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Effect of background color and shelters on female pigmentation in the ornamental red cherry shrimp *Neocaridina davidi* (Caridea, Atyidae)

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Abstract

Neocaridina davidi, the "red cherry" shrimp, is becoming popular in trade markets because of the reddish coloration of females. The aim of this study was to analyze the impact of background substrate color, and the presence of shelters on *N. davidi* female pigmentation and astaxanthin content. In the first experiment juveniles were assigned to one of three treatments: white, red, or black background substrate. After 90 days, females exposed to the black background showed higher astaxanthin content and relative carapace colored area than those exposed to white and red backgrounds. The second experiment evaluated the presence of shelters with white or black backgrounds. Juveniles were assigned to one of four treatments: white background with shelters (WS), white background without shelters (WWS), black background with shelters (BS), and black background without shelters (BWS). After 90 days the presence of shelters did not influence either the color nor total astaxanthin content. Females exposed to BWS and BS had higher astaxanthin content and relative carapace colored area than those exposed to WWS and WS. Growing *N. davidi* with black substrates could be a low cost and easy method to improve its economic value. Furthermore, this species could be cultivated as a pigment-enriched food source for other aquatic species.

KEYWORDS
astaxanthin, background color, *Neocaridina davidi*, ornamental aquaculture, shelters

1 | INTRODUCTION

Crustacean coloration is determined by organic components called pigments, located within the cuticle or within chromatophores beneath the cuticle (Bagnara & Hadley, 1973).The major pigments of carideans are carotenoids, and the most important is astaxanthin, a xanthophyll with a strong antioxidant activity (Palozza & Krinsky, 1992), which produces a bright red color in the animals (Bauer, 2004). It is also considered to be a protector against UV light stress (Kobayashi & Okada, 2000). Carotenoids are not synthesized by crustaceans, and must be obtained from diets (Boonyaratpalin, Thongrod, Supamattaya, Britton, & Schlipalius, 2001). Crustacean coloration represents an ecological and physiological adaptation to their habitat (e.g., as a camouflage), and can be modulated by a variety of factors such as background substrate color, water quality, photoperiod, temperature, carotenoid content in the diet, and light intensity among others (Rao, 1985). In some shrimp species such as *Palaemon serratus* (Chassard-Bouchaud, Genofre, & Noel, 1973), *Penaeus monodon* (Tume, Sikes, Tabrett, & Smith, 2009; Wade et al., 2012), *Litopenaeus vannamei* (Parisenti et al., 2011), and *Palaemonetes vulgaris* (Brown Jr, 1934), dark bottoms promote the expansion of pigments while light bottoms induce pigment concentration within chromatophores.

The dispersion, concentration, and pigment content inside chromatophores are responsible for such color changes (Duarte, Flores, & Stevens, 2017). Two different mechanisms, mediated by hormones, are implicated in this type of response: physiological or morphological. In the first one, rapid reversible changes are produced over minutes and hours in pigment distribution (dispersion or concentration), and in the second one slower changes are produced over days and weeks, involving pigment content changes, and the alteration in type and number of chromatophores (Auerswald, Freier, Lopata, & Meyer, 2008; Bauer, 2004; Detto, Hemmi, & Backwell, 2008; Fingerman, 1969; Robison & Charlton, 1973).

Ornamental aquaculture has increased significantly in recent years, and a large number of freshwater shrimp have been the focus of the aquarium trade (Werner, 2003). The "red cherry" shrimp *Neocaridina davidi* (Bouvier, 1904) is a freshwater species inhabiting streams and lakes in Asia (Cai, 1996), and has been increasingly targeted for the aquaculture industry as an ornamental species because of the striking reddish pigmentation exhibited by females. This species can be easily maintained in aquaria and has many biological characteristics that make it suitable for cultivation, such as high tolerance to starvation and a wide range of water temperatures; additionally they can tolerate cultivation with a high density of conspecifics (Baliña, Temperoni, López Greco, & Tropea, 2018; Pantaleão et al., 2015; Tropea, Stumpf, & López Greco, 2015; Vazquez, Delevati-Colpo, Sganga, & López-Greco, 2017). On the other hand, this species has a short life cycle with direct development and females produce several successive spawnings with the same brood quality (Marciano, Tropea, & López Greco, 2018; Sganga, Tropea, Valdora, Statti, & López-Greco, 2018; Tropea & López Greco, 2015). Despite all the studies mentioned above, there is scarce information about modulating factors of *N. davidi* color that might enhance its commercial value in the aquaculture market.

In this study, we tested the hypothesis that black and red substrates promote pigment dispersion, whereas white substrate induces pigment concentration. On the other hand, we tested the hypothesis that shelter absence causes a stress condition in the culture of *N. davidi*, because of an increase in the exposure to extrinsic parameters, such as UV radiation and predation, thus inducing pigment dispersion in female chromatophores.

The aim of the current study was to investigate the role of background substrate color, and the presence of artificial shelters on *N. davidi* female pigmentation through a photographic analysis of the carapace, and quantification of total content of astaxanthin, as a means to obtain a better knowledge of the environmental factors that control body

color in this species. Moreover, this study would help understand simple ways to enhance the ornamental value of this shrimp species, thus providing valuable information to the aquaculture industry.

2 | MATERIALS AND METHODS

2.1 | Experiment one—Effect of background color

For the first experiment, eight ovigerous females of *N. davidi* were obtained from a reproductive stock. Each female was individually placed in a 18 × 12.5 × 12 cm^3 plastic aquarium containing 1.8 L of dechlorinated tap water, and without background substrate under the following conditions: water temperature 27 ± 1° C (pH 7.5, hardness 80 mg/L as $CaCO_3$ equivalents), continuous aeration, a photoperiod of 14 L: 10D and a light intensity <200 lx. Each aquarium was also provided with ~1.6 g of Java moss (*Vesicularia* sp.) used as a shelter. Water was completely replaced once a week. The animals were fed daily *ad libitum* with commercial balanced food for tropical fish (Tetracolor®, Tetra GmbH, Melle, Germany) with an approximate composition as follows: minimum crude protein 47.5%, maximum crude fiber 2.0%, minimum crude fat 6.5%, maximum moisture 6.0%, minimum phosphorus 1.5%, minimum ascorbic acid 100 mg/kg and with a carotenoid content of 0.38 μg/mg. The total carotenoid content was determined spectophotometrically by a method modified after Renstrøm, Borch, and Liaaen-Jensen (1981) and Torrissen and Naevdal (1984). This commercial food exhibits optimal results in terms of growth, reproduction, and survival in laboratory conditions (Baliña et al., 2018; Marciano et al., 2018; Sganga et al., 2018; Vazquez et al., 2017). The rearing conditions mentioned above were based on previous studies with this species (Tropea et al., 2015; Tropea & López Greco, 2015).

After 15–20 days of egg incubation, 30 newly hatched juveniles from each mother were randomly assigned to one of the three treatments: white, red, or black background substrate color using a randomized block design in which each litter was considered a block. The experimental unit was an 18 × 12.5 × 12 cm^3 plastic aquarium with a 1-cm thickness layer of colored ornamental gravel, and under the same experimental conditions mentioned above. Thus, each plastic aquarium represented a replicate (n = 8 replicates per treatment, adding up to 24 experimental units), with 10 sexually undifferentiated colorless juveniles (subreplicates) fed daily ad libitum with Tetracolor®. At the end of the 90-day period all shrimp were sexed, and survival rate was calculated (counting the total number of animals, males and females per treatment). Females were photographed on the left side of the carapace for color measurement, their body weight was also recorded (precision 0.01 mg), and were sacrificed after being cold-anesthetized for 5 min. Finally, they were lyophilized for astaxanthin determination. Males were not analyzed for color measurement and astaxanthin quantification, because of the lack of pigments in their bodies.

2.2 | Experiment two—Effect of background color + shelters

For the second experiment, 15 ovigerous females were obtained from a reproductive stock and were placed into a plastic aquarium of 33.5 × 25 × 19 cm^3 containing 8 L of dechlorinated tap water, and maintained under the same experimental conditions mentioned in experiment one. At hatching, juveniles were randomly assigned to one of the four treatments: white background with shelters (WS), white background without shelters (WWS), black background with shelters (BS), and black background without shelters (BWS). Based on the results of the first experiment, it was decided to assay only white and black background substrates. Shelters were small PVC tubes (five tubes per replicate) 5 cm long and 2 cm in diameter. For white background white PVC tubes were used, and for black background, black PVC tubes. As in the first experiment, each replicate (n = 8 replicates per treatment, adding up to 32 experimental units) contained 10 sexually undifferentiated colorless juveniles (subreplicates) fed daily *ad libitum* with Tetracolor®. Java moss was not provided to avoid its use as shelter. At the end of the 90-day period the same variables as in trial one were measured only in females.

2.3 | Color measurement

A digital picture of the left side of each female's carapace (the body's largest colored area of the animal, and the most suitable to analyze) was taken using a Nikon Coolpix P340 (Nikon® Imaging Japan Inc, Tokyo, Japan), under a stereoscopic microscope with a ×20 magnification (Carl Zeiss Stemi 2000-C). The camera was set to Macro Close-Up mode. Pictures were taken at a shutter speed of 1/15 to 1/30 with an aperture of F3.7 and the flash turned off. The camera angle was adjusted to minimize the reflective surface. Pictures were stored in a 4,000 × 3,000 pixel format on a "fine" quality setting. Animals were prepared for the photograph by wiping excess water off the carapace, and to avoid parallax distortions all photographs were taken setting the specimens in the center of the image, while most of the image margins were not occupied by the carapaces. All images were taken under light conditions provided by a 36 W-fluorescent lamp daylight (6,500 K). Similar fluorescent lamps were used in the experimental room. Colored area was quantified in millimeters, and relativized to the total photographed carapace area, using the Image-Pro Plus® software (version 4.5.0.29). Pereopods, ocular peduncles, and rostrum were not considered in the color measurement.

2.4 | Astaxanthin quantification

The total astaxanthin content was extracted according to Gu, Deming, Yongbin, Zhigang, and Feirong (2008) modified by Calvo, Simoes, López-Aguiar, and Capella (2016). All lyophilized female shrimp from each replicate were gently pounded in a porcelain mortar, and 25–30 mg of the total amount of dry biomass was utilized for the pigment quantification. After that, 14 ml of acetone was added maintaining the solution in an ultrasound bath for 10 min. The samples were centrifuged at 5,000 rpm for 10 min in a refrigerated centrifuge (4°C) to obtain the carotenoids in the supernatant. Next, it was concentrated under vacuum until acetone was entirely evaporated. Total astaxanthin concentration was determined spectrophotometrically (BGM Labtech, Optima Fluostar) in DMSO at 485 nm and calculated according to a standard curve of astaxanthin (Sigma-Aldrich, Argentina, 98% purity). All procedures, including extractions, were performed under conditions of low-light intensity in a darkened laboratory. The yields of the astaxanthin extractions were expressed as micrograms of pigment per mg dry weight of female shrimp. All determinations were performed in triplicate.

2.5 | Statistical analyses

For the first experiment, female weight, relative colored area, and astaxanthin content were compared among treatments (white, red, and black background substrate color) using linear mixed models. Litter was considered as a random factor. For the second experiment two factors were analyzed (background substrate color and shelter) using a two-way analysis of variance. Female weight was used as covariate in both experiments. The number of replicates was eight in both assays. Results were expressed as means ± *SD*, and those showing significant overall differences were subjected to post hoc Tukey's test. All statistical analyses were performed using the software InfoStat version 2016 (Di Rienzo et al., 2016), and tests were carried out at 95% significance level.

3 | RESULTS AND DISCUSSION

3.1 | Experiment one

The survival rate was >80% in all treatments at the end of the experiment, but it was higher in shrimp reared on the red background compared with those exposed to the white background (type-II Wald chi-square test: x2=6.6, df = 2, p = .036). Nevertheless, these significant differences were not biologically meaningful (only 12%), as under laboratory conditions a percentage greater than 80 is considered as high. There was no difference between the average female

weight among the different backgrounds (F = 1.66, df = 2, p = .227) (Figure 1a). Female shrimp exposed to the black background substrate showed a left side relative colored carapace area three times higher, than those exposed to white and red background (F = 19.63, df = 2, p = .0002), and we did not find differences between females from white and red backgrounds (Figure 1b). The covariate was not statistically significant (F = .57, df = 1, p = .46). A lighter reddish coloration was expected in animals exposed to the white substrate, because some caridean species, like *Paleomons serratus* (Chassard-Bouchaud et al., 1973) and *Crangon septemspinosa* (Bauer, 2004), acquire a lighter coloration over a white background surface to match its color, possibly as a camouflage strategy for hiding from predators (Merilaita & Lind, 2005). The same plasticity is also found in the shore crab *Carcinus maenas* (Stevens, 2016). Color plasticity is considered an important feature of many species, and may allow individuals to exploit a wider array of habitats (Duarte, Stevens, &

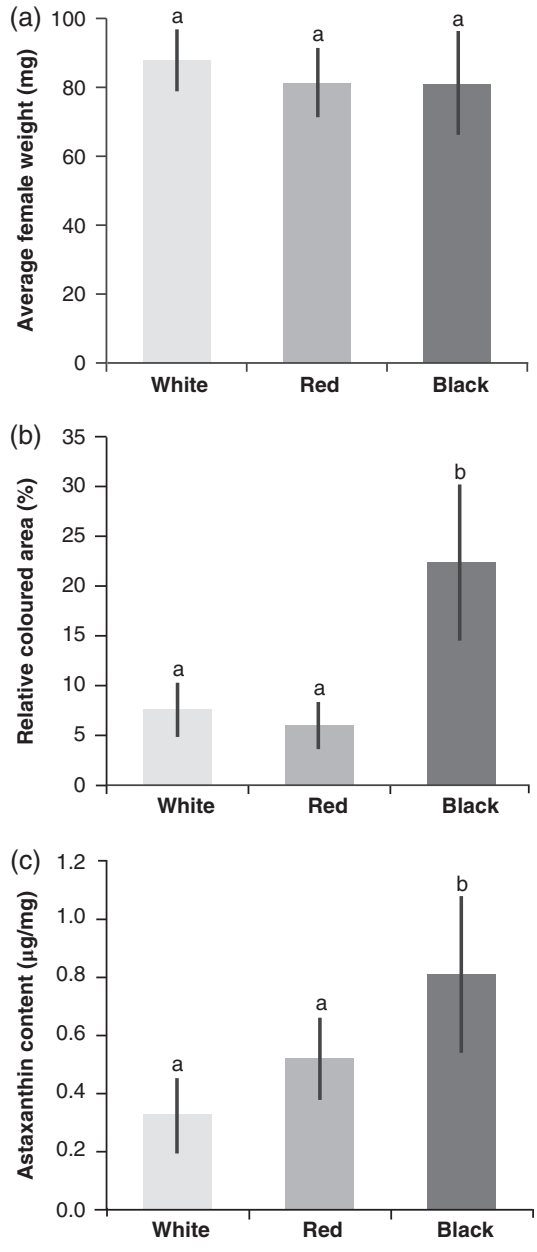

FIGURE 1 Average female weight (mg) (a), relative colored area of carapace left side (%) (b), and astaxanthin content (µg/mg) (c) of *N. davidi* (mean ± *SD*), after 90 days exposure to one of the three different background color substrates (white, red, or black). Treatments with different letters differ significantly (p < .05)

Flores, 2016) through long-term changes associated with phenotypic plasticity and development (see Stevens, 2016 for revision).

The differences in the size of pigmented areas among treatments could be easily distinguished in female photographs (Figure 2). A close examination of the carapace revealed that the highly colored shrimp from the black substrate had pigments more uniformly distributed, compared to the lighter shrimp from the white and red substrate, which showed dense concentrations of pigments in their chromatophores. In *P. vulgaris* a higher pigment concentration was observed after the animal was taken from a black background and placed upon a white one (Brown Jr, 1934). Also the brown shrimp *Crangon crangon* were paler on a white background and darker on a black background (Siegenthaler, Mastin, Dufaut, Mondal, & Benvenuto, 2018)

In our experiment, most animals exposed to the white substrate showed a greater number of white chromatophores with pigment expansion, than the female shrimp exposed to black and red backgrounds, although this feature was not quantified (Figure 3). Moreover, a more intense red coloration was expected in the female shrimp exposed to the red background than those exposed to the white substrate, but this did not occur. By contrast, *Lysmata boggessi* shrimp cultured in red tanks had a more intense red coloration than those exposed to white tanks, evidenced through digital image analysis (Calvo et al., 2016).

The total astaxanthin content was significantly higher in *N. davidi* female shrimp growingon a black background than those animals growing on red and white background substrates (F = 17.53, df = 2, p = .0004) (Figure 1c). The covariate was not statistically significant (F = 2.44, df = 1, p = .15). Brown, Jr. (1934) also observed, in *P. vulgaris*, an increase in the quantities of the red pigments ("pigment formation") and a decrease in white pigments ("pigment

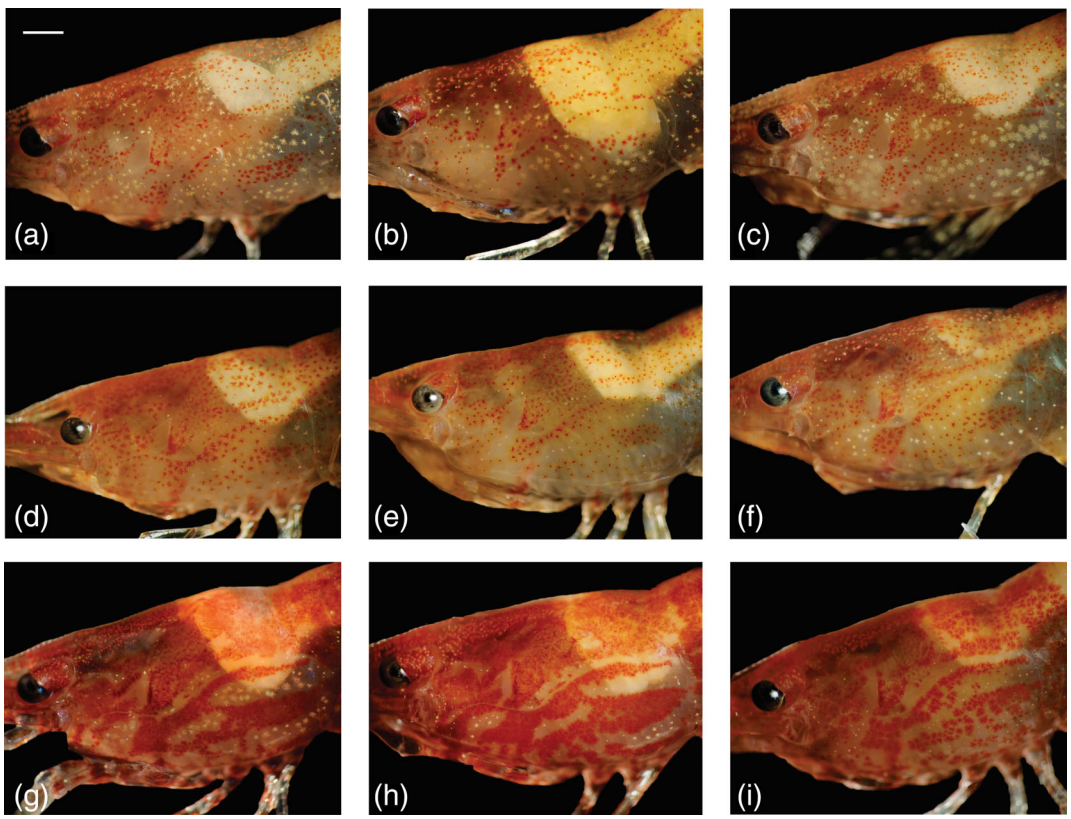

FIGURE 2 Photographs showing the left side of *N. davidi* female's carapace grown in white (a–c), red (d–f), and black (g–i) background substrate for 90 days. Scale bar = 7 mm (for all photographs)

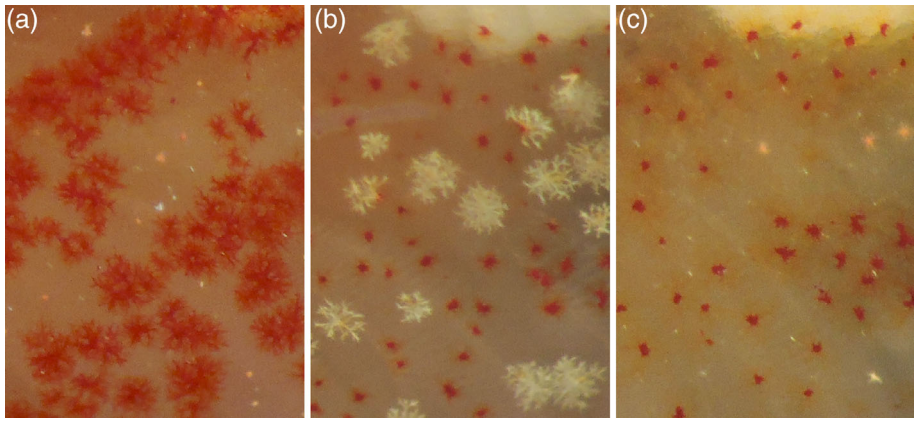

FIGURE 3 Photographs showing in detail pigment dispersion and concentration within chromatophores in mature *N. davidi* females in black background (a), white background (b), and red background (c)

destruction") when it was exposed to a black background. In the same way, animals placed upon a white background showed an increase in the amount of white pigment, and a decrease in red pigment. According to this author there is a possibility that the pigment formation and destruction could be controlled by the same pigmentary effector hormones that are responsible for the pigment's migration control inside chromatophores (physiological response). Furthermore, in the Hawaiian ghost crab, *Ocypode ceratophtalma*, chromatophore destruction was measured after transfer of the animals from black to white background and, conversely, those crabs transferred from white to black background showed an increase of chromatophores (Green, 1964).

On the other hand, Tume et al. (2009) and Wade et al. (2012) found pigment concentration or pigment dispersion in the chromatophores of *P. monodon* when growing in white or black tanks, respectively, but they observed similar astaxanthin content between animals grown in the two color tanks mentioned above. Moreover, Wade et al. (2012) also found in *P. monodon*, that when animals were exposed to dark substrates, they showed a higher accumulation of crustacyanin (a multimeric protein complex) together with an increase of free astaxanthin inside their expanded chromatophores. On the contrary, when animals were exposed to white substrates, the crustacyanin protein was depleted and astaxanthin was retained in an esterified form inside the constricted chromatophores.

Additionally, the background tank color modified the color of *L. vannamei* shrimp, but did not interfere significantly in the carotenoid total amount accumulated in these animals (Parisenti et al., 2011).

Our study displayed similar findings as Laohavisuti and Ruangdej (2014), but some differences were found in the results: when exposing adult *N. davidi* males and females together to red and black backgrounds during 2 months, they attained a greater body weight and a higher carotenoid total amount, than shrimp reared on a white background substrate. Differences with the present study could be related to the more extended evaluation period (3 months), and to the fact that recently hatched colorless juveniles were used in our experiment instead of adults. So the effect of color substrate could be different during growth and female acquisition of color (long-term exposition during the life cycle), than on adult shrimp. Additionally, the present study included a photographic analysis for detecting differences of color change in females.

Crustacean coloration is mainly dependent on the qualitative and quantitative pigments present in the epidermal chromatophores, and the pigmented layer of the epidermal exoskeleton (Meyers, 2000). A diet rich in carotenoids is very important for the observation of morphological changes in shrimp, and for this reason all animals in these experiments were fed with carotenoid contents in their diet.

The results obtained in the first part of the study indicate that the background substrate color induces the expansion and contraction of pigments within chromatophore cells. Furthermore, the substrate color interferes significantly with the total astaxanthin levels accumulated in the animal, and in the quantity and distribution of

chromatophores. Thus, the two well-known mechanisms (physiological and morphological) would seem to be participating in female color change.

Several studies have shown that morphological color change involves chemical modification of pigments (e.g., redox reactions), anabolism, and catabolism of integument pigments (Insausti & Casas, 2008, Umbers et.al 2014). Nevertheless, the mechanisms of the morphological response are poorly understood (Wade, Melville-Smith, Degnan, & Hall, 2008). On the other hand, Babak (1913) postulated the existence of a relationship between the physiological and morphological responses: after pigments remain concentrated or dispersed within chromatophores for a prolonged period, the pigment quantity and/or number of chromatophores decreases or increases, respectively (this is known as Babak's law). Based on this information, it could be possible that a physiological response uninterrupted for a long period (which in this case means being exposed to a dark background) could trigger a greater expression of the genes encoding the enzymes involved in the pathway for biosynthesizing astaxanthin from B-carotene. Also dark backgrounds could enhance the carotenoid mechanism of transport, which would lead to an increase in the metabolism of lipoproteins (known as lipid and carotenoid carriers according to Ando & Tanaka, 1996). The existence of scavenger receptors (transmembrane lipoprotein receptors) has been studied in *Drosophila* and in the silkworm *Bombyx mori*. These receptors recognize the lipoproteins, facilitating the carotenoid movement into the cell (Kiefer, Sumser, Wernet, & von Lintig, 2002; Sakudoh et al., 2013; Toews, Hofmeister, & Taylor, 2017). Regarding this information, another hypothetical explanation for the increasing amount of astaxanthin in those *N. davidi* females exposed to the dark bottoms could be because of a greater expression of these receptors. More detailed studies are required to elucidate how dark backgrounds promote an increment in astaxanthin amounts in *N. davidi* females.

We found these results highly valuable considering that *N. davidi* could be used as a natural carotenoid supplementation for species of farmed fish or other aquatic species with ornamental purposes. The greatest benefit of diets fortified with astaxanthin (such as the strong antioxidant properties, the improvement of the immune system, and the color enhancement) is that it is indispensable in early stages of development of fish and crustacean species, as well as under intensive culture conditions where stress factors play a significant role (Meyers, 2000). The minimum required levels of astaxanthin vary among species. In the red porgy *Pagrus pagrus* for instance, it was estimated that an adequate supplement involves astaxanthin esterified in a concentration between 13 and 25 mg/kg (Tejera et al., 2007). On the other hand, the false clown anemonefish *Amphiprion ocellaris*, requires a minimum of 80–160 mg/kg dietary astaxanthin concentration to achieve a more orange-red coloration (Ho, O'Shea, & Pomeroy, 2013), whereas Atlantic salmon fry, *Salmo salar*, need a minimum dietary astaxanthin level of 5.1 mg/kg to achieve maximum growth and survival during the start-feeding period (Christiansen, Lie, & Torrissen, 1995).

3.2 | Experiment two

The survival rate was also >80% in all treatments at the end of the experiment. It was similar between black and white background (p = .082, Z = −1.741, df = 2), and among those animals reared with or without shelters (p = .082, Z = −1.741, df = 2). Regarding average female weight, no significant differences were found between background colors (F = 0.07, df = 1, p = .799), and among those replicates with or without shelters (F = 0.64, df = 1, p = .431) (Figure 4a). Female shrimp exposed to black substrate had three times more relative colored area on the left side of the carapace, than those exposed to white substrate (F = 226.54, df = 1, p < .0001), but no significant differences were found between shelter treatments for this variable (F = 1.87, df = 1, p = .182) (Figure 4b). The covariate was not statistically significant (F = 0.008, df = 1, p = .93). The total astaxanthin content was two times higher in those female shrimp reared on black background color, than those animals grown on white background color (F = 22.25, df = 1, p = .0001), but again no significant differences were found between shelter treatments for this variable (F = 0.009, df = 1, p = .924) (Figure 4c). The covariate was not statistically significant (F = 2.362, df = 1, p = .137). The interaction between both factors was not significant for any of the tested variables (survival rate, Z = 1.451, df = 1, p = .147, average female weight, F = 1.46, df = 1, p = .238, astaxanthin content, F = 0.231, df = 1, p = .635, and relative colored area F = .088, df = 1, p = .77).

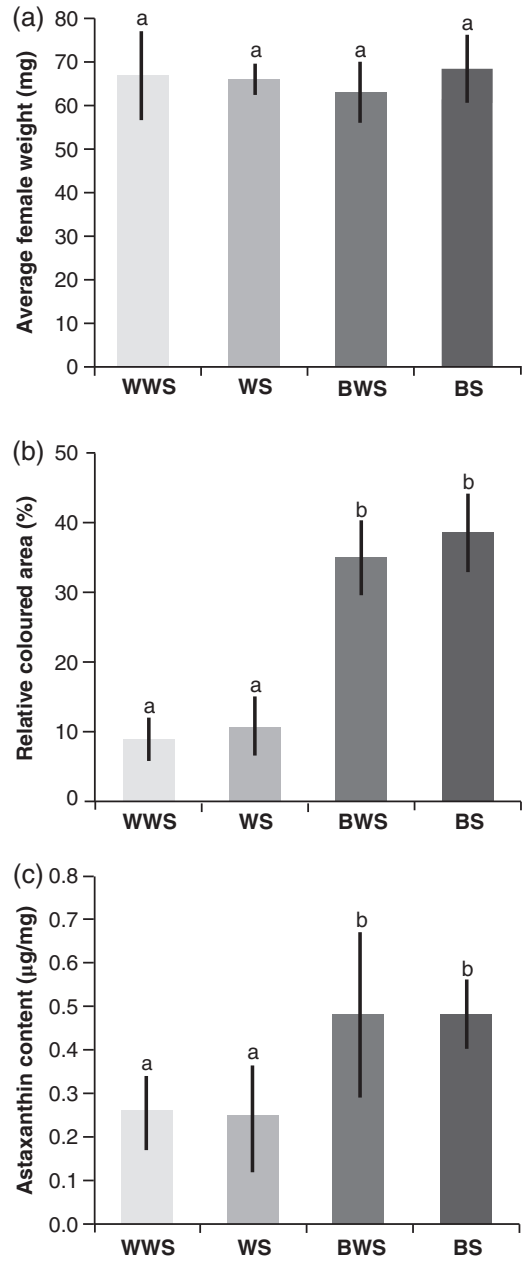

FIGURE 4 Average female weight (mg) (a), relative colored area of carapace left side (%) (b), and astaxanthin content (µg/mg) (c) of *N. davidi* (mean ± *SD*), after 90 days of exposure to one of the four treatments: black background with shelters (BS), black background without shelters (BWS), white background with shelters (WS), and white background without shelters (WWS). Treatments with different letters differ significantly ($p < .05$)

Thus, suggesting that the presence of shelters did not influence the color and the astaxanthin total content of *N. davidi* females, but, moreover, as in the first experiment, the background color had a very important effect in these variables.

Cultured organisms are continuously exposed to physical–chemical stressors (such as variation in water temperature, pH, salinity levels, dissolved oxygen, and the presence of ammonium and nitrites), and biological stressors, such as pathogen infections (Abad-Rosales, Betancourt-Lozano, Vargas-Albores, & Roque, 2011; de la Vega et al., 2007), and some of them might influence the body coloration of organisms (Bernal Rodríguez, García, Ponce-Palafox, Spanopoulos-Hernández, & Puga-López, 2017). Also, the excess luminosity is considered a stress factor. In *Homarus americanus* and *L. vannamei* body color was increased by intense light illumination (Tlusty, Metzler, Huckabone, Suanda, & Guerrier, 2009; You et al., 2006). The principal purpose of astaxanthin accumulation may be to avoid damage caused by

excess light, especially ultraviolet light. Also, *Macrobrachium tenellum* exposed to higher light intensities (between 1,500 and 3,500 lx) increased the number of expressing chromatophores, compared with those animals exposed to lower light intensities (between 30 and 600 lx) (Vega-Villasante, Martínez-Ochoa, García-Guerrero, & Arrona-Ortiz, 2015). Moreover, Auerswald et al. (2008) observed in the Antarctic krill, *Euphausia superba*, a morphological color change associated with the seasonal changes in light regime: in the animals caught in summer the astaxanthin content and the number of chromatophores was 450 and 250–480% higher, respectively, than in those sampled in winter.

There are several studies about the presence, availability, and different types of shelters affecting variables such as growth, survival, aggression, cannibalism, and so on in several crustacean species (Calvo, Tomas, & López Greco, 2013; James, Tong, & Paewai, 2001; Jones & Ruscoe, 2001; Marshall, Warburton, Paterson, & Mann, 2005; Mintz, Lipcius, Eggleston, & Seebo, 1994; Savolainen, Ruohonen, & Tulonen, 2003), but no data has been reported yet on whether the presence of shelters influences body color in shrimp. In our study, we expected greater body pigmentation in *N. davidi* females raised without shelters because of the higher exposure to extrinsic parameters such as UV radiation, high light intensities and predation. Nevertheless, as we did not find any differences in astaxanthin content and female pigmentation between the absence and presence shelter treatments, we believe that those stressful parameters were not strong enough to trigger an extra morphological response. Based on the results found by Vega-Villasante et al. (2015) in the caridean species *M. tenellum*, we assume our laboratory experimental light intensity (<200 lx) was too low to be considered a stress factor to the animals.

In the fish *Rhamdia quelen*, the color tank combined with the presence of an appropriate shelter, reduced the magnitude and duration of the stress response evaluated in terms of cortisol concentrations (Barcellos et al., 2009). Moreover, the use of black shelters in rearing tanks is necessary for reducing stress and aggression among *Macrobrachium rosenbergii* postlarvae in the hatchery (Kawamura, Bagarinao, Yong, Fen, & Lim, 2017).

3.3 | Conclusions

The results of this study have demonstrated that the black background substrate is capable of increasing the astaxanthin accumulation in chromatophores obtained from diet, as well as significantly increase the characteristic red color of *N. davidi* female shrimp. Because animal coloration affects its visual appeal, this information could be useful to substantially improve its economic value in the aquaculture market, as an attractive and colorful ornamental species with an easy and inexpensive rearing methodology. Despite this fact, these results are very valuable, considering that *N. davidi* could be cultivated as a food source or dietary supplement for other aquatic species requiring high levels of pigments (e.g., fish for human consumption or as ornamental species).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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