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Research Article

Effect of commercial diets on growth performance and stress response in tomato clownfish (*Amphiprion frenatus*) juveniles: a first approach

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ABSTRACT. Clownfish or anemone fish (*Amphiprion* spp.) are among the most commercialized marine ornamental fish species in the world. The present study evaluated three commercial aquaculture diets (A, 40% crude protein (CP), 9% crude fat (CF); B, 45% CP, 15% CF; C, 38% CP, 6% CF) on the growth performance and response to stress of tomato clownfish *Amphiprion frenatus* juveniles during six weeks. Growth performance was evaluated through final weight and length, weight gain, condition factor, specific growth rate, and feed conversion factor. Stress response was assessed using novel environment and mirror individual stress tests, performed in two sets in the second and fifth weeks. Results demonstrated that diet B not only significantly increased ($P < 0.05$) fish growth performance and improved feed conversion rate, but also raised activity and swimming speed ($P < 0.05$) in the novel environment test and the number of attack attempts in the mirror test ($P < 0.05$), compared to diets A and C. Additionally, tomato clownfish showed consistent behavioral responses over time. These results are of interest for the ornamental fish aquaculture industry to select adequate diets and to develop protocols to improve welfare of this fish species in captivity.

Keywords: clownfish; ornamental fish culture; commercial diets; growth performance; stress response or stress coping styles

INTRODUCTION

Amphiprion genus is among the most commercialized marine ornamental fish species in the world. This fish species, commonly known as clownfish or anemone fish, is highly appreciated in the ornamental industry due to its colors, easy adaptability to rearing conditions, and relatively high reproduction success in rearing conditions (Olivotto et al. 2011, Biondo 2017). Over the last two decades, the genus *Amphiprion* has been the subject of research. Results have exposed essential aspects of their biology, such as embryology and ontogeny (Yasir & Qin 2007, Anto & Turingan 2010), the effect of stocking density on growth (Chambel et al. 2015), reproduction and fecundity (Varghese et al. 2009, Holtsworth et al. 2017). These advantages have contributed to the development of captive breeding protocols to reduce dependence on wild-caught specimens and, therefore, support a more sustainable ornamental fish trade. Nevertheless, some gaps con-

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cerning the optimization of culture conditions, including nutrition, welfare, and growth performance during the juvenile phase, remain comparatively limited (Cañedo-Orichuela et al. 2023).

A wide range of commercial feeds for ornamental fish species is currently available on the market with highly disparate dietary compositions and nutritional formulations. An overall lack of precision on targeted fish species and of information on their feeding habits in wild conditions (Sicuro 2017), making difficult the selection of appropriate feeds for rearing a specific fish species, like tomato clownfish (*Amphiprion frenatus*), which is one of the most popular species in ornamental fish industry (Hoff 1996), a marine and omnivore species that feeds on a mixed diet of algae, zooplankton, small crustaceans and other small invertebrates in the wild, and is, at the same time, an algal grazer and opportunistic feeder, scavenging for food from their host anemones and in the water column (Khoo et al. 2018, Kobayashi et al. 2025). Most of the current knowledge on quantitative and qualitative nutritional requirements of ornamental fish arose mainly from research carried out on aquaculture fish species (Webster & Lim 2002, Carr et al. 2023, Sugiuria 2025) or from a few studies on freshwater ornamental fish species (Sales & Janssens 2003, Velasco-Santamaría & Corredor-Santamaría 2011, Saint-Erne 2024, Trejo-Sánchez et al. 2025). Studies for marine ornamental fish remain comparatively scarce, although some studies have demonstrated the importance of diet composition on growth and performance. For example, Díaz-Jiménez et al. (2020) reported that dietary lipid and protein levels of 10 and 43 g kg^{-1}, respectively, promoted growth in juvenile false clownfish (*Amphiprion ocellaris*).

In contrast, Varghese & Raj (2021) showed that protein level (45%) improved growth performance and feed utilization in the sebae anemonefish (*Amphiprion sebae*). Nevertheless, information regarding the nutritional requirements of *A. frenatus* remains particularly scarce. To date, only a limited number of studies have evaluated the effects of dietary composition on growth performance, reproductive behavior, and physiological responses in this fish species (Schmidt et al. 2016, Moncayo-Gómez et al. 2020).

On the other hand, most studies assessed ornamental fish nutritional requirements on growth performance, physiological processes related to digestibility and feed efficiency (Velasco-Santamaría & Corredor-Santamaría 2011) or evaluated the effect of dietary pigments-fortified diets on coloration and pigmentation as accepted global parameters for ornamental fish quality (Khatoon et al. 2010, Rout et al. 2013, Simhachalam et al. 2015). However, almost no research has examined the impact of diet on ornamental fish welfare, such as stress responses and adaptability to captivity. Therefore, assessing the effect of different commercial feeds to optimize growth performance and overall fitness of *A. frenatus* in captivity is of great interest for the ornamental fish industry to improve fish growth, health, and welfare, thanks to the selection of diets whose formulation would be as close as possible to fish requirements, and would allow reducing production costs related to feeds.

Nonetheless, the effect of fish nutrition on their adaptive response to stress in captivity has been evidenced in many works, where properly balanced diets, adjusted to species-specific dietary requirements of fishes, were demonstrated to benefit their energy utilization, health, welfare, and behavioral response to cope with stress in captivity (Fletcher 1997, Lall & Tibbetts 2009, Herrera et al. 2019). In this context, Herrera et al. (2019) have reviewed the physiological effects of dietary compounds on stress mitigation in fish culture and highlighted the role of proteins and amino acids, lipids and fatty acids, carbohydrates and vitamins (mostly vitamin C and E), and even additives and specific extracted molecules (e.g. amino acids, nucleotides, polysaccharides, etc.). For instance, Hooley et al. (2014) studied the effect of dietary protein and lipid levels on stress tolerance in tilapia (*Oreochromis niloticus*) juveniles cultured in a high-intensity recirculating-water system. De la Roca et al. (2020) also documented differences in the enzymatic activity of energy utilization between wedge sole (*Dicoglossa cuneata*) with distinct stress coping styles. Kennedy-Luz et al. (2012) reported that different protein levels were also significantly related to air exposure resistance and survival in *O. niloticus* fingerlings. Esmaeili et al. (2022) described that Oscar (*Astronotus ocellatus*) fed high dietary lipid content and exposed to early-life mild stressors grew faster, presented higher health status and survival after an acute confinement stress test than fish fed lower lipid contents and without stress exposure. Lastly, Sánchez-Velázquez et al. (2024) reviewed how feeding ingredients influence stress responses, metabolic and cellular markers, growth rate, and oxidative stress in fishes. However, most of the research about fish welfare has been performed on aquaculture species (Stevens et al. 2017), and from the few studies on ornamental fish species, even less studies reported an association between fish welfare and dietary compounds; some examples are lipid levels on physiological stress in *A. ocellatus* (Esmaeili et al. 2022), β-glucans levels

on intestinal health in angelfish (*Pterophyllum scalare*) (De Lima et al. 2024) or β-carotenes on immune responses in platy fish (*Xiphophorus maculatus*) (Abdollahi et al. 2019). To our knowledge, no study has reported evidence of the impact of ornamental fish nutrition on the behavioral response to stress in captivity. Hence, the present study aimed to evaluate the effect of three commercial diets with different protein and crude fat contents on growth performance and stress resistance in tomato clownfish (*A. frenatus*) juveniles.

MATERIALS AND METHODS

Ethics approval

The number of individuals and handling procedures used in this study were approved by the Regional Committee of Bioethics in Nayarit, Mexico (Document reference: 096/CEB/2017). Authors considered the Three Rs guidelines (Guide for the Care and Use of Laboratory Animals) to minimize the suffering and pain inflicted on fish during trials.

Fish rearing and experimental conditions

Two hundred tomato clownfish juveniles were obtained from a commercial aquarium located in Toluca, Mexico. Before the experiments, fish were acclimated and maintained in the Aquaculture lab facilities of the Nayarit Center for Innovation and Technology Transference (UEGIA-CENITT-UAN, by its Spanish acronym) for three months. A total of 144 juvenile fish, with a mean weight and length of 0.45 ± 0.14 g and 2.81 ± 0.33 cm, were randomly selected and distributed into nine 65 L rectangular tanks (16 fish per tank), at a density of 0.24 fish L^{-1} or a biomass of 0.11 g L^{-1}. Tanks were connected to a recirculation aquaculture system, including mechanical and biological filtration. Levels of ammonia, nitrites, and nitrates were monitored every other day with an aquarium test kit (API Marine kit®; USA) to keep values of $NH_3 < 0.01$ mg L^{-1}, $NO_2 < 0.3$ mg $L^{-1,}$ and $NO_3 < 10.0$ mg L^{-1}, respectively. Water temperature and dissolved oxygen were measured daily in the morning using a multi-parameter oxygen meter (Extech® Model 407510; USA) to maintain temperature at 27.0 ± 1.5°C and dissolved oxygen at 5.8 ± 0.8 mg L^{-1}. Photoperiod was fixed to 14:10 h light/dark (14:10 h, natural photoperiod during summer season in Tepic, Mexico).

Experimental diets

Three commercial aquaculture diets (Table 1) were assessed on growth performance and behavioural patterns under stressful situations in clownfish juveniles: diet A (formulated for marine ornamental juveniles fish species) with 40.3% of crude protein (CP) and 9.0% of crude fat (CF); diet B (formulated for aquaculture marine and carnivore finfish species) with 45.3% CP and 15.0% CF; and diet C (formulated for aquaculture omnivore fish species) with 38.5% CP and 6.0% CF. Selected diets were homogenized to a diameter of 1 mm, according to fish mouth size. Confidentiality of brands of the commercial diets employed was respected to avoid any conflict of interest (following criteria of Chambel et al. 2015).

Feeding trial and sample procedure

Juvenile fish were hand-fed until apparent satiation twice a day (at 8:00 and 13:00 h) for six weeks. To accurately estimate the amount of food consumed per tank and to maintain optimal water quality, uneaten food observed at the bottom of the tank was siphoned out, dried, and weighed, then subtracted from the total amount of food offered to estimate food intake. Growth was evaluated at the beginning and weekly until the end of the feeding trial by measuring total length with a caliper (Luzeren®; 0-6" ± 0.01) and weight with a laboratory scale (Adam® Model HCB 1002; 1,000 g × 0.01 g). All organisms were sampled for morphometric measurements at each sampling point.

Proximate analysis

The proximate composition (%) of experimental diets was determined using officially standardized methods (AOAC 1995). Moisture was quantified by subtracting sample dry weight, measured after 24 h in a convection oven at 100°C, from sample wet weight. Total nitrogen content was determined by the micro-Kjeldahl method, and CP was estimated by multiplying the results by a 6.25 factor. CF was determined by the Soxhlet method using petroleum ether solvent extraction. Ash content was estimated by incinerating samples for 8 h in a muffle furnace at 550°C. Nitrogen-free extract (NFE) was calculated by subtracting CP, CF and ash percentages from 100% of the feed sample. Diet gross energy content was assessed based on CP, CF and NFE content, using equivalents of 23.64, 39.54 and 17.15 MJ kg^{-1}, respectively (Blaxter 1989) (Table 1).

Survival and growth performance

Sampled data were initial and final length, weight, and number of fish, while survival (S, %), weight gain (WG, g), relative weight gain (RWG, %), specific growth rate (SGR), Fulton's condition factor (K), and thermal growth coefficient (TGC, $g^{1/3}$ $°C^{-1}$ d^{-1}) were calculated according to the following formulae:

Table 1. Proximate composition of the three experimental diets supplied to *Amphiprion frenatus* juveniles (dry matter basis).

Variables	Experimental diets		
	A	B	C
Dry matter (%)	92.7	95.5	93.9
Crude protein (%)	40.3	45.3	38.5
Crude fat (%)	9.0	15.0	6.0
Ash (%)	7.7	9.9	15.6
Nitrogen free extract + crude fiber (%)	43.0	29.8	39.9
Energy content (MJ. kg^{-1})	20.46	21.75	18.32

(1) Survival (S, %) = (final number of fish / initial number of fish) × 100

(2) Weight gain (WG, g) = W_f - W_i), where W_f: final body weight (g) and W_i: initial body weight (g).

(3) Relative weight gain (RWG, %) = [(W_f - W_i) / W_i] × 100.

(4) Specific growth rate (SGR, % d^{-1}) = 100 × [(Ln W_f - Ln W_i)] / t, where t: assay duration (42 days).

(5) Fulton's condition factor (K) = 100 × [W_f / L_f], where L_f: final length^3 (cm)

(6) Thermal growth coefficient (TGC, $g^{1/3}$ °C^{-1} d^{-1}) = {[(W_f (g))$^{1/3}$ - (W_i (g))$^{1/3}$] / (T × t)} × 1,000, where T: average water temperature (27°C) and t: assay duration (42 days).

Behavioral stress test responses

Thirty-nine tomato clownfish juveniles (13 per treatment) were subjected to two common stress tests used in aquaculture: a novel environment test and a mirror test (Castanheira et al. 2015). Both tests were applied in sequence, and twice: on the second (run 1) and fifth week (run 2) of the experiment, following criteria described by Ibarra-Zatarain et al. (2020).

Novel environment test: It consisted of placing fish individually in a rectangular plastic tank (30 cm length × 19 cm width × 15 cm high) filled with water from the rearing tank (renewed for each fish) and gridded at the bottom into squares of 3 cm^2. A camera (Swann®; 2Kseries-1080p; model SWDKV-845804) was fixed 40 cm above the tank to video-record fish behavior for 180 s. Five behavioral variables were analyzed: (i) total distance travelled (TDT, cm) by each fish; (ii) total number of squares crossed (TSC) by each fish; (iii) minimum speed (MinS, cm s^{-1}); (iv) medium speed (MedS, cm s^{-1}) and (v) maximum speed (MaxSm cm s^{-1}) of fish during the test (adapted from Hosoya et al. 2008, Benhaïm et al. 2012, Ibarra-Zatarain et al. 2015).

Mirror test: It consisted of placing fish individually in a plastic container (20 cm length × 14 cm width × 9 cm tall), in which a mirror was on one of the tight sides to reflect the fish's image and which was divided into four equal areas (1 to 4, 1 being the closest to the mirror and 4 the farthest). Five behavioral variables were analyzed for 180 s: (i to iv)) time (TM1, TM2, TM3, TM4, in s) spent by fish in each area 1 to 4; (v) total number of attack attempts (TAA) made by each fish against its own reflect, denoted by the erection of dorsal fins and bites of the mirror (adapted from Way et al. 2015, Kohda et al. 2019).

Analyses on behavioral parameters

All behavioral parameters from both tests were analyzed with an image processing program in MATLAB (V2015a). Overall, videos were transformed to RGB24 (red-green-blue), and the red and green channels were used to contrast fish from the background. Then, video analysis was performed into four stages: 1) frame lecture: a video reader function in MATLAB was used for sequential analysis, in which videos were adjusted to a 480×460 resolution in RGB24 (red × green × blue) and each pixel was transformed to 8 bit; 2) video processing: red and green colors were used to contrast fish from background, then a fit-geotrand function was applied to correct frames and improve the projection of images; 3) fish identification: a local thresholding segmentation method was dynamically applied to images and each frame was adjusted using the formula: [0.25×max(R/G) - max(R/G)], afterwards, the region props function was used to estimate the area, perimeter, and centroid of the segmented object; 4) movement analysis: grids centroids (squares equal to 3 cm^2) and video frames were set to analyze fish movement in relation to centroid of grids. Methodology and image processing were based on Radke (2009) and Gonzalez et al. (2020).

Table 2. Growth performance variables of *Amphiprion frenatus* juveniles fed the three commercial feeds (mean ± standard error of mean, n = 3). Superscript letters and bold *P*-values indicate statistical differences.

Growth performance variables	Commercial diets			*P*-value
	A	B	C	
Survival (%)	95.8 ± 7.2	95.8 ± 7.2	95.8 ± 7.2	1.000
Final weight (g)	0.52 ± 0.02^{b}	0.63 ± 0.02^{a}	0.55 ± 0.02^{b}	**0.001**
Final length (cm)	2.90 ± 0.05^{b}	3.09 ± 0.05^{a}	2.91 ± 0.05^{b}	**0.005**
Weight gain (g)	0.04 ± 0.03	0.14 ± 0.03	0.06 ± 0.04	0.150
Relative weight gain (%)	7.85 ± 6.77	28.25 ± 5.91	13.08 ± 8.43	0.177
Fulton's condition factor (K)	2.17 ± 0.08	2.15 ± 0.06	2.27 ± 0.08	0.277
Specific growth rate (% d^{-1})	0.33 ± 0.15	0.69 ± 0.13	0.51 ± 0.18	0.251
Thermal growth coefficient	0.027 ± 0.014	0.066 ± 0.013	0.043 ± 0.018	0.177

Statistical analyses

Data were expressed as mean ± standard error of the mean (SEM) and significant differences were set at $P < 0.05$. Statistical analyses were performed with SPSS V25. All data were tested for normality using the Shapiro-Wilk test and homoscedasticity using Levene's test. Subsequently, to determine differences among diet treatments for growth performance (S, W_f, L_f, WG, RWG, K, SGR, TGC) and behavioral variables (TDT, TSC, MinS, MedS, MaxS, TM1, TM2, TM3, TM4, TAA), one-way analyses of variance (ANOVA) were applied on data respecting conditions of normality and homogeneity of variance, followed by Tukey's *post-hoc* tests when significant differences were found. Kruskal-Wallis tests were performed on data that did not meet the normality and homoscedasticity conditions. A Pearson's correlation analysis was run between, on one hand, weight and length, and on the other hand, all behavioral responses from the novel environment and mirror tests, to validate that behavioral responses were inherent to fish size or weight. To analyze the consistency of behavioral responses in both stress tests between runs 1 and 2, Student *t*-tests were applied to each behavioral variable, independently for each of the three diets.

RESULTS

Survival and growth performance

S and K were not significantly different between dietary treatments ($P > 0.005$). Fish fed diet B showed significantly higher final weight ($P < 0.001$) and length ($P = 0.005$) than fish fed diets A and C. Fish fed diet B also tended to present higher WG, RWG, SGR and TGC than fish fed diets A and C. However, these differences were not significantly different between dietary treatments.

Behavioral stress test responses

Neither weight nor length were significantly correlated with any of the behavioral variables, for either the novel environment or the mirror test ($P > 0.05$). The consistency in behavioral patterns of tomato clownfish was demonstrated over time, since no significant differences were found between runs 1 and 2 for any of the 10 behavioral parameters assessed and for any of the three different diets tested ($P > 0.05$).

Novel environment test: Although no significant differences were detected in TDT and TSC by fish between diets ($P > 0.005$) in neither of the runs, fish fed diet B presented significantly higher MinS ($P = 0.028$ and 0.023), MedS ($P = 0.006$ and 0.023) and MaxSm ($P = 0.002$ and < 0.001) than fish fed diet A in both runs 1 and 2, respectively (Table 3).

Mirror test: Tomato clownfish fed diet B spent significantly more time in the closest area to the mirror (area 1) than fish fed with diets A and C, for both runs 1 and 2 ($P = 0.004$ and 0.003, respectively), while those fed diet A spent significantly more time in the central areas of the container (areas 2 and 3) than fish fed diets B and C, for both runs 1 (area 2: $P = 0.032$; area 3: $P =$ 004) and 2 ($P < 0.001$, for both areas 2 and 3) (Table 3). Finally, fish fed diet C spent significantly more time in the farthest area from the mirror (area 4) than fish fed diets A and B, for both runs 1 and 2 ($P = 0.049$ and $P =$ 0.021, respectively). Regarding the number of attack attempts, fish fed diet B tended to perform a higher number of bites and fin erections than juveniles fed diets A and C in run 1. However, this trend was not significantly different ($P > 0.05$), while it was signifi-

Table 3. Behavioral variables of *Amphiprion frenatus* juveniles fed the three commercial feeds for runs 1 and 2 (mean ± standard error of mean, n = 13). Superscript letters and bold *P*-values indicate statistical differences between diets within the same run.

	Run 1 (on week 2)				Run 2 (on week 5)			
	Commercial diets			*P*-value	Commercial diets			*P*-value
	A	B	C		A	B	C	
Novel environment test								
Total distance traveled (cm)	967.75 ± 60.07	1134.23 ± 83.42	1098.46 ± 78.88	0.136	989.31 ± 85.55	1144.85 ± 69.26	1079.77 ± 39.72	0.275
Total squared crossed	322.58 ± 20.91	385.77 ± 26.24	366.15 ± 26.29	0.080	329.77 ± 28.52	381.62 ± 23.09	359.92 ± 13.24	0.275
Minimum speed (cm s^{-1})	1.14 ± 0.12^{b}	1.62 ± 0.10^{a}	1.40 ± 0.08^{ab}	**0.028**	1.00 ± 0.12^{b}	1.36 ± 0.07^{a}	1.11 ± 0.07^{ab}	**0.023**
Medium speed (cm s^{-1})	7.89 ± 0.68^{b}	11.44 ± 0.79^{a}	10.24 ± 1.00^{ab}	**0.006**	8.08 ± 0.83^{b}	11.25 ± 1.10^{a}	8.33 ± 0.56^{ab}	**0.023**
Maximum speed (cm s^{-1})	19.08 ± 0.95^{b}	26.09 ± 1.22^{a}	22.64 ± 1.62^{ab}	**0.002**	21.44 ± 1.90^{b}	34.58 ± 2.16^{a}	21.95 ± 1.18^{b}	**<0.001**
Mirror test								
Time spent in area 1 (s)	107.37 ± 6.34^{b}	152.94 ± 6.62^{a}	118.20 ± 7.32^{ab}	**0.004**	109.69 ± 5.58^{b}	149.72 ± 7.65^{a}	115.76 ± 14.12^{b}	**0.003**
Time spent in area 2 (s)	22.13 ± 2.53^{a}	13.02 ± 3.41^{ab}	12.01 ± 2.56^{b}	**0.032**	21.12 ± 2.27^{a}	13.44 ± 5.10^{b}	9.35 ± 1.98^{c}	**0.004**
Time spent in area 3 (s)	21.65 ± 2.22^{a}	3.37 ± 0.87^{b}	5.20 ± 1.72^{b}	**<0.001**	20.35 ± 2.15^{a}	3.06 ± 0.88^{b}	6.71 ± 1.89^{b}	**<0.001**
Time spent in area 4 (s)	28.77 ± 4.78^{b}	10.22 ± 3.12^{c}	44.27 ± 16.97^{a}	**0.049**	27.47 ± 3.41^{b}	13.53 ± 3.03^{b}	47.74 ± 14.82^{a}	**0.021**
Total number of attack attempts	24.92 ± 2.10	31.54 ± 2.49	22.77 ± 5.28	0.211	20.54 ± 1.20^{ab}	30.00 ± 4.61^{a}	15.54 ± 2.10^{b}	**0.006**

cantly higher in fish fed diet B and significantly lower in fish fed diet C in run 2 ($P = 0.006$).

DISCUSSION

Growth performance

Food acceptance by fish was appropriate, and no severe mortality or disease was detected in fish from any of the three diets assessed during the whole feeding trial. Therefore, differences in fish growth performance between dietary treatments were likely due to feed composition and their metabolic utilization by fish. In this context, fish fed diet B, with the highest amounts of protein (45% CP) and lipid (15% CF), exhibited higher growth performance than fish fed the other diets with lower contents (diet A: 40% CP, 9% CF; diet C: 38% CP, 6% CF). Both proteins and lipids are essential macronutrients for overall structural growth: the first one for building muscle mass and tissue synthesis, while the second one serves as the primary, energy-dense source for metabolic processes and essential fatty acids during the growing phase of individuals in rearing conditions (Carr et al. 2023, Desouky et al. 2024).

Fish are metabolically prepared to use protein as a preferential source of energy rather than lipids and carbohydrates (Cowey 1995). However, because protein content in fish diets usually constitutes the largest production cost in aquaculture (Watanabe 2002), minimizing protein while optimizing growth may be achieved by considering not only absolute amounts of dietary protein and non-protein energy (i.e. lipids and carbohydrates), but also their relative proportions. An adequate protein/lipid ratio allows sparing dietary protein for energy by using other sources instead, and allocating proteins to the synthesis of new proteins for growth (De Silva et al. 1991, Khan et al. 2019), thereby optimizing fish feed. On the contrary, inadequate protein/lipid ratios might induce to deficiencies in feed gross energy, in their utilization and in fish growth, as illustrated by Chambel et al. (2015), where a higher SGR was observed in percula clownfish *Amphiprion percula* juveniles fed a diet with 41% CP and 9% CF compared to those fed higher protein content (until 52% CP) but lower lipid content (8% CF). On the contrary, common clownfish *A. ocellaris* juveniles fed a diet containing 43% CP and 10% CF were shown to grow significantly faster than those fed a commercial diet containing lower protein (33% CP) and higher lipid (15% CF) contents (Díaz-Jiménez et al. 2020). In paradise fish *(Macropodus opercularis),* the optimal SGR was found in fish fed between 40 and 45% CP and 10% CF (Dadgar et al. 2020), while in striped damsel *(Dascyllus aruanus)* juveniles, optimal SGR were calculated for fish fed 46.4% CP and 5% CF (Vijayagopal et al. 2008). As for anemona clownfish (*A. percula*) juveniles, fish fed a commercial diet with a protein content (39.4% CP) similar to diet A (40% CP) and lower than diet B (45% CP) of the present study, but with lipid content (11% CF) between diet A (9% CF) and diet B (15% CF), showed higher SGR than *A. frenatus* juveniles from any dietary treatment of the present study, at similar fish initial weight, fish density, trial duration and feed level at apparent satiation (Lupatsch et al. 2013). In addition to the structural role of lipid and protein in growth, all these studies exemplified the double role of proteins and lipids for structural and energetical purposes and their importance in te relationship between fish growth performance and available dietary energy, calculated as the sum of the mass of macronutrients multiplicated by their respective heat of combustion, with lipids contributing approximatively more than twice to energy than proteins (Blaxter 1989, Glencross et al. 2007).

Behavioral stress test responses

Stress responses were observed to be consistent over time (between runs 1 and 2), in agreement with the definition of stress coping styles (SCS) proposed by Koolhaas et al. (2010): a coherent pattern of behavioral and physiological stress responses that is consistent over time and across situations, representing a fundamental, genetically driven trait in animals to cope differently with stressful situations. SCS reveals adaptive and evolutionary inter-individual variability in stress responses, ranging from proactive or bold animals (highly explorative, active, and aggressive) to reactive or shy ones (using freezing, avoidance, and fleeing strategies), as pointed out in the present study. It has been demonstrated that genetic and epigenetic factors mediate this inter-individual variability (Rey et al. 2016, Hoots et al. 2026). Moreover, individual responses to stressful situations might be adaptive and correlated with nutrition, energy utilization, and genetics, as reported by Careau & Garland (2012), Crespel et al. (2024), and Hoots et al. (2026).

In this study, tomato clownfish juveniles fed a diet with high protein and lipid contents (diet B: 45% CP, 15% CF) displayed a trend to an overall higher explorative behavior in the novel environment test and to a more aggressive attitude in the mirror test, than fish fed diets with lower protein and lipid contents (diet A: 40% CP, 9% CF; diet C: 38% CP, 6% CF). Similarly, angel fish (*Pterophyllum scalare*) juveniles fed

decapsulated *Artemia* cysts with higher levels of protein (54% CP) than commercial flakes or pellets (43 and 45% CP, respectively) were reported to present a higher tolerance to an osmotic stress challenge (García-Ulloa & Gómez-Romero 2005). As well, an optimal protein dietary level of 32% was found to enhance stress resistance (lower cortisol levels and aspartate aminotransferase activity) and immunity (lower serum complement component 3 and 4 concentrations) under a high temperature challenge in blunt snout bream (*Megalobrama amblycephala)* fry, compared to lower (28 and 30%) and higher (43 and 36%) protein levels (Habte-Tsion et al. 2017). On the other hand, excessive dietary protein levels (55% CP) have been associated with decreased air exposure resistance and survival in *O. niloticus* fingerlings (Kennedy-Luz et al. 2012) and with lower survival to confinement test in *A. ocellatus* juveniles (51% CP compared to 38% CP) (Zare et al. 2024). Even a negative impact of sub-optimal dietary nitrogen formulations has been documented on fish amino acid metabolism, physiological stress responses and welfare (Conceição et al. 2012). Protein levels employed in the present study might not be elevated enough to observe this detrimental effect on survival, growth or stress response in this fish species.

However, most studies evaluating the effect of feed composition on stress response have considered dietary lipids as a main factor. Dietary lipids not only provide metabolic energy, but also influence physiological processes involved in stress adaptation and neural function. For instance, *A. ocellatus* exposed to early-life mild stressors was shown to grow faster and have higher health status and survival after an acute confinement stress test when fed a high dietary lipid content, than fish fed lower lipid contents and without stress exposure (Esmaeili et al. 2022). In addition, many studies pointed out the impact of dietary essential fatty acid composition on stress response in aquaculture fishes. Although further studies assessing the effect of the fatty acid profile of the three commercial diets on *A. frenatus* behavior would be needed to be able to provide avenues for understanding better the causal relationship between the diets and the differences in explorative behavior and aggressiveness observed, the role of eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and araquidonic acid (ARA, 20:4n-6) should be highlighted. In particular, gilthead seabream (*Sparus aurata*) juveniles fed higher contents of lecithin and EPA showed significantly higher resistance to handling stress and to temperature and salinity stress tests (Liu et al. 2002). Senegalese sole (*Solea senegalensis*) juveniles fed enriched live prey with cod oil-based emulsion (rich in DHA, EPA and with low ARA/DHA and ARA/EPA ratios) displayed higher activity and took higher risks than fish fed enriched live prey with vegetal-oil emulsions (Ibarra-Zatarain et al. 2015). The particular beneficial effect of dietary high ARA content on fish stress response has been reported in guppies (*Poecilia reticulata*) fed algae-enriched diets, which showed enhanced stress resistance after a salinity test (Dagar et al. 2009) as well as in European sea bass (*Dicentrarchus labrax*) juveniles, which presented higher growth and stress tolerance to a handling manipulation than fish fed usual ARA doses (Atalah et al. 2011).

Finally, the importance of proteins and amino acids, of lipids and fatty acids, particularly ARA for the synthesis of eicosanoids (especially prostaglandins) and neurotransmitters (catecholamines, glutamate, gamma-aminobutyric acid, serotonin) involved in the regulation of immune system, hypothalamic-pituitary-axis reactivity, cortisol and corticosterone secretion and release to the blood circulatory system has been proved in many studies and thoroughly reviewed in Herrera et al. (2019) and Ciji & Akhtar (2021), highlighting the role of these stress-attenuating biomolecules on improving resistance to environmental stressors, stress coping abilities and stress mitigation in fishes. In this sense, it is worth highlighting that further studies are needed to bring a more holistic approach to the current preliminary study and to understand mechanisms underlying the effect of the dietary composition on fish behavior (activity and aggressiveness), specifically in the assessment of physiological stress indicators, such as cortisol, glucose, or lactate.

CONCLUSIONS

Thus, the result of the present investigation suggested that diets with highest protein and lipid amounts assessed not only promote growth and survival of tomato clownfish (*A. frenatus*) juveniles held in captivity, but also impacted on the behavioral responses to environmental stressors, by demonstrating that fish fed with diet B exhibited higher overall activity and aggressive tactics that resembled proactive-reactive coping abilities in tomato clownfish in response to stressful situations. Nonetheless, further research is needed to understand the underlying mechanisms of the interconnection between nutrition and behavior in this species, opening a new field of research. Relying on diets that enable greater adaptation to stress in captivity for ornamental species would be of great interest for the ornamental aquaculture sector in Mexico.

Credit author contribution

A. Boglino: experimental design, sampling, data analysis, writing, reviewing, and editing the manuscript; J.P. Rivera-Caicedo: data analysis, software, data validation; Z. Ibarra-Zatarain: conceptualization, methodology, sampling, student supervision, writing original draft and editing, project administration and funding acquisition.

Conflict of interests

The authors declare no conflict of interest.

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