



Arabian Killifish (*Aphaniusdispar*): Assessment of growth performance and survival on different feeds in seawater tanks and potential use in Pakistan

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Abstract: The aim of this study was to examine the growth performance and feed preference of Arabian killifish, *Aphaniusdispar* (mean initial body weight 7.4 ± 0.6 g and length 3.1 ± 0.8 cm) fed upon seven types of feeds (A, B, C, D, E, F and G) for sixty days in laboratory. Feed was given twice at 2% of their total body weight. The experiment was conducted in seven (I, II, III, IV, V, VI and VII) identical seawater tanks having capacity of 75 liters each and optimum conditions were maintained for water temperature (25°C to 28°C), pH (6.5-7.5), dissolved oxygen (5 mg/l-6 mg/l), salinity (28‰ - 32‰), ammonia (<0.2 ml/l), nitrite (<0.1 ml/l), alkalinity (2.3-2.6 meq/l) and total hardness (2.3-2.6 mg/l). It was observed that maximum growth was achieved with feed-E and feed consumption was excellent. Feed-A was found to be least preferable. Rate of consumption of feed-F was observed to be quite well without a significant loss, whereas the rate of consumption of feed-D was better than that of feed-B, C and G. All the fishes remained healthy and no mortality was observed throughout the study period. In conclusion, *A. dispar* has significant potential as an aquaculture species for commercial and economical benefits like bait sellers would be able to get benefit by fulfilling the demand of anglers; decrease the pressure of catching small size edible fish which is further used for fishmeal production.

Keywords: *Aphaniusdispar*, killifish, food preference, growth, artificial feed.

1. INTRODUCTION

The Arabian killifish (*Aphaniusdispar*, Rüppell 1829) of the family Cyprinodontidae are widely seen in waters around the Persian Gulf, the Red sea, Arabian Peninsula, the eastern Mediterranean sea (Hamied, *et al.*, 2020), Southern sea of Pakistan and Indian ocean (Freyhof, *et al.*, 2017). The natural breeding period of *A. dispar* was recorded during March to September. They have strong affinity towards mosquito larvae and can revolutionize biological control in regions affected by mosquito related diseases and is considered to be omnivorous (Lotan and Ben-Tuvia, 1996; Walker, 2002; Ghosh and Dash, 2007).

Generally, the tenacity of *A. dispar* is high that it is eurythermal and euryhaline in nature (Al-Akel and Suliman, 2011). Arabian killifish has such a remarkable resilience that it is able to withstand certain degrees of inorganic and organic pollution (Homski, *et al.*, 1994; Saeed *et al.*, 2015) while thriving and surviving in low levels of oxygen. Its major habitats are coastal lagoons, but it has the tendency to flourish in waters found inland due to possible migration from coast (Reichenbacher *et al.*, 2009) (Haq and Yadav, 2011)

2011). Some of the Killifish might reach up to 50 mm to 70 mm in size. They have a more slender body and have very unique pigmentation pattern consisting of flank bars and blotches (Hamied, *et al.*, 2020).

Fish feeds that are manufactured are critical for economic use of space, time and facilities of aquaculture. Manufactured foods exhibit two major objectives; firstly, in order to give sufficient nutrients for growth of fish, and to gain profit for the aquaculture sector. It is evaluated that six million tons of finfish and shellfish are produced annually around the globe via aquaculture (FAO, 2020). The production can be significantly enhanced by optimal feeding, selective breeding and better water quality (Žák, *et al.*, 2020). In order to ascertain feed conversion efficiency we need to accurately determine the feed consumption rate. The factor of growth is very comprehensive principle in order to determine fish responses to experimental diets. The higher consumption rate is the more fishes would be able to grow and thus it would prove to be successful experimental diet. Factors that would affect the fish feed intake are water quality, density of the pellet, fish size, stocking density, and the feed ration level (Imsl and *et al.*, 2003; Abbas and Siddiqui, 2007).

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In order to gain information regarding the dynamics of fishes near Karachi, we conducted a research to observe the growth performance and food preference of *A. dispar*. Selection of this species is due to their frequent abundance in catch; very little work has been done on this species previously in Pakistan except (Shoaib, *et al.* 2013) and Khan and Qureshi (1996); high Adaptability has been done in tanks or aquarium and transported live at considerable distances; form a primary food chain as well. Furthermore, they described that Arabian killifish can be used as a live baitfish for larger fish and utilized in fishmeal production. In this study, some energetic feeds were prepared by locally available ingredients and subsequently tested on this fish to assess their growth performance in seawater.

2. MATERIAL AND METHODS

A total of sixty days laboratory experiment was conducted at Seed Production Unit Hawks bay, during the period of July to August, 2016 to investigate the rate of consumption of different food components and their effect on the growth of Killifish fingerlings.

Fish collection, holding and acclimatization

Fingerlings of *A. dispar* (one month old) were procured from Sandspit brackish waters by using a cast net. After acclimatization for 10 days, 105 specimen of similar size and weight were selected and stocked in seven well planted identical tanks (size, 12×24×10 inch) with the water capacity of 75 liters. Fifteen fishes with the mean initial body weight of 7.4 ± 0.6 g and body length of 3.1 ± 0.8 cm were stocked into seven separate tanks (two replicates) labelled as I, II, III, IV, V, VI, and VII, respectively. Each tank was well aerated for oxygenation and contained natural seawater and tanks were siphoned daily after providing compound feed. Weight (in grams) and total length (in centimeter) of individual fish was measured fortnightly. Water quality was maintained at optimum level and measured daily throughout the study course; water temperature (Mercury thermometer), Dissolved oxygen (DO meter, Yellow spring instrument), Salinity (Atago hand refract meter), pH (pH meter), Total Hardness (EDTA titration method), Alkalinity (Methyl orange titration method), Nitrite (Azo dye photometric method), Ammonia (Indophenol derivative photometric method). Fish health and mortalities were inspected daily in this period.

Diet and feeding regime

Seven diets (Table 1) were formulated and labelled as (A, B, C, D, E, F and G) consisted of worm-like pellets (size 3mm) and different composition. Throughout the experiment, feed were given by hand of their 2% body weight, twice daily at 09.00 and 15.00

hours for two months. During harvesting, the final weight of each specimen was taken in water filled glass beaker using top loading balance (Sortorius ET 214S), length was measured from head to tail by ordinary scale in cm.

Statistical assessment

Data was analyzed through the SPSS software (Version 19). Length-weight [L/W] relationship was described (Zar, 1996) by this equation $\{W = aL^b\}$.

3. RESULTS AND DISCUSSION

Water quality was found optimum in range during the study period. Specifically, temperature did not differ more than two degree during the experiment and found within the suitable range from 25°C to 28°C. Dissolved Oxygen level remained within the range of 5 mg/l to 6 mg/l, pH was around 6.5 to 7.5, salinity was between 28 ‰ to 32 ‰ which is a safe level for most of the fishes. Ammonia and Nitrite values were found less than 0.2 mg/l and 0.1 mg/l, respectively. Hardness and Alkalinity were maintained as 70 mg/l to 140 mg/l and 2.3 mm/l to 2.6 mm/l which are optimum level for fish growth. Green (2013) mentioned similar range of water quality for Gulf killifish as mentioned in our study. Although, (Burger *et al.* 2018) specified that Gulf Killifish are capable to tolerate a wide range of water quality conditions in low salinity and freshwater ponds. Likewise, Frenkel and Goren (2000) and Abbas and Siddiqui (2007) investigated the effects of water quality on the growth of *A. dispar* and *T. jarbua* under identical laboratory condition. However, during breeding trial under laboratory conditions on killifish species *N. furzeri* recommended maintaining water quality by the same level as mentioned in our work (Polačik, *et al.*, 2016).

The growth measurement was observed in terms of mean initial and final body length and weight and total weight gain (Table 2). The result showed that fish grew slowly at initial rearing period due to new environment adaptation but after two weeks a visible increment in weight and length was observed as correspondingly described by Abbas and Siddiqui (2007). It was evaluated that feed-E consisting of 50% shrimp mean + 50% Premix^b was the best choice for consumption as seen by the net weight gain (1.25 ± 0.5 g and 3.25 ± 1.0 cm) of all fishes in Tank-V being both highest amongst all other types of tanks and feeds. Similarly, Frenkel and Goren (2000) and Polačik, *et al.* (2016) reared killifish fry in laboratory condition, provided dry and frozen feed with live (*Artemianaupli*) and found significant growth in a particular time. Recently, Burger *et al.* (2018) experimented on the growth and survival of killifish *F. grandis* and narrated that this species can be reached to the marketable sizes about 3 to 5 grams within 4 to 8 weeks, respectively.

Tank-VI showed second highest weight gain and net growth of all fish (1.2 ± 0.4 g and 3.05 ± 0.8 cm). The feed consumption for Tank-VI was feed-F containing 20% shrimp meal + 80% Premix^b. It is worth noting that all the top 3 growths and weight gains shown by fish had different varying levels of shrimp meals and Premix^b in the feeds. Tank-IV had third highest weight gain amongst different tanks of about (1.15 ± 0.3 g and 2.9 ± 0.6 cm) which contained 80% shrimp meal + 20 % Premix^b. Tanks-III and VII had similar level of weight gain and net growth of about (1.1 ± 0.2 g; 2.95 ± 0.9 cm and 3.0 ± 0.4 cm), respectively. Although, Tank-I and II had observed lowest weight gain and net growth which contain fishmeal with 20% and 50% Premix^a. These results showed that shrimp meal with Premix^b had better results than other mixture (fish meal+Premix^a). However, Abbas and Siddiqui (2007) used 40% protein containing diet (Fishmeal based) for rearing *T. jarbua* fish in captivity and obtained high growth performance with (39 juveniles/ 0.06m^3) stocking density of fish. Although, growth variability was observed on *Perca fluviatilis* by (Melard, *et al.*, 1995) and (Popper, *et al.*, 1992) on *Sparus aurata* in captivity. Although, (Polačik, *et al.* 2016) mentioned that feeding directly affects on the growth and fecundity pattern of killifish, *N. furzeri* and artificial feed for this species is not presently available for *A. dispar* as in our present study. Recently, (Zak *et al.*, 2020) successfully weaned killifish, *N. furzeri* by using commercial dry pellets (BioMar INICIO) during 12 to 21 dph age and found significant consumption rate on dry pellets from 21 dph onwards.

Pearson correlation showed the relationship between different types of feeds compositions and net growth of the killifish (**Table 3**). This clearly shows that, there is no significant relationship between the net growth and nutritional value which in turn gives us a very prominent insight in understanding that the net growth is more linked to feed preference by fish rather than its nutritional value. Fishes fed feed-E in Tank-V showed significantly higher growth as compared to other groups. Even though the null hypothesis is rejected the P value of Pearson correlation showed mild negative relationship between net growth and crude proteins % and net growth and moisture %. There is weak level of negative relationship between net growth and crude fiber % and mild positive relationship between net growth and crude fats % (Abbas 1999) and Abbas and Siddiqui (2007).

No fish mortality was observed throughout the study course and comparable to the outcomes of Frenkel and Goren (2000) and Burger *et al.* (2018). Regression on body length and weight described the optimal

Growth performance ($b=3$) of experimental fish in our study which is equivalent to the findings of (Abbas 1999) (Abbas and Siddiqui 2007).

4. CONCLUSION AND RECOMMENDATIONS

An appropriate selection of ingredients and adequate supply of balance diet is of prime importance for the production of feed to support potential growth of fish. In our two months trial period we sustained one month old killifish in seawater tanks by providing different formulated feeds and no mortality was observed. Generally, taking into consideration the present work method is not specific and it needs to collect other data to evaluate the results with different stocking density of fish, ration level and feeding ratio with low salinity to freshwater medium. In Pakistan, *A. dispar* is not cultured artificially and has significant potential as an aquaculture species for commercial and economical benefits like bait sellers would be able to get benefit by fulfilling the demand of anglers; decrease the pressure of catching small size edible fish which is further used for fishmeal production; due to their color pattern the ornamental industry can also be flourished; owing to larvivorous nature this species is ideal to control malaria in Pakistan and further used as a lab model animal to investigate toxicity research and in other disciplines.

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Novelty Statement

Outcomes of present work will be advantageous to aquaculture sector through basic knowledge of feed preference and growth of *A. dispar* on pelleted feeds as no previous literature was found on this species in Pakistan.

Author's Contribution

Rahat Rukhsana designed and performed the experiment in laboratory and prepared manuscript. Shahnaz Rashid conceived the idea of this manuscript. Asma Fatima explored recent literatures, modified and revised the text of manuscript. Ghulam Abbas conducted statistical analysis of the data, and reviewed last version for publication.

Conflict of interest

Declared no conflict of interest among authors.

Table 1. Preparation and chemical composition (DM basis) of different feeds used in 60-day trial period.

Feed	Composition	Crude Protein %	Crude Fats %	Moisture %	Crude Fiber%
A.	80% Fish meal +20% Premix ^a	51.5	5.58	10.16	2.82
B.	50% Fish meal +50% Premix ^a	37.7	5.41	10.4	4.79
C.	20% Fish Meal +80% Premix ^a	24.32	5.4	10.64	6.76
D.	80% Shrimp Meal+20% Premix ^b	37.55	4.52	6.9	12.88
E.	50% Shrimp Meal+ 50% Premix ^b	27.87	4.94	8.25	8.8
F.	20% shrimp meal+ 80% Premix ^b	18.2	5.48	9.6	4.73
G.	50% Premix A+ 50% Premix ^b	13.57	5.74	10.65	5.4

Premix^a: Wheat bran (40%), Wheat flour (40%), Wheat germs (18%), & Fish oil (2.0%).

Premix^b: Wheat flour (40%), Rice flour (20%), Corn flour (20%), Potato starch (8.0%), Dried milk (10%), & Fish oil (2.0%).

Table 2. Initial body weight, final body weight, weight gain, net growth and survival rate of Killifish (*Aphanius dispar*) fed different investigational diets.

Tank+ feed	Average initial body weight (g)	Average initial body length (cm)	Average final body weight (g)	Average final body length (cm)	Weight gain (WG, g)	Net growth (cm)	Survival rate (S, %)
I + A	7.5±0.4 ^a	3.1±0.6 ^a	8.45±1.0 ^a	6.2±0.5 ^a	0.95±0.3 ^a	3.01±1.2 ^a	100
II+ B	7.44±0.5 ^a	3.1±0.8 ^a	8.44±1.1 ^a	6.0±0.8 ^a	1.0±0.1 ^a	2.9±1.0 ^a	100
III+C	7.45±0.6 ^a	3.15±0.5 ^a	8.55±0.5 ^a	6.1±0.4 ^a	1.1±0.2 ^a	2.95±0.9 ^a	100
IV+D	7.42±0.4 ^a	3.2±0.3 ^a	8.57±0.8 ^a	6.1±0.5 ^a	1.15±0.3 ^a	2.9±0.6 ^a	100
V+E	7.48±0.4 ^a	3.15±0.4 ^a	8.73±1.0 ^b	6.4±0.5 ^b	1.25±0.5 ^b	3.25±1.0 ^b	100
VI+F	7.48±0.3 ^a	3.15±0.5 ^a	8.68±0.9 ^b	6.2±1.0 ^a	1.2±0.4 ^b	3.05±0.8 ^a	100
VII+G	7.42±0.2 ^a	3.2±0.9 ^b	8.52±0.4 ^a	6.2±0.8 ^a	1.1±0.2 ^a	3.0±0.4 ^a	100

Weight gain (WG, g) = final body weight - initial body weight;

Survival rate (S, %) = (Final no. of fish / Initial no. of fish) x 100.

Values characterizes mean ±S.D (02 replicates); Similar value has similar superscripts which means non-significant result.

Table 3. Pearson's Correlations between the net growth and biochemical composition of the experimental feeds.

	Crude proteins %	Crude fats %	Moisture %	Crude fibre %	Net growth %
Pearson Correlation	-0.023	0.030	-0.122	-0.150	1
Net growth					
Sig. (2-tailed)	0.961	0.948	0.795	0.748	
N	07	07	07	07	07

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

REFERENCES:

- Abbas, G. (1999). Optimum feeding rate of juveniles' red snapper, *Lutjanus argentimaculatus* in seawater tanks. Indian J Anim. Sci. 69: 458-461.
- Abbas G. and P.J.A. Siddiqui. (2007). Effect of stocking density on growth and survival of the juvenile crescent perch, *Therapon jarbua* (forsskal 1775) reared in seawater tanks. Int. J. Biol. Biotech, 4: 73-81.
- Al-Akel A. S. and E. M. Suliman. (2011). Biological control agent for mosquito larvae: Review on the killifish, *Aphaniusdispar* (Rüppel, 1829). Afr. J. Biotechnol, 10: 8683-8688.
- Burger W. S., S. W. Ramee, C. M. Culpepper, C. C. Green, and P. J. Allen. (2018). Demonstration of survival, growth, and reproduction of Gulf killifish in freshwater ponds. N. Am. J. Aquac, 80: 88-96.
- FAO (Food and Agricultural Organization of the United Nations) (2020). The State of World Fisheries and Aquaculture. In: Sustainability in action. FAO, Rome. 922Pp.
- Frenkel V. and M. Goren. (2000). Factors affecting growth of killifish, *Aphaniusdispar*, a potential biological control of mosquitoes. Aquaculture, 184: 255-265.
- Freyhof J and M. Geiger. (2017). *Aphaniuskruppi*, a new killifish from Oman with comments on the *A. dispar* species group (Cyprinodontiformes: Aphaniidae). Zootaxa, 4338: 557-573.
- Ghosh SK, and A.P. Dash. (2007). Larvivorous fish against malaria vectors: A new outlook. Trans. of the Roy. Soc. Trop. Med. Hyg, 101: 1063-1064.
- Green C. (2013). Intensive (non-pond) culture of Gulf Killifish. SRAC publication; no. 1202.
- Hamied A., Q. Alnedawy, A. Correia, C. Hacker, M. Ramsdale, H. Hashimoto and T. Kudoh. (2020). Identification and Characterization of Highly Fluorescent Pigment Cells in Embryos of the Arabian Killifish (*Aphaniusdispar*). Iscience, 23: 101674.
- Haq S. and R. S. Yadav. (2011). Geographical distribution and evaluation of mosquito larvivorous potential of *Aphaniusdispar* (Rüppell), a native fish of Gujarat, India. J. Vector Borne Dis, 48: 236.
- Homski D., M. Goren, and A. Gasith. (1994). Comparative evaluation of the larvivorous fish *Gambusia affinis* and *Aphaniusdispar* as mosquito control agents. Hydrobiologia, 284: 137-146.
- Imsland, A. K., A. Gunnarsson, Foss and S. O. Stefansson. (2003). Gill Na⁺-ATPase activity, plasma chloride and osmolality in juvenile turbot *Scophthalmus maximus* reared at different temperatures and salinities. Aquaculture, 218: 671-683.
- Khan M. A. and N. Qureshi. (1996). Population dynamics of killifish *Aphaniusdispar* (Ruppel) in Sandspit area off Karachi. Pak. J. Mar. Sci, 5: 41-46.
- Lotan R. and A. Ben-Tuvia. (1996). Distribution and reproduction of killifish *Aphaniusdispar* and *A. fasciatus* and their hybrids in the Bardawil Lagoon on the Mediterranean coast of Sinai, Egypt. Isr. J. Zool, 42: 203-213.
- Melard C., P. Kestemont, and E. Baras. (1995). First results of European *Perca fluviatilis* intensive rearing in tanks: effect of temperature and size grading on growth. Bull. Fr. Piscic. 336: 19-27.
- Polačik M., R. Blažek, and M. Reichard. (2016). Laboratory breeding of the short-lived annual killifish *Nothobranchius furzeri*. Nature Protocols, 11: 1396-1413.
- Popper D. M. O. Golden, and Y. Shezifi. (1992). Size distribution of juvenile gilthead sea bream *Sparus aurata*, practical aspects. Isr. J. Aquacult. Bamidegh, 44: 147-148.
- Reichenbacher B., E. Kamrani, H. R. Esmaeili, and A. Teimori. (2009). The endangered cyprinodont *Aphanius ginaonis* (Holly, 1929) from southern Iran is a valid species: evidence from otolith morphology. Environ Biol Fish, 86: 507Pp.
- Saeed S., N. AlNaema, and E. J. Febbo. (2015). Arabian killifish (*Aphaniusdispar*) embryos: A model organism for the risk assessment of the Arabian Gulf coastal waters. Environ Toxicol Chem, 34: 2898-2905.
- Shoaib N., P. J. Siddiqui, and H. Khalid. (2013). Toxicity of synthetic pyrethroid pesticides, fenpropathrin and fenvalerate, on killifish *Aphaniusdispar* juveniles. Pak. J. Zool, 45: 1160-1164.
- Walker K. (2002). A review control methods for African Malaria vectors, PhD Thesis, Environmental health project, US Agency for Inter. Development. Washington DC. 20525: 54.
- Žák J., I. Dyková and M. Reichard. (2020). Good performance of turquoise killifish (*Nothobranchius furzeri*) on pelleted diet as a step towards husbandry standardization. Scientific Reports, 10: 1-11.
- Zar I. H. (1996). Biostatistical Analysis. Prentice-Hall Inc., New Jersey, 662.