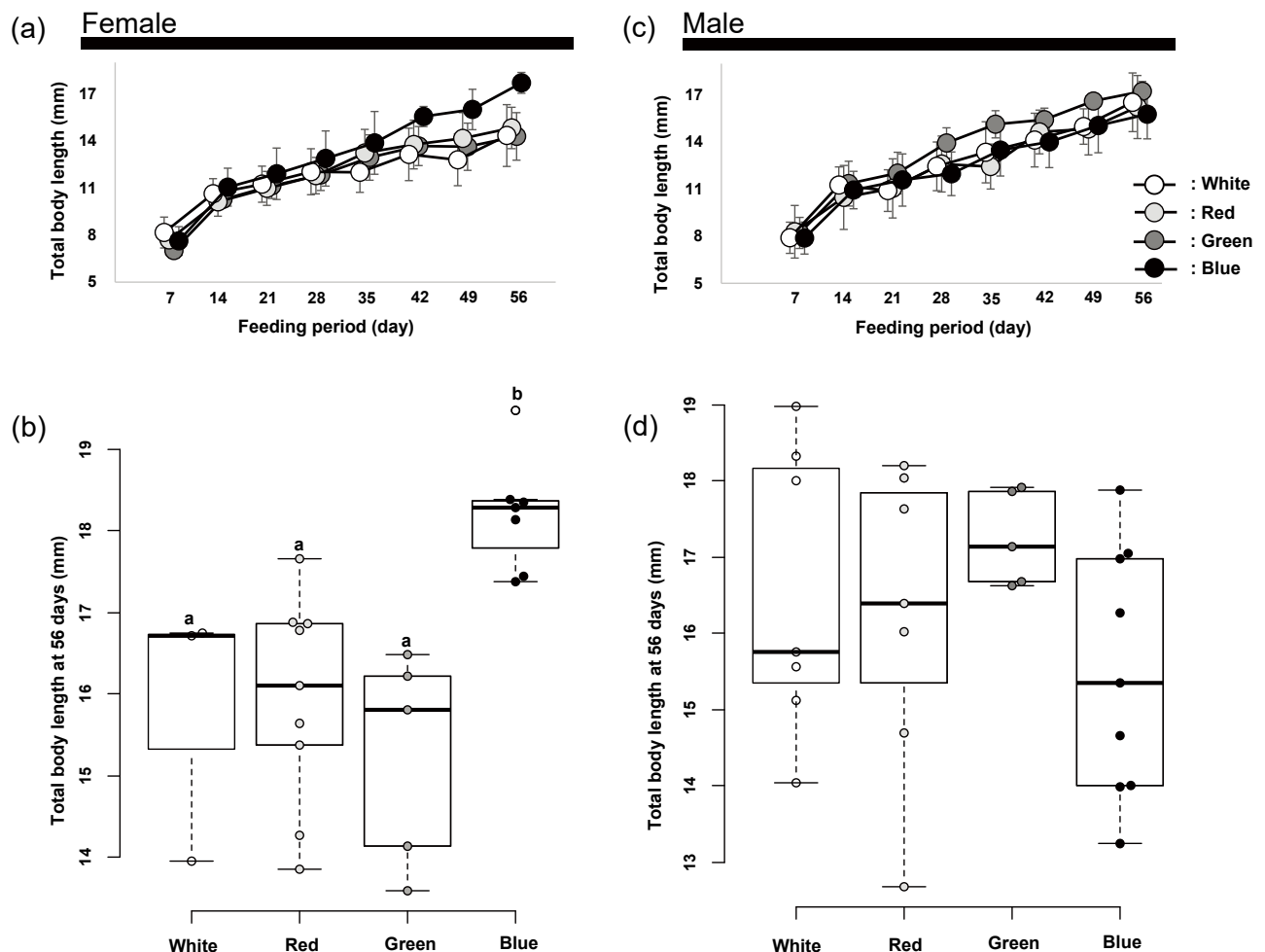
In all rearing experiments, the proportion of living female and male juveniles on day 56 was not statistically different among the four light colors (Table 1).

## DISCUSSION

### Impacts of blue light on the crayfish larvae

Our current experiments revealed that specific light wavelength, especially blue light, affects growth in *P. clarkii* larvae as well as other marine decapod species (Guo et al. 2011; Wang et al. 2003). Interestingly, unlike marine decapods, our data suggested that *P. clarkii*

shows both sexual and seasonal differences in growth in response to blue light. This is a first finding to prove that there is a substantial relationship between specific light wavelength and growth with seasonal differences. Although red swamp crayfish generally exhibit year-round breeding, the spawning season of the population used in this study was only 4–5 months (from the end of May to October) because their habitat is paddy fields (Fig. 4). They remain underground to overwinter during drought seasons. Our data demonstrated the following: (1) during the spawning season (natural growing period), blue light promoted the growth of females faster than males, and (2) during the overwintering season, blue light suppressed growth in both females and males although females were more sensitive. Despite a wealth of strenuous chronic rearing experiments and electrophysiological studies to show the effects of light



**Fig. 3.** Results of summer (experiment 2, round 1). Left (a, c) and right (b, d) panels are females (a, b) and males (c, d), respectively. Upper panels show the time-course growing patterns with error bars showing standard deviation. Each point was measured once a week over eight weeks. Black, dark grey, light grey and white indicate blue, green, red, and white LED lights, respectively. Lower panels show a comparison of total body length among the four LED light conditions at the end of the experiment (day 56). Different lowercase letters denote significant differences (one-way ANOVA post hoc Tukey-Kramer test,  $p < 0.05$ ). The number of individuals are shown in table 1.

colors on decapod crustaceans, less attention has been paid to sexual differences. Here we revealed that female juveniles are more sensitive to blue light than males in terms of growth.

### Effect of blue light on growth of *P. clarkii* in winter (Experiment 3, December to January)

At the beginning of the experiment, the number of individuals was 36 and 30 in rounds 1 and 2, respectively. Growth pattern seemed to be suppressed by blue light from days 35–42 after initial exposure in both female and male groups (round 1 shown in Fig. 5 upper and round 2 in Fig. S1). On day 56, this suppression was clearer (round 1 shown in Fig. 5 lower left and round 2 in Fig. S2).

### Relation between sensitivity of specific light wavelength and developmental stages

Previous studies have shown that different

decapod species exhibit different sensitivity to the light spectrum at various developmental stages (Fanjul-Moles and Fuentes-Pardo 1988; Forward and Gronin 1979), and the impacts of different light conditions on feed and growth of crustaceans were different (Guo et al. 2011; Wang et al. 2003). In terms of light effects on crayfishes, it is less known, although crayfishes have been used as a model organism of photoreceptor study using the traditional electrophysiology (see below). Several previous attempts demonstrated that a specific light wavelength, especially blue or green, substantially affected the growth of decapod crustaceans (Emmerson et al. 1983; Wang et al. 2003). Moreover, it has been reported that while *P. clarkii* younger than 4 weeks after hatching showed greater sensitivity at short wavelengths (blue to UV range), they also showed a total lack of responsiveness to long wavelengths (yellow to red range), and that this sensitivity shifted to long wavelengths when they grew to the adult stage (Fanjul-Moles and Fuentes-Pardo 1988), indicating the existence of two independent photoreception systems

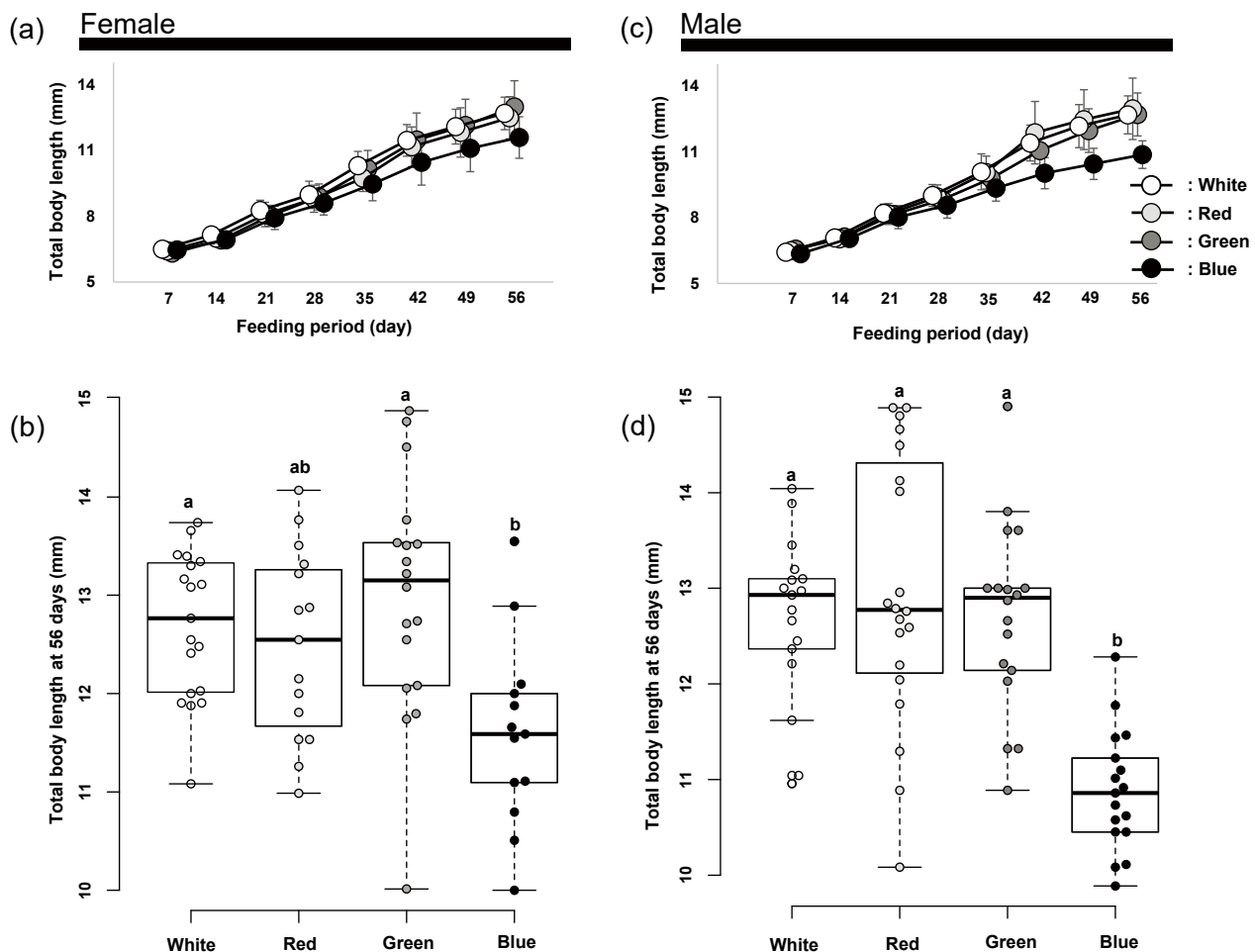


Fig. 4. Illustrated summary of this study. Exp-1, Exp-2, and Exp-3 are early spring, summer, and winter seasons, respectively.



for detecting short and long wavelengths. Interestingly, our data showed that apparent differences in growth curves in response to each color light occurred after 5–6 weeks. These results suggest that blue light perception for 4 weeks after hatching influences juvenile growth.

### Plastic sensitivity of blue light to seasonal changes

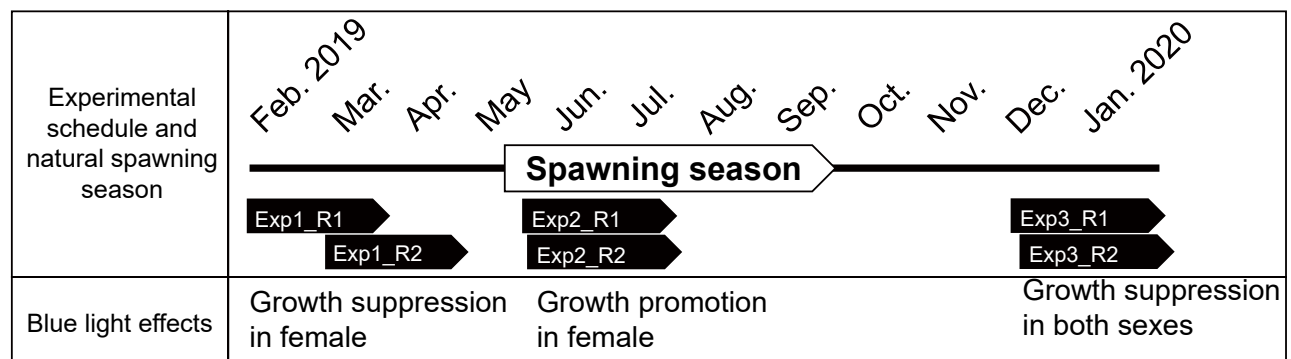
Various experiments have demonstrated that opsin (photoreceptor) gene expression varies in response to seasonal changes as follows: opsin expression levels are different in damselfish species between summer and winter (Stieb et al. 2016); opsin gene expression changed in response to photoperiodic changes in zebrafish (Matos-Cruz et al. 2011) and stickleback (Shao et al. 2014); LWS (long-wavelength sensitive) opsin regulated seasonal changes in color perception in medaka (Shimmura et al. 2017). Compared with teleost fishes, a few reports are available in crustaceans about seasonal variation of opsin gene expression, for example, gene expression of LW (long-wavelength) opsin shows seasonal variation in somatic tissues in both female and male oriental river prawn *Macrobrachium nipponense* (Li et al. 2018). Freshwater crayfishes such as *P. clarkii* are known to have three distinct photo-sensitivity systems—(1) in the retina of compound eyes (Rodríguez-Sosa et al. 2017), (2) non-visual photoreceptors in the supraesophageal ganglion (referred to as the brain) (Sullivan et al. 2009), and (3) caudal photoreceptors in the sixth abdominal ganglion (Rodríguez-Sosa et al. 2008)—and are used as a suitable model species to study physiological functions (Sánchez-Hernández et al. 2018). Moreover, several studies identified cryptochrome (blue and ultraviolet absorbing photoprotein)-immunoreactive

cells in the medulla-terminalis-hemiellipsoidal complex in the eyestalk and the anterior margin of the median protocerebrum in the brain (Fanjul-Moles et al. 2004), while blue-sensitive pigment was identified in caudal photoreceptors (Rodríguez-Sosa et al. 2008). Based on this knowledge, ensuing experiments will investigate which photo-sensitive system is involved in larval growth and if there are sexual differences.

In this study, no sex-biased proportions of surviving individuals after an eight-week experiment were observed, suggesting that a specific wavelength could not induce sex reversal and that sex-specific lethality in red swamp crayfish is unlike that in medaka fish (Hayasaka et al. 2019). Although our study revealed that seasonal and sexual differences in blue light-triggered growth promotion or suppression in the red swamp crayfish, their physiological and ecological significance remain unclear. Relevant next experimental steps are to observe the molting interval, quantitative relation between feed intake and residual feed, and identification of molecular clues causing growth differences in red swamp crayfish reared under different light conditions.

### CONCLUSIONS

Our eight-week rearing experiments under specific light colors demonstrated that blue LED light promoted or suppressed larval growth in the red swamp crayfish *P. clarkii*, highlighting sexual and seasonal differences. In the crayfish, embryogenesis takes place in the egg, which is attached to the mother's pleon, indicating that embryogenesis can be conducted *in vitro*. In fact, embryonic developmental staging and optimization of rearing conditions have been examined (Alwes and



**Fig. 5.** Results of winter (experiment 3, round 1). Left (a, c) and right (b, d) panels are females (a, b) and males (c, d), respectively. Upper panels show the time-course growing patterns with error bars showing standard deviation. Each point was measured once a week for eight weeks. Black, dark grey, light grey, and white indicate blue, green, red, and white LED lights, respectively. Lower panels show a comparison of total body length among the four LED light conditions at the end of the experiment (day 56). Different lowercase letters denote significant differences (one-way ANOVA post hoc Tukey-Kramer test,  $p < 0.05$ ). Number of individuals is shown in table 1.

Scholtz 2006; Jin et al. 2019a). Based on this technical information, an investigation of blue light effects on embryogenesis will be necessary. Our findings provide a research opportunity to understand how red swamp crayfish can adapt to various habitable niches from the point of view of light color perception, and the development of a more effective aquaculture system can be applied to not only crayfish but also other commercially available decapod crustaceans using a specific light environment. To date, *P. clarkii* has been introduced not only in several states in the continental USA, but also in many other countries in Asia, Africa, and Europe for freshwater aquaculture (Loureiro et al. 2015), and its aquaculture industry is currently growing in the USA, China, and Spain (Souty-Grosset et al. 2016). Our current findings of sex-specific and seasonal blue light effects on *P. clarkii* using may contribute to the establishment of a more efficient aquaculture system.

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**Authors' contributions:** All authors considered the experimental design. KT and KU conducted the experiments. KT performed the statistical analysis and wrote the first draft of the manuscript. All authors approved the final manuscript.

**Competing interests:** The authors declare that they have no conflicts of interest.

**Availability of data and materials:** All data are provided within the manuscript and supplemental materials.

**Consent for publication:** Not applicable.

**Ethics approval consent to participate:** The present study complies with current Japanese laws.

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## Supplementary materials

**Fig. S1.** Time-course growing patterns of the three experiments (round 2) with error bars showing standard deviation. Each point was measured once a week for eight weeks. Black, dark grey, light grey, and white correspond to blue, green, red, and white LED lights, respectively. (download)

**Fig. S2.** Comparison of total body length among four LED light conditions in the three experiments (round 2). The left and right panels are females and males, respectively. Different lowercase letters denote significant differences among the four LED light conditions (one-way ANOVA post hoc Tukey-Kramer test,  $p < 0.05$ ). (download)