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Research Article

The coloration of *Neocaridina davidi* (Bouvier, 1904) (Caridea, Atydae) fed with live microalgae *Haematococcus pluvialis* and the cyanobacteria *Spirulina* (*Arthrospira*) *platensis*

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ABSTRACT. *Haematococcus pluvialis* synthesizes and accumulates astaxanthins, while *Spirulina* (*Arthrospira*) *platensis* produces phycocyanins. The ornamental shrimp *Neocaridina davidi* is commercially important for its variety and intensity of coloration. We evaluate the effect on the coloration of *N. davidi* by consuming *H. pluvialis* and *S. platensis* as a food supplement to preserve their nutritional quality. *H. pluvialis* was subjected to stress to obtain aplanospores with astaxanthin. Live microalgae were included in gelled spheres to provide them as a supplement to three-day-old *N. davidi* juveniles with different phenotypes: wild (NdW), red (NdR), and blue (NdB). The experiment involved the commercial food supply and spheres with or without microalgae for 31 days. Chromatophores present in the uropod of each phenotype were examined, their number and expansion estimated, and the area covered by optical density (OD). Results show that *H. pluvialis* reached a greater astaxanthin accumulation on day 30. The coloration of the NdR and NdB phenotypes improved by consuming microalgae, increasing the OD in the uropod. NdB phenotype showed better coloration when consuming *S. platensis* (56.2%), while the NdR phenotype was more favored when consuming *H. pluvialis* (74.2%). The chromatophores expanded their area by doubling in the NdR and NdB phenotypes, where *H. pluvialis* increased its intensity four times in NdR. The number of chromatophores was significant only in NdR with *S. platensis*. In conclusion, gelling is a viable method to preserve the nutritional quality of live microalgae and transfer bioactive compounds that improve the coloration of *N. davidi*.

Keywords: *Neocaridina davidi*; bioactive compounds; ornamental shrimp; dietary supplements; pigments; uropod; optical density

INTRODUCTION

The commercial importance of microalgae is due to their nutritional content, as they contain polyunsaturated fatty acids (PUFAs), sterols, proteins, polysaccharides, vitamins, and pigments. In addition, its photosynthetic efficiency and rapid growth rate allow the accumulation of bioactive compounds useful for their biotechnological application in the pharmaceutical, food, nutraceutical, cosmetic and aquaculture industries (Hemaiswarya et al. 2011, Gong & Bassi 2016, Shah et al. 2016, Han et al. 2019, Pérez-Legaspi et al. 2019, Machado-Sierra et al. 2021). Microalgae are capable of synthesizing and accumulating commercially important pigments such as chlorophylls (a-e), carotenoids (β-carotenes, astaxanthins, lutein, lycopene, and canthaxanthin), and phycobiliproteins (phycocyanins, allophocyanins and phycoerythrins)

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(Hemaiswarya et al. 2011, Gong & Bassi 2016, Pérez-Legaspi et al. 2019). In addition, microalgae, due to their nutritional content, offer benefits to the aquatic organisms that consume them since they stimulate the immune, nervous, and endocrine system, favoring their development, coloration and survival, being a key piece for the direct nutrition of fish larvae, crustaceans and mollusks (Conceição et al. 2010, Hemaiswarya et al. 2011, Shah et al. 2016, Lim et al. 2017, Rosas et al. 2018, Han et al. 2019, Pérez-Legaspi et al. 2019, Zhang et al. 2019, Ma et al. 2020, Machado-Sierra et al. 2021).

The microalgae *Haematococcus pluvialis* and *Spirulina* (*Arthrospira*) *platensis* are commonly used to enhance development and increase immune response (Macias-Sancho et al. 2014, Ma et al. 2020). *H. pluvialis* is a chlorophyte capable of synthesizing secondary carotenoids, mainly astaxanthin, through carotenogenesis; this microalga is considered the main natural source of astaxanthin since it accumulates large amounts (>30 g kg^{-1} of dry biomass) after being exposed to stress conditions (Shah et al. 2016, Lim et al. 2017, Pérez-Legaspi et al. 2019). Astaxanthin is a pigment used in aquaculture as an additive to improve the coloration of fish and crustaceans, indicating better quality and acceptance for the consumer (Hemaiswarya et al. 2011, Lim et al. 2017). *S. platensis* is a cyanobacteria used as an additive in aquaculture due to its high nutritional value as a source of protein (55-70%), fatty acids, vitamins, and pigments such as chlorophylls, carotenoids, and phycobiliproteins (Macias-Sancho et al. 2014, Kohal et al. 2017, Rosas et al. 2018, Zhang et al. 2019). The content of pigments supplied through the diet influences the pigmentation of crustaceans, in addition to being modulated by factors such as substrate color, water quality, photoperiod, temperature, and light intensity (Tomas et al. 2019). Algal biomass of high nutritional quality is slightly used due to its high cost and low availability, so inert feed based on grains or fish meat is more used for its low cost despite limiting the growth rate and decreasing survival (Hemaiswarya et al. 2011, Ma et al. 2020). There are alternatives to supply microalgae, such as pastes, freezing, air drying, and spray; however, its nutritional quality is altered, affecting water quality and promoting bacterial proliferation by broken cells (Hemaiswarya et al. 2011). Therefore, it is necessary to look for alternatives that preserve the biochemical characteristics of microalgae to provide the highest nutritional quality of live food to the aquatic organisms that consume them.

Fishmeal protein is the most expensive nutrient in the shrimp farming diet (Macias-Sancho et al. 2014). *Spirulina* has been proven to be a dietary supplement for shrimp farming, improving its development with benefits that optimize its cost and yield (Kohal et al. 2017). Even the replacement of 75% of *S. platensis* as an alternative protein favors the survival and development of the shrimp *Penaeus vannamei*, benefiting its immune system (Macias-Sancho et al. 2014). One of the most popular and on-demand ornamental shrimps in the aquarium market is *Neocaridina davidi* due to its diversity of attractive colors and ease of cultivation (Kohal et al. 2017, Vazquez et al. 2017, Sganga & López-Greco 2019, Tomas et al. 2019, 2020). It can also be used as live food for other ornamental aquatic species (Sganga & López-Greco 2019, Tomas et al. 2020) and even grow in high densities without affecting its growth (Vazquez et al. 2017). Including 10% of *S. platensis* in their diet can optimize their reproduction, growth, and survival (Kohal et al. 2017). The type of diet supplied influences the biochemical composition of *N. davidi* females, mainly in their lipid and carotenoid content, as well as their coloration and intensity (Tomas et al. 2020). Although maternal pigmentation does not influence the nutritional quality of their descendants (Sganga & López-Greco 2019), the black substrate does influence their astaxanthin content and coloration intensity, as well as the dispersion of their chromatophores as a response to the background color (Tomas et al. 2019). However, crustaceans depend on their diet to obtain carotenoids because they cannot synthesize them *de novo*. It is convenient to supply foods rich in pigments during their cultivation to improve their coloration, biochemical composition, and economic value (Lim et al. 2017, Pérez-Legaspi et al. 2019, Tomas et al. 2019, 2020).

The coloration of crustaceans depends on the concentration and dispersion of chromatophores. These are pigmented cells in the epidermis under a translucent exoskeleton (Flores & Chien 2011, Siegenthaler et al. 2017, Tomas et al. 2019). Chromatophores can form chromatosomes, establishing a base of chromatic arrangements that manifest their exterior coloration (Flores & Chien 2011, Siegenthaler et al. 2017). Most of the research focused on the coloration of *N. davidi* includes the analysis of carotenoids, astaxanthin, and dispersion of chromatophores on the carapace of adults of the red cherry phenotype (Sganga & López-Greco 2019, 2020, Tomas et al. 2019, 2020) despite of the wide variety of colors offered by this ornamental shrimp, other phenotypes have not been considered. Flores & Chien (2011) evaluated the dispersion of chromatosomes in three phenotypes of *N. denticulata*,

suggesting that the most appropriate area to monitor coloration changes is the uropod, in addition to proposing it as a non-invasive method to describe, quantify, and measure more accurately the chromatophores and chromatosomes. Therefore, in this study, we assess the effect of live microalgae *H. pluvialis* and *S. platensis* by gelling as a supplement in the diet of three different phenotypes of the ornamental shrimp *N. davidi* to analyze their coloration through the dispersion of their chromatophores and chromatosomes present in the uropod.

MATERIALS AND METHODS

Microalgae culture

The microalgae *Haematococcus pluvialis* and the filamentous cyanobacteria *Spirulina* (*Arthrospira*) *platensis* were used; both species were obtained from the microalgae collection of the Centro de Investigaciones Biológicas del Noroeste S.C. (CIBNOR). The cultivation of *S. platensis* was carried out with foliar fertilizer (Bayfolan Forte®) at a concentration of 0.2 mL L^{-1} and sodium bicarbonate (1.5 g L^{-1}) in sterile purified water (Purikor®), maintaining constant illumination of 4000 lx and temperature of 26 ± 2°C. The most appropriate concentration of foliar fertilizer (Bayfolan Forte®) in sterile distilled water was searched to obtain sufficient biomass of *H. pluvialis* in the germinal phase (mobile green vegetative cells) during its cultivation, involving 0.1, 0.2, 0.6, 0.8, 1.0 and 1.2 mL L^{-1}, respectively. A temperature of 18 ± 2ºC and constant illumination of 4000 lx were maintained in a lighting chamber until sufficient algal biomass was obtained. Subsequently, the biomass was harvested through a filter paper (Whatman N°1, particle retention 11 µm), placed in a Petri dish, and exposed the microalgae to the illumination of 36,000 lx, temperature of 26 ± 2ºC, keeping the filter paper hydrated with 4.5 mg L^{-1} of dipotassium phosphate (K_2HPO_4) (J.T. Baker USA) in distilled water until its desiccation on a wet basis. Thus, inducing carotenogenesis in *H. pluvialis* for the synthesis of astaxanthin by stress conditions and obtaining mature aplanospores according to the protocol of Orosa et al. (2000). The cell count of *H. pluvialis* was performed by Neubauer's chamber (Labor Loptik) to record the number of aplanospores obtained until achieving the highest cell density.

Gelling of microalgae

To supply the live microalgae and preserve their nutritional content, they were included by species in gelled spheres. In addition, negative control without microalgae was considered. Previously, the microalgal biomass was rinsed five times with sterile distilled water, centrifuging at 3000 revolutions for 5 min to remove the fertilizer residues in the supernatant to avoid damaging them. The microalgae were concentrated in a total volume of 30 mL with sterile purified water. Sodium alginate Alginus® (1.2 g 150 mL^{-1}), sodium citrate (3 g L^{-1}) and commercial food (0.5 g 20 mL^{-1}) specific to Neocaridine Probiotik® (47% protein, fat 16%, fiber 1.2%, ash 12%, moisture 10%) were added. For the elaboration of spheres, 250 µL aliquots were transferred with the help of a Pasteur pipette to a solution of calcium chloride (8 g L^{-1}) with purified water, maintaining a constant agitation using a magnetic stirrer plate IKA® Ceramag Midi to avoid conglomerates, obtaining spheres of 4.2 ± 0.3 mm in diameter. All ingredients used were food grade.

Neocaridina davidi culture

Adult *N. davidi* ornamental shrimp of different phenotypes were obtained from a pet store (aquarium): wild (NdW), fire red (NdR), and diamond blue (NdB). These were acclimatized for 12 h by dripping in 40-L fish tanks with previously cycled water. The organisms were kept at a temperature of 28 ± 2ºC and the illumination of 3000 lx with a photoperiod of 12:12 h (light:dark). Thirty percent of water refills were performed each week to maintain the pH at 7.5, which was monitored with the help of a portable potentiometer (HANNA pH211). At the same time, the other parameters were analyzed by colorimetry (Master Test Kit Nutrafin®) comprising hardness (120 mg L^{-1}), dissolved oxygen (5.5 mg L^{-1}), NH_4 (<0.2 mg L^{-1}), NO_3^- (<1 mg L^{-1}) and NO_2^- (<0.3 mg L^{-1}). In addition, aeration was supplied by a canister-type filter (Boyu Ef05), and 0.5 cm of silica sand was included as a substrate, as well as aquatic plants of java fern ornament (*Microsorum pterophus*) occupying an area of 10 cm^2 and 15 floating plants known as a soft frog (*Limnobium laevigatum*) per fish tank. Finally, the commercial feed was supplied for Neocaridine Probiotik® every three days. After a period of adaptation and quarantine, individuals (15 females: 5 males) of each phenotype with the most homogeneous coloration (except the wild phenotype) and size (2-3 cm) were selected for reproduction. The males were separated from the females in 2 L outdoor maternity baskets (Ideas Marinas®) for three months. They were then gathered and synchronized their reproduction and spawning to obtain enough juveniles of the same age to use them as organisms for the test.

Experimental design

Three-day-old juveniles of *N. davidi* of each phenotype were obtained to perform the bioassays, placing 11 individuals (n = 11, without sex distinction) according to the phenotype in outdoor maternity baskets (Ideas Marinas®) by four replicates (n = 44), allowing the entry of culture water through the recirculation of the same water. The bioassays comprised the following treatments: NdW, NdR, and NdB fed with a type of sphere according to the microalgae (*H. pluvialis* or *S. platensis*), in addition to including negative control (spheres without microalgae) for each phenotype. Three spheres with gelled microalgae were added per treatment every three days. In contrast, on the other days, commercial food was provided for Neocaridine, concluding on day 31, considering changes in the dispersion and concentration of the chromatosome. Food scraps, feces, and molts were collected daily before feeding by siphoning every 24 h. At the end of the experiment, individuals of each phenotype were collected and subjected for 1 min into ice water to immobilize them and lower their body temperature to avoid loss of coloration due to stress. Subsequently, the animals were placed in a slide with excavation to observe the chromatophores and chromatosomes in their uropods and obtain digital images with modifications according to the protocol of Flores & Chien (2011). The images were captured using an epifluorescence optical microscope (Leica DM 4000) and an image analysis system (Leica Application Suite® Version 4.12.0). Finally, the animals were acclimatized by drip to 26ºC to return them to the fish tanks. The images of the uropods were analyzed using the open-access software ImageJ v.1.64. The area of the uropods and chromatosomes was measured (µm) to obtain the percentage of optical density on the chromatosome for each treatment. The number of chromatophores present in the uropod per treatment was quantified. Therefore, we considered chromatosome dispersion and concentration the dependent variable, while the presence of microalgae or cyanobacteria in the diet was considered the independent variable. With the data obtained, one-way analysis of variance (ANOVA) and Tukey *post-hoc* comparison tests with a *P*-value < 0.05 were performed using the Statistica v.7.0 software (StatSoft Inc. 2004).

RESULTS

The largest amount of biomass obtained from the microalgae *H. pluvialis* in the green phase was at a concentration of 0.8 mL L^{-1} of Bayfolan Forte® at 24 days. Carotenogenesis in *H. pluvialis* began on the third day, finding Pamella-like cells with accumulation of astaxanthin in transition to aplanospores (Fig. 1b). In contrast, the largest number of aplanospores with accumulated astaxanthin was achieved at 30 days (Fig. 1f). The ornamental shrimp *N. davidi* accepted to consume the gelled spheres with live microalgae as a supplement, registering 100% survival in all treatments. After the period of supplementation with gelled spheres, it was observed that both the NdR and NdB phenotypes improved their coloration by consuming spheres with live microalgae, revealing that the NdB phenotype showed better coloration when consuming *S. platensis* (56.2%). In comparison, the NdR phenotype was more favored when consuming *H. pluvialis* (74.2%) due to the increase in the number and intensity of chromatophores in the body (Fig. 2) as well as in uropods (Fig. 3) registering higher percentages of optical density for these treatments compared to the NdW phenotype (Table 1). In Table 1, the highest number of chromatophores of the NdR phenotype was recorded with *S. platensis*, followed by *H. pluvialis*.

In contrast, the NdW phenotype showed more chromatophores with *H. pluvialis*. Finally, the NdB phenotype did not show significant differences in the number of chromatophores when consuming spheres with or without microalgae. On the other hand, the area of chromatosomes increased significantly twice as much when consuming spheres with live microalgae, observing that *H. pluvialis* favors the NdW phenotype and with greater intensity (four times), the NdR phenotype, while *S. platensis* favors the NdB phenotype followed by the NdR phenotype. However, there is no significant difference ($P < 0.05$), showing a relationship with the area of chromatosomes, more than doubling in the three phenotypes, registering the highest value when the NdR phenotype consumed *H. pluvialis*. The number of chromatophores only increased in the NdR phenotype, being significant when consuming the cyanobacteria *S. platensis* (Table 1).

DISCUSSION

The consumption of live microalgae through gelled spheres contributes to the improvement of the coloration of the ornamental shrimp *N. davidi*, depending on its phenotype and the microalgae or cyanobacteria supplied (Fig. 2). In our study, we consider gelling as a viable and cheap alternative to transfer live microalgae taking care the integrity of their biochemical composition to supply good quality

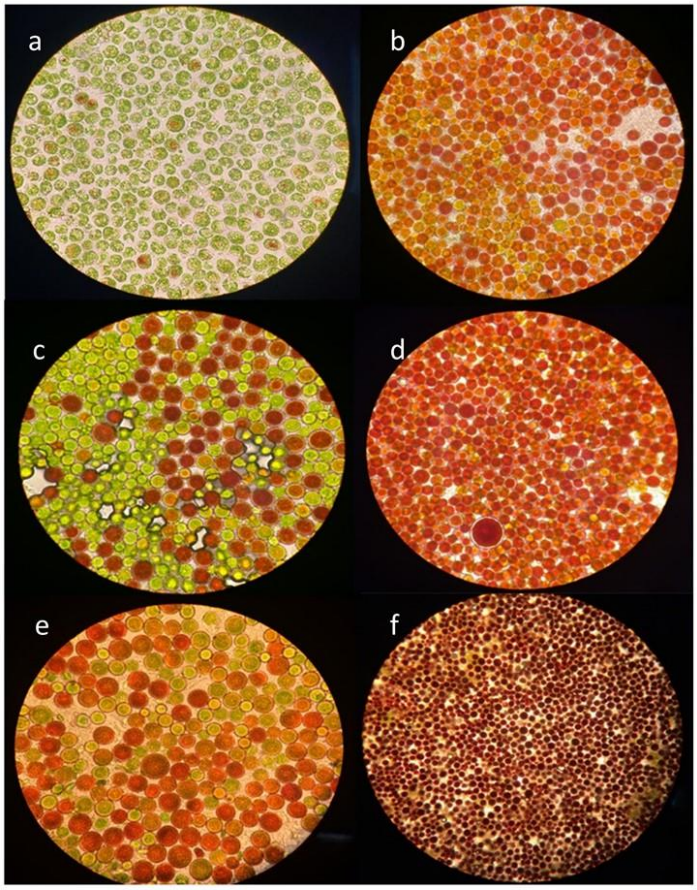

Figure 1. Carotenogenesis of *Haematococcus pluvialis*. a) Green pamella vegetative cells initiating carotenogenesis (day 3); b-d) cells in transition to aplanospore with the accumulation of astaxanthin (days 7-9); e-f) aplanospore cell with the accumulation of astaxanthin (days 19 and 30).

nutrients as a food supplement; which might be related to 100% of the survival of the juveniles used. In this way, it is possible to obtain essential nutrients for the development, survival, and fecundity of crustaceans, such as proteins and PUFAS of the linolenic (n-3) and linoleic (n-6) families (Tomas et al. 2020). In addition, live microalgae offer high nutritional value, attractiveness, and better digestibility than inert food (Hemaiswarya et al. 2011), which contributes to improving the immune response and health of the organism displaying better phenotypic coloration (Duarte et al. 2017). Therefore, obtaining nutrients from live microalgae by gelling contributes to the improvement of the coloration of the ornamental shrimp *N. davidi* (Figs. 2-3) since the main pigmentation of caridean-type crustaceans is due to carotenoids, mainly astaxanthin (Tomas et al. 2019), which they obtain from the diet since they do not synthesize it *de novo* (Lim et al. 2017, Pérez-Legaspi et al. 2019, Tomas et al. 2019, 2020).

S. platensis and *H. pluvialis* were cultivated using the foliar fertilizer Bayfolan Forte®, achieving enough biomass at low cost (1 L at US$ 9.82, using 1 mL L^{-1}). We obtained the largest amount of aplanospores with astaxanthin accumulated after 30 days of exposure in the stress conditions for *H. pluvialis*, according to Orosa et al. (2000), Shah et al. (2016), Lim et al. (2017) and Pérez-Legaspi et al. (2019) who mention that stress conditions such as high irradiation, nitrate deficiency and excess phosphates can induce green vegetative Pamella cells, to transition cells of aplanospora type to mature aplanospores with high concentration of astaxanthin in lipid droplets showing bright red color (Fig. 1).

The coloration intensity in the NdR and NdB phenotypes of *N. davidi* was favored by consuming live microalgae (Fig. 2). Therefore, consuming pigments from live microalgae influences the color intensity of *N. davidi* agrees with Tomas et al. (2020), who mentions that consuming carotenoids such as asta-xanthin favors the pigmentation of *N. davidi* red cherry,

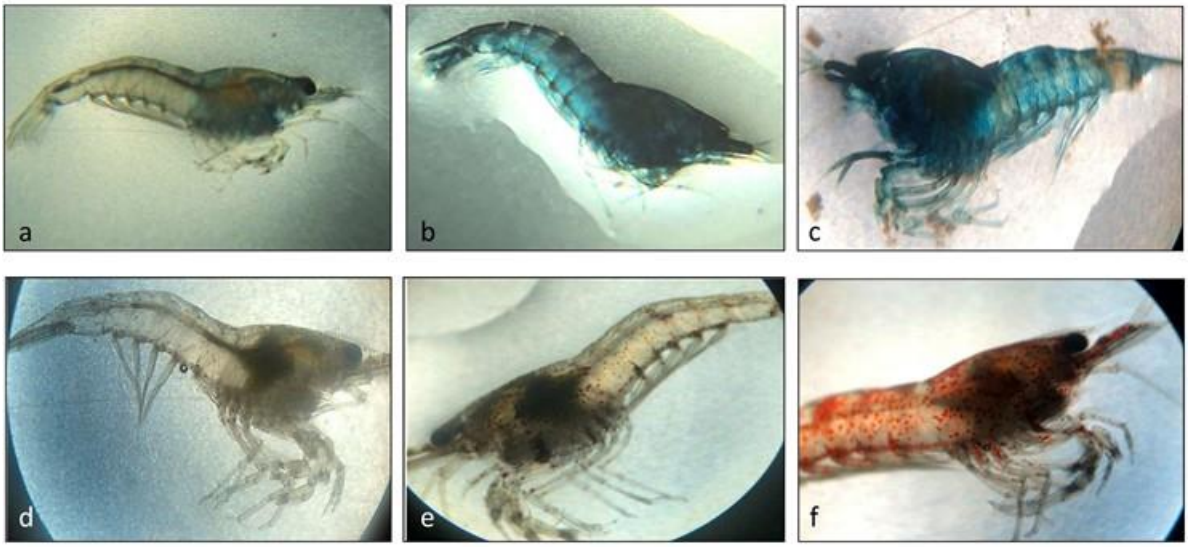

Figure 2. Phenotypes of *Neocaridina davidi* fed with live microalgae as a supplement. Diamond blue phenotype (NdB): a) fed with spheres without microalgae, b) fed with *Spirulina* (*Arthrospira*) *platensis*, c) fed with *Haematococcus pluvialis*. Fire red phenotype (NdR): d) fed with spheres without microalgae, e) fed with *S. platensis*, f) fed with *H. pluvialis*.

but *S. platensis* is unsuitable for improving its reddish coloration. Most studies evaluating the coloration of *N. davidi* comprise the content of total carotenoids such as astaxanthin and chromatophore dispersion in the carapace of adults of the red cherry phenotype (Sganga & López-Greco 2019, 2020, Tomas et al. 2019, 2020). However, several phenotypes of *N. davidi* may be influenced by various factors that modulate its pigmentation, such as habitat type, seasonality, temperature, radiation, and even age and sex; in addition, the distribution of chromatophores can vary according to the region of the body where the flat shape of the uropod is a good option that allows observing with greater detail and precision the pigmentation (Flores & Chien 2011). Although visual stimulation is important, interaction with diet may have a greater influence on crustacean larvae's coloration and astaxanthin content (Duarte et al. 2017, Tomas et al. 2019). We decided to evaluate the effect of the live microalgae *H. pluvialis* and *S. platensis* using the uropod to analyze the dispersion of chromatophores and chromatosomes in three phenotypes of *N. davidi* during their first 31 days of development, taking care of their integrity and loss of color during their manipulation. Our results agree with Flores & Chien (2011), who suggest that the uropod is an adequate structure to evaluate changes in the coloration of crustaceans since it allows the observation of chromatophores and chromatosomes; in addition to being a non-invasive method with the possibility of recovering the organisms analyzed.

The pigment content, concentration, and dispersion of chromatophores are responsible for the coloration of invertebrates as crustaceans (Duarte et al. 2017, Tomas et al. 2019). The NdW phenotype of *N. davidi* has a weak coloration with slightly green tones. In our study, it was possible to identify in the uropod of this phenotype the presence of different chromatophores of different colors (Fig. 3), that is, melanophores (pigment melanin brown or black), erythrophores, xanthophores (red and yellow with pteridines and carotenoids), and leucophores or iridophores that give blue or white coloration due to the reflection of light (Duarte et al. 2017). Therefore, the uropod allows for the detailed observation of the pigmentation and development of chromatophores because it is weakly calcified, poorly sclerotized, and not pigmented (Flores & Chien 2011). In addition, the diversity of chromatophores observed in the NdW phenotype suggests that *N. davidi* can acquire any color depending on various factors where the diet slightly stimulates the intensity of the chromatophores. Thus, the change in coloration is determined by changes in the state, proportion, abundance, and arrangement of the type of chromatophores, as well as by the interaction of combined effects (Duarte et al. 2017).

In the NdR phenotype that did not consume microalgae as a supplement (control), it is possible to distinguish that red chromatophores are distributed along the uropod. Tomas et al. (2020) mention that the consumption of *S. platensis* is not suitable for impro-

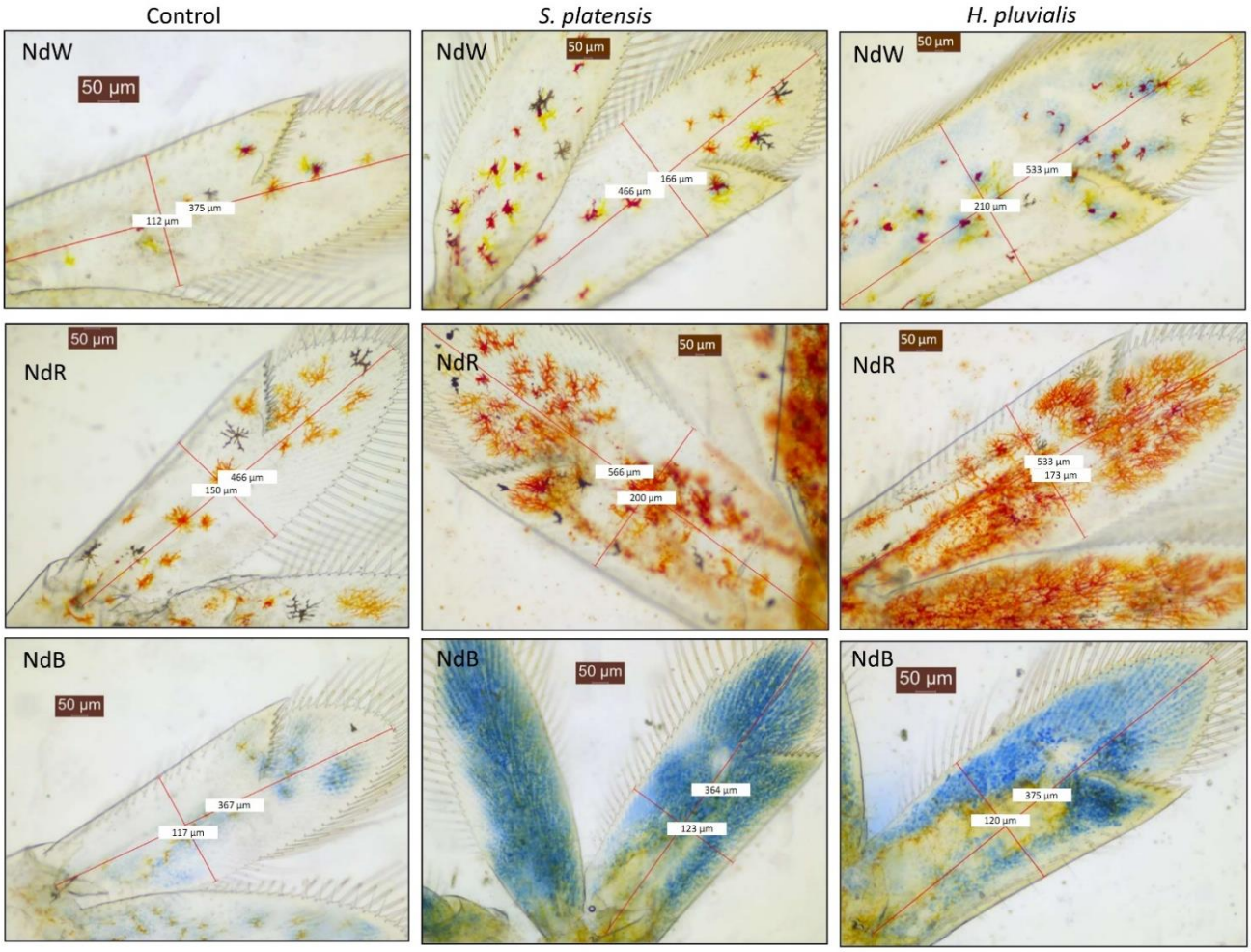

Figure 3. Analysis of the uropod chromatophores of three phenotypes: wild (NdW), fire red (NdR), and diamond blue (NdB) of *Neocaridina davidi* fed with live microalgae (*Spirulina* (*Arthrospira*) *platensis* and *Haematococcus pluvialis*), and without microalgae (control).

ving the reddish color of *N. davidi* red cherry. However, in the NdR phenotype analyzed, we observed that when consuming this cyanobacterium, the intensity, dispersion, and extension of its red chromatophores increased by double, forming chromatosomes compared to shrimp that did not consume "control" microalgae (Fig. 3, Table 1). This discrepancy might be due to the NdR phenotype instead of red cherry. The body region, or even the supply of live cyanobacteria instead of inert food, was analyzed. On the other hand, by supplying the *H. pluvialis* microalgae to shrimp of the NdR phenotype, it is possible to cover almost the entire uropod due to the extent that reaches the area of the chromatosomes (Fig. 3, Table 1) because the aplanospores of *H. pluvialis* contain astaxanthin and other pigments such as canthaxanthin, echinone, and lutein; which can accumulate in tissues such as skin and muscle of those organisms that consume them, favoring their pigmentation and acquiring a bright and attractive coloration (Hemaiswarya et al. 2011, Shah et al. 2016, Lim et al. 2017, Pérez-Legaspi et al. 2019, Tomas et al. 2020).

By analyzing the uropod of the NdB phenotype that consumed *S. platensis*, it is possible to distinguish that its chromatophores spread to form large areas of chromatosomes that reach to cover almost the entire uropod compared to shrimp that did not consume "control" microalgae. Consuming *H. pluvialis* stimulated pigmentation and chromatosome formation in greater proportion than the control but with less intensity than when consuming cyanobacteria (Fig. 3, Table 1). Probably because *Spirulina* improves the attractability and palatability of the feed as a food effector promoter or chemoattraction agent for shrimp

Table 1. Analysis of the uropod of three different phenotypes of *Neocaridina davidi* fed with *Spirulina* (*Arthrospira*) *platensis* and *Haematococcus pluvialis*. NdB: *Neocaridina davidi* blue, NdR: *Neocaridina davidi* red, NdW: *Neocaridina davidi* wild.

Phenotype	Treatment	Optical density (%)	Chromatophores number	Chromatosome area (ppp)
NdW	Control	30.28 ± 4.11	27.43 ± 9.39	1861.92 ± 514.66
	S. platensis	32.09 ± 4.38	28.75 ± 9.40	4032.96 ± 1089.67
	H. pluvialis	52.59 ± 7.05	38.31 ± 10.86	4690.96 ± 875.29
NdR	Control	32.73 ± 5.69	27.75 ± 7.38	6976.96 ± 2920.98
	S. platensis	48.41 ± 8.22	59.12 ± 22.81	11425.24 ± 3978.58
	H. pluvialis	74.24 ± 23.80	42.12 ± 10.76	24745.00 ± 3900.6
NdB	Control	37.74 ± 20.80	24.75 ± 10.62	2614.72 ± 1952.25
	S. platensis	56.20 ± 5.74	26.06 ± 6.11	5296.48 ± 3810.95
	H. pluvialis	51.06 ± 15.19	26.81 ± 10.55	5101.08 ± 3986.56

by improving their coloration (Kohal et al. 2017); in addition, its content of pigments present in *S. platensis* such as phycocyanins, as well as vitamins A and E, phenolic compounds and fatty acids that contribute to the improvement of the phenotypic coloration of crustaceans (Macias-Sancho et al. 2014, Kohal et al. 2017, Machado-Sierra et al. 2021). Therefore, it is convenient to supply pigments in the diet of crustaceans to increase their coloration, which depends on the quality and quantity of pigments in the chromatophores (Tomas et al. 2020).

In this study, it is possible to recognize that the pigments present in microalgae can stimulate the dispersion and intensity of chromatophores and the formation of chromatosomes in the ornamental shrimp *N. davidi*, depending on the phenotype, which is possible by gelling live microalgae as it helps preserve their biochemical composition and transfer quality nutrients to the organisms that consume them. It also contributes to larval development by improving their coloration as an alternative that favors their commercialization as an ornamental species. In conclusion, the pigments and other nutrients present in the live microalgae supplied by gelling to ornamental shrimp *N. davidi* favor the intensity and dispersion of their chromatophores, improving their coloration, which depends on the species of microalgae and the phenotype of shrimp used. As previously shown, astaxanthin of *H. pluvialis* stimulates the red phenotype (NdR), while phycocyanins of *S. platensis* induce the blue phenotype (NdB).

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