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Growth Performance, Molting Frequency and Carapace Coloration of Two Different Size Classes of Red Swamp Crayfish (*Procambarus clarkii*) Fed with Different Diets

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ABSTRACT

In this study, the effects of three different commercial aquarium feeds were tested on the growth performance, molting frequency and carapace coloration of red swamp crayfish (*Procambarus clarkii*) in two different size classes (4 and 5 cm). For this purpose, six experimental groups were formed according to crayfish size (S1: Size 1 and S2: Size 2) and feed type (BF: bottom fish food, CF: cichlid food and CG: crustacean granules) as S1BF, S1CF, S1CG, S2BF, S2CF and S2CG, and the crayfish were fed with these diets for 12 weeks. The final mean weight (FMW) of the S1CG was higher than the S1BF ($P<0.05$). The final mean total length (FMTL) and final mean carapace length (FMCL) of S2BF were the lowest ($P<0.05$). No significant differences were recorded in the feed conversion ratios (FCR), specific growth rates (SGR) and survival rates (SR) of red swamp crayfish in two different size classes ($P>0.05$). The lowest cheliped injury and cannibalism rates were found in the crayfish fed with crustacean feed in both size classes. The mean molting frequencies (MMF) of the S1CG and S2CG were statistically higher than the S1CF and S2BF, respectively ($P<0.05$). S2CG has the highest final lightness (L^*) value ($P<0.05$). The final redness (a^*) and final yellowness (b^*) values of the S1BF and S2BF groups were the lowest ($P<0.05$). The results showed the positive effects of crustacean feed on all the tested parameters in both size classes. Further studies are needed to investigate the efficient use of species-specific rations in the crayfish species.

Keywords: Cambaridae, crayfish feeding, aquarium feed, growth performance, pigmentation

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Farklı Yemlerle Beslenen İki Farklı Boy Sınıfındaki Kırmızı Bataklık Kerevitlerinin (*Procambarus clarkii*) Büyüme Performansı, Kabuk Değiştirme Frekansı ve Karapas Renklenmesi

Öz: : Bu çalışmada, iki farklı boy sınıfındaki (4 ve 5 cm) kırmızı bataklık kerevitlerinin (*Procambarus clarkii*) büyüme performansı, kabuk değiştirme frekansı ve karapaks renklenmesi üzerine üç farklı ticari akvaryum yeminin etkileri test edilmiştir. Bu amaçla kerevit boyutuna (S1: Boy 1 ve S2: Boy 2) ve yem tipine (BF: dip balığı yemi, CF: çiklit yemi ve CG: krustase yemi) göre S1BF, S1CF, S1CG, S2BF, S2CF ve S2CG olmak üzere altı deney grubu oluşturulmuştur ve kerevitler bu yemlerle 12 hafta boyunca beslenmiştir. S1CG'nin son ortalama ağırlığı, S1BF'den daha yüksektir ($P<0,05$). S2BF'nin son ortalama total boy uzunluğu ve son ortalama karapaks uzunluğu en düşüktür ($P<0,05$). Kırmızı bataklık kerevitlerinin yem dönüşüm oranlarında, spesifik büyüme oranlarında ve hayatta kalma oranlarında iki farklı boy sınıfında önemli farklılıklar kaydedilmemiştir ($P>0,05$). Her iki boy sınıfında da krustase yemiyle beslenen kerevitlerde en düşük kıskaç kaybı ve kanibalizm oranları bulunmuştur. S1CG ve S2CG'nin ortalama kabuk değiştirme frekansı, sırasıyla S1CF ve S2BF'den istatistiksel olarak daha yüksektir ($P<0,05$). S2CG, en yüksek son parlaklık (L^*) değerine sahiptir ($P<0,05$). S1BF ve S2BF gruplarının son kırmızılık (a^*) ve son sarılık (b^*) değerleri en düşüktür ($P<0,05$). Sonuçlar, krustase yeminin her iki boyut sınıfında test edilen tüm parametreler üzerindeki olumlu etkilerini göstermiştir. Kerevitlerde türe özel yemlerin verimli kullanımı ile ilgili daha fazla çalışmaya ihtiyaç vardır.

Anahtar kelimeler: Cambaridae, kerevit besleme, akvayum yemi, büyüme performansı, pigmentasyon

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Introduction

Red swamp crayfish (*Procambarus clarkii*) originated in north-eastern Mexico and Louisiana, USA. During the last decade, cultivation of this species has been widespread in both ornamental and commercial aquacultures because of their resistant and tolerant characteristics (Cruz and Rebelo 2007), attractive behaviors, and appealing coloration and the species is the most cultivated crayfish in the world (FAO 2019). But unfortunately, the red swamp crayfish was reported as an invasive animal from inland waters of many countries of the world (GISD 2021). In Türkiye, this species became more popular in the aquarium sector in the last quarter of the 2000s. Although, there is no common culture of red swamp crayfish in Türkiye, the species is one of the most available crayfish in the aquarium sector.

With the increase in the number of species demanded in the aquarium sector, research on ornamental aquaculture has gained importance. Basics in ornamental aquaculture have primarily originated from conventional aquaculture (Sicuro 2018). For example, the biggest variety of raw materials and dietary additives widely used in ornamental aquaculture is similar to conventional. However, fast development in ornamental aquaculture has caused an ever-increasing demand for enhanced and profitable aquafeeds for the cultivation of finfish and invertebrate species (Boonyaratpalin et al. 2001). Today, ornamental aquafeeds are marketed in a wide spectrum according to the specific characteristics of the species, i.e., living in freshwater, saltwater, brackish water, etc., feeding as carnivorous, omnivorous, herbivorous, etc., being a vertebrate or an invertebrate, or surface and bottom feeding (Sicuro 2018).

Nutrition is extremely important, stating second only to water quality, in the culture of aquatic species (Asoka and Hettiarachchi 2004). The feeding behavior of an animal is critical both in terms of determining its nutritional status and growth and reproductive activities (Hughes 1993), but it also gives information about the place of the animal in the food chain (Poon et al. 2010). Benthic animals have important functions in the food chain and therefore in the ecosystem. For instance, crayfish species, extensively identified as ecosystem engineers, have been known for detritivorous and omnivorous feeding characteristics (Rodríguez-Serna et al. 2010). Plant and detrital resources contain dietary pigments, trace elements and energy (Brown 1995). Therefore, the including these resources in formulated feeds is very important in terms of vital parameters. The number of aquafeeds formulated by commercial companies with raw materials, such as fish and fish derivatives, cereals, vegetable protein extracts, various vegetables, yeasts and algae for ornamental crustaceans is increasing, currently. However, there is no clear information that reveals the adequacy level of commercial feeds.

Color is one of the significant parameters for the price in ornamental aquaculture (Sicuro 2018). Coloration is the primary determining criterion not only for fish but also for both freshwater and marine invertebrates. For that matter, Kaldre et al. (2015) mentioned the importance of carapace color not only for ornamental aquaculture, but also for crayfish cultivated for consumption. Aquarium enthusiasts and seafood consumers are decided by brightness and vibrant pigmentation to the freshness, quality and health of the animal (Boonyaratpalin et al. 2001). Color differentiation in crayfish depends on several factors, including morphological characteristics, genetics, environmental conditions, and diet (Wade 2010; Kaldre et al. 2015). In ornamental aquaculture, dietary additives, including fatty acids, ascorbic acid, alpha-tocopherol and carotenoids are widely used in aquarium feeds for the enhancement of coloration (Güroy et al. 2012).

The efficiency of commercial feeds on several crayfish species, including Mexican crayfish, *Procambarus llamasi* (Rodríguez-Serna et al. 2010), marbled crayfish, *Procambarus fallax* f. *virginalis* (Kaldre et al. 2015) and Australian red claw crayfish, *Cherax quadricarinatus* (Karadal and Türkmen 2012) are studied previously. However, no research was found on the red swamp crayfish with commercially available aquafeeds. Therefore, the aim of the present study was to evaluate the effects of different commercial ornamental fish and crustacean feeds on the growth performance, molting frequency and carapace coloration of red swamp crayfish (*P. clarkii*) in two different size classes (4 and 5 cm). Because the most popular sizes of these crayfish in the aquarium trade are usually around 4-5 cm, the effects of study parameters were tested in both size groups.

Materials and Methods

Ethical Approval

The present study was carried out in accordance with animal welfare and the ethics of experiment. This study complied with the Guidelines of the EU Directive 2010/63/EU for animal experiments.

Rearing Systems and Crayfish

The study was carried out in the Tropical Aquaculture Laboratory, Faculty of Fisheries, İzmir Katip Çelebi University, İzmir, Türkiye. Two different sizes (Size 1 of 4.00±0.06 cm and Size 2 of 5.13±0.03 cm in total lengths) of red swamp crayfish (*P. clarkii*), reared in the Tropical Aquaculture Laboratory, were stocked in 10 L plastic containers

connected to two recirculating sump systems (300 L each) and adapted for 14 days. A submersible pump (Aquawing AQ6000) placed in glass sump aquarium (140 × 50 × 22 cm) circulated the freshwater and fresh air was supplied to all containers by an electromagnetic air pump (Resun ACO-001), continuously. PVC pipes with 5 cm diameter were placed to containers as much as the number of individuals. The 20% water was removed from the sump aquarium twice a week and chlorine-free tap water was added to the system. The photoperiod was maintained as 12:12 (light:dark). Crayfish were weighed individually at the initial and the end of the trial.

Experimental Design and Diets

Three different commercial ornamental finfish and crustacean feeds were used in the study (Table 1). Crayfish were fed once a day near satiation and uneaten food was removed from the containers after 1.5 h from feeding by siphoning and weighed. Six experimental group were formed with two sizes of crayfish (S1 and S2) and three different feeds, including Art Akua Bottom Fish Food (BF), AHM Natural Cichlid Granulat (CF) and Tetra Crusta Granules (CG) as S1BF, S1CF, S1CG, S2BF, S2CF and S2CG. The experiment was carried out in 24 plastic containers in four replications for 12 weeks and 8 crayfish were stocked in each container.

Table 1. Proximate compositions of commercial diets used in the experiment

Composition (%)	Art Akua Bottom Fish Food	AHM Natural Cichlid Granulat	Tetra Crusta Menu
Crude protein	50.00	40.34	44.00
Crude lipid	10.00	5.08	11.00
Crude fiber	3.00	1.11	2.00
Crude ash	7.00	6.92	-
Moisture	7.00	6.29	8.00

Water Parameters

An external heater (Hydor ETH 300) is connected to the circulation pump outputs of the systems. Thus, the temperature of a system was kept constant throughout the trial. The water parameters, including temperature, pH and dissolved oxygen of the freshwater in plastic containers were checked daily with AZ 84051 Combo Water Quality Meter during the study. Mean values were recorded as 24.58±0.17 °C for temperature, 7.42±0.14 for pH and 8.97±0.42 ppm for dissolved oxygen.

Evaluation of Growth Performance

Growth performance data were obtained by biweekly weight, total and carapace length measurements. Before weighing, each crayfish was collected from containers and dried on filter paper to remove water from branchiostegites and appendages. Crayfish were weighed in bulk with an electronic balance (KERN PCB 2500-2, precision of ±0.01 g). Total length measurements were performed from the rostrum tip to the telson end whereas the carapace lengths were measured from the tip of the rostrum to the posterior edge of the carapace using a digital caliper. At the beginning of the experiment, the same weight of commercial feeds was weighed for each plastic container and were stocked to small boxes, and the crayfish were fed with these feeds during the trial. The feed intake of the crayfish was recorded by weighing the feeds in these boxes during the biweekly measurements. Growth parameters, including feed intake (FI), feed conversion ratio (FCR), specific growth rate (SGR) and survival rate (SR) were calculated according to following formulae:

FI (g) = average of the total feed given to each experimental group during the study,

FCR = Feed intake / Weight gain,

SGR (%/day) = 100 x ([Ln Final crayfish weight] - [Ln Initial crayfish weight]) / Experimental days.

Survival and Cannibalism Rate

The number of crayfish that died, injured or lost their claws, and died from cannibalism during the study were recorded according to their tank numbers and trial groups. The survival rate (SR), cheliped injury rate (CIR) and cannibalism rate (CR) were determined with following calculations at the end of the trial:

SR (%) = 100 x (Number of total crayfish - Number of dead crayfish) / Number of total crayfish,

CIR (%) = 100 x Number of crayfish cheliped injured / Number of total crayfish,

CR (%) = 100 x Number of crayfish died due to cannibalism / Number of total crayfish.

Monitoring of Molting Frequency

At the start of the experiment, each crayfish carapace in the plastic containers was individually marked with nail polish in different colors from the right side (Ramalho et al. 2010). The crayfish were checked daily and shells (if any) in the containers

were recorded according to their color. For determining intermolt period, newly molted crayfish was marked again from the same area with the same color of nail polish. New marks on individuals were checked until the shell hardened. Numbers of molting in the experimental groups were recorded and after two days from each molt, weight and total length were measured for collecting molt increment data. The mean molting frequency was calculated with the following formula (Chen and Chen 2003):

$$MMF = [(n_1 \times 1) + (n_2 \times 2) + (n_3 \times 3) + \ldots\ldots + (n_k \times k)] / \text{total number of crayfish}.$$

Molting parameters, including mean molting number (MMN), intermolt period (IMP), weight increment at molt (WIM) and length increment at molt (LIM) were calculated according to the following formulae:

$$MMN = \text{Total number of molting} / \text{Crayfish number},$$

$$IMP\ (\text{days}) = \text{Time of } n+1 \text{ molt} - \text{Time of } n \text{ molt},$$

$$WIM\ (\%) = 100 \times (\text{Weight after molting} - \text{Weight before molting}) / \text{Weight before molting},$$

$$LIM\ (\%) = 100 \times (\text{Length after molting} - \text{Length before molting}) / \text{Length before molting}.$$

Carapace Coloration Measurement

Color measurements were taken from all crayfish at the beginning and end of the experiment in order to obtain coloration data. Measurements were taken from the carapace region of the crayfish on a flat surface with a colorimeter (Color Muse, Variable Inc., Tennessee, USA) (Dang et al. 2021). The measurements were performed on top surface (4 mm) of carapace of each crayfish. The colorimeter was set to take absolute measurements in the L^*, a^*, b^* measuring mode (CIE 1976). L^* is the lightness variable (where white: 100 L^* and black: 0 L^*), a^* is the redness where $+a^*$ stands for red, and $-a^*$ stands for green, and b^* is the yellowness where $+b^*$ stands for yellow, and $-b^*$ stands for blue.

Statistical Analysis

The Shapiro-Wilk W and Levene tests were subjected to verify normality and homogeneity of variance before further analysis was undertaken, respectively. One-way analysis of variance (ANOVA) was performed for the analysis of the data of growth performance, molting frequency and carapace coloration. Differences between the experimental groups were ranked Tukey's multiple range test. All means were presented with standard errors (±SE). For statistical assessment of the study data, a statistical software (Statgraphics Centurion XVI, Statpoint Technologies Inc., The Plains, VA) was used (Zar 1999). Differences were considered significant at the 95% confidence interval.

Results

Growth performance parameters of red swamp crayfish fed with different commercial feeds in two different size groups are given in Tables 2 and 3. The final mean weight (FMW) of the S1CG was higher than the S1BF ($P<0.05$). The final mean total length (FMTL) of the S1CG was the highest among the S1 experimental groups ($P<0.05$). There were no significant differences in final mean carapace length (FMCL), feed conversion ratio (FCR) and specific growth rate (SGR) of S1 groups ($P>0.05$). Although there was no statistical difference in the FMWs of S2 groups, the S2CG was higher than the other groups. The FMTL and FMCL of S2BF was the lowest ($P<0.05$). There was no significant difference in FCR and SGR of S2 groups ($P>0.05$). Feed intakes (FI) of the experimental groups were statistically increased as BF, CF and CG for two different sizes, respectively ($P<0.05$). No significant differences were recorded in the survival rates (SR) of red swamp crayfish in two different size classes ($P>0.05$).

Table 2. Growth performance of Size 1 (S1) red swamp crayfish fed with different commercial ornamental finfish and crustacean feeds for 12 weeks

	S1BF	S1CF	S1CG
Initial mean weight (g)	1.52±0.02	1.53±0.01	1.52±0.03
Final mean weight (g)	3.69±0.08^{a}	3.77±0.12^{ab}	4.16±0.10^{b}
Initial mean total length (cm)	3.98±0.10	4.03±0.11	4.00±0.10
Final mean total length (cm)	5.62±0.10^{a}	5.59±0.10^{a}	5.87±0.11^{b}
Initial mean carapace length (cm)	2.02±0.03	1.99±0.04	2.03±0.01
Final mean carapace length (cm)	2.76±0.05	2.81±0.04	2.90±0.08
Feed intake (g)	2.63±0.02^{a}	2.83±0.02^{b}	3.15±0.05^{c}
Specific growth rate (%/day)	1.06±0.04	1.07±0.04	1.20±0.03
Feed conversion ratio	1.21±0.04	1.20±0.07	1.14±0.06

Different letters in the same line indicate statistically significant differences ($P<0.05$) among the groups

Table 3. Growth performance of Size 2 (S2) red swamp crayfish fed with different commercial ornamental finfish and crustacean feeds for 12 weeks

	S2BF	S2CF	S2CG
Initial mean weight (g)	3.53±0.02	3.51±0.01	3.50±0.02
Final mean weight (g)	8.77±0.14	8.98±0.22	9.11±0.05
Initial mean total length (cm)	5.10±0.05	5.15±0.05	5.12±0.04
Final mean total length (cm)	6.29±0.06[a]	6.51±0.07[b]	6.63±0.12[b]
Initial mean carapace length (cm)	2.49±0.07	2.54±0.03	2.51±0.02
Final mean carapace length (cm)	3.06±0.04[a]	3.21±0.05[b]	3.29±0.09[b]
Feed intake (g)	5.68±0.06[a]	6.28±0.07[b]	7.18±0.05[c]
Specific growth rate (%/day)	1.08±0.02	1.12±0.03	1.14±0.01
Feed conversion ratio	0.96±0.03	1.05±0.02	1.03±0.04

Different letters in the same line indicate statistically significant differences (P<0.05) among the groups

Survival, cheliped injury and cannibalism rates of two different sizes of red swamp crayfish fed with commercial diets are shown in Figures 1, 2 and 3, respectively. The SRs of the S1 groups were 59.38, 75 and 62.63% for the BF, CF and CG, respectively and 84.38% for the BF and CF and 93.75% for the CG in the S2 groups (P>0.05 among their size classes). The CIRs of the S1BF, S1CF, S1CG, S2BF, S2CF and S2CG were found as 46.88, 40.63, 34.38, 28.13, 25 and 18%, respectively (P>0.05 among their size classes). The CR of S1BF was 21.88%, S1CF and S2CF were 15.63%, S1CG and S2BF were 12.5%, and S2CG was 6.25% (P>0.05 among their size classes).

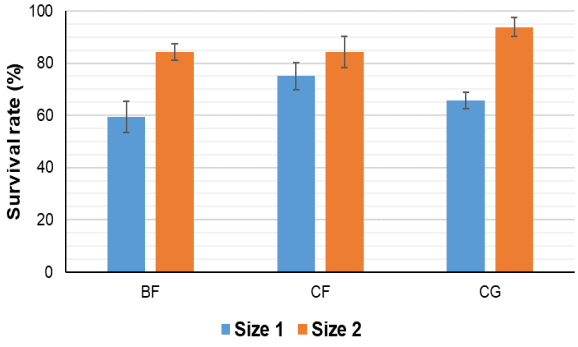

Figure 1. Survival rates at the end of the trial of two different size classes of red swamp crayfish fed with different commercial ornamental finfish and crustacean feeds. Error bars (±SE) of each experimental group were shown in the column chart.

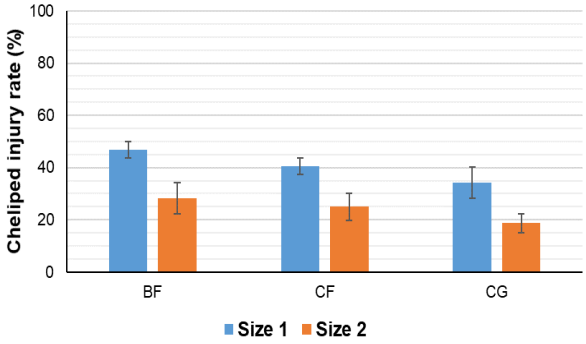

Figure 2. Cheliped injury rates at the end of the trial of two different size classes of red swamp crayfish fed with different commercial ornamental finfish and crustacean feeds. Error bars (±SE) of each experimental group were shown in the column chart.

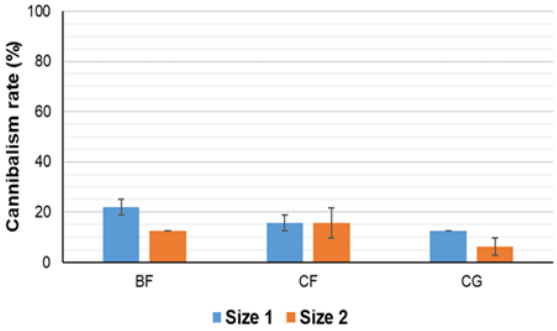

Figure 3. Cannibalism rates at the end of the trial of two different size classes of red swamp crayfish fed with different commercial ornamental finfish and crustacean feeds. Error bars (±SE) of each experimental group were shown in the column chart.

Molting numbers and parameters of red swamp crayfish fed with different ornamental fish and crustacean feeds in two different size classes are detailed in Tables 4 and 5. The highest molting numbers were recorded at two and three times in S1 (20 specimens for each) and one time in S2 (60 specimens), irrespective of experimental groups. The mean molting frequency (MMF) and mean number of molting (MNM) of the S1CG were statistically higher than the S1CF ($P<0.05$). The MMF and MNM of the S2CG were higher than the S2BF ($P<0.05$). The IM of the S1CF and S2BF were the highest among their size classes ($P<0.05$). While the WIM and LIM increased in the order of BF, CF, CG in both groups, a statistical difference was found in the WIM of only the S1 groups ($P<0.05$).

Table 4. Molting parameters of Size 1 (S1) red swamp crayfish fed with different commercial ornamental finfish and crustacean feeds for 12 weeks

		S1BF	S1CF	S1CG
n		19	24	21
Number of molting	1	1	5	0
	2	6	11	3
	3	6	6	8
	4	4	2	6
	5	2	0	4
Mean molting frequency		3.00±0.26^{ab}	2.23±0.13^{a}	3.52±0.29^{b}
Mean number of molting		14.25±2.02^{ab}	13.25±0.48^{a}	18.50±1.85^{b}
Intermolt period (days)		28.69±2.54^{a}	38.06±2.40^{b}	24.40±2.10^{a}
Weight increment at molt (%)		48.56±1.43^{a}	51.40±1.11^{ab}	54.69±1.46^{b}
Length increment at molt (%)		22.02±1.63	23.47±1.48	24.69±1.42

Different letters in the same line indicate statistically significant differences ($P<0.05$) among the groups

Table 5. Molting parameters of Size 2 (S2) red swamp crayfish fed with different commercial ornamental finfish and crustacean feeds for 12 weeks

		S1BF	S1CF	S1CG
n		27	27	30
Number of molting	1	23	19	18
	2	3	5	6
	3	1	3	5
	4	0	0	1
Mean molting frequency		1.18±0.09^{a}	1.41±0.07^{ab}	1.65±0.19^{b}
Mean number of molting		8.00±0.71^{a}	9.50±0.65^{ab}	12.25±1.11^{b}
Intermolt period (days)		72.08±5.17^{b}	59.91±3.16^{a}	53.40±7.37^{a}
Weight increment at molt (%)		82.85±1.41	84.80±1.57	86.12±1.06^{b}
Length increment at molt (%)		32.70±1.32	34.96±1.71	36.95±2.35

Different letters in the same line indicate statistically significant differences ($P<0.05$) among the groups

The initial and final carapace coloration parameters of the red swamp crayfish used in the experiment are listed in Tables 6 and 7. There was no statistical difference in the lightness (L^*) of S1 groups ($P>0.05$). The final redness (a^*) and final yellowness (b^*) values of the S1BF were the lowest ($P<0.05$). S2CG has the highest lightness (L^*) value ($P<0.05$). The final redness (a^*) and final yellowness (b^*) values of the S2BF were the lowest ($P<0.05$). However, significant increases were noted through the initial and final values of the lightness (L^*) of the S1BF, S1CF, S1CG, S2CF and S2CG, the redness (a^*) and yellowness (b^*) of the S1CF, S1CG, S2CF and S2CG groups ($P<0.05$). Also, statistical decreases were recorded in the initial and final values of the lightness (L^*) and yellowness (b^*) of the S1BF and the redness (a^*) of the S1BF and S2BF groups ($P<0.05$).

Table 6. Carapace coloration of Size 1 (S1) red swamp crayfish fed with different commercial ornamental finfish and crustacean feeds for 12 weeks

	S1BF	S1CF	S1CG
Initial lightness (*L)**	39.62±0.39^{A}	39.75±0.49^{A}	39.02±0.39^{A}
Final lightness (*L)**	41.75±0.43^{B}	41.35±0.52^{B}	41.74±0.72^{B}
Initial redness (*a)**	25.02±0.83^{B}	24.55±0.97^{A}	24.87±0.87^{A}
Final redness (*a)**	17.72±0.81$^{a, A}$	25.72±0.53$^{b, B}$	26.06±0.51$^{b, B}$
Initial yellowness (*b)**	23.63±0.63^{B}	23.83±0.54^{A}	23.49±0.56^{A}
Final yellowness (*b)**	20.46±0.92$^{a, A}$	26.02±0.61$^{b, B}$	25.51±0.47$^{b, B}$

Different small letters in the same line and capital letters in the same column indicate statistically significant differences (P<0.05) among the groups and between the initial and final parameters in each, respectively

Table 7. Carapace coloration of Size 2 (S2) red swamp crayfish fed with different commercial ornamental finfish and crustacean feeds for 12 weeks

	S2BF	S2CF	S2CG
Initial lightness (L*)	40.12±0.36	40.11±0.48A	40.17±0.26A
Final lightness (L*)	40.58±0.33a	43.70±0.66b, B	45.01±0.56c, B
Initial redness (a*)	26.79±0.75B	27.74±1.09A	28.76±1.05A
Final redness (a*)	20.69±0.81a, A	30.87±1.70b, B	33.64±1.23b, B
Initial yellowness (b*)	22.51±0.30	23.29±0.50A	22.21±0.38A
Final yellowness (b*)	21.97±0.27a	24.77±0.49b, B	25.07±0.64b, B

Different small letters in the same line and capital letters in the same column indicate statistically significant differences (P<0.05) among the groups and between the initial and final parameters in each, respective

Discussion

The effects of different commercial aquarium feeds (bottom fish food, cichlid food and crustacean granules) on growth performance, molting frequency and carapace coloration of 4 and 5 cm red swamp crayfish (*P. clarkii*) were investigated in the present study. The growth performance regarding final mean weight (FMW) and final mean total length (FMTL) of crayfish have been enhanced with crustacean granules, while bottom food was the lowest. Although the protein content of bottom food was higher (50%) than cichlid (40.34%) and crustacean (44%) foods in our study, low protein feeds (cichlid and crustacea) attained higher growth rates. Similar results were reported in the previous study conducted on Mexican crayfish (*P. llamasi*) by Rodríguez-Serna et al. (2010). The authors formed six experimental feeding groups with different kinds of farm animal feeds (rabbit, turkey, pig, tilapia, shrimp and trout) and they stated that high protein contents were in the trout and shrimp feeds with 43.2 and 38%, respectively. The highest FMW, weight gain (WG) and specific growth rate (SGR) were recorded in the group fed with shrimp feed in this previous study and the authors explained that this situation was possible due to lower stability in the water. Obviously, there are main aspects to take into when formulating the species-specific feed for ornamental crayfish in order to balance between dietary requirements and stability. Also, Goldblatt et al. (1980) identified that this is a nutrient loss problem caused by leaching in crustaceans according to their slow-eating ability. Furthermore, growth of crayfish is also significantly affected by the culture conditions, type of food and dietary ingredients (Jones et al. 2000). However, Kaldre et al. (2015) declared that the weight and length gain of marbled crayfish (*P. fallax* f. *virginalis*) was higher among crayfish fed with astaxanthin-rich discus feed, whilst Harpaz et al. (1998) stated that the growth performance of the Australian red claw crayfish (*C. quadricarinatus*) was not influenced by dietary carotenoids. These results clearly show that there may be important ingredients in the structure of the formulated feed, apart from the protein ratio. For instance, Erol et al. (2017) fed the 2.5 cm narrow-clawed crayfish (*Astacus leptodactylus*) with a 55% protein ratio of trout feed and the authors recorded the highest total and carapace lengths in this group. But we found the highest FMTL and FMCL in the red swamp crayfish fed with crustacean feed among the S1 groups. This, of course, is related to the effectiveness of the species-specific feed. In addition, the shape and structure of feed are the significant criteria for accessibility in the bottom of the water in

crustaceans. Karadal and Türkmen (2012) reported that Australian red claw crayfish (*C. quadricarinatus*) are easier to reach because granule feed sinks faster than flake and stick forms, and this situation directly affects their growth performance. Consequently, all this demonstrates the importance of considering all the needs of the animal when formulating specific feeds.

Survival is vital in crayfish cultivation because of the cannibalism problem (Taugbøl and Skurdal 1992). Rodríguez-Serna et al. (2010) reported 100% survival rate in 1.16 g Mexican crayfish (*P. llamasi*) fed with six different farm animal feeds. Also, Kaldre et al. (2015) stated 89% and 78% survival for 1.15 and 2.45 g marbled crayfish (*P. fallax* f. *virginalis*) fed with carp and discus feeds, respectively. In our study, the Size 1 group, similar to previous studies, has a lower survival rate (ranging from 59.38-75%) than these mentioned results. The reason for this is thought to be related to species behavior. The survival rate in cultured animals has a complex mechanism that is affected by many variables. Previous studies have revealed that growth, survival and cannibalism of crayfish are impressed by various factors, such as water parameters (temperature, dissolved oxygen, etc.), photoperiod, feeding regime, shelter presence, density, competition and species and size differentiations (Mazlum and Eversole 2005; Farhadi et al. 2014; Yu et al. 2020). For instance, Blank and Figler (1996) demonstrated the interspecific dominance of red swamp crayfish (*P. clarkii*) over white river crayfish (*Procambarus zonangulus*) in shelter competition. It can be said that this situation, which is based on competition between species belonging to the same genus, will directly affect the survival rate of white river crayfish (*P. zonangulus*). Based on this example, it is very important to evaluate factors such as food competition and feeding regime in terms of differences between species. However, in many crustaceans, limited shelter, low availability of food, unstable feeding, high stocking density, species' behaviors not only cause aggressiveness but also increase cheliped injury or loss, and even cannibalism in culture conditions (Elgar and Crespi 1992; Taugbøl and Skurdal 1992; Figiel and Miller 1995; Savolainen et al. 2004; Marshall et al. 2005; Yu et al. 2020). In this study, although there was no statistical difference in the cheliped injury and cannibalism rates, the lowest rates were recorded in the groups fed with crustacean feed in both size classes. As mentioned by Farhadi et al. (2014), food presence and proper feeding are significant for maintaining cannibalism in crayfish species. Furthermore, cannibalism in crayfish is closely related to molting, which is their vulnerable period (Taugbøl and Skurdal 1992; Farhadi et al. 2014) and it also has been detected during inter- and postmolt stages (Elgar and Crespi 1992). Therefore, as the molting period increases, the rate of cannibalism is expected to decrease, as in the results between two different size classes in this study.

In crustaceans, molting is a part of the growth and development (Ghanawi and Saoud 2012) and is an energy-intensive process (Raviv et al. 2008). Therefore, similar results with growth performance were recorded on the molting frequency of Size 2 red swamp crayfish fed with different commercial feeds used in the study. However, molting frequency of Size 1 crayfish fed with bottom food was found to be higher, although statistically similar to those fed with cichlid feed. This can be explained as an increase in the molting frequency due to the fact that it is more frequent in the early periods (Barki et al. 1997). Fockedey (2005) declared the intermolt period (IM) may increase or decrease by the quality of food. In our study, lower IMs were noted in CG groups in both size classes. However, Paglianti and Gherardi (2004) reported no significant differences in weight increment at molt (WIM) and length increment at molt (LIM) in red swamp crayfish (*P. clarkii*) fed with different plant and animal derived natural food sources. Although our findings are similar to the results of the previous study, except for the WIM in the S1 groups, it is much higher than the mentioned study. Previous findings provided evidence for the positive effects of species-specific formulated feeds on molting parameters, as in this study.

Coloration is a key feature in the selectivity of an aquarium species in the industry and indirectly displays the value. However, diet is the one of major factors that directly affected to the coloration in finfish and shellfish. In crustaceans, carapace coloration primarily depends on two main characteristics: proper carotenoid type (predominantly astaxanthin) for the species and carotenoid level in the formulated diet (Négre-Sadargues et al. 2000; Kaldre et al. 2015; Wade et al. 2017). In addition, Tanaka (1978) has recommended that decapods should be fed carotenoid-included diets for obstructing the color fading. Commercial company of the crustacean feed used in this study stated that the granules contained nutritious carotenoids offer a varied and balanced feeding. Although the commercial company has not presented the exact ration of the feed, it is clearly seen that it is quite effective on the carapace coloration according to the results obtained in the study. These results point those two sizes of red swamp crayfish could successfully utilize the dietary carotenoids from crustacean feed to enhance the lightness and redness of carapace. Lightness (L^*), redness (a^*) and yellowness (b^*) identified as CIELAB color space are significant parameters to assess the coloration in

crustaceans (Long et al. 2017). In this study, these parameters indicate the same statistical levels between cichlid and crustacean feeds, but higher values in crustacean feed. As it is known that there are various carotenoids in cichlid feed, this can be explained as the fact that the crustacean feed has a more balanced ration. However, these parameters also showed that the bottom food led to the carapace color even paler than the initial color rather than improving it. Although the bottom food contains natural carotenoid sources, including krill, gammarus and squid meals, and *Schizochytrium* sp., *Chlorella* sp. and *Spirulina* sp. algae, this situation is thought to be due to nutrient leaching and that the unsuitable feed stability for crustaceans.

In conclusion, red swamp crayfish showed a great enhancement during the feeding with commercially formulated crustacean feed and positive results were revealed when regarding to meet the demand of both aquaculture and aquarium industries. But it should be noted that it is the most expensive feed used in the study. Since feed is the biggest expense in ornamental fish sector, it is very important to maintain profitability in high-capacity productions. Therefore, the use of the crustacean feed in the final product stage is recommended in terms of accelerating growth performance, improving body coloration and balancing profitability.

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Distribution and Some Diagnostic Properties of *Capoeta damascina* (Valenciennes, 1842) in Streams of the Ceyhan and Seyhan River Basins, Türkiye

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ABSTRACT

This study determined the distribution and some diagnostic characteristics of *Capoeta damascina*, which belongs to the Cyprinidae family, in the Ceyhan and Seyhan River basins. *C. damascina* individuals were detected in 21 of 25 Ceyhan River Basin stations and 12 out of 15 in the Seyhan River basin stations. *C. damascina* show a wide distribution in the Ceyhan and Seyhan River basins. It has been determined that *C. damascina* lives at altitudes between 125 m (Hemite Stream) and 1620 m (Söğütlü Stream) in the Ceyhan River basins, and in streams at altitudes ranging from 165 m (Çakıt stream) to 1758 m (Sarız Stream) in the Seyhan River basins. The average total length of *C. damascina* individuals (n: 218) caught in the Ceyhan River Basin is 14.72 cm, and their average weight is 40.12 g. In the Seyhan River basin, the average total length of *C. damascina* individuals (n: 74) was found to be 17.31 cm, and their average weight was 72.24 g. The mean number of line lateral scales was 71, the number of gill rakers was 23, and the number of pharyngeal teeth was 4:3:2-2:3:4.

Keywords: *Capoeta damascina*, distribution, diagnostic, Ceyhan River, Seyhan River

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Ceyhan ve Seyhan Nehir Havzalarındaki Akarsularda *Capoeta damascina* (Valenciennes, 1842)'nın Dağılımı ve Bazı Diagnostik Özellikleri, Türkiye

Öz: Bu çalışmada, Cyprinidae familyasına ait *Capoeta damascina*'nın Ceyhan ve Seyhan nehir havzalarındaki dağılımı ve bazı diagnostik özellikleri belirlenmiştir. Ceyhan Nehir havzasında 25 istasyondan 21'inde, Seyhan Nehir havzasında ise 15 istasyondan 12'sinde *C. damascina*'nın varlığı tespit edilmiştir. *C. damascina*, Ceyhan ve Seyhan Nehir havzalarında çok geniş bir dağılım göstermektedir. Ceyhan Nehir havzasında 125 m (Hemite Çayı) ve 1620 m (Söğütlü Çayı) arasındaki rakımlarda, Seyhan Nehir havzasında ise 165 m (Çakıt Suyu) ile 1758 m (Sarız Suyu) arasında değişen rakımlardaki habitatlarda yaşadığı belirlenmiştir. Ceyhan Nehir havzasında yakalanan *C. damascina* bireylerinin (n: 218) ortalama total boyları 14,72 cm, ortalama ağırlıkları ise 40,12 g'dır. Seyhan Nehir havzasında bulunan *C. damascina* bireylerinin (n: 74) ise ortalama total boyları 17,31 cm, ortalama ağırlıkları ise 72,24 g olarak tespit edilmiştir. Ortalama line lateral pul sayısı 71, solungaç diken sayısı 23 olup farinks diş sayısı ise 4:3:2-2:3:4 şeklindedir.

Anahtar kelimeler: *Capoeta damascina*, dağılım, diagnostik, Ceyhan Nehri, Seyhan Nehri

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Introduction

The genus *Capoeta* has a wide geographical distribution in northern India and China, Afghanistan, Turkestan, the Aral Sea, the Middle East, and Anatolia (Türkmen et al. 2002; Alp et al. 2005; Kaya et al. 2019). This genus comprises 36 nominal species, with 18 species reported from Turkey (Çiçek et al. 2022). There are many studies on the *Capoeta* species (Alp et al. 2005; Alwan 2010; Alwan et al. 2016a; Alwan et al. 2016b; Bektaş et al. 2017; Turan et al. 2022). Turan et al. (2022), revaluated the species belonging to the genus

Capoeta in Anatolia based on genetic data. The *Capoeta damascina* species group occurs in the entire Levant, Mesopotamia, the Orontes, Iran, and the southern and eastern parts of Turkey (Zareian and Esmaili 2017). It is also stated that *C. damascina* is in the Mesopotamian group (Bektaş et al. 2019). *C. damascina* is commonly found in the Orontes, Ceyhan, and Seyhan river basins (Geldiay and Balık 2009; Kaya et al. 2019; Bayçelebi 2020). *C. damascina* individuals in the Ceyhan and Seyhan River basins were reported as *Capoeta capoeta angorae* in studies conducted by Alp et al. (2005). Later, taxonomic and molecular studies by Alp et al. (2020), Alwan (2010), Alwan et al. (2016b) and Turan et al. (2022) demonstrated that the species *C. angorae* found in the Ceyhan and Seyhan River basins is strictly synonymous with *C. damascina*.

C. damascina is widely found in the Ceyhan and Seyhan river basins and has economic importance. In the Ceyhan and Seyhan basins, Geldiay and Balık (2009); Bayçelebi (2020), Kaya (2019) and Turan et al. (2022) have records of *C. damascina* from various localities. These studies were generally reported a few locations from the basins. Today, there are many reservoirs and hydroelectric power plants (HPP) in the Ceyhan and Seyhan River system. For example, there are 9 reservoirs and 50 small hydroelectric power plants (HPP) in the Ceyhan River system (Alp et al. 2020). Most of these hydroelectric power plants (HPP) do not have fish passes (Alp et al. 2020). It is inevitable that these adverse conditions will cause habitat loss in the reproductive migration (Alp et al. 2020) *C. damascina* populations. There is no study in the literature that determined the extensive distribution areas of *C. damascina* in the Ceyhan and Seyhan basins. In this study, it is aimed to set a step for future studies by revealing the regional distribution and some diagnostic features of *C. damascina* in the Ceyhan and Seyhan basins.

Materials and Methods

This study was carried out in streams of the Ceyhan and Seyhan River basins between April 2014 and May 2016, the locations of the streams and reservoir are shown in Figure 1.

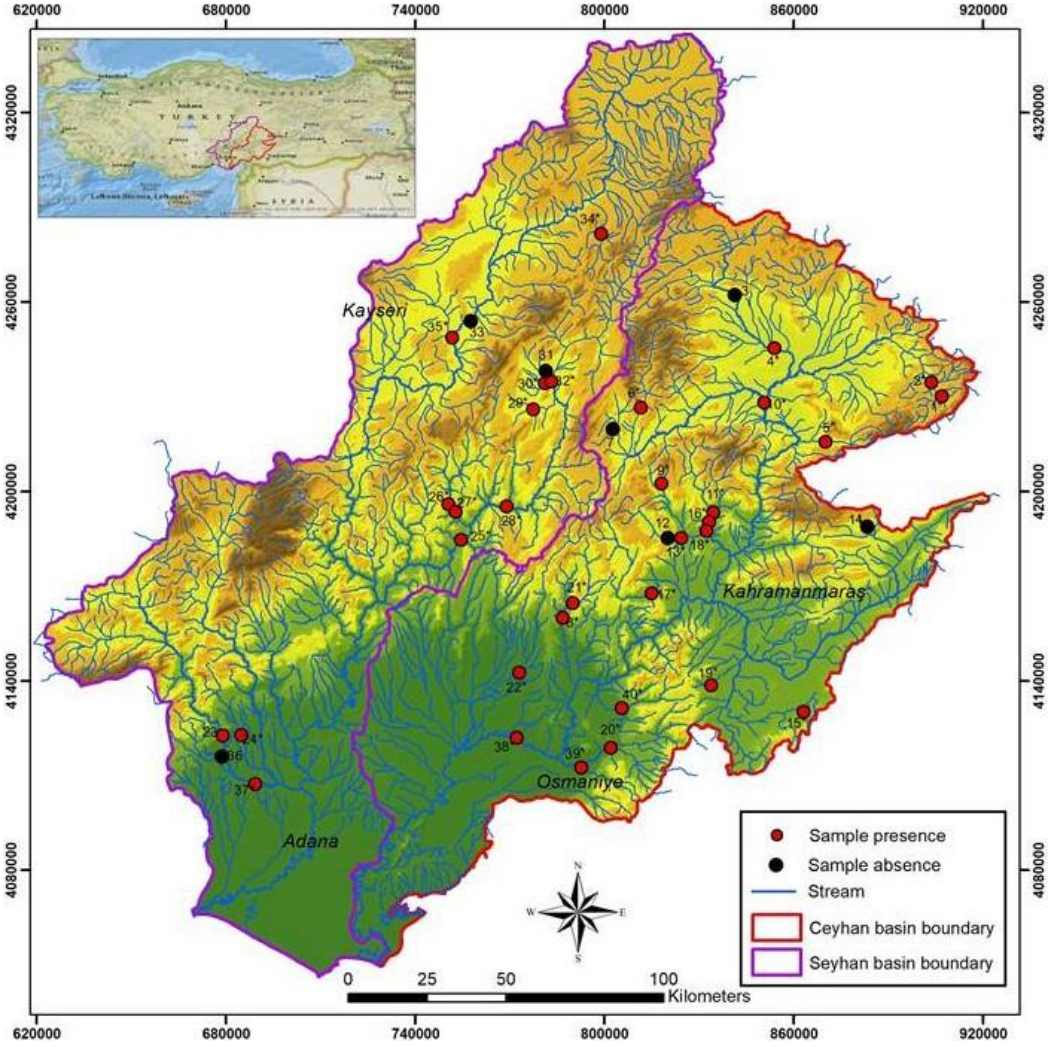

Figure 1. Distribution of *C. damascina* in streams in the Ceyhan and Seyhan basins

The Ceyhan River is one of the important rivers of Türkiye, and it arises from the mountains at an altitude of approximately 2200 m in the Göksun, Elbistan, and Afşin districts of Kahramanmaraş and is fed by springs and streams. The Ceyhan River forms a wide delta in Çukurova and empties into the Iskenderun Bay. The most important streams of the Ceyhan River are Söğütlü, Nergele, Hurman, Kömür, Törbüzek, Fırnız, Aksu, Savrun, Yarpuz, Akçasu and Hemite.

The Seyhan Basin is located in the south of Türkiye and the eastern Mediterranean district. This basin is to be nourished by two main streams called Zamantı and Göksu. North of Gövdeli Mountain (2719 m), where the west part of the East Taurus Mountains is to be named Uzunyayla (Sunkar 2008). This basin has been surrounded by mountainous areas with over 2000 m average altitude from the north, south, and west. Streams in the upper basin of the Zamantı Stream have a meander structure. Especially, stream where Uzunyayla district of Zamantı stream is covered with vascular plant flora. Besides, an important branch of the Zamantı Stream is Karagöz. In addition, Zamantı Stream joins Göksu near Feke. Göksu is one of the most heavily flowing branches of the Seyhan River. It takes its source from Tahtalı Mountains around Tufanbeyli. It merges with Sarız Stream, passes through narrow and deep valleys around Feke and merges with Kapuzbaşı waterfalls to mix with the Seyhan River. In addition to the Seyhan River, it is also fed by streams such as Çakıt, Üçürge, Feke, Himmetli, İncedere and Kalasuyu.

Fish samples were caught by using an electroshocker, separating and tension nets in rivers, and nets with 18x18 mm, 22x22 mm, and 32x32 mm mesh size in lakes and reservoirs. The GPS coordinates of the sampling stations were recorded (Table 1).

Table 1. Sampling stations and their GPS coordinates

Stations	Streams and reservoirs	Altitude	Longitude	Latitude
1*	*Söğütlü Stream	1620	37.63361	38.11694
2*	*Söğütlü Stream	1474	37.60028	38.15028
3	Hurman Stream 1	1258	36.90028	38.43361
4*	*Hurman Stream 2	1145	37.04575	38.28699
5*	*Nergele Stream	1213	37.21694	38.00028
6*	*Kömür Stream,	1417	36.55028	38.13361
7	Terbüzek Stream	1390	36.45028	38.06694
8*	*Keşiş Stream	401	36.24499	37.54226
9*	*Tekir Stream	1125	36.61726	37.91461
10*	*Ceyhan River	1233	37.00028	38.13361
11*	*Tekir Stream	750	36.80028	37.80028
12	Fırnız Stream	920	36.63251	37.75838
13*	*Fırnız Stream	690	36.68361	37.75028
14	Aksu Stream	1125	37.35028	37.76694
15*	*Aksu Stream	464	36.90028	37.16694
16*	*Zeytin Stream	698	36.78361	37.80028
17*	*Körsulu Stream	560	36.56736	37.60261
18*	*Menzelet Reservoir	588	36.77332	37.77489
19*	*İmalı Deresi	649	36.76694	37.33361
20*	*Akçasu Stream	264	36.40028	37.16694
21*	*Karaçay Stream	127	36.28361	37.58361
22*	*Savrun Stream	295	36.88361	37.58361
23	*Çakıt Stream	165	35.01694	37.23361
24*	*Üçürge Stream	195	35.08361	37.23361
25*	*Feke Stream 1	518	35.90028	37.76694
26*	*Feke Stream 2	719	35.83361	37.86694
27*	*Feke Stream 3	593	35.86694	37.85028
28*	*Himmetli Creek	729	36.05731	37.86524

Table 1. Continue

29*	*Güzelim Stream	1376	36.16694	38.13361
30*	*İncesu Stream	1367	36.21694	38.20028
31	Kalasuyu Stream	1350	36.21694	38.23361
32*	*Göksu Stream	1355	36.23361	38.21694
33	Zamantı Stream 1	1768	36.66694	38.63361
34*	*Sarız Stream	1758	36.43361	38.63361
35*	*Zamantı Stream 2	1350	35.88111	38.35249
36	Karaisalı Stream	148	35.00028	37.18361
37*	*Çakıt Stream	83	35.13361	37.08361
38*	*Hemite Stream	125	36.06694	37.18361
39*	*Yarpuz Stream	126	36.30028	37.11694
40*	*Sabun Creek	223	36.45028	37.26694

(*Stations where *C. damascina* is distributed)

The sampled fish were brought to the Hydrobiology Research Laboratory in 5-liter plastic containers in a 4 % formaldehyde solution. Then, the height measurements of the fish were made with a digital calliper with a precision of 0.01 mm, and the weight measurements were made with a digital scale with a precision of 0.01 g. The stations where fish sampling is done and the localities where *C. damascina* individuals are caught are shown on the map in Figure 1.

The body of *C. damascina* is spherical, spindle-shaped, and covered with scales. The scales are not large. The head is broad, the nose is blunt, and the mouth is ventral. They have short double whiskers. The lobes of the caudal fin are pointed and the fork is deep. The back of the body is gray-brown, and the abdomen and flanks are yellowish white (Figure 2). It is therefore referred to as "Yellow Fish" in the area and is significant for both reactionary and commercial fishing in streams that are connected to the river system. The *C. damascina* species spawns in the spring, when the males' heads are covered in tiny white reproductive tubercules (Alp et al. 2020).

Figure 2. *C. damascina* specimen from the Fırnız stream

Results and Discussion

C. damascina individuals were found in 12 out of 15 different stations in the Seyhan River basin and 21 out of 25 stations in the Ceyhan River basin. Individuals of *C. damascina* have been found in the Ceyhan River basin, Hurman, Nergele, Kömür, Söğütlü, Fırnız, Körsulu, Tekir, Zeytin, Aksu, Keşiş, İmalı, Karasu, Sabun, Savrun, Yarpuz, Hamus, Hemite and Menzelet Reservoir (Figure 1, Table 1). In the Seyhan River basin, *C. damascina* was found from Çakıt, Üçürge, Feke, İncesu, Sarız, Güzelim, and Zamantı Stream (Figure 1; Table 1). *C. damascina* individuals were not observed in the 3rd, 7th, 14th, 31st, and 33rd stations.

The average total length of *C. damascina* individuals (n: 218) founded in the Ceyhan River basin was 14.72 cm, and their average weight was 40.12 g. The smallest identified individual was 8.27 cm and 6.4 g; the largest individual had a total length of 23.4 cm and 139.14 g. The average total length of *C. damascina* individuals (n: 74) caught in the sampling localities in the Seyhan River basin was 17.31 cm, and their average weight was 72.24 g. The smallest individual

was 9.68 cm and 10.7 g, and the largest individual was 27.67 cm and 225.8 g. Average length and weight distributions of *C. damascina* populations founded in the rivers belonging to the Ceyhan and Seyhan River basins are given in Table 2.

Table 2. The total lengths (cm) and total weights (g) of *C. damascina* from the stations of Seyhan and Ceyhan River basins (n: Number of fish, TL: Total Length (cm), W: Weight (g), SD: Standard Deviation)

Seyhan River Basin							
Stations	**n**	**Mean TL (cm)**	**Min.-Max.**	**SD**	**Mean W(g)**	**Min.-Max.**	**SD**
Çakıt Stream	11	14.13	9.68-16.85	2.46	37.30	10.7-58.4	16.21
Zamantı Stream	14	21.24	16.36-27.08	3.45	119.67	49.92-207.9	56.72
İncesu Stream	6	12.37	10.83-13.33	0.93	22.28	18.9-28.6	4.18
Sarız Stream	8	19.52	16.38-21.46	1.64	89.82	57.7-113.5	19.89
Güzelim Stream	4	18.58	14.08-27.67	6.26	91.62	34.5-225.8	91.09
Feke Stream	11	20.06	14.08-27.63	4.05	130.73	34.5-225.8	59.70
Himmetli Stream	14	16.33	12.25-21.70	3.22	54.75	21.9-123.0	33.63
Göksu Stream	6	12.37	10.83-13.33	0.93	22.28	18.9-28.6	4.18
Ceyhan River Basin							
Hurman Stream	17	12.73	10.00-15.90	1.30	26.94	13.80-43.60	8.31
Nergele Stream	13	12.01	10.30-13.10	0.77	18.30	11.20-24.60	3.61
Kömür Stream	13	16.10	12.60-19.00	2.09	49.20	27.87-82.47	17.06
Söğütlü Stream	8	11.84	10.33-14.08	1.06	31.19	24.62-47.59	6.94
Fırnız Stream	18	18.49	12.82-22.50	2.86	64.65	25.68-101.95	22.68
Körsulu Stream	15	16.60	14.55-18.89	11.18	52.24	32.97-68.06	9.40
Tekir Stream	44	15.25	10.00-23.4	3.36	47.36	12.07-139.14	30.73
Zeytin Stream	14	14.86	8.27-21.07	3.92	37.05	20.05-70.25	19.36
Aksu Stream	16	14.61	9.5-20.80	2.85	36.00	10.80-90.80	20.93
Keşiş Stream	3	14.63	14.10-15.10	0.50	49.04	40.76-57.85	8.55
İmalı Stream	4	16.51	13.78-21.69	3.57	86.25	56.00-126.00	31.41
Menzelet Dam	14	19.76	12.00-29.22	5.35	11.82	10.04-13.90	1.05
Karaçay Stream	5	12.31	10.59-14.84	1.95	26.62	16.22-42.16	11.68
Sabun Stream	4	12.61	11.22-13.72	1.03	30.88	21.62-37.41	6.67
Savrun Stream	6	13.48	10.43-19.12	3.20	46.52	19.83-110.90	33.81
Yarpuz Stream	5	12.68	10.45-14.49	1.47	33.99	12.87-46.71	12.91
Hamus Stream	10	11.58	8.60-14.50	1.69	18.38	6.40-31.50	7.39
Hemite Stream	9	13.47	12.90-15.10	0.73	34.11	28.00-46.40	6.23

The meristic features of *C. damascina* in the Ceyhan and Seyhan basins are given in Table 3. Accordingly, the number of scales on the lateral line of *C. damascina* varied between 65 and 78. The pharyngeal teeth are in a 4:3:2-2:3:4 order (Table 3). The number of gill rakers is between 22-24. Kaya et al. (2019) state that the number of scales on the line lateral of *C. damascina* varies between 66 and 76, and the number of gill rakers is between 20 and 25, which is similar to our research findings in terms of meristic properties.

Table 3. Meristic characteristics of *C. damascina* samples in Ceyhan and Seyhan River basins (n: number of fish, X: mean value; Min: minimum, Max: maximum; SD: standard deviation)

		n	**X**	**Min.**	**Max.**	**SD**
Dorsal fin	Spine	35	2	2	3	0.29
	Soft ray	35	9	7	10	0.56
Anal fin	Spine	30	2	1	2	0.25
	Soft ray	30	6	5	8	0.73
Pectoral fin	Spine	28	1	1	2	0.48
	Soft ray	28	13	11	16	1.19
Ventral fin	Spine	28	1	1	1	
	Soft ray	28	8	7	9	0.58
Line lateral scale		55	71	65	78	3.91
Line lateral dorsal		14	13	12	15	1.03
Line lateral ventral		14	11	8	13	1.49
Gill rakers		14	23	22	24	0.73

According to literature, one of the most common fish species in the Ceyhan River basin is *C. damascina* (Alp et al. 2020). There are currently 50 small hydroelectric power plants (HPP) in the Ceyhan River basin, in addition to large reservoirs like Berke Reservoir, Aslantaş Reservoir, Sır Reservoir, Kılavuzlu Reservoir, Menzelet Reservoir, Kartalkaya Reservoir, and Adatepe Reservoir (Alp et al. 2020). Large reservoirs like Yedigöze, Çatalan and Seyhan Reservoir, as well as smaller hydroelectric power plants, can be found in the Seyhan River basin. According to Alp et al. (2020), *C. damascina* populations migrated toward the upper basins of the streams during the spawning season.

The spawning habitats and migration of *C. damascina* will inevitably be impacted as a result of this situation. Domestic, recreation, and trout farming activities also contribute to the pollution of the Aksu, Fırnız, Törbüzek, Söğütlü and Hurman streams. Additionally, there are quarries for sand and gravel, irrigation ponds, etc. in the field of study. Additionally, there are a number of activities that harm fish populations, such as the fact that *C. damascina* populations will inevitably be negatively impacted by these unfavourable conditions.

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Oxidative Damages of Two Neonicotinoid Pesticides to *Arthrospira platensis* (Gomont)

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ABSTRACT

In this study, chlorophyll-*a* amount, OD 560 and antioxidant parameters (total SOD, APX, GR, MDA, H_2O_2 and Proline) were determined in order to understand the effects of Thiacloprid and Imidacloprid on *Arthrospira platensis* Gomont. Both Imidacloprid and Thiacloprid applications showed significant reductions in growth rate and chlorophyll-*a* content of *A. platensis* cultures with dose-dependent manner when the days and concentrations were compared each other. SOD activity significantly decreased in the Imidacloprid application while Thiacloprid caused a significant increase only at 75 µg mL^{-1} concentration. APX activity significantly increased in the Imidacloprid and Thiacloprid applications at 50 µg mL^{-1} and 35 µg mL^{-1} concentrations, respectively. Imidacloprid treatment increased GR activity at 20 and 30 µg mL^{-1} concentrations while GR activity increased at 15, 25 and 35 µg mL^{-1} Thiacloprid concentrations. The MDA content of *A. platensis* cultures did not change with Imidacloprid or Thiacloprid applications. The H_2O_2 content did not change at all different Imidacloprid concentrations. However, the H_2O_2 content decreased at 15 µg mL^{-1} and increased at 45 and 75 µg mL^{-1} Thiacloprid concentrations. Free proline content increased in the Imidacloprid and Thiacloprid applications at 100 µg mL^{-1} and 75 µg mL^{-1} concentrations, respectively. These neonicotinoid pesticides cause oxidative stress in *A. platensis* cells.

Keywords: *Arthrospira platensis*, oxidative stress, antioxidant, thiacloprid, imidacloprid

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Neonikotinoid Pestisitin *Arthrospira platensis*'e (Gomont) Oksidatif Zararları

Öz: Bu çalışmada Thiacloprid ve Imidacloprid'in *Arthrospira platensis* Gomont üzerindeki etkilerini anlamak için klorofil-*a* miktarı, OD 560 ve antioksidan parametreler (toplam SOD, APX, GR, MDA, H_2O_2 ve Prolin) belirlenmiştir. Hem İmidacloprid hem de Thiacloprid uygulamaları, *A. platensis* kültürlerinin büyüme hızında ve klorofil-*a* içeriğinde doza bağlı olarak günler ve konsantrasyonlar karşılaştırıldığında önemli azalmalar göstermiştir. Imidacloprid uygulamasında SOD aktivitesi önemli ölçüde azalırken, Thiacloprid sadece 75 µg mL^{-1} konsantrasyonunda önemli bir artışa neden olmuştur. APX aktivitesi, sırasıyla 50 µg mL^{-1} ve 35 µg mL^{-1} konsantrasyonlarında İmidacloprid ve Thiacloprid uygulamalarında önemli ölçüde artmıştır. İmidakloprid uygulaması GR aktivitesini 20 ve 30 µg mL^{-1} konsantrasyonlarında artırırken, GR aktivitesini 15, 25 ve 35 µg mL^{-1} Thiacloprid konsantrasyonlarında arttırmıştır. *A. platensis* kültürlerinin MDA içeriği, İmidacloprid veya Thiacloprid uygulamaları ile değişmemiştir. H_2O_2 içeriği herhangi bir imidakloprid konsantrasyonunda değişmemiştir. Ancak H_2O_2 içeriği 15 µg mL^{-1}'de azalmış, 45 ve 75 µg mL^{-1} Thiacloprid konsantrasyonlarında ise artmıştır. Serbest prolin içerikleri sırasıyla 100 µg mL^{-1} ve 75 µg mL^{-1} konsantrasyonlarında Imidacloprid ve Thiacloprid uygulamalarında artmıştır. Bu neonikotinoid pestisitler, *A. platensis* hücrelerinde oksidatif strese neden olmaktadır.

Anahtar kelimeler: *Arthrospira platensis*, oxidative stress, antioxidant, thiacloprid, imidacloprid

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Introduction

The usage of pesticides is still the most preferred method of agricultural struggle against diseases and pests despite having long-lasting destructive effects on environmet, non-target organisms, and humans (Karahan et al. 2018). Pesticides can be transported

by atmospheric precipitation, farmland, sewage in various centers, hazardous waste disposal waters, even over long distances by air (Tankiewicz et al. 2010). This situation is caused by the contamination of the water resources by pesticides (Malato et al. 2001). When pesticides enter the aquatic environment, they continuously reduce the quality of groundwater and surface water being essential drinking water resources for the majority of the world population (Tankiewicz et al. 2010). This critical problem has attracted the interest of scientists due to its considerable toxicity in physical and biochemical processes. The accumulation of this contamination and toxicity to organisms can lead to irreversible changes and hazards (Daneshvar et al. 2007; Tankiewicz et al. 2010)

Neonicotinoids are the most important class of synthetic insecticides produced in recent years, and these insecticides are nicotine derivatives (Bolboaca and Jaentschi 2005; Jeschke et al. 2011, Casida and Durkin 2013). These water-soluble compounds, the most widely used insecticides in the world, can be taken up by plants and consumed by non-target organisms (Morrissey et al. 2015; Wood and Goulson 2017). Aquatic organisms, such as terrestrial organisms, may be susceptible to these compounds, although they attract significantly less attention (Wood and Goulson, 2017). The neonicotinoid group insecticides are classified as N-nitroguanidines (like as imidacloprid) and N-cyanoaminides (like as thiacloprid) (Bolboaca and Jaentschi 2005).

Imidacloprid (1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine) is the first and most widely used neonicotinoid insecticide developed as a neurotoxin affecting the central nervous system of the insects (Akbaş et al. 2014). Imidacloprid is used for leaf treatment of plants such as grain, cotton, wheat, legumes, potatoes, some fruits, grass and vegetables, and it is also applied against harmful insects in soil, seeds, trees, and animals (BCPC 2004). It can interfere in the irrigation system (Franco et al. 2009). Its toxicity is high due to some features such as the ability of transport from agricultural areas to surface water, widespread use, and persistence in water (US EPA 2008; Starner and Goh 2012).

Thiacloprid [3-(6-chloro-3-pyridinylmethyl)-2-thiazolidinylidene] cyanamide is a neonicotinoid insecticide that affects the nervous system of insects (Matsuda et al. 2005; Matsuda et al. 2009; Kocaman et al. 2014). Thiacloprid is widely used in orchards and vegetables to combat aphids, as well as seed coating in corn and some crops (Schuld and Schmuck 2000; Schmuck et al. 2001). Like other neonicotinoids, Thiacloprid is highly soluble in water and potentially contaminates surface water following precipitation events (water solubility is 185 mg L^{-1})(EPA 2003). According to some scientific studies, Thiacloprid is also harmful to freshwater invertebrates (Beketov and Liess 2008; Beketov et al. 2008; Morrissey et al. 2015). Therefore, it is important to evaluate the concentrations of which these chemicals are toxic to aquatic organisms (Tisler et al. 2009)

A lot of physical, chemical, and biological environmental factors cause oxidative stress by affecting the production of reactive oxygen species (ROS) in plants and cyanobacteria (Smirnoff 1993; Hendry 1994; Bartosz 1997, Olga et al. 2003). Under normal conditions, concentrations of oxygen radicals are kept at low levels by the activity of antioxidants (Asada 1984). The imbalance between the production of ROS and the activity of antioxidants leads to oxidative damage (Del-Rio et al. 1991; Del Vos et al. 1992; Smirnoff 1993). These active oxygen species are inactivated by enzymes such as superoxide dismutase, catalase and peroxidase and by antioxidant molecules such as organic chemicals proline, ascorbate, and carotenoids in cellular systems. Free radicals can damage the major cellular components such as lipids, proteins, carbohydrates and nucleic acids (Olga et al. 2003). In addition, another group of pesticides, such as herbicides, has been shown to produce singlet oxygen and other active oxygen species in various regions of the photosynthetic electron transport chain and cause oxidative stress in cells (Halliwell 1987).

The aim of this study is to explore the changes in the growth parameters of *Arthrospira platensis* being a phototrophic primary producer and to evaluate the aspects of the oxidative stress by neonicotinoids.

Materials and Methods

Algae culture and treatment

A. platensis-M2 was obtained from the Soley Microalgae Institute (California, USA) (Culture collection No: SLSP01). Algae were grown in Spirulina Medium (Aiba and Ogawa 1977) under axenic conditions. 20 mL algal cultures were inoculated to 180 mL culture medium in Erlenmeyer flask and were allowed to grow under the conditions of 93 µmol photons m^{-2} s^{-1} photosynthetically available radiation in 12:12 h light/dark cycle at 30±1 ºC during 10 days. At the end of 10 days, cultures were renewed and, all the flasks contained 50 mL algal culture. The commercial formulation of Thiacloprid and Imidacloprid (240 g L^{-1}, 350 g L^{-1}; respectively, EC, Sakarya, Turkey) were used in all bioassays and prepared in distilled water. Various concentrations of Imidacloprid and Thiacloprid were added to the culture medium. The range of concentrations was determined with preliminary range-finding bioassays according to the EC50 value (the concentration of a drug that gives half-maximal response) for growth parameters (Sebaugh 2011).

Cell growth and chlorophyll-*a* assay

Optic density (OD) of microalgae was measured spectrophotometrically over a period of 7 days under control and stressed conditions taking absorbance at 560. To determine the growth rate, OD560 absorbances were measured spectrophotometrically for 7 days and EC50 values were calculated with OriginPro 8.5 programme. Chlorophyll-*a* contents were estimated by methanol extraction and were measured spectrophotometrically for 7 days (MacKinney, 1941).

Antioxidant enzyme activities

On the 7th day of the study, 2 mL culture solutions from the control and treated samples were centrifuged at 14.000 rpm for 20 min at 4°C, and obtained pellets were kept at -20 °C until enzyme activity measurements. Pellets were crushed with liquid nitrogen and suspended in specific buffers with proper pH values for each enzyme. The protein concentrations of algal cell extracts were determined according to Bradford (1976), using bovine serum albumin (BSA) as a standard.

The superoxide dismutase (SOD) activity was determined by the method of Beyer and Fridovich (1987), based on the photoreduction of NBT (nitroblue tetrazolium). The pellets (0.2 g) were extracted in 1.5 mL homogenization buffer containing 100 mM K_2HPO_4 buffer (pH 7.0), 2% PVP, and 1 mM Na_2EDTA. After centrifugation at 14.000 rpm for 20 min at 4°C, the resulting supernatants were used to measure SOD activity. The reaction mixture consisted of 100 mM K_2HPO_4 buffer (pH 7.8) containing $9.9x10^{-3}$ M methionine, $5.7x10^{-5}$ M NBT, 1% Triton X-100 and enzyme extract. The reactions were started by the addition of 0.9 µM riboflavin and the mixture was exposed to light with an intensity of 375 µmole m^{-2} s^{-1}. After 15 min, the reactions were stopped by switching off the light and absorbance was read at 560 nm. The SOD activities were calculated by a standard graphic and expressed as unit mg^{-1} protein.

The ascorbate peroxidase (APX) activity was determined according to Wang et al. (1991) by estimating the decreasing rate of ascorbate oxidation at 290 nm. APX extractions were performed in 50 mM Tris–HCl (pH 7.2), 2% PVP, 1 mM Na_2EDTA, and 2 mM ascorbate. The reaction mixtures consisted of 50 mM K_2HPO_4 buffer (pH 6.6), 2.5 mM ascorbate, 10 mM H_2O_2, and enzyme-containing 100 µg protein in a final volume of 1 mL. The enzyme activities were calculated from the initial rate of the reaction using the extinction coefficient of ascorbate (E = 2.8 mM cm^{-1} at 290 nm).

The glutathione reductase (GR) activity was measured with the method of Sgherri et al. (1994). Extractions were performed in 1.5 mL of suspension solution containing 100 mM K_2HPO_4 buffer (pH 7.0), 1 mM Na_2EDTA, and 2% PVP. The reaction mixtures (total volume of 1 mL) contained 100 mM K_2HPO_4 buffer (pH 7.8), 2 mM Na_2EDTA, 0.5 mM oxidize glutathione (GSSG), 0.2 mM NADPH and enzyme extract containing 100 µg protein. The decrease in absorbances at 340 nm were recorded. The corrections were made for the non-enzymatic oxidation of NADPH by recording the decrease at 340 nm without adding GSSG to the mixture. The enzyme activities were calculated from the initial rate of the reaction after subtracting the non-enzymatic oxidation using the extinction coefficient of NADPH (E = 6.2 mM cm^{-1} at 340 nm).

Determination of malondialdehyde and hydrogen peroxide

The malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content were determined by the method of Heath and Packer (1968). 0.2 g pellets were homogenized in 3 mL of 0.1% TCA (4°C), and centrifuged at 4100 rpm for 15 min, and the supernatants were used in the subsequent determination. 0.5 mL of 0.1 M Tris–HCl pH 7.6 and 1 mL of TCA–TBA–HCl reagent (15% w/v) (TCA–0.375% w/v, TBA–0.25 N HCl) were added into the 0.5 ml of the supernatant. The mixtures were heated at 95°C for 30 min and then quickly cooled in the ice bath. To remove suspended turbidity, the mixtures were centrifuged at 4100 rpm for 15 min, then the absorbances of supernatant at 532 nm were recorded. Non-specific absorbances at 600 nm were measured and subtracted from the readings recorded at 532 nm. The MDA contents were calculated using its extinction coefficient of 155 mM^{-1} cm^{-1}. Hydrogen peroxide content was determined in the assay mixture consisting of 0.5 mL of 0.1 M Tris–HCl (pH 7.6). 1 mL of 1 M KI were added to 0.5 mL of supernatant. After 90 min, the absorbances were recorded at 390 nm.

The proline content determination

The proline content was determined by the method of Weimberg et al. (1987). 0.1 g pellets were homogenized in 10 ml of 3% aqueous sulphosalicylic acid and the homogenates were incubated in the hot water bath at 95 °C for 30 minutes. Samples were cooled and centrifuged at 4100 rpm for 10 min. Two milliliters of the extract reacted with 2 mL of acid–ninhydrine and 2 mL of glacial acetic acid for 1 h at 100°C. The reaction mixtures were extracted with 4 mL toluene. The chromophores containing toluene were separated, and the absorbances were recorded at 520 nm.

Statistical analysis

The differences between the control and treated samples were analyzed by one-way ANOVA, taking $P < 0.05$ as significant according to LSD. Three replicate cultures were used for each treatment. The mean values ± SE were given in Figures.

Results

Imidacloprid and Thiacloprid applications showed significant reductions in OD560 and chlorophyll-*a* content *of A. platensis* cultures with dose-dependent manner when the days and concentrations were compared each other (Fig1 and 2).

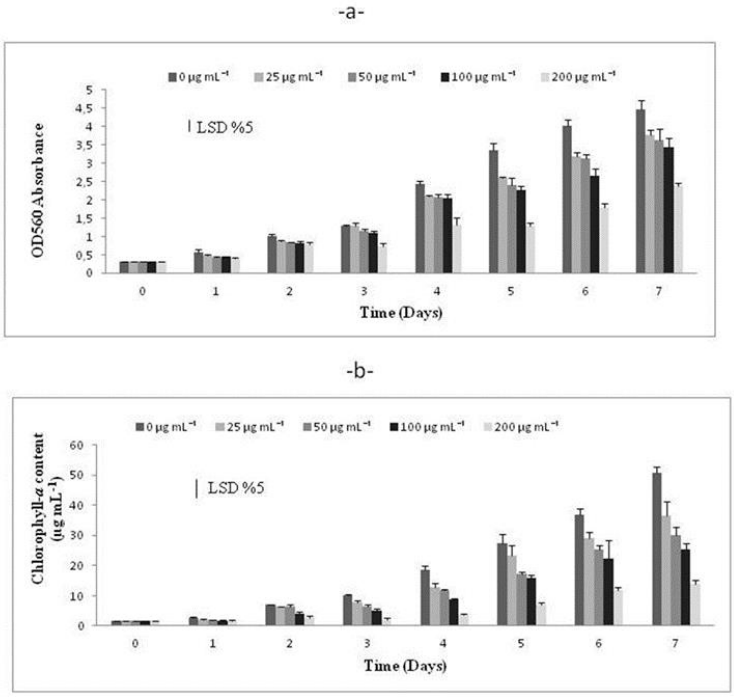

Figure 1. Biomass values (a) and (b) chlorophyll-a content of Arthrospira platensis supplemented with 0-200 µg mL $^{-1}$ Imidacloprid concentrations during 7 days. Data are the means ± SE of three replicates.

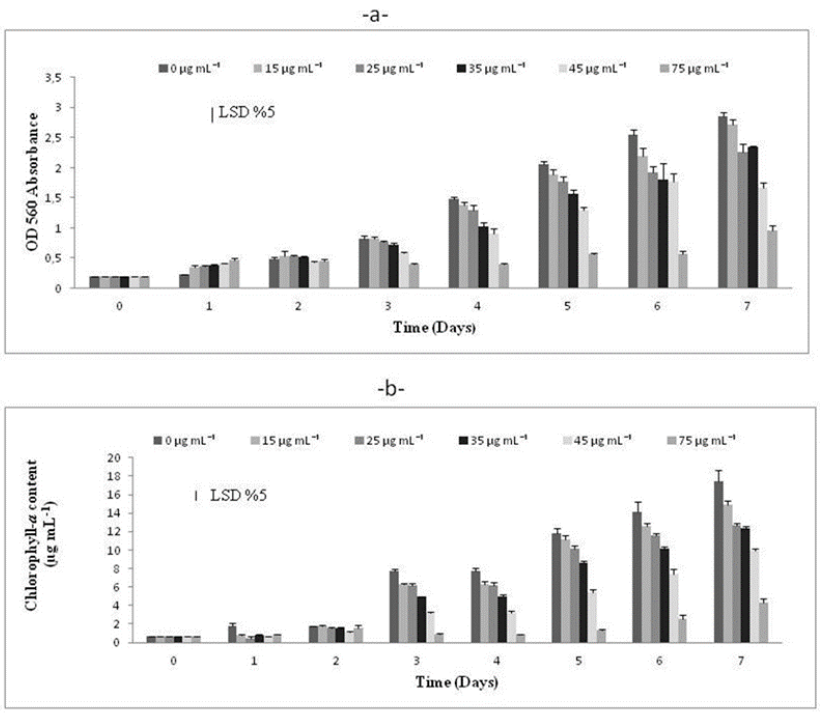

Figure 2. Biomass values(a) and (b) chlorophyll-*a* content of *Arthrospira platensis* supplemented with 0-75 µg mL^{-1} Thiacloprid concentrations during 7 days. Data are the means ± SE of three replicates.

SOD activity significantly decreased in the Imidacloprid application at all concentrations (10, 20, 30, 40, and 50 µg mL^{-1}) (Fig 3a) ($p < 0.05$), while Thiacloprid caused a significant increase in only 75 µg mL^{-1} concentrations (Fig 4a) ($p < 0.05$). APX activity significantly increased in the Imidacloprid and Thiacloprid applications at 50 µg mL^{-1} (Fig 3b) and 35 µg mL^{-1} (Fig 4b) concentrations, respectively ($p < 0.05$). Imidacloprid treatment increased GR activity at 20 and 30 µg mL^{-1} concentrations (Fig 3c) ($p < 0.05$), while Thiacloprid application increased statistically at 15, 25 and 35 µg mL^{-1} concentrations compared to control (Fig 4c) ($p < 0.05$).

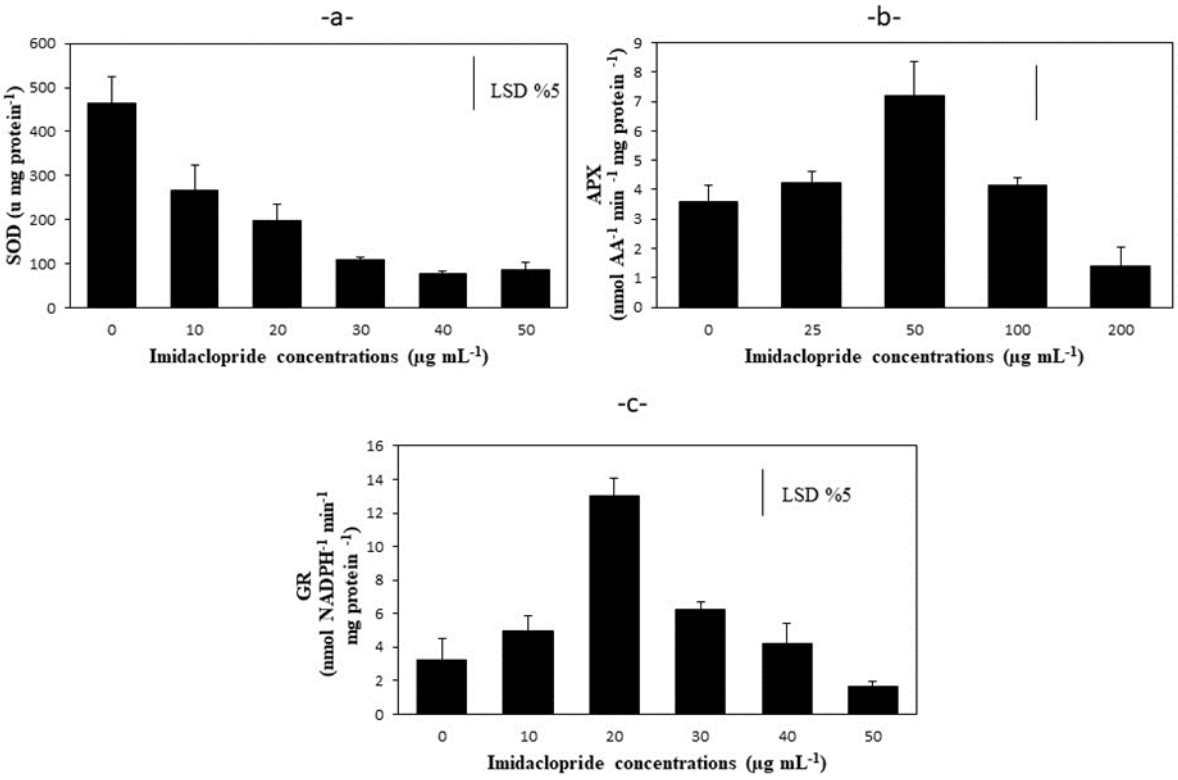

Figure 3. Total superoxide dismutase (SOD) (a), ascorbate peroxidase (APX) (b) and glutathione reductase (GR) (c) activities of *A. platensis* supplemented with Imidacloprid concentrations. Data are the means ± SE of three replicates. Mean values in columns are significantly different at the 5% level according to the least significant differences (LSD) Test.

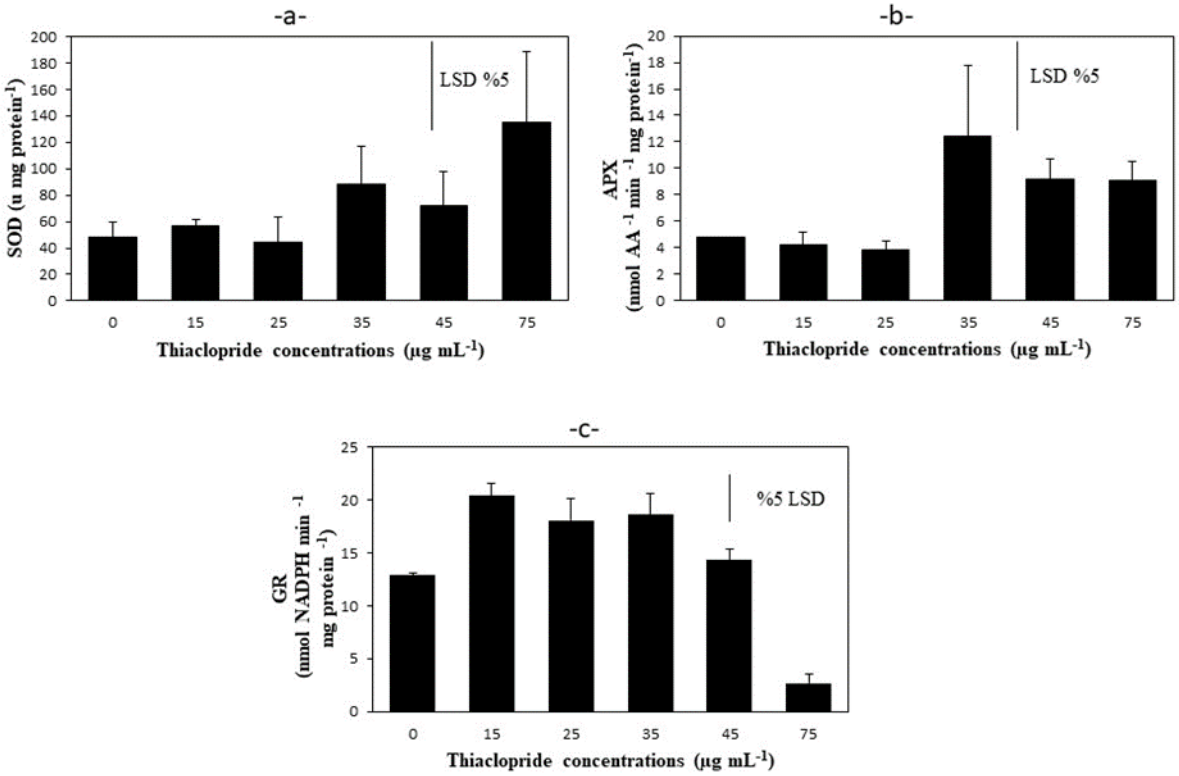

Figure 4. Total superoxide dismutase (SOD) (a), ascorbate peroxidase (APX) (b) and glutathione reductase (GR) (c) activities of *A. platensis* supplemented with Thiacloprid concentrations. Data are the means ± SE of the three replicates. Mean values in columns are significantly different at the 5% level according to the least significant differences (LSD) Test.

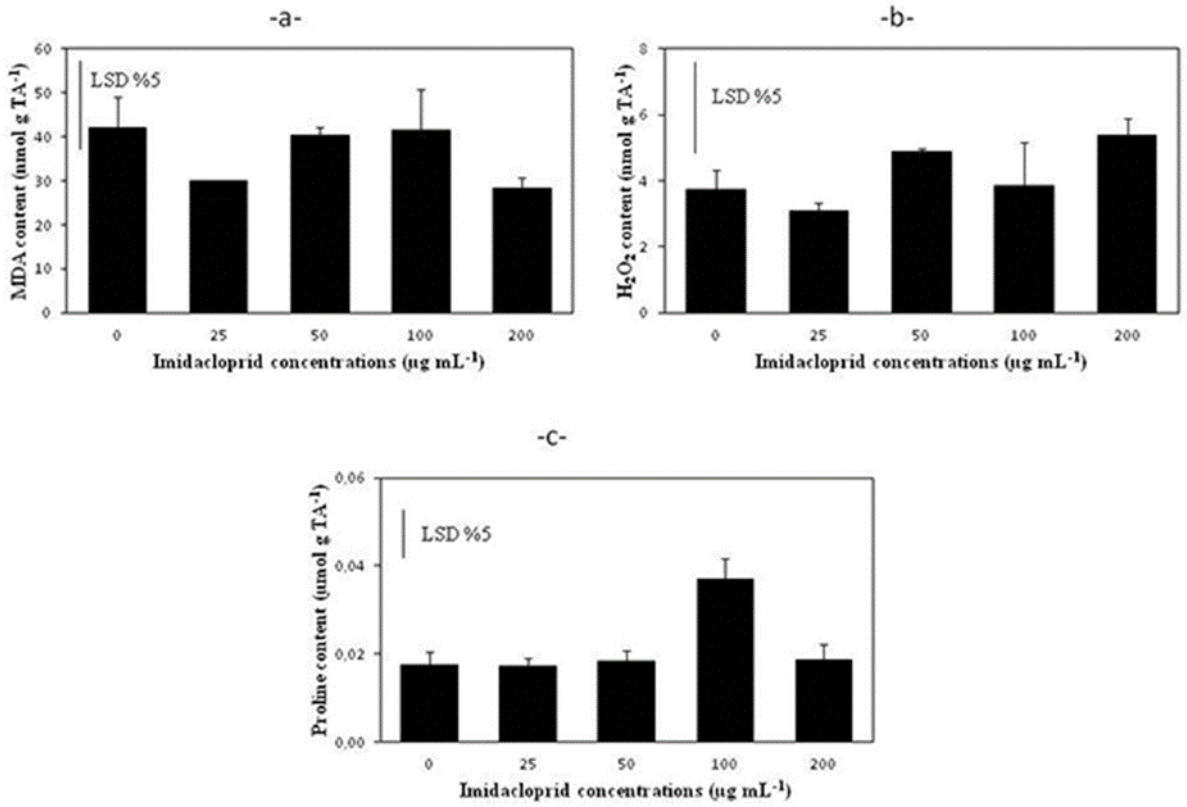

Figure 5. Malondialdehyde (a), hydrogen peroxide (b) and proline (c) contents of *A. platensis* supplemented with Imidacloprid concentrations. Data are the means ± SE of the three replicates. Mean values in columns are significantly different at the 5% level according to the least significant differences (LSD) Test.

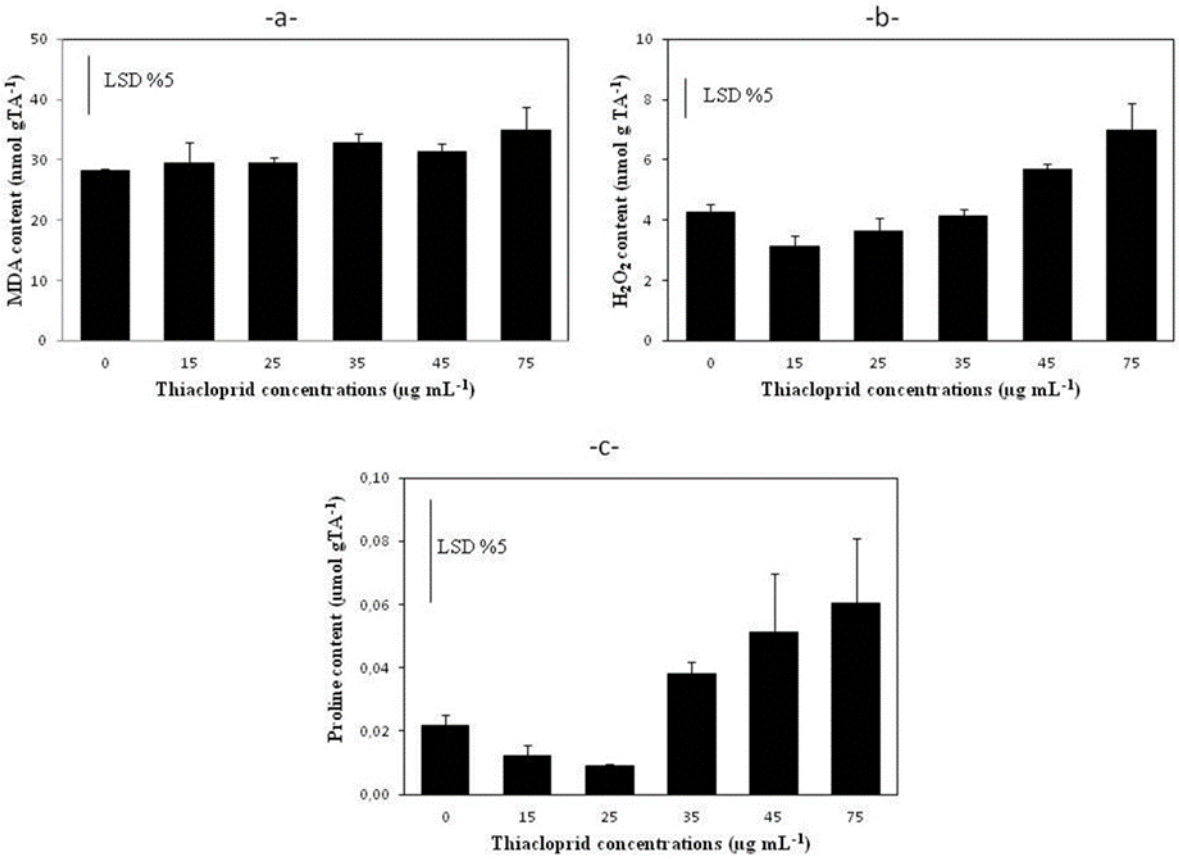

Figure 6. Malondialdehyde (a), hydrogen peroxide (b) and proline (c) contents of *A. platensis* supplemented with Thiacloprid concentrations. Data are the means ± SE of three replicates. Mean values in columns are significantly different at the 5% level according to the least significant differences (LSD) Test.

Discussion

In this study, Imidacloprid and Thiacloprid effects were investigated with some parameters such as OD560, chlorophyll-*a* content, some antioxidant enzyme activities (superoxide dismutase, ascorbate peroxidase and glutathione reductase) and H_2O_2, MDA and proline on *A. platensis*. Malev et al. (2012) evaluated the toxicity of Imidacloprid on *Desmodesmus subspicatus* and specified that Imidacloprid has harmful effects on non-target microorganisms. They reported that algal inhibition was significant at 127.8 and 255.6 mg L^{-1} concentrations compared to the control group. The data obtained by Malev et al. (2012) are similar to the effects of Imidacloprid on *A. platensis* in our study. The studies about Thiacloprid on algal toxicity are limited in the literature. Therefore, our work will fill the blank in the literature.

SOD is an antioxidant enzyme being responsible from detoxifying superoxide radicals produced in algal cells under stress conditions (Tunca 2020). When the pollution increases in the environment, the cellular detoxification system is stimulated and SOD synthesis rate and / or activity increases. These changes occur faster in molecular cell levels than growth and reproduction process (Rabinowich and Fridovich 1985). Therefore, changes in SOD activity are sensitive biomarkers to environmental pollution (Li et al. 2005). In our study, the total SOD activity significantly increased at the highest Thiacloprid concentration (75 µg mL^{-1}) on *A. platensis*. SOD activity have been investigated in many algae due to evaluation of various pesticide effects (Prasad et al. 2005; Galhano et al. 2010; Qian et al. 2008; Qian et al. 2009; Salman et al. 2016; Li et al. 2005; Kumar et al. 2014). According to the previous studies, SOD activity increased by the pesticide application, which increased the production of $\cdot O_2^-$ and other free radicals.

In our study it was observed that Imidacloprid caused significant reductions in chlorophyll-*a*. The loss of photosynthetic metabolism may have caused significant reductions in SOD enzyme activity or vice versa. Liu et al. (2015) reported that Azoxystrobin inhibits the SOD activity (19-300 µg L^{-1}) on *Chlorella vulgaris*. They suggested that degradation of SOD enzyme structure is caused by Azoxystrobin, and thus algal growth may be inhibited.

GR is effective in the detoxification of H_2O_2 in plant cells due to functions in the Haliwell-Asada pathway (Bray et al. 2000). GR catalyzes the last step

of the ascorbate-glutathione pathway. GR enzyme activity was significantly increased at 20 and 30 µg mL^{-1} concentrations in Imidacloprid treatment, when GR enzyme activity was significantly increased at 15, 25 and 35 µg mL^{-1} Thiacloprid concentrations. Mofeed and Mosleh (2013) observed that GR activity increased with fenhexamid and atrazine application on *Scenedesmus obliquus*. It is concluded that this enzyme displayed an essential role in the detoxification of these pesticides. The increases in GR activity may have occurred to neutralize the $ROS^{\cdot}$ APX uses ascorbic acid as an electron donor to eliminate harmful H_2O_2 (Verma and Dubey 2003). In our study, Imidacloprid treatment significantly increased APX activity at 50 µg mL^{-1} but decreased at 200 µg mL^{-1}. Previous studies have reported that APX activity was induced due to increased oxidative stress (Weckx and Clijsters 1996). The high concentrations of salicylic acid and 2,6 dichloroisoniconitic acid have been reported to inhibit APX activity (Durner and Klessig 1995). According to the results of these studies, Imidacloprid pesticide increases APX enzyme activity by causing oxidative stress at low concentrations, but it inhibits APX enzyme at higher concentrations due to breaking down the enzyme structure.

Thiacloprid treatment showed a statistically significant increase in APX activity at 35 µg mL^{-1}, whereas it did not change at other concentrations (15, 25, 45 and 75 µg mL^{-1}). The increase in APX enzyme activity can be explained by the increase in GR enzyme activity at a similar concentration.

There was no significant change in the MDA content by Imidacloprid applications, and this situation was parallel the results of H_2O_2 assay. Chen (2020) explained that MDA and H_2O_2 content may have been prevented by other antioxidant responses caused by sulfonamides- induced oxidative stress in *Chlorella vulgaris*. In addition, proline may inhibit cell membrane damage and MDA amount. Siripornadulsil et al. (2002) reported that Cadmium treatment did not change the MDA content of transgenic *Chlamydomonas reinhardtii* strain producing proline at high concentrations and they reported that proline could prevent free radical damage by acting as an antioxidant.

Thiacloprid pesticide application showed no significant change in MDA content. Jiménez et al. (1998) reported that the MDA content did not change during the senescence, but the H_2O_2 content increased in *Pisum sativium*. They attributed this situation to MDA metabolization in mitochondria. According to Thaicloprid application, the increase in the proline content may have contributed to the unchanged MDA content.

The proline content of *A. platensis* cultures increased statistically at 75 µg mL^{-1} Thiacloprid concentration when it was increased statistically in at Imidaclopride concentration of 100 µg mL^{-1} compared to the control. Proline may have increased according to pesticide accumulation (Fatma et al. 2007; Duval et al. 1999; Galhano et al. 2011; Kumar et al. 2008; Choudhary et al. 2007; Kumar et al. 2014).

In conclusion, the changes in antioxidant enzyme activities and other parameters varied according to the pesticide type and used concentrations. This difference arises from the ability of the applied pesticide to produce ROS in different ratios. Neonicotonoid pesticides have irreversible damages on prokaryotic primary producers. Therefore, these pesticides should be used with caution.

Acknowledgemet

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Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

Conflict Of Interest

The authors confirm that there is no conflict of interest in the present study.

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Length based-stock assessment and Spawning Potential Ratio (LB-SPR) of exploited tilapia species (*Coptodon zillii*, Gervais, 1848) in Lake Volta, Ghana

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ABSTRACT

Length based stock assessment parameters and LBSR of *C. zillii* in Lake Volta were investigated for the purpose of sustainable management. The lengths of 517 individuals were measured from the lake from January to December 2020 and analyzed using TropFishR. The von Bertalanffy parameters including asymptotic length (L_∞), growth rate (K), and growth performance index (Φ) were estimated as 30.4 cm, 0.38 per year, and 2.73 respectively. Total mortality rate (Z), natural mortality rate (M) and fishing mortality rate (F) were 4.58 per year, 0.89 per year and 3.96 per year respectively. The exploitation rate (E) was highly above the exploitation rate at the maximum sustainable yield (Emax) which shows that the fishery of the stock is facing high unsustainable fishing pressure. Based on the findings of the study, it is recommended that fishing pressure be reduced and, mesh size regulation be strictly enforced to ensure sustainability of the stock.

Keywords: *C. zillii*, growth, mortality, TropFishR, Lake Volta, LB-SPR

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Introduction

Native to Africa and the south-western Middle East, are three key taxonomic groups including *Oreochromis*, *Sarotherodon*, and *Coptodon* (Gophen, 2016). They inhabit a variety of fresh and less commonly brackish water habitats, from shallow streams and ponds through the rivers, lakes, and estuaries (Soliman et al. 2017; Komolafe 2008). Tilapia is the common name of more than 70 fish species belonging to the family Cichlidae which undergo under three genera (*Sarotherodon*, *Oreochromis*, and *Tilapia*) (Meyer 2002). It is known that the family Cichlidae have a high economic significance in tropical inland waters in Africa, and they play important role in the ecology of freshwater bodies (Ikomi and Jessa 2003).

Coptodon zillii (Gervais, 1848) is distinguished by its ability to adapt to freshwater, brackish, and hyaline conditions (Mohamed and Abood 2020; Eddine et al. 2016). In Africa, its distribution extends from Morocco and Egypt in the North, Côte d'Ivoire and Nigeria in the West to the Democratic Republic of Congo in Central Africa, reaching a maximum length of 26.0 cm, 289 g weights, and can live for about seven years and, usually found in water depth of up to 1m (Dadebo et al. 2014). It can tolerate a wide range of temperature and salinity and can utilize aquatic vegetation. The family Cichlidae plays an important role in commercial fisheries and aquaculture worldwide. The total landing of cichlid fishes in the world was about 1.6 million tons in 2016 (FAO 2018), and it was the second most important fish in fish farming in the world after carps, with a production of 6.3 million tons in 2018 (FAO 2019). Despite the importance of *C. zillii* and its widespread occurrence, information about its reproductive biology and dynamics is scarce. Nonetheless, in Ghana, numerous studies have been done on the growth, biology, and physiology of *C. zillii* (e.g., Atindana et al. 2014), but there are few studies (e.g., Danson and Apegyah 2009) on its stock assessment.

Stock assessment by Sparre and Venema (1992) provides advice on the optimum exploitation of aquatic living resources. Hilborn and Walters (1992) defined it as a method that involves the use of various statistical and mathematical expressions to make quantitative predictions about the reactions of fish populations to alternative management choices

(Abdul and Omonyi 2011). So, this study was undertaken to provide the basic data on some aspects of the stock assessment including growth parameters, mortality parameters, and exploitation rate for its sustainable management in Ghana.

Materials and Methods

Study Area

The study was undertaken in some selected fishing communities in Yeji which geographically is located between longitude 0º10' and 1º05W and latitude 8º8' and 8º20'N. The selected fishing communities included Tonka, Vutideke, Brekente, and Fante Akura (Figure 1). With a population of 28,515, Yeji is the capital of Pru District in the Brong-Ahafo Region (GSS, 2014). The selection of these sampling inland fishing communities was based on geographical isolation and the level of fishing activities based.

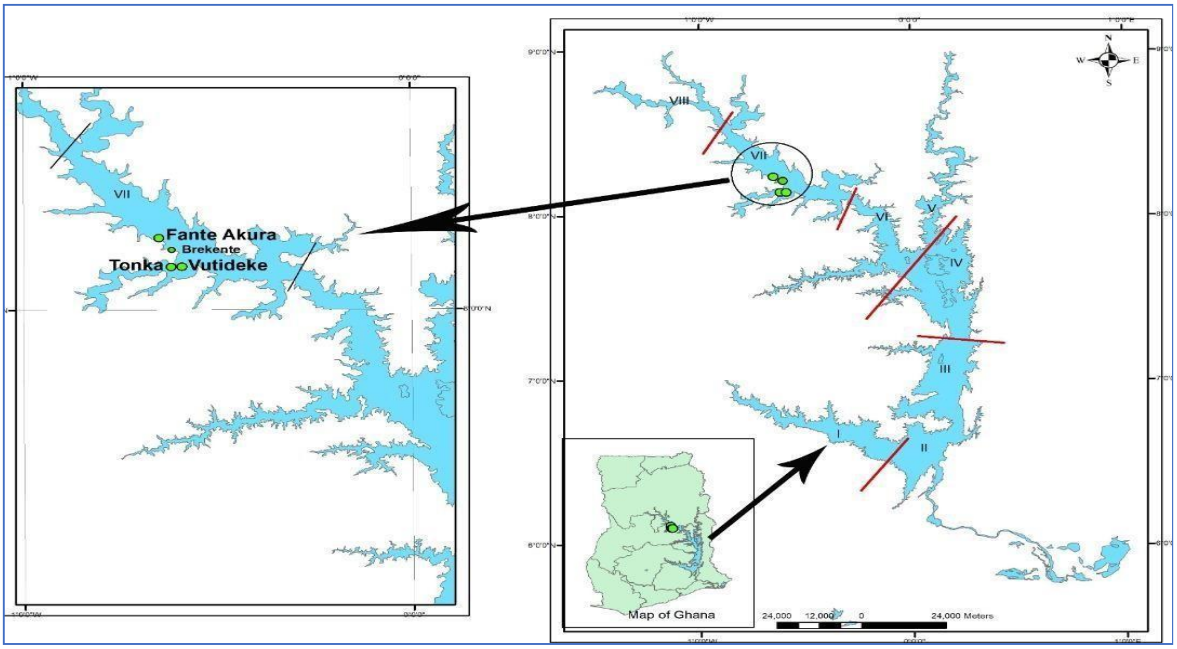

Figure 1. Map showing the study areas within Stratum VII of the Volta Lake, Ghana

Data Collection

Samples of *C. zillii* from Lake Volta, Ghanawere collected from fishermen who landedtheir catch at any of the four sampling locations, over twelve (12) months (i.e., January 2020 to December 2020). Species were identified in-situ using Dankwa et al. (1999) and Lowe-McConnell and Wuddah (1972) identification keys. Measurements of length and weight were performed using a 100 cm graduated wooden measuring board and to the nearest gram of 0.1 g using the digital balance.

Estimation of Parameters Growth Parameters Formulas

Growth parameters including curvature parameter (*K*), asymptotic length (L_∞), growth performance index and theoretical age at length zero (t_o) the growth performance index (Φ') were estimated using the ELEFAN_ SA. Longevity (t_{max}) of the species was calculated as $t_{max} = 3/K$ (Anato 1999). The growth performance index was estimated as $\Phi' = 2\log L_\infty + \log K$ (Munro and Pauly, 1984). T -zero (t_o) $\text{Log}_{10}(-t_0) = -0.3922 - 0.2752 \log_{10} L_\infty - 1.038\log_{10} K$ (Pauly, 1984).

Mortality Parameters

The total mortality (*Z*) was estimated using linearized length-converted catch curve (Sparre and Venema, 1992). The estimation of natural mortality rate (*M*) followed, $M = 4.118K^{0.73}L\infty^{-0.333}$ (Then et al. 2015). Fishing mortality (*F*) was calculated as Z – M (Qamar et al. 2016). The exploitation rate (*E*) was calculated as F/Z (Georgiev and Kolarov 1962).

Virtual Population Analysis (*VPA*)

VPA is a method that allows for the reconstruction of the population from total catch data by age or length. The initial step was to estimate the terminal population (*Nt*), followed by the successive estimation of F values and finally, the population sizes are computed for each length class (midpoint). These procedures were estimated using the VPA method (Pope 1972).

Yield per recruit

The relative yield-per-recruit was estimated using the knife-edge method (Beverton and Holt 1957).

Length based spawning ratio

The length spawning potential parameters including the length at maturity, gear selectivity at Lc_{50} and Lc_{95} and spawning potential ratio (SPR)

were determined using barefoot ecologist toolbox application software by CSIRO (2020) that are commonly used and access directly through a public link via http://barefootecologist.com.au/lbspr.

Results

Length distribution

Table 1 shows the length distribution of *C. zillii* with February, May, July, August and December recording the highest number of samples (i.e N = 53 samples), whereas in September the lowest number of samples were recorded (i.e., N = 24 samples). Overall, 517 samples of *C. zillii* were recorded during the study period. The mean, minimum and maximum lengths of *C. zillii* were estimated as 10.4 cm, 4.7 cm and 22.7 cm respectively.

Table 1. The length-frequency distribution data of *C. zillii*

Length Class (cm)	2020-01-01	2020-02-02	2020-03-03	2020-04-04	2020-05-05	2020-06-06	2020-07-07	2020-08-08	2020-09-09	2020-10-10	2020-11-11	2020-12-12
6	0	3	0	0	0	1	0	0	1	0	0	0
8	6	4	1	7	0	5	0	27	7	5	2	15
10	19	5	24	10	15	4	8	15	5	18	21	20
12	6	16	17	5	18	6	32	5	10	12	13	17
14	3	17	4	3	14	10	7	2	0	8	3	0
16	1	7	1	1	5	1	5	3	1	4	4	0
18	0	1	1	0	0	0	1	1	0	0	1	0
20	0	0	0	0	0	0	0	0	0	0	0	1
22	0	0	0	0	0	1	0	0	0	0	0	0
24	0	0	0	0	1	0	0	0	0	0	0	0
Total	35	53	48	26	53	28	53	53	24	47	44	53

Restructured length frequency of *C. zillii* with superimposed growth curves is shown in Figure 2. The asymptotic length (L_∞) was 30.4 cm with a growth rate (K) of 0.57 per year. Growth performance index (Φ') was 2.73. The longevity (t_{max}) was approximately seven (7) years. The age at zero length (t_o) was estimated as 0.38 years. The Powell Wetherall plot showed Z/K to be 3.03 with a 95% confidence interval of 2.83 – 3.24 (Figure 3).

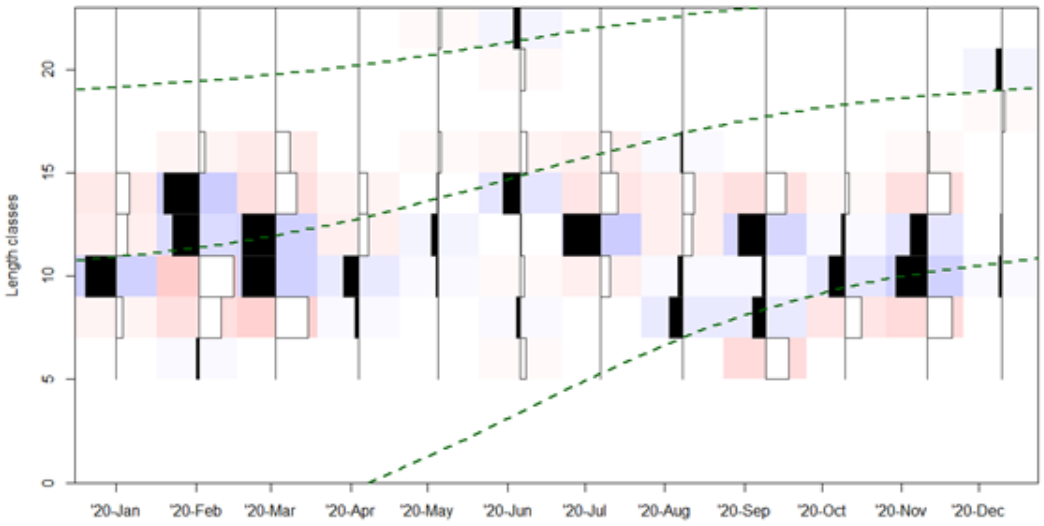

Figure 2. Reconstructed length-frequency distribution with growth curves.

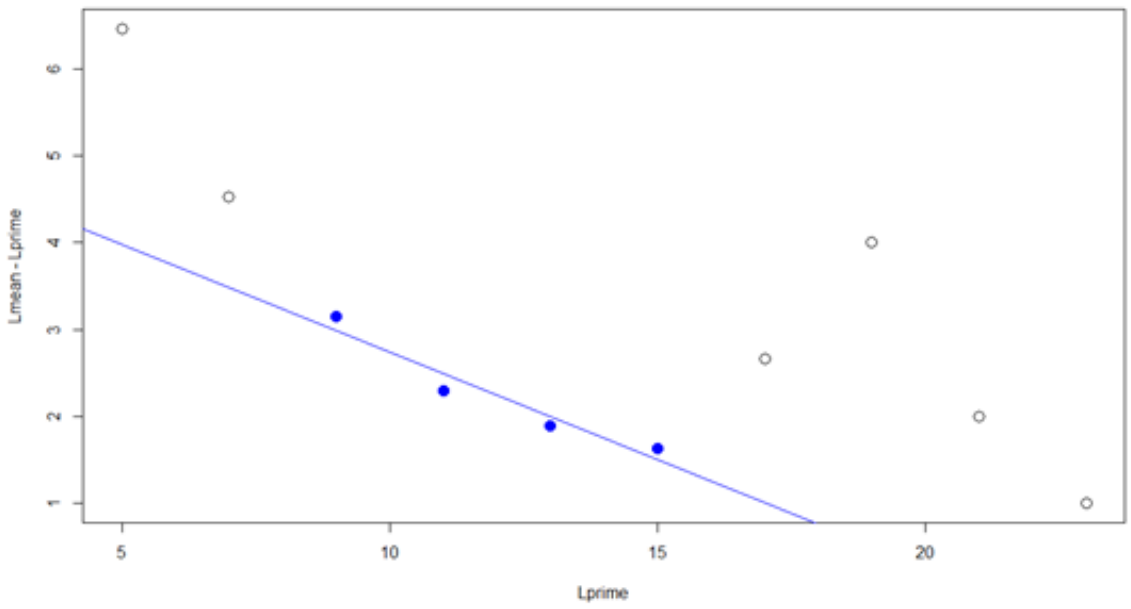

Figure 3. Powell Wetherall Plot of *C. zillii*

Mortality parameters

The linearized length-converted catch curve was used for the estimation of instantaneous total mortality (*Z*) as shown in Figure 4. The total mortality rate (*Z*) was calculated as 4.58 ± 0.68 per year. The natural and fishing mortality rates were estimated at *M*= 0.89 per year and *F* = 3.65 per year respectively. The current exploitation rate (*E*) was obtained at 0.81.

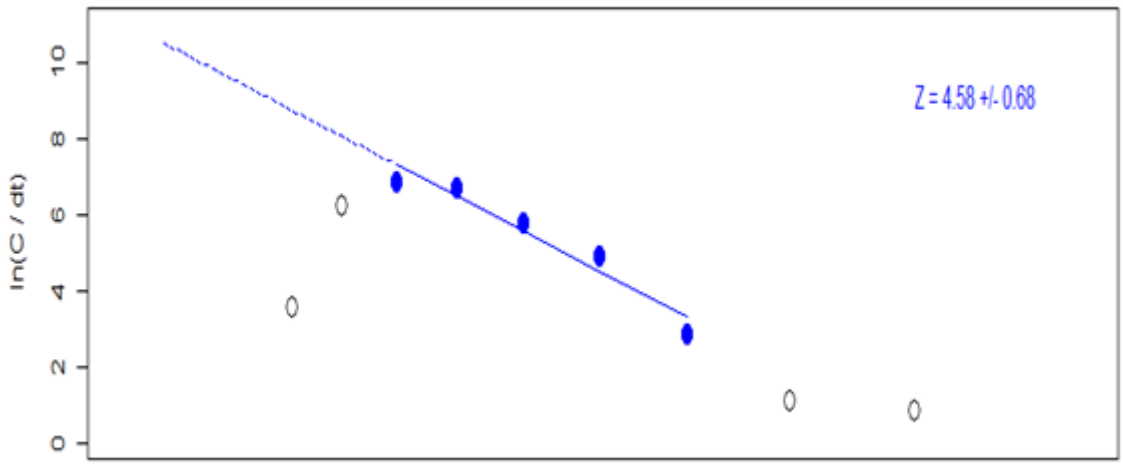

Figure 4. Linearized length-converted catch curve for total mortality estimation (Z).

Virtual Population Analysis

The virtual population analysis of *C. zillii* is shown in Figure 4. Individuals within the range of 16 cm experienced the highest level of exploitation (catch = $7x10^5$ per year). Natural losses were the highest among individuals within the length range of 12 cm. Surviving individuals in the stock exhibited a declining trend with rising fishing pressure. The highest number of survivors in the stock was observed in the length range of 8 cm. Fishing effort was the highest (F = 4.67 per year) on individuals within the length range of 16 cm and the lowest (*F* = 0.04 per year) on individuals at length range of 8 cm (Figure 5).

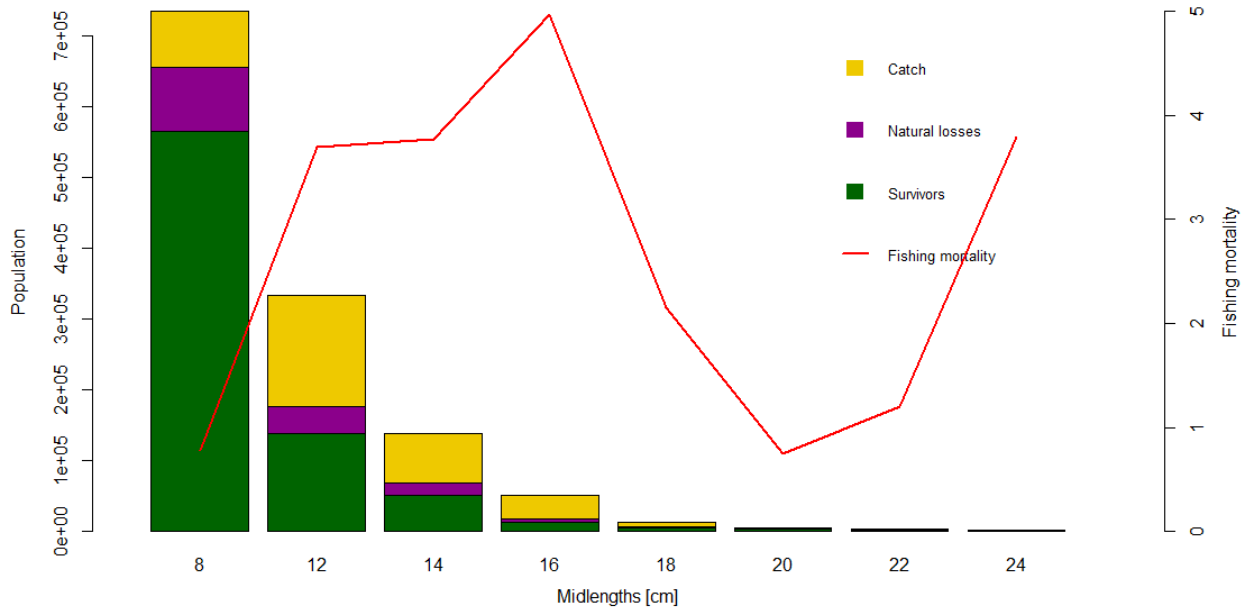

Figure 5. Length structured virtual population analysis of *C. zillii* in Lake Volta

Relative yield per recruit

The plot of relative yield per recruit against fishing mortality showed that the indices for sustainable yield were 0.43 for $F_{0.5}$ and 0.66 F_{msy} as indicated in Figure 6a. The corresponding F0.5 and Fmsy was estimated 0.68 per year and 1.70 per yield.

The isopleth plot in Figure 6b shows that 50% of catch are retained in the gear at a high fishing mortality rate.

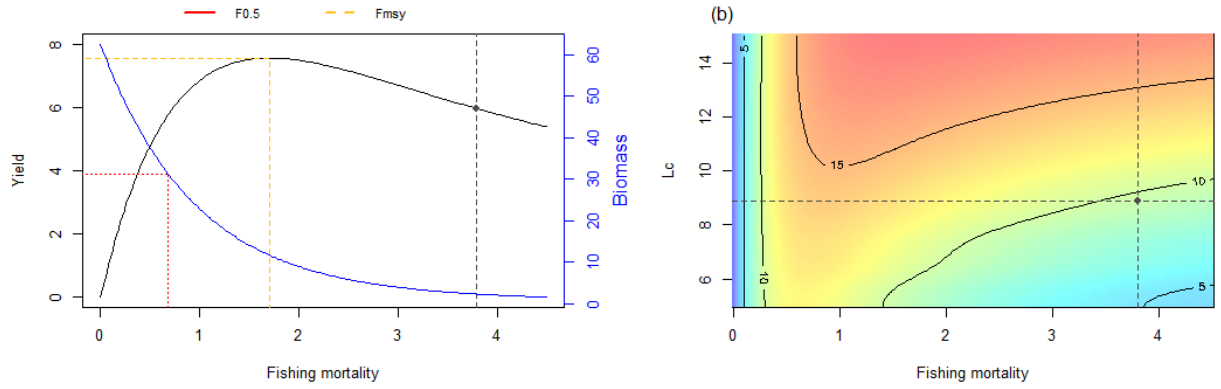

Figure 6. a) Yield-per-recruit plot of *C. zillii* in the Lake Volta. b) Isopleth plot of *C. zillii*

Length based spawning ratio

Observation from Figure 7 shows that the length at first maturity of *C. zillii* was approximately 9.5 cm. The corresponding lengths at capture (Lc) for *C. zillii* were estimated as Lc_{50} = 8.2 cm, and Lc_{95} = 10.4 cm (Figure 7). The Length Based-Spawning potential ratio (LB-SPR) analysis of *C. zillii* was 4% for the total population (Figure 8).

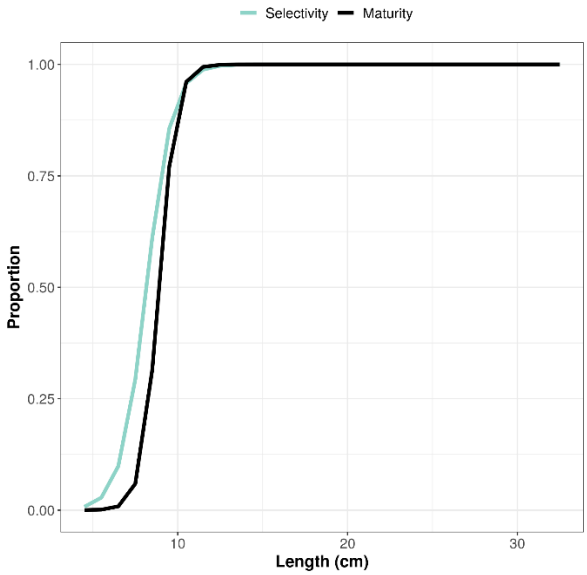

Figure 7. Length at first at maturity (Lm_{50}) and selectivity (Lc_{50})

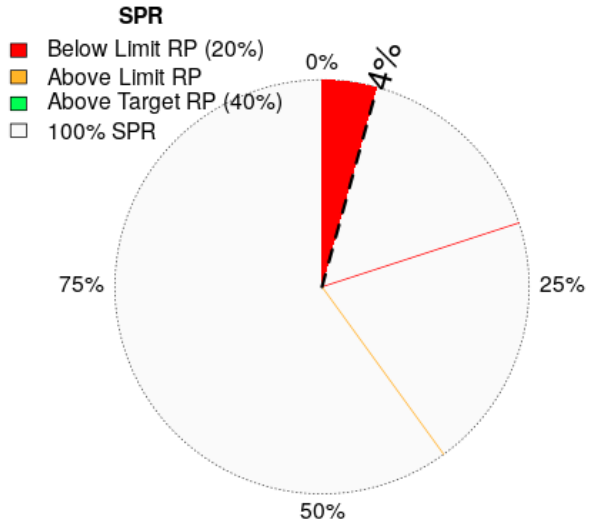

Figure 8. Percentage of SPR of *C. zillii*

Discussion

The growth rate for *C. zillii* estimated for the study was 0.57 per year, relatively higher than estimates from other studies. For instance, Mohamed and Abood (2020), Mehanna (2004), Uneke and Nwani (2014) recorded 0.32 per year, 0.49 per year and 0.46 per year from Iraq, Egypt and Nigeria respectively. Studies by Adeyemi and Akomb (2012) from Nigeria and Mehanna (2004) recorded higher asymptotic length values (i.e., 34.5 cm and 33.5 cm) than recorded from the current study (i.e., 30.4 cm). Nonetheless, the growth performance index from the study (i.e., 2.73) compared favourably with estimates from Adeyemi and Akomb (2012) and Mehanna (2004) who documented 2.72 and 2.74. Factors such as variation in ecological parameters of the habitats, metabolic activity, availability of feed items, genetic constitution of the individuals, fishing pressure and sample size retrieved for analysis (Mohamed and Abood 2020; El-Kasheif et al. 2015) could be the reason for the variation in growth rates. The growth rate from the present study (0.57 per year) signified that *C. zillii* in Lake Volta is a fast-growing species. This characteristic of the species could be a compensation for the existing high level of mortality (Adeyemi and Akombo 2012). This is evinced by the Z/K > 1 which suggests that the population is a mortality dominated one.

From the present study, the fishing mortality rate (F = 3.69 per year) was greater than the natural mortality rate (M = 0.89 per year). Again, the fishing mortality surpassed the fishing rate at the maximum sustainable yield (F_{max} = 1.70 per year). These findings suggest that the population of *C. zillii* in the Lake Volta is exposed to high fishing pressure. Furthermore, the estimated fishing mortality rate from the study was greater than estimates from other locations (i.e., Uneke and

Nwani (2014) and Mohamed and Abood (2020) recorded 2.02 per year and 0.68 per year). This explains that the impact of fishing activities on this species appears to be extremely higher than in these locations and can possibly lead to collapse of its population in the future. In support of this, the current exploitation rate E = 0.81 was significantly higher than the exploitation at the maximum sustainable yield (E_{max} = 0.67).

The estimated length at first capture from the current study for *C. zillii* was 8.20 cm, lower than the length at first maturity (Lm_{50}) of 9.5 cm. This suggests that many of the individuals of this species are not given the opportunity to spawn at least once before becoming vulnerable to the fishing gear. In such cases, growth overfishing maybe present with recruitment overfishing becoming possible in the future.

Length-based VPA analysis showed that the number of individuals of the species subjected to natural losses as well as the number of survivors declined as they matured. This clearly shows that recruitment into the stock may be reduced to the barest minimum in the future if proper measures are not put in place. The SPR was lower than the limit reference point of 20% which shows low proportion of mature stock to be recruited into the population. The decrease in the SPR level maybe assigned to the decline in selectivity and matured stock. The threshold value of SPR is 40% which can be accepted as a proxy for the Maximum Sustainable Yield (MSY) for recruitment overfishing in a less resilient fish population. This alludes to the earlier claim that recruitment overfishing within the population is possible in the future.

The study has shown that *C. zillii* from Lake Volta is a fast-growing species with a growth rate of 0.57 per year. Findings from the mortality parameters suggests that species is overexploited ($E > E_{max}$). Furthermore, findings from the SPR, Lm_{50} and Lc_{50} revealed that population of the species is exposed to growth overfishing and consequently recruitment overfishing which could have serious implications on food security and economic wellbeing of dependent households. Summarily proper fisheries management measures such as mesh size regulations should be put in place in order to ensure sustainable exploitation of the species.

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Koi (*Cyprinus carpio*) ve Japon Balıklarında (*Carassius auratus*) Betanodavirus Varlığının Araştırılması

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ÖZ

Nodaviridae familyasında yer alan *betanodavirus*'un neden olduğu Viral nervöz nekrozis (VNN) ya da bir diğer adıyla Viral ensefalopati-retinopati (VER) dünyanın pek çok yerinde hem deniz balıklarında hem de tatlı su balıklarında görülür. Larval ve yavru boylarda, nadiren de yetişkin balıklarda hastalığa neden olmaktadır. Özellikle yetiştiriciliği yapılan balık türlerinde önemli ekonomik kayıplara neden olduğu için son yıllarda enfeksiyonla ilgili araştırmalara odaklanılmıştır. Bu çalışmada, Antalya bölgesinde yetiştiricilik faaliyeti gösteren akvaryum işletmelerinde Koi ve Japon balığı türlerinde *betanodavirus* varlığı araştırılmıştır. Üç farklı akvaryum işletmesinden her bir türü temsilen en az 30'ar adet olmak üzere toplam 180 balık örneklendi ve Real Time RT-PCR (RT-qPCR) metodu ile test edildi. Çalışma sonunda hiçbir örnekte betanodavirus RNA'sına rastlanmadı. Bu çalışma ile Türkiye'de koi ve japon balığı türlerinde betanodavirus varlığı moleküler tekniklerle ilk defa araştırılmıştır.

Anahtar kelimeler: Betanodavirus, Japon balığı, Koi, Real Time RT-PCR (RT-qPCR), Türkiye

MAKALE BİLGİSİ

ARAŞTIRMA MAKALESİ

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Investigation of *Betanodavirus* Presence in Koi (*Cyprinus carpio*) and Goldfish (*Carassius auratus*)

Abstract: Viral nervous necrosis (VNN), also known as Viral encephalopathy-retinopathy (VER), which is caused by *betanodavirus* in the *nodaviridae* family, is seen in both marine and freshwater fish in many parts of the world. It causes disease in larvae and juveniles, rarely in adult fish. Since it causes significant economic losses, in the recent years the research has been especially focused on the infections related to it. In this study, the presence of betanodavirus was investigated in Koi and goldfish species in aquarium facilities operating in the Antalya region. A total of 180 fish, at least 30 representing each species, were sampled from three different aquarium facilities and tested by the Real Time RT-PCR (RT-qPCR) method. At the end of the study, *betanodavirus* RNA was not found in any of the samples. With this study, the presence of *betanodavirus* in koi and goldfish species in Türkiye was investigated for the first time by the molecular techniques.

Keywords: *Betanodavirus*, Goldfish, Koi, Real Time RT-PCR (RT-qPCR), Türkiye

Alıntılama

Oğuz U, Kaplan M, Kalaycı G. 2023. Koi (*Cyprinus carpio*) ve Japon Balıklarında (*Carassius auratus*) Betanodavirus Varlığının Araştırılması LimnoFish. 9(1): 37-42. doi: 10.17216/LimnoFish.1141342

Giriş

Akvaryum balığı yetiştiriciliğinde koi ve japon balıkları, üretimi en çok yapılan balık türleri arasında ilk sıralarda yer almaktadır. Ayrıca ülkemizin sıcak iklime sahip bölgelerinde giderek artan sayıda akvaryum balığı yetiştiricilik tesisleri açılmaktadır. Ancak akvaryum balığı yurtiçinde talebe karşın arzın çok az bir kısmı ülkemizde yetiştiricilik yolu ile sağlanırken halen farklı ülkelerden ithalat ile talep karşılanmaya çalışılmaktadır (Kılıçerkan ve Çek 2011; Pala ve Yılmaz 2020). Üretimin giderek artmasına paralel olarak hastalıklar ile mücadele de önem kazanmaktadır. Bu doğrultuda balıklarda enfeksiyonlara yol açan patojenlerin belirlenmesi geleceğe yönelik araştırmalar için temel oluşturması bakımından önem arz etmektedir.

Betanodavirus, *Nodaviridae* familyasında yer alan zarsız, ikosahedral simetrili, 25-30 nm boyutunda, tek sarmallı, pozitif polariteli, iki segmentli RNA [RNA1 (3.1 kb) ve RNA2 (1.4 kb)] genomunu içeren bir virustur (Nishizawa vd. 1997). RNA1 genomik bölgesi virusun replikasyonundan sorumlu olup, RNA2 genomu ise konakçı spesifitesi ve tropizmden sorumlu genom segmentleridir (Nagai ve Nishizawa 1999; Panzarin vd. 2014). RNA2 genomik bölgesindeki değişken T4 sekansına göre gerçekleştirilmiş filogenetik analize göre etkenin dört genotipi tanımlanmıştır. Bunlar sırasıyla SJNNV (Stripped jack nervous necrozis virus), TPNNV

(Tiger puffer nervous necrozis virus), BFNNV (Barfin flounder nervous necrozis virus) ve RGNNV (Red-spotted grouper nervous necrozis virus) genotipleridir (Nishizawa vd. 1997). Ayrıca su sıcaklığı ile hastalık semptomlarının görülmesi arasında da güçlü bir korelasyon vardır (Iwamoto vd. 2000).

Horizontal bulaşta kontamine su, en önemli etmenlerdendir. Bunun yanında canlı balık yemleri (rotifer, artemia) ve kurtçuklar ile balıkçıl kuşların da rezervuar olabileceği düşünülmektedir (Vázquez-Salgado vd. 2020). Virusun yetişkin balıkların gonadlarında da bulunabildiğine dair çalışmalar mevcut olduğundan vertikal bulaşmadan da söz edilebilir (Mushiake vd. 1994, Dalla Valle vd. 2000).

VNN, balıkların önemli viral enfeksiyonlarındandır ve üreticilerin büyük çaplı ekonomik kayıplar yaşamalarına neden olmaktadır. Dünya genelinde 120'den fazla balık türünde virusun izole edildiği bildirilmiştir (Chi vd. 2003; Furusawa vd. 2007; Maltese ve Bovo 2007; Bovo vd. 2011; Vendramin vd. 2013). Virus; beyin, medulla spinalis, optik sinir ve retinal dokulara affinite göstermektedir. Yapılan histolojik muayenelerde etkenin beyin ve retina dokularında vakuolleşmelere yol açtığı bu nedenle balıklarda anormal yüzme davranışları ve görme kaybı gibi sinirsel semptomların ortaya çıktığı bildirilmektedir (Nishizawa vd. 1997). Ayrıca betanodavirusların akuatik çevreye oldukça dirençli olduğu, hem tatlı su balıklarında hem deniz balıklarında enfeksiyona neden olabileceği bildirilmektedir (Vendramin vd. 2013). Etkenin tanısında Real Time RT-qPCR yöntemi ile viral nükleik asit tespitinin spesifitesinin diğer tanı yöntemlerine oranla daha yüksek olduğu bildirilmiştir (Anonim 2019).

Bu çalışmanın amacı, akvaryum balığı yetiştiriciliği fazlaca yapılan Koi (*Cyprinus carpio*) ve Japon Balığı (*Carassius auratus*) türlerinde *betanodavirus* varlığının RT-qPCR yöntemi ile moleküler olarak araştırılmasıdır.

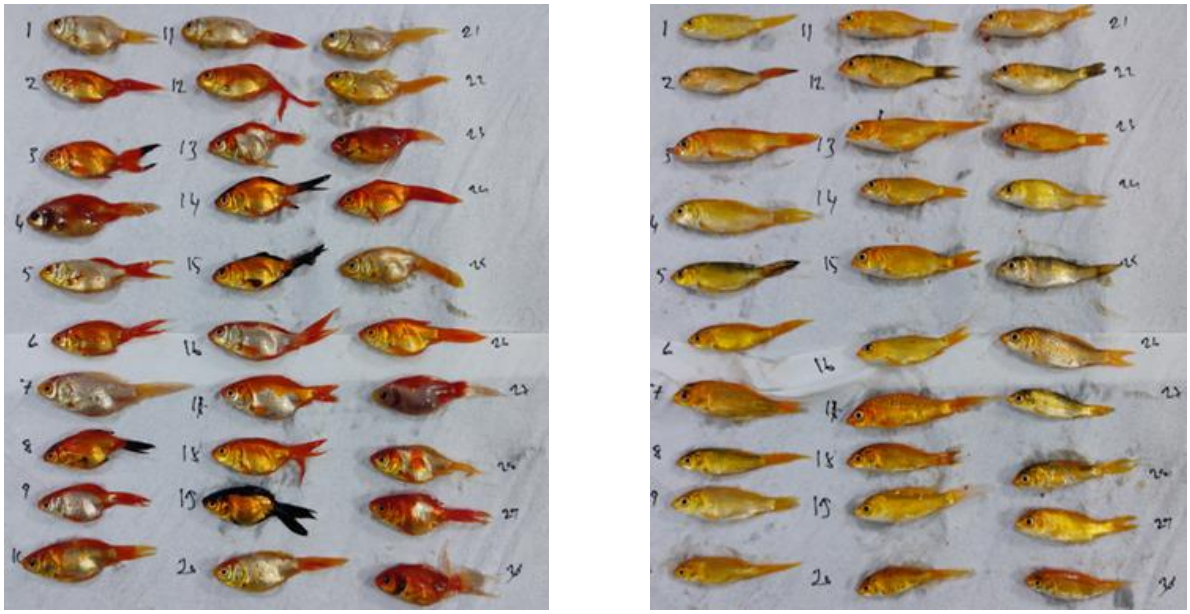
Materyal ve Metot

Örneklerin Temini

Araştırmada kullanılacak koi ve japon balığı türlerinden Antalya ilinde yetiştiricilik faaliyeti gösteren 3 farklı akvaryum işletmesinden her bir türden 30'ar adet olmak üzere toplamda 180 adet balık numunesi alınmıştır (Çizelge 1). Örneklerin alındığı işletmelere A, B ve C kodları, Japon balığı için 'J', Koi balığı için 'K' kodları verilmiştir. Örneğin; A işletmesinden alınan 10 numaralı Japon balığı numunesine verilen kod, AJ10 olarak oluşturulmuştur. Ayrıca balık refahı açısından çalışmaya başlamadan önce yerel etik komitesinden Etik Kurul onayı alınarak çalışmaya başlanıldı. Numune alırken özellikle anormal yüzme davranışı gösteren letarjik balıklar tercih edildi. Alınan balık numunelerinden Japon balıklarının ortalama boyu 7 cm, Koi balıklarının ise ortalama boyu 8 cm olarak ölçülerek kaydedildi. Numunelerin alındığı havuz sıcaklığı Koi balığı için 23 °C, Japon balığı için ise 24°C olarak kaydedilmiştir.

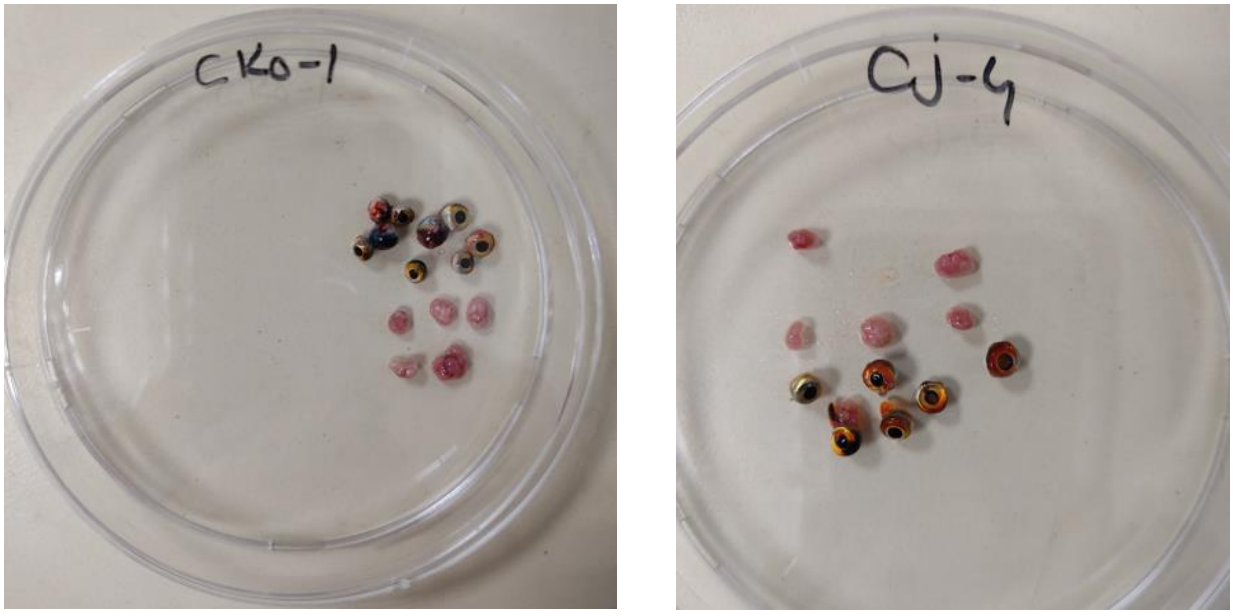
Örneklerin Hazırlanması

Uygun şartlarda alınan numunelere ötenazi (fenoksietanol) uygulanarak bilgiler kaydedildi (Şekil 1). Her 5 balık bir örnek olacak şekilde balıkların beyin ve gözleri alınarak steril petrilere konuldu (Şekil 2). Steril makas ve pensten oluşan set, her 5 balık için ayrı ayrı kullanıldı. Her bir petri kutusundaki numuneler steril kum ve daha önceden %2 fötal dana serumu – %1 antibiyotik antimikotik solüsyon ilave edilen glutaminli L-15 vasat kullanılarak homojenize edildi. Elde edilen homojenizatlara 3500 rpm'de +4 °C'de 15 dakika santrifüj işlemi uygulandı. Elde edilen süpernatantlar 0.45 µm filtreden geçirilerek 2 ml hacimli dondurma ampullerine taksim edilerek ekstraksiyon işlemi yapılana kadar -80°C'de stoklandı.



Şekil 1. Üç farklı yetiştirme işletmesinden alınan Koi (*Cyprinus carpio*) ve Japon balığı (*Carassius auratus*) numuneleri

Figure 1. Koi (*Cyprinus carpio*) and goldfish (*Carassius auratus*) samples from three different breeding farms



Şekil 2. Koi (*Cyprinus carpio*)'ye ait beyin ve göz organları (sol) ile Japon balığına (*Carassius auratus*) ait beyin ve göz organlarının steril petriye alınması (sağ).

Figure 2. Removal of brain and eye organs of Koi (*Cyprinus carpio*) (left) and brain and eye organs of goldfish (*Carassius auratus*) in sterile petri dishes (right).

Nükleik asit Ekstraksiyonu ve RT-qPCR

Nükleik asit ekstraksiyonunda, ticari ekstraksiyon kiti (MagNA Pure LC Total Nucleic Acid Isolation Kit, Roche, Germany) kullanılmış olup, ekstraksiyon işlemi otomatik ekstraksiyon cihazı (Roche MagNA Pure LC System) kullanılarak üreticinin talimatları doğrultusunda yapıldı.

Amplifikasyonda, ticari kit (Real Time Ready Virus Master, Roche, Germany) kullanıldı. OIE (2019)'nin önerdiği ve Panzarin (2010) tarafından geliştirilen, virusun RNA2 segmentinin T4 değişken bölgesine göre (69bp) dizayn edilen **RNA2 FOR**: CAA-CTG-ACA-RCG-AHC-ACA-C, **RNA2 REV**: CCC-ACC-AYT-TGG-CVA-C ve **RNA2 Probe**: TYC-ARG-CRA-CTC-GTG-GTG-CVG primer prob setleri kullanıldı. Bu çalışmada, Panzarin ve ark. (2010) tarafından geliştirilen, Kaplan ve Karaoğlu (2021)'in laboratuvar ortamında optimize ettiği metod kullanılmıştır.

RNA ekstraksiyonu ve RT-qPCR işlemlerinde pozitif kontrol virus olarak Istituto Zooprofilattico Sperimentale delle Venezie, Dipartimento di

Ittıopatologia, Italy'den sağlanan Balık Hastalıkları Ulusal Referans Laboratuvarı İzmir/Bornova Veteriner Kontrol Enstitüsü Viroloji Bölümü stoklarında bulunan referans betanodavirus suşu (475/198) kullanıldı.

Bulgular

İncelemesi yapılan her iki türe ait (Japon balığı ve Koi balığı) toplam 180 balık numunesinde *betanodavirus* RNA'sı tespit edilmemiştir (Çizelge 1).

Çizelge 1. Örnekleme yapılan yetiştirme tesisleri, örneklere ait bilgiler ve sonuçlar

Table 1. Sampling breeding facilities, information about the samples and the results

Sıra	İl/İlçe	İşletmeye Verilen Kod	Tür	Örnekleme Tarihi	Örnek Kodu	Örnek Sayısı	Sonuç
1	Antalya/Döşemealtı	A	Koi	20.12.2021	AK-1/30	30	Negatif
2	Antalya/Döşemealtı	A	Japon	20.12.2021	AJ-1/30	30	Negatif
3	Antalya/Serik	B	Koi	21.12.2021	BK-1/30	30	Negatif
4	Antalya/Serik	B	Japon	21.12.2021	BJ-1/30	30	Negatif
5	Antalya/Kepez	C	Koi	22.12.2021	CK-1/30	30	Negatif
6	Antalya/Kepez	C	Japon	22.12.2021	CJ-1/30	30	Negatif

Tartışma ve Sonuç

Son yıllarda gerek deniz kafes balıkçılığı gerekse tatlı su balığı yetiştiriciliği yapılan işletmelerde patojen viral etkenlerin neden olduğu hastalıklar daha çok ortaya çıkmaktadır. Bu durum karşısında koruyucu hekimlik uygulamaları dünden bugüne daha fazla önem arz etmektedir.

Viral Nervöz Nekrozis hastalığı, larval dönemdeki ve juvenil diye adlandırılan yavru balıklarda % 100'e varan mortalite oranı ile özellikle deniz balıklarında ölümlere neden olmaktadır. Dünyada hızla yayılım gösteren *betanodavirus* salgınları son yıllarda ülkemizde de yetiştiricilik tesisleri ile kuluçkahanelerde de görülmeye başlamıştır (Kalaycı vd. 2016; Kaplan ve Karaoğlu 2021; Kaplan vd. 2021; Kaplan vd. 2022).

Ülkemizde 2011 yılında Mersin'de kültürü yapılan bir levrek işletmesinde *betanodavirus*'a ilk kez rastlanılmıştır (Özkan Özyer vd. 2014). Ege bölgesinde faaliyet gösteren levrek kuluçkahanesinden ise 2014 yılında VNN hastalığının belirtilerini gösteren balıklar örneklenmiş ve bu balıklarda da *betanodavirus* pozitifliği tespit edilmiştir (Kalaycı vd. 2016). Akdeniz bölgesinde bulunan bir kuluçkahanede levreklerde *betanodavirus*'a rastlanılmış olup yapılan filogenetik analiz sonucu virusun RGNNV genotipi olduğu tespit edilmiştir (Kaplan ve Karaoğlu 2021). Karadeniz bölgesinde ise levreklerde tespit edilmiş ve yine RGNNV genotipi olduğu belirlenmiştir (Kaplan vd. 2021). Ege bölgesinde çiftlik şartlarında yetiştiricilik yapan bir işletmede ise çipura türü balıklarda *betanodavirus* tespit edilmiş olup ilk kez reasortant bir suşa (RGNNV/SJNNV) rastlanılmıştır (Kaplan vd. 2022).

Hindistan'da 2006 yılında yapılan bir araştırmada asemptomatik Japon balıklarında (*Carassius auratus*) *betanodavirus* tespit edilmiştir (Jithendran vd. 2011). Asya ülkelerinde akvaryum balığı olarak yetiştirilen medaka türü (*Oryzias latipes*) balıkların da yapılan araştırmalarda *betanodavirus*'a karşı duyarlı olduğu ve virusa maruz bırakılan balıklarda klinik semptomların gözlemlendiği tespit edilmiştir (Furusawa vd. 2006). Son yıllarda yapılan çalışmalarda *betanodavirus*'ların tatlı su türlerinde de birçok araştırmacı tarafından bildirilmiş olması, araştırmaların bu yönde de yapılması gerektiği kanısını güçlendirmektedir (Keawcharoen vd. 2015; Bandín ve Souto 2020). Tatlı su türlerinden Rus Karaca Mersin balığı (*Acipenser gueldenstaedtii*), Yılan balığı (*Anguilla Anguilla*), Japon balığı (*Carassius auratus*), Lepistes (*Poecilia reticulata*), Tilapya (*Oreochromis niloticus*) ve Yayın balığı (*Parasilurus asotus*) gibi türlerde *betanodavirus* tespit edilmiştir (Chi vd. 2003; Athanassopoulou vd. 2004; Bigarré vd. 2009; Jithendran vd. 2011; Bandín ve Souto 2020). Ayrıca suyun tuzluluk oranının hastalığın ortaya çıkışı ya da viral replikasyonda herhangi bir bağlantısının olmadığı da bildirilmektedir (Bovo vd. 2011). Bu durum her ne kadar betanodavirusların deniz balıklarında daha çok görülse de tatlı su türlerinin de enfeksiyona açık olduğunu ve yeni türlerde de yayılım gösterebileceğini desteklemektedir. Su sıcaklığı ve balık türlerinin çeşitliliği konakçı durumunu doğrudan etkilemekte ve farklı genotiplerin ortaya çıkmasına ve hatta reasortant suşların (RGNNV/SJNNV, SJNNV/RGNNV) da salgınlara neden olabileceğini göstermektedir (Toffan vd. 2017, Panzarin vd. 2012). Virusun genomunun segmentli yapısı türler arasında mutasyona sebebiyet verip, virusun yeni balık türleri için de tehdit olabileceğini düşündürmektedir (Hegde vd. 2003). *Betanodavirus* salgınlarının deniz balıklarının yanı sıra son yıllarda tatlı su türlerinde de görüldüğü bildirilmektedir (Hegde vd. 2003; Gomez vd. 2006). Bu sebeple hem deniz balıklarında hem de

tatlı su türlerinde daha çok araştırmaya ihtiyaç duyulmaktadır.

Son otuz yıl içinde betanodaviruslar ile ilgili çok fazla araştırma makalesi yayımlanmıştır. Ancak; virüs konakçı ilişkileri, türler arasında genotipik değişimler, çevresel koşullar, hastalığın yayılımı ve küresel ısınmanın da virusun epidemiyolojisine etkisi de dahil çok fazla araştırmaya ihtiyaç vardır (Bandín ve Souto 2020). Giderek daha geniş coğrafyalarda yayılım gösteren *betanodavirus* enfeksiyonlarının sadece deniz balıklarında değil, tatlı su yetiştiriciliği yapılan kuluçkahane ve tesislerde de tespit ediliyor olması araştırma çalışmalarının bu yönlere de evrilmesi gerektiğini göstermektedir. Ornamantel balıklar olarak adlandırılan tatlı su akvaryum türlerinin kısıtlı, dar ve küçük kapasiteli alanlarda farklı türlerin yakın ilişkide olduğu düşünüldüğünde *betanodavirus* gibi birçok viral hastalığın bulaşması daha kolay olacaktır. Bu sayede virusa duyarlı olan türlere yeni türlerin eklenmesi de kaçınılmaz olacaktır.

Sonuç olarak bu çalışma ile Türkiye'de akvaryum balıklarında *betanodavirus* varlığı ilk kez araştırılmıştır ve 3 farklı akvaryum balığı yetiştiren işletmeden alınan koi ve japon balığı türlerinde *betanodavirus* tespit edilmemiştir. Akvaryum balıklarının *betanodavirus* epidemiyolojisindeki rollerinin daha iyi anlaşılması için, ileride farklı türleri de içeren daha geniş çalışmaların yapılmasının yararlı olacağı düşünülmektedir.

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Insect Larval Meal as A Possible Alternative to Fish Meal in Rainbow Trout (*Oncorhynchus mykiss*) Diets: Black Soldier Fly (*Hermetia illucens*), Mealworm (*Tenebrio molitor*)

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ABSTRACT

This study was conducted to determine the effect on growth performance and intestinal histomorphology of the rainbow trout (*Oncorhynchus mykiss*) fed diets including black soldier fly (*Hermetia illucens*) prepupae meal (HI) and mealworm (*Tenebrio molitor*) larvae meal (TM) used instead of fish meal. Six diets with HI and TM at three inclusion levels (10%, 20% and 30%) and a control diet based on fish meal were prepared. Test diets were encoded as control, HI10, HI20, HI30, TM10, TM20 and TM30. Fish (average initial weight of 34.17±0.88 g) were randomly placed (30 for each) in 500 L fibreglass tanks. Each of the seven diet treatments was tested in triplicated tanks. Fish were fed by hand at a level of 2.5% of body weight three times a day for 90 days. Results demonstrated that the growth performance and intestinal histomorphology were significantly affected by black soldier fly (HI) prepupae meal substitution ($p<0.05$). HI prepupae meal used instead of the fish meal had a negative effect on the growth performance, but not on the intestinal villi length. In addition, intestinal villi width decreased in fish on diets containing 10% or 20% HI prepupae meal. The growth performance and intestinal histomorphology were significantly affected by diets including TM. The diets containing 20% and 30% TM meal significantly decreased growth performance variables, but intestinal villi length increased. The results suggest that mealworm meal (10%) can be included in diets of rainbow trout at a level of 10% instead of fish meal without adversely affecting growth performance. Future studies should be expanded using a highly defatted TM and HI larvae meals.

Keywords: Aquaculture, trout, nutrition, growth, villus

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Gökkuşağı alabalığı (*Oncorhynchus mykiss*) yemlerinde balık ununa alternatif olarak böcek unu: Siyah asker sineği (*Hermetia illucens*), Un kurdu (*Tenebrio molitor*)

Öz: Bu çalışma balık unu yerine siyah asker sineği (*Hermetia illucens*) prepupa unu (HI) ve un kurdu (*Tenebrio molitor*) larva unu (TM) ile beslenen Gökkuşağı alabalığı (*Oncorhynchus mykiss*)'nın büyüme performansı ve bağırsak histomorfolojisi üzerine etkisini belirlemek için yapılmıştır. Bu amaçla, üç farklı seviyede (%10, %20 ve %30) HI ve TM içeren altı diyet ve balık unu esaslı bir kontrol diyeti test edilmiştir. Test diyetleri sırasıyla Kontrol, HI10, HI20, HI30, TM10, TM20 ve TM30 olarak kodlanmıştır. Balıklar (ortalama başlangıç ağırlığı 34,17±0,88 g) 500 L'lik fiberglas tanklarda (112x112 cm kare, 40 cm derinlikte) rastgele (her biri için 30) dağıtılmıştır. Yedi muamele 3 tekerrürlü olarak denenmiştir. Balıklar 90 gün boyunca günde üç kez vücut ağırlığının %2,5'i oranında elle beslenmiştir. Büyüme performansı ve bağırsak histomorfolojisi ile ilgili veriler siyah asker sineği (HI) prepupae unu ikamesinden önemli ölçüde etkilendiğini göstermiştir ($p<0,05$). Balık unu yerine kullanılan BSF prepupa unu büyüme performansı üzerinde olumsuz bir etki göstermişt ancak bağırsak villus uzunluğu üzerinde olumlu etki göstermiştir. Ek olarak, %10 veya %20 HI prepupa unu içeren diyetle beslenmede bağırsak villus genişliği azalmıştır. Büyüme performansı ve bağırsak histomorfolojisi, diyetsel un kurdu unu düzeylerinden önemli ölçüde etkilenmiştir. Zira, %20 ve %30 un kurdu unu içeren diyetlerle beslemede balıkların büyüme performansı azalırken, bağırsak villus uzunluğu artış göstermiştir. Sonuçlar, büyüme performansı üzerinde herhangi bir olumsuz etki göstermeksizin balık unu yerine un kurdu ununun (%10) kullanılabileceğini göstermiştir. Bununla birlikte, ilerideki çalışmaların yağı yüksek oranda alınmış un kurdu ve siyah asker sineği larva unu kullanımı üzerine genişletilmesi önerilerilebilir.

Anahtar kelimeler: Yetiştiricilik, alabalık, besleme, büyüme, villus

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Introduction

Aquaculture is the fastest-growing food-producing sector globally, with worldwide finfish production increasing by 90% during the last decade (Rimoldi et al. 2019). The expansion of the aquaculture industry means an increase in the need for high-quality fishmeal, especially for carnivorous species (Secci el al. 2019). One of the most critical issues that threatens the sustainability and further growth of intensive aquaculture of carnivorous species is the dependence on fishmeal and fish oil in aquafeeds (Cardinaletti et al. 2019). Given that this resource is now limited with the decrease in global availability and the rising price, the aquaculture industry is forced to find more sustainable and cost-effective alternatives to fish meal (Secci el al. 2019). Protein-rich vegetable raw materials used in practical diets for carnivorous fish species have many drawbacks such as relatively low protein content, unbalanced essential amino acid profiles, low palatability, presence of antinutrients and competition with other food-feed industry sectors. Nutritional strategies are being developed to find other alternatives to fishmeal and improve the use of plant protein-based diets (Magalhaes et al. 2017). Fishmeal and soybean meal widely used as protein sources in aquaculture feeds have become unsustainable due to the progressive depletion of wild marine fish stocks and the considerable environmental cost of protein-rich plant cultivation. The most promising animal alternatives to fish meal are nonruminant by-product meals and insect meals (Rimoldi et al. 2019). Compared to conventional animal protein, insects have several advantages such as cultivability on discarded organic by-products with low water input, high feed conversion efficiency, emission of low levels of greenhouse gases and ammonia, few animal welfare issues, and low risk of transmitting zoonotic infections (Magalhaes et al. 2017). Edible insects are considered potential feed source because they contain quality protein and other nutrients (Su et al. 2017). Insects grow and reproduce quickly, have high feed efficiency, do not compete with humans and other farm animals, and are part of the natural diet of many species (Iaconisi et al. 2019). Yellow mealworm (*Tenebrio molitor*) is among authorized insect species for nutrition, and it feeds on several grains, flour, and derived products. Yellow mealworm larvae can be easily cultured with low-nutritive plant and animal waste products, and they are commercially used as pet food for birds and reptiles or fishing baits.

T. molitor larvae meal having 47–60% protein and 31–43% lipids (Henry et al. 2018) was used as a feed ingredient for several fish species such as gilthead sea bream (*Sparus aurata*) (Iaconisi et al. 2019; Piccolo et al. 2017), yellow catfish (*Pelteobagrus fulvidraco*) (Su et al. 2017), rainbow trout (*Oncorhynchus mykiss*) (Chemello et al. 2020; Henry et al. 2018), common catfish (*Ameriurus melas*) (Roncarati et al. 2015). Taking into account the fish growth performance and the diets nutrient utilization, *T. molitor* is suitable for the partial replacement of fish meal in aquafeeds for a variety of fish species such as African catfish, tilapia, yellow catfish, common catfish, rainbow trout, European sea bass, gilthead sea bream, blackspot sea bream, and red seabream (Iaconisi et al. 2019). The attention of the aquafeed industry has mainly been directed to the use of insects since compared to other animal protein sources, they, in particular, flies show several advantages (Rimoldi et al. 2019). In recent years, research and risk assessment studies have also been carried out on the larvae of the *Hermetia illucens* (Dumas et al. 2018). Its potential as a feed ingredient has been reported for poultry, pigs, Atlantic salmon, channel catfish and blue tilapia, Nile tilapia, rainbow trout and turbot (Stadtlander et al. 2017). The crude protein and crude lipid contents of *H. illucens* larvae meal can vary from 40 to 54% and 15 to 49% (dry matter basis), respectively, depending on the substrates used to grow the insect larvae and processing methods (Dumas et al. 2018). A major drawback in using *H. illucens* meal is its lack of long-chain polyunsaturated fatty acids such as EPA and DHA and the presence of chitin. When 30% full-fat *H. illucens* prepupae meal was used in channel catfish and 15% in rainbow trout, a comparable growth performance to fish fed on fish meal-based diets was observed (Cardinaletti et al. 2019).

Many monitoring indices such as growth performance, diet digestibility, liver enzyme activities (Chemello et al. 2020), gut microbiota (Rimoldi et al. 2019), fillet fatty acid profile (Secci et al. 2019), blood biochemistry, liver and intestine histology, gene expression of cytokines (Cardinaletti et al. 2019), immune response, intestinal antioxidant enzymes (Henry et al. 2018) have been carried out in studies on rainbow trout. Even a study was also conducted on the possible effect of soldier fly on villus length and width (Dumas et al. 2018). However, no study has been found on villi histomorphology of TM, and the information obtained about both insect types is quite limited. With this study, the use of alternative insect meal in fish diets will contribute to the investigation of the gut absorption capacity. More studies are required to better understand the nutritional values of HI and TM for rainbow trout. Therefore, this study was planned to determine the effects of diets containing full-fat HI

and TM meals substitution of fishmeal on growth performance and intestinal histomorphology in rainbow trout (*O. mykiss*).

Materials and Methods

Fish and Trial Design

This study was carried out in a freshwater research unit of Fisheries Research Institute, Elazığ, Türkiye. Rainbow trout (*O. mykiss*) used in the study were obtained from a private commercial farm located in Elazığ. After 21 days of adaptation, fish with an initial average weight of 34.17±0.88g were randomly distributed to 500 L (112x112 cm) experimental tanks with 40 cm depths. Each experimental tank was stocked with 30 fish. Seven experimental groups (Table 1), including a control diet, three *T. molitor* meal diets with TM inclusion levels of 10, 20 and 30% (coded as TM10, TM20, TM30, respectively) and three *H. illucens* prepupae meal diets with 10, 20 and 30% HI (coded as HI10, HI20 and HI30), respectively were tested in the study. All ingredients except the fish oil were mixed and extruded, and then fish oil penetrated with vacuum coating to feeds prepared. Trial feeds were prepared in a private enterprise after the rations were calculated. Feeds with 4 mm inner diameter were kept at room conditions and in a cool environment during trial period. Fish were fed by hand 2.5% of body weight three times a day (08:30, 12:30 and 16:00) for 90 days. Each experimental treatment was tested in triplicate. Water temperature (14.13±1.75ºC), pH (8.60±0.32), oxygen (8.39±0.64 mg/l) and mortality were checked daily. The water flow rate to each tank was adjusted to change the total volume 20 times per day. The tanks were daily cleaned by siphoning. 14 hours of darkness and 10 hours of light were applied. Fish were slightly anesthetised with 50 mg L^{-1} benzocaine (Oswald 1978) after by 16 hr of starvation for analyses. At the end of the experiment, fish were randomly selected from each tank and analyzed for growth performance and histomorphologic measurements.

Table 1. Formulation and proximate composition of experimenteal diets (%)

Ingredients, %	Control	HI10	HI20	HI30	TM10	TM20	TM30
Fish meal[1]	30	20	10	0	20	10	0
Soybean meal	21	21	21	21	21	21	21
Sunflower meal	9	9	9	9	9	9	9
Pea protein	6	6	6	6	6	6	6
Wheat gluten	4	6.5	8.9	11.4	5.4	6.8	8.2
Wheat flour	9	8.4	7.9	7.3	8.8	8.6	8.4
Corn gluten	9	9	9	9	9	9	9
Black soldier fly[2]	0	10	20	30	0	0	0
Mealworm[3]	0	0	0	0	10	20	30
Fish oil	11	9.1	7.2	5.3	9.8	8.6	7.4
Vitamin mix[4]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral mix[5]	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin C	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Proximate composition							
Crude protein	46.22	46.23	46.18	46.20	46.23	46.22	46.22
Crude lipid	15.35	15.36	15.38	15.39	15.34	15.35	15.36
Ash	8.77	8.81	7.71	7.50	6.60	8.62	8.54
Moisture	4.66	4.34	4.11	4.31	4.17	4.44	4.42

1 Fish meal was a mixture of European sprat (*Sprattus sprattus)* and Atlantic herring (*Clupea harengus*) meals containing 65 % of crude protein and 8.5 % of crude lipid.

2 Black soldier fly contained approximately 53 % protein and 24 %lipid

3 Mealworm contained approximately 45 % protein and 31 % lipid

4 Supplied the following (mg/kg diet): Vitamin A, 20000 IU, vitamin D 2000 IU, vitamin E 200 mg, vitamin K3 12 mg, vitamin B1 20 mg, vitamin B2 30 mg, calcium D pantothenate 50 mg, vitamin B6 20 mg, vitamin B12 0.05 mg, niacin 6 mg, folic acid 0.5 mg, biotin 200 mg, vitamin C 200 mg, inositol 300 mg

5 Supplied the following (mg/kg diet): coper sulphate 10 mg, manganese oxide 50 mg, cobalt mono-carbonat 0.15 mg, zinc oxide 50 mg, calcium iodate 0.8 mg, sodium selenite 0.15 mg, ferric sulphate 50 mg

Diet Composisiton Analysis

Analysis of each diet was performed according to the standard methods of Helrich (1990): crude protein by Kjeldahl procedure, crude lipid by Soxhlet method, ash by drying in a muffle furnace and moisture using a forced-air oven.

Growth Indices

Growth performance was calculated using the following equations.

Spesific growth rate (SGR) % = 100 x [(In Final weight – In Initial weight) / days]

Weight gain (WG) = (In Final weight – In Initial weight)

Feed conversion ratio (FCR) = (Feed intake / Weight gain)

Survival rate (SR) = 100 x (Final number of fish / Initial number of fish)

Histomorphological Analysis

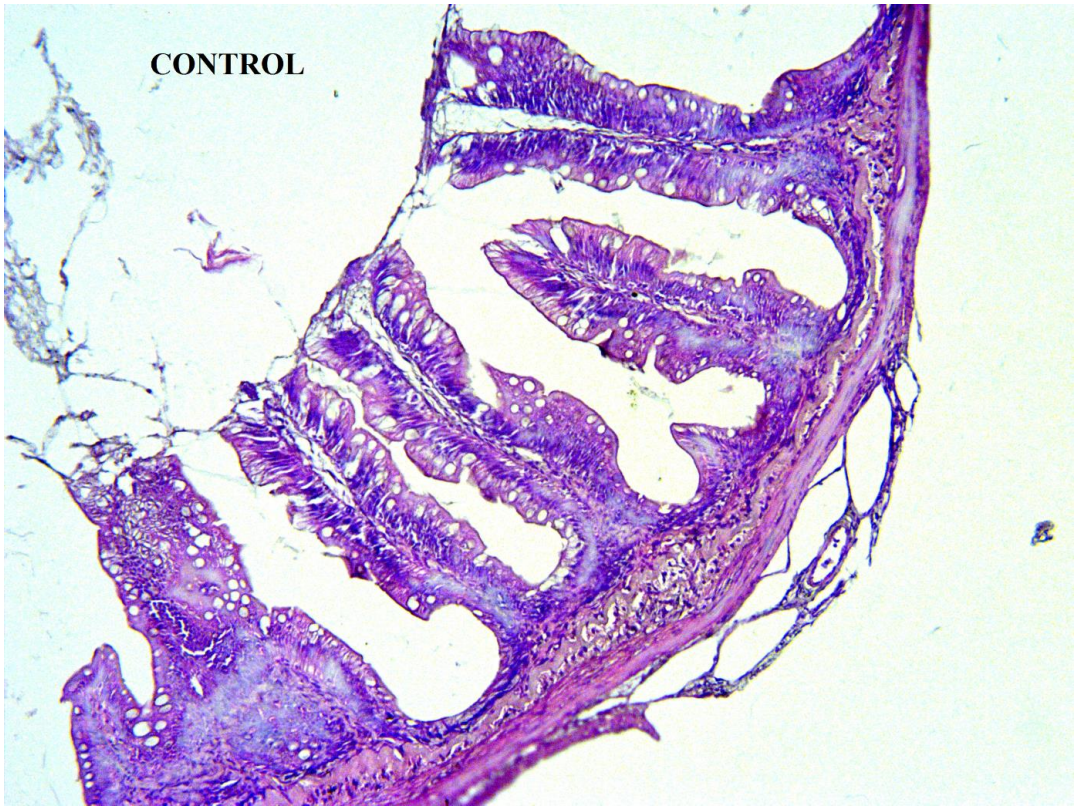
In histomorphological studies, tissues were cut as 1.0 cm pieces and placed into 10% formalin for dehydration. In the dehydration process, tissues were placed into cassettes and embedded in paraffin blocks. Then, cooled paraffin blocks were cut with a microtome equipped with the blade at 5µm. The tissue samples were transferred into slides and stained with hematoxylin and eosin solutions. Finally, stained samples were analyzed with ZEISS Primostar HD Light microscope and evaluated using an image processing system. Muscularis thickness, villi length (VL) and villi width (VW) were measured from ten fish from each group for histomorphologic analyses.

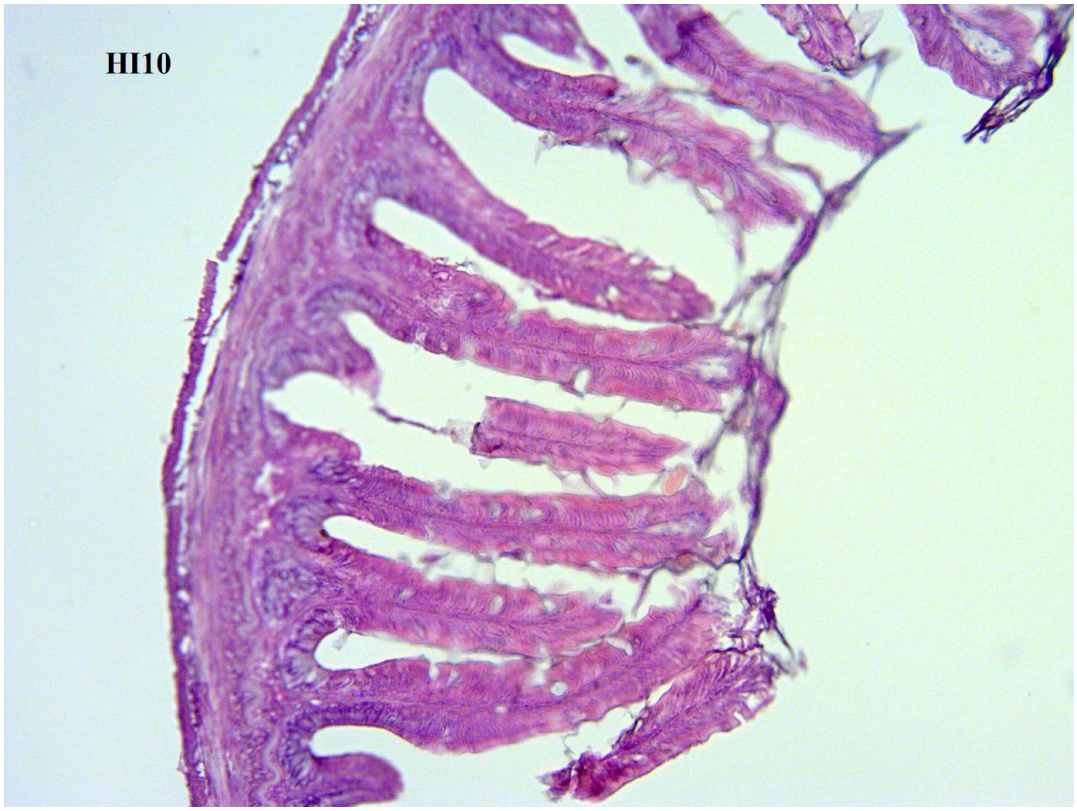
Data Analysis

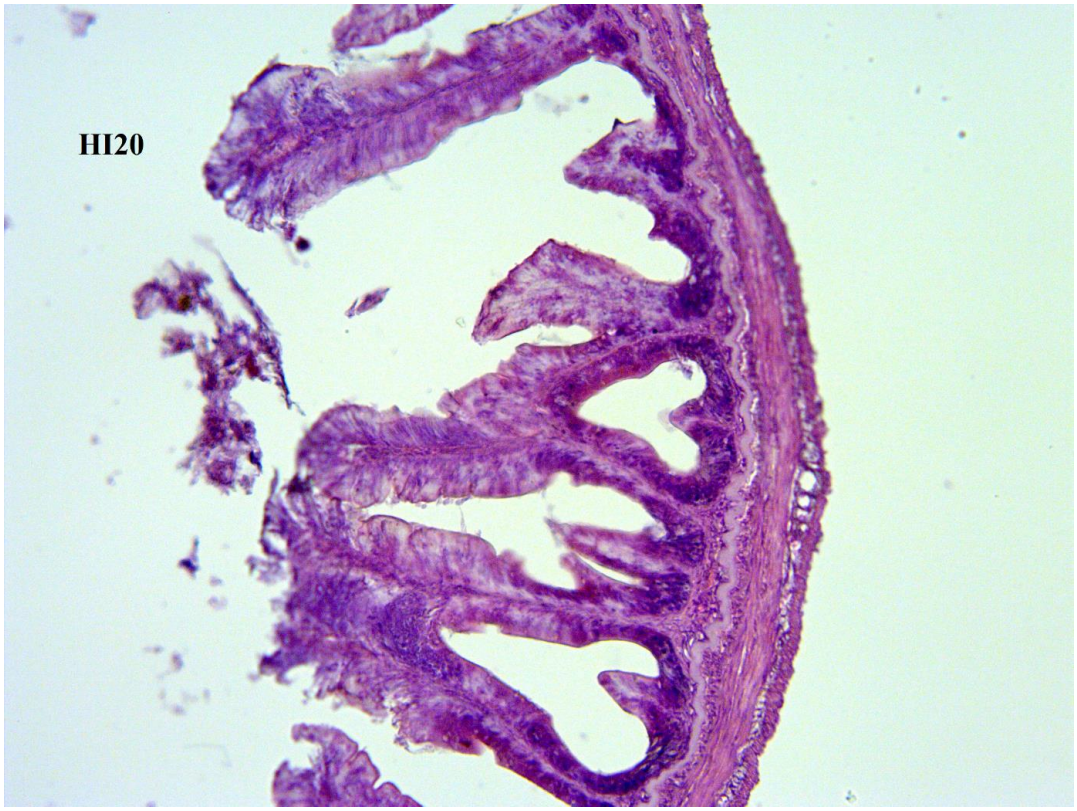
Results were reported as means with standard errors. Data were submitted to one-way analysis of variance with SPSS 15. Duncan's multiple range test was performed for the significance of differences of means among the groups. The data were considered significant at the $P<0.05$ level. Two-way ANOVA analysis showed that the growth performance and intestinal histomorphology affected both insect meal and levels.

Results

Results are shown in Table 2, Table 3 and Figure 1. The growth performance and intestinal histomorphology were significantly influenced by dietary TM, and HI meal supplementation ($P<0.05$). Black soldier fly meal significantly decreased the growth performance, including FBW, FCR, WG and SGR. However, HI meal at a level of 10% did not affect feed intake. Besides, FI, FW, FCR, WG and SGR in the group fed with control diet were similar to those fed with dietary TM at a level of 10% ($p>0.05$). TM meal at a level of 30% significantly decreased the growth performance. Additionally, TM meal at a level of 20% significantly decreased the FW, WG and SGR. The growth performance decreased with the increase of dietary TM levels.




HI10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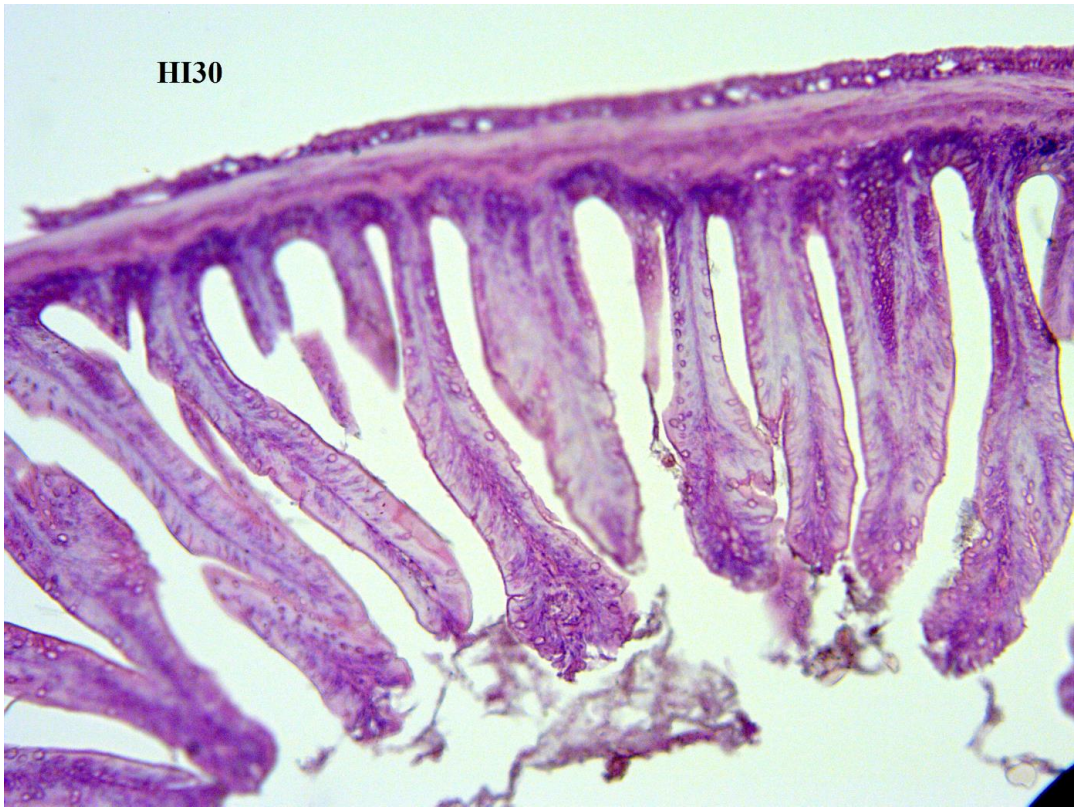
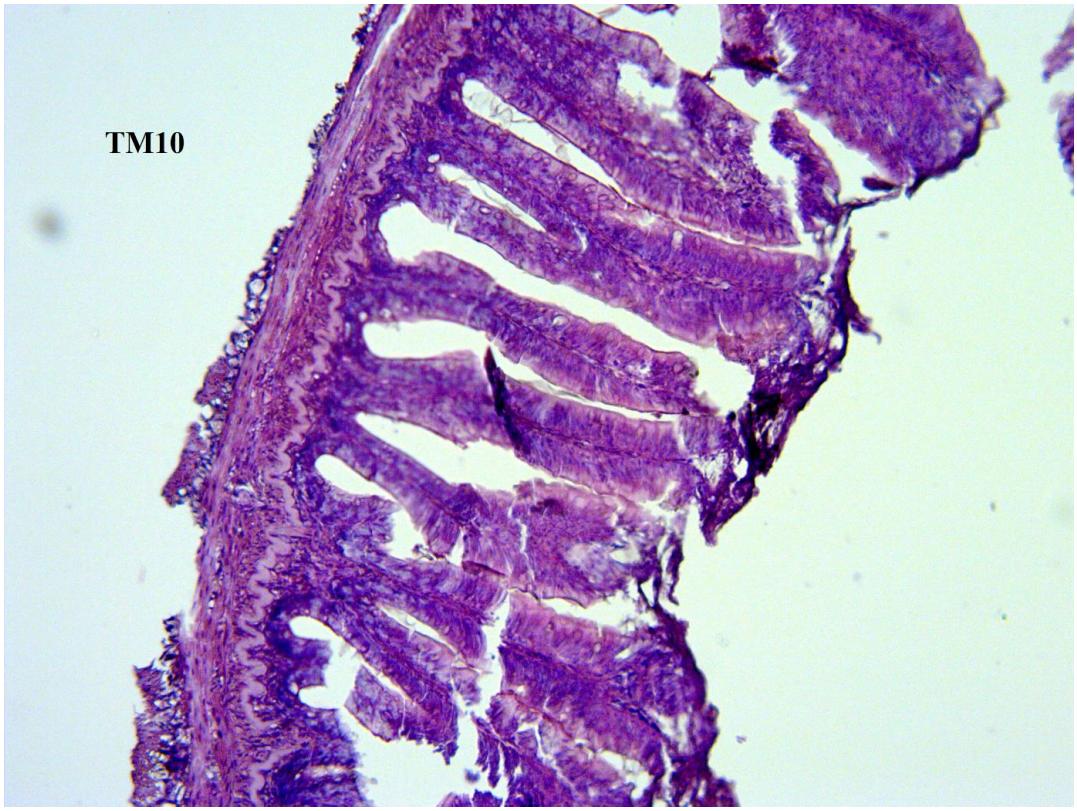

HI30


TM10

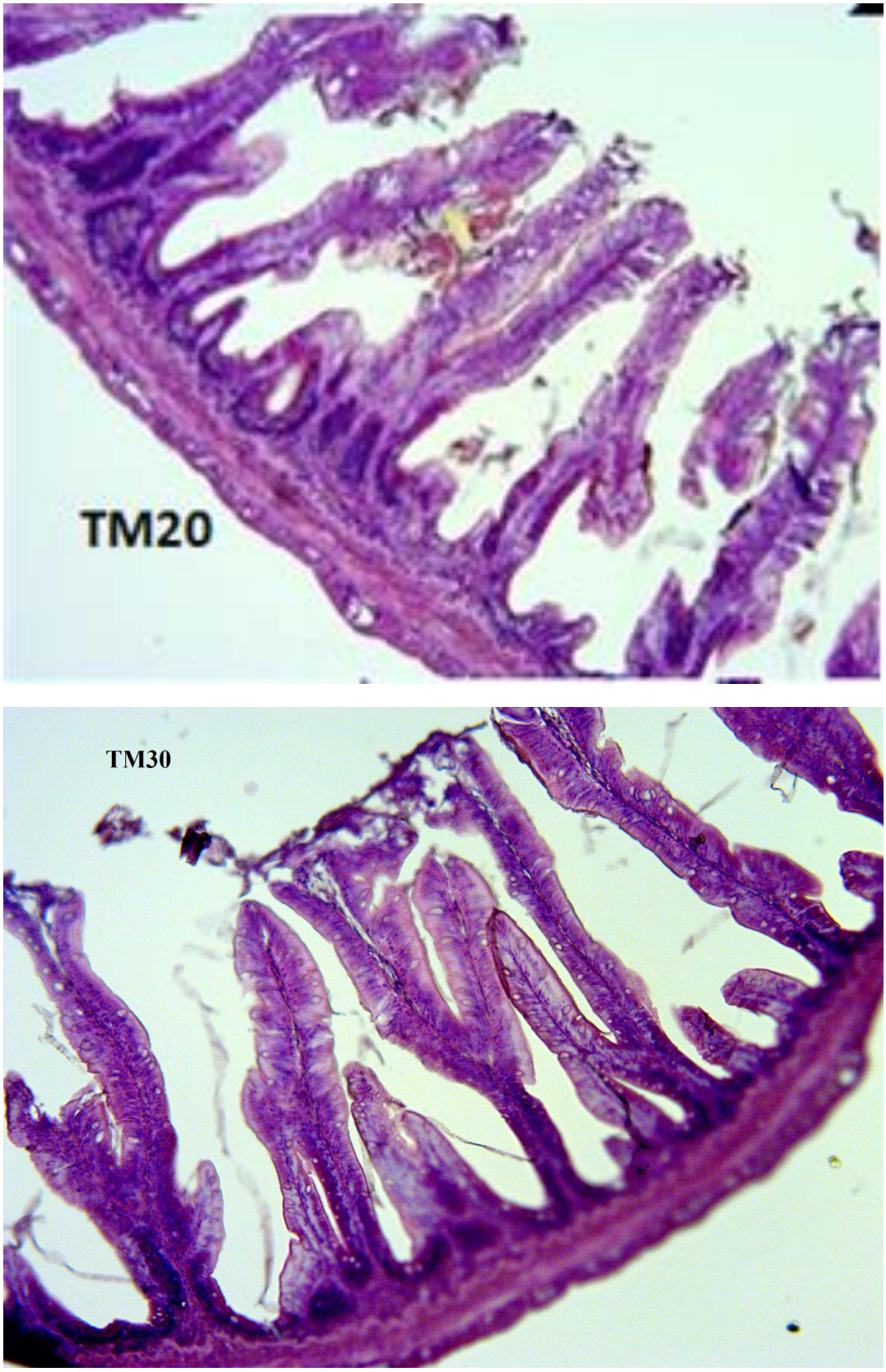


Figure 1. Intestinal histology of Rainbow trout fed with mealworm meal and Black soldier fly meal (H&E, 10X)

Table 2. Growth performance of Rainbow trout fed mealworm meal and black soldier fly meal

Items	Control	HI10	HI20	HI30	TM10	TM20	TM30	Two-way anova variances		
								A	B	AXB
IW	34.33±0.47	34.00±0.50	34.03±0.05	34.31±0.95	34.22±0.95	34.13±0.51	34.33±0.62	0.939	0.920	0.988
FI	126.98±6.4^{a}	121.17±3.99^{a}	103.92±2.28^{b}	107.63±2.84^{b}	124.89±3.82^{a}	121.60±1.81^{a}	90.66±0.94^{c}	0.958	0.000	0.000
FW	158.92±4.2^{a}	133.25±3.8^{b}	107.15±4.04^{d}	122.83±1.15^{c}	152.49±1.26^{a}	135.30±4.41^{b}	91.16±2.27^{e}	0.000	0.000	0.000
FCR	1.09±0.10^{a}	1.30±0.02^{a}	1.53±0.08^{b}	1.23±0.06^{ab}	1.12±0.04^{a}	1.29±0.06^{ab}	1.89±0.16^{c}	0.445	0.009	0.000
WG	124.58±4.6^{a}	99.25±3.39^{b}	73.12±4.03^{d}	88.51±2.07^{d}	118.27±0.42^{a}	101.17±4.37^{b}	56.83±2.28^{e}	0.000	0.000	0.000
SGR	1.70±0.04^{a}	1.52±0.02^{b}	1.27±0.04^{d}	1.42±0.04^{c}	1.66±0.02^{a}	1.53±0.04^{b}	1.09±0.03^{e}	0.005	0.000	0.000
SR	82.22±2.94	82.22±4.01	84.44±1.11	90.00±1.92	83.33±1.93	84.45±2.22	81.11±2.94	0.483	0.571	0.143

Means with different superscript letters in a row are significantly different at $p<0.05$. IW (g): Initial body weight, FW (g): Final body weight, FI (g): Feed intake, FCR: Feed conversion ratio. A: First independent variable (insect meal), B: Second independent variable (levels), AxB: Interaction of two independent variables.

Table 3. Intestinal histomorphology of Rainbow trout fed mealworm meal and black soldier fly meal

Items	Control	HI10	HI20	HI30	TM10	TM20	TM30	Two-way anova variances		
								A	B	AXB
Villi length	443.24±12.60^{b}	458.89±14.04^{b}	446.30±8.31^{b}	442.66±14.60^{b}	454.67±13.08^{b}	501.21±9.77^{a}	514.22±19.10^{a}	0.001	0.244	0.015
Villi width	83.80±4.03^{a}	70.61±3.80^{b}	69.20±3.04^{b}	83.98±3.46^{a}	73.51±1.85^{b}	74.73±1.56^{ab}	68.66±3.88^{b}	0.010	0.308	0.003
Serosa	31.35±2.41^{ab}	20.18±1.01^{c}	26.68±2.39^{b}	31.89±2.88^{ab}	36.89±2.82^{a}	31.31±1.57^{ab}	30.63±2.15^{ab}	0.002	0.839	0.371
Muscularis	45.52±4.04^{bc}	33.02±1.68^{d}	41.80±2.14^{c}	49.55±3.52^{bc}	66.29±3.29^{a}	54.15±3.06^{b}	48.19±2.19^{bc}	0.000	0.851	0.000
Submucosa	24.40±2.58^{ab}	21.48±0.67^{b}	22.60±1.33^{b}	22.72±1.44^{b}	29.07±1.78^{a}	26.45±1.28^{ab}	25.96±1.91^{ab}	0.001	0.432	0.001

Means with different superscript letters in a row are significantly different at $p<0.05$. A: First independent variable (insect meal), B: Second independent variable (levels), AxB: Interaction of two independent variables.

Intestinal villi width of fish fed with control diet were similar to those fed with dietary HI30. Similarly, muscularis thickness of fish fed with control diet was similar to those on HI20 and HI30. When intestinal histomorphological results were examined, groups fed with 20% and 30% TM had higher VL than the control group. While the highest VW was seen in the group fed with the control diet, the highest muscularis thickness was obtained in the group fed with TM10.

Discussion

Insect meal is a good candidate as a substitute for fishmeal in aquaculture feeds, and research on their use in fish feeds has revealed encouraging results (Roncarati et al. 2015). Among these potential sources, mealworm is considered a good alternative for the partial replacement of fish meal in the different fish species (Chemello et al. 2020). Besides, Terova et al. (2021) found that without causing negative effects on rainbow trout gut microbial communities, mealworm larvae meal is a valid alternative animal protein to replace fish meal in the aquafeeds. The addition of mealworm larvae meal instead of the fish meal does not have a negative effect on the growth performance of sea bream (*S. aurata*) (Piccolo et al. 2017), rainbow trout (*O. mykiss*) (Chemello et al. 2020), Yellow catfish (*P. fulvidraco*) (Su et al. 2017). African catfish fed a diet including 80% mealworm meal of fish meal showed a good growth and feed efficiency (Su et al. 2017). Although insect meal added to rainbow trout feeds had no effect on weight gain, feed conversion ratio and specific growth rate were significantly higher in TM0 since feeding rate was significantly higher in TM0 than TM50 (Belforti et al. 2015). In our study, *T. molitor* did not have a negative effect on the growth performance when included 10%. Studies on the effects of mealworm larvae meal on intestinal physiology in fish are lacking. Our results obtained with 20% and 30% mealworm were similar to those of Zadeh et al. (2019), who found that mealworm increased villi length in jejunum supplemented Japanese quails (*Coturnix japonica*). Besides, our results with mealworm at the level of 20% and 30% agree with the results of Roncarati et al. (2015), who reported that feeding with a dietary mealworm in substitution of 50% of fish meal decreased the final body weight of rainbow trout.

Black soldier fly has been recognized as a potential candidate ingredient due to its rich protein and lipid contents (Katya et al. 2017). However, the successful inclusion of black soldier fly into aquatic feeds depends on the fish species and the characteristics of insect derivatives (Dumas et al. 2018). Jia and Hing (2017) reported that black soldier fly prepupae meal alone could substitute fish meal as a protein source in the fish diet. Similarly, Magalhaes et al. (2017) found that BSF can be included in the diets of European sea bass juveniles up to 19.5%, replacing 45% of fish meal. Similar results were also obtained in juvenile mirror carp (*Cyprinus carpio* var. specularis) (Xu et al. 2020) and rainbow trout (Cardinaletti et al. 2019; Stadtlander et al. 2017). Magalhaes et al. (2017) stated that the addition of high levels (45%) of black soldier fly to the diet reduces the growth performance of channel catfish, *Ictalurus punctatus*, rainbow trout and turbot, *Scophthalmus maximus*. However, Stamer et al. (2014) reported that black soldier fly meal with 75% fish meal substitution decreased growth performance, including FW, WG, SGR and FCR. Similarly, in our study, dietary inclusion of full-fat black soldier fly meals significantly decreased growth performance. In a previous study, Dumas et al. (2018) reported that feeding with 6.6% and 13.2% black soldier fly larvae meal did not affect the growth performance of rainbow trout. In an additional study, Katya et al. (2017) reported that feeding with diets formulated to replace fish meal using processed black soldier fly larval meal at 100% decreased FCR, SGR and WG in juvenile barramundi (*Lates calcarifer*). Diets containing 6.6%, 13.2% and 26.4% Black soldier fly larva meal had no impact on villi length and villi width of the posterior intestine of rainbow trout, but villi in the anterior intestine of trout fed 26.4% black soldier fly larva meal was significantly lower compared to the control diet (Dumas et al. 2018). In our study, intestinal villi length in the middle section did not change, while villi width was significantly reduced when fish fed with diets containing10% and 20% black soldier fly larvae prepupae meal. We used full-fat insect meal to test diets in the study. Therefore, the difference in the results obtained in the studies may be due to adding partially defatted insect meal to the diet.

In conclusion, dietary 10% TM meal can be used instead of fish meal without adversely affecting growth performance. Moreover, diets containing 20 and 30% mealworm meal may have a potential to increase intestinal villi length. However, to obtain more effective results from the studies to be carried out on mealworm and black soldier fly meal, it is necessary to expand the experimental studies by highly reducing the lipid content of mealworm and black soldier fly larvae meal.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes and feed legislation.

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