



Development of *Pangasius hypophthalmus* polyculture production in Pakistan when cultured with freshwater and Chinese carps

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Abstract

Pond aquaculture has been developed to increase the production of the *Pangasius hypophthalmus*, an important aquatic species; it plays a pivotal role in providing food, fish, nutrition, income, and livelihoods for various value-chain actors in Pakistan. However, this method is mainly used in intensive monoculture, and little knowledge is available on polyculture approaches in Pakistan. Therefore, this study was conducted to evaluate the growth and production of fish at the different species composition in polyculture. The experiment was conducted for 90 days at the Department of Fisheries and Aquaculture UVAS, Lahore, Pakistan. There were three treatment groups, each with three replications. In all treatments, the stocking density of pangas was similar at different species compositions. All the ponds were subjected to the same regime of feeding and fertilization. Fortnightly random sampling was done to adjust the feeding rate. In this study, the *Pangasius hypophthalmus*, showed significant variations with a final weight of 385.34 g, in T3, and 318.50 g, in T1 compared to others. %Weight gain and % SGR of Pangasius were comparatively higher among other fishes in T1 and T3. The FCR of pangas was recorded as 1.9 in T1 and 1.30 in T3, which shows a better result than the control and other groups. In addition, the polyculture of the pangas influenced the nitrate level, which was increased in the high-density group compared to low density and control. At the end of the trial, we observed non-significant variations in cortisol and glucose levels among all treatments, which indicates that there was no stress on Pangasius in the polyculture system. Due to positive effects on growth parameters, it is concluded that the polyculture of pangas with only Indian and mixed (Indian and Chinese carp) could be tried to enhance the economic benefits in Pakistan.

Keywords Polyculture · *Pangasius hypophthalmus*. Carps · Growth · Stress

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Introduction

The availability of quality protein to feed the continuously increasing population is one of the major concerns of the current era. Furthermore, this growing population is adding more pressure to the existing food resources and food supply. Global mean per capita seafood consumption has been the highest since records began in the 1960s. In 2019, half of the worldwide seafood supply came from the rapidly expanding aquaculture sector. Further increase in per capita consumption expects doubling in the volume increase of aquaculture by 2050. Expansion in aquaculture can improve nutritional security for millions of people and provide significant potential as a “low stressor” component of integrated feeding systems (Jin et al. 2023). For this purpose, seafood is a reasonable substitute to provide quality protein supplements in the human diet (Soria et al. 2022). With increasing public awareness of environmental issues, the focus has been shifted to the ecological effects of fish culture methods. Aquaculture uses various culturing environments, such as cages, ponds, etc. (FAO 2018).

Pond polyculture is an effective method to enhance the efficiency and sustainability of production systems (Dumont et al. 2013; Wang et al. 2023). Polyculture can improve the functional ability of a system by taking advantage of coexistence and interactions between the species (Altieri et al., 2014). It is different from monoculture, where only single species of fish can be stocked. Animal diversity in polyculture systems, improves its efficiency through better utilization of food resources and reduces food losses. Furthermore, species diversity minimizes the level of waste by recycling the co-products (provided by the other species or by optimum utilization of space) (Ibrahim and Naggar, 2010; Patel et al., 2023). Interestingly, polyculture can also be applied as an alternative to drug treatments by promoting direct biotic interactions, such as cleaner fish (Brooker et al. 2018). It may increase fish yields if one species improves food accessibility for the other species, so the selection of species is very important in polyculture to optimize profitability and quality. Previously, successful polyculture using different species has been utilized in China almost 1000 years ago and is now spread all over the world, especially in Southeast Asia (Saikia et al., 2023; Lutz 2003; Azad et al., 2004; Baska et al. 2009; Wang et al. 2019; Hossain & Haque, 2019; Hossain et al. 2022; Xiong et al., 2023).

Although aquaculture has huge potential for Pakistan’s fisheries industry and its development (Shahzad et al. 2022). However, compared to other countries, aquaculture production in Pakistan is not up to the required level. Poor economic conditions of the fish farmers and outdated fish farming techniques and practices are the main reasons for low production (Rehman et al. 2019). At present, Indian major carp and Chinese carps are the dominant culturable species in the present polyculture system of Pakistan. The introduction of the catfish *Pangasius hypophthalmus* prevailing culture system is of utmost importance to increase per-acre fish production and quality protein to cater for the protein demand of the growing population of the country (Mehboob et al., 2017; Ehsan et al., 2023). Its culture has increased in numerous South Asia and East Asian countries due to its size, taste, high meat proportion (absence of intramuscular bones), high disease resistance, and tolerance against a wide range of environmental parameters. Therefore, this species is designated as an attractive and sustainable fish (Mehboob et al., 2017).

It is generally believed that the polyculture of *P. hypophthalmus* with native species would be successful and profitable in Pakistan due to its fast growth, wider tolerance to changes in water quality, disease resistance, and comparatively high market price. Another important fact is that this fish can easily be acclimatized to artificial feed due to its omnivorous nature. Now

a day, this species is culturing in freshwater ponds separately. However, potential polyculture advantages for *P. hypophthalmus* remain theoretical since very limited knowledge has begun to scratch polyculture implementation in ponds. To fulfill this gap, we conducted the first report on the consequences of pond polyculture of *P. hypophthalmus* by comparing growth, proximate, and stress responses when cultured with Indian and Chinese carp to achieve sustainable higher production in Pakistan.

Materials and methods

Ethical statements

The study was conducted in earthen ponds at the Department of Fisheries and Aquaculture, the University of Veterinary and Animal Sciences, Ravi Campus, Pattoki.

Experimental design

The experiment was carried out in earthen ponds with an area of 200 m² each for 90 days. There were three different treatment replicates (having a mixture of pangas with Indian and Chinese carp) and one control treatment (only pangas) with three replicates. Before the experiment, all the ponds were sun-dried, drained for 15 days, cleaned by liming, and then manured with organic and inorganic fertilizers (cattle manure, DAP) urea, and poultry manure at 45 kg (50% of cattle dung), 2.5 kg DAP, and 1.25 kg urea/pond before filling water. The purpose of fertilization was to boost the primary productivity in earthen ponds. All the ponds were filled with tube well water up to 1.5 m in depth. The same-size of fingerlings of *Pangasius hypophthalmus*, Indian major carps (*Labeo rohita* and *Cirrhinus mrigala*), and Chinese carps (*H. molitrix*, *C. idella*, and *C. carpio*) were used as experimental fish species. All these fish species were collected from a local fish hatchery and farm, UVAS, block C, Ravi Campus, except *P. hypophthalmus*, which was procured from the private fish farmer, district Bahawalnagar who imported them from Thailand.

A total of 400 fish/ponds of different species were stocked in a polyculture system with *P. hypophthalmus*, Indian major carps (*L. rohita*, *C. mrigala*), and Chinese carps (*H. molitrix*, *C. idella*), and common carp. The fish samples were weighed and measured at the time of stocking and after every fortnight 10 fish samples of each fish species were captured by dragnet. The study trial was grouped into three treatments having different species composition and one control, each with three replicates as follows: *P. hypophthalmus*: *L. rohita*: *C. mrigala* were 1:1:0.5 ratio in treatment (T1), *P. hypophthalmus*: *H. molitrix*: *C. idella*: *C. carpio* were 1:0.5:1:0.5 ratio in treatment (T2), *P. hypophthalmus*: *L. rohita*: *C. mrigala*: *H. molitrix*, *C. idella*: *C. carpio* were 1:1:0.5:0.5:1:0.5 ratio in treatment (T3), and only *P. hypophthalmus* were stocked in control that illustrated in Table 1. Feed containing 30% crude protein (C.P.) procured from Oryza organics (Table 2) was given @ 3% fish wet body weight and readjusted periodically after every fortnight interval.

Table 1 Stocking density of fish species in treated ponds

Fish species	T1	T2	T3	Control
Rohu	160	-	90	-
Grass carp	-	133	88	-
Silver carp	-	67	44	-
Pangasius	160	133	90	400
Mori	80	-	44	-
Common carp	-	67	44	-
Total	400	400	400	400

Table 2 Oryza organic feed composition

Ingredient	CP % of ingredients	Inclusion level	Contribution of CP%
Fish meal	56.72	4	2.26
Soybean meal	46.60	27	12.58
Rice polish	14	15	2.10
Wheat bran	12	10	1.2
Maize gluten	28.50	10	2.85
Canola meal	38.00	15	5.7
Wheat	12	5.0	0.6
Maize	16	10	1.6
DCP	-	0.5	0.5
Antioxidants	-	0.1	0.1
Lysine-meth	-	0.5	0.5
Vitamin		2.9	-
Total	100	100	30.00

Fish growth studies

Fish growth parameters were recorded before stocking, viz. initial weight (g) and length (cm). Other parameters, such as final weight, net weight gain, percent weight gain, SGR%, and FCR, were calculated as follows:

Percent weight gain (PWG, %) = $100 \times (\text{Final weight} - \text{initial weight}) / \text{initial weight}$.

Specific growth rate (SGR, % day⁻¹) = $100 \times (\ln \text{Wt} - \ln \text{Wi}) / t$

Feed conversion ratio (FCR) = feed intake (g, dry weight) / weight gain (g, wet weight)

Physicochemical parameters

Physico-chemical parameters such as temperature, dissolved oxygen, pH, electric conductivity (EC), total dissolved solids (TDS), salinity, and light penetration were controlled each day with a digital meter, whereas nitrates were on a weekly basis by HANNA Nitrate Test Kit HI3874.

Proximate analysis

Five fish from each group were randomly sampled, and the muscle from each group was frozen until being used to analyze whole-body proximate composition by following the method of Liu et al. (2020). Proximate composition was determined following the methods of the Association of Official Analytical Chemists (AOAC 2006). All samples were dried in a vacuum oven (Model: 524 Precision Scientific, USA) at 105 °C for 18 h to determine the dry matter. Crude protein was determined by a protein analyzer (Leco FP528, St. Joseph, USA). Samples were digested in sulfuric acid (15 mL) at a high temperature (415 °C) in the presence of potassium sulfate and copper sulfate (catalyst). Crude fat was measured by the ether extraction method using Soxtec System H.T. (Soxtec System HT6, Tecator, Sweden). Ash contents were determined by incinerating 1 g of the samples in a muffle furnace (Thermolyne, Dubuque, IA, USA) at 550 °C overnight.

Gut contents analysis

Four fish from each group were harvested randomly by drag net for gut contents analysis. Fish were brought into the lab, sacrificed, and their whole guts were separated and well-preserved in 10% formalin solution. The gut contents of each fish were studied under a microscope following the protocol by Umamaheswari (2015). The data were analyzed by following the method described by Khabade (2015).

Blood collection and plasma cortisol analysis

Five fish per treatment were collected by dragnet. Blood was sampled from the caudal vasculature of five fish per group using 1 ml heparinized syringes and stored at 4 °C. Then the blood samples were centrifuged at $956 \times g$ for 10 min at 4 °C to separate the serum for biochemical indices analysis. The cortisol level was determined by the radioimmunoassay method followed by Kobayashi and Mikuni (1987) and confirmed by commercial kits. The glucose level was measured by assay kits as previously described (Hassid and Abraham, 1957).

Statistical analysis

The recorded raw data were investigated to determine the normality of distribution and the homogeneity of variances between experimental groups by using Kolmogorov–Smirnov. Next, data were statistically examined by the general linear model and one-way analysis of variance (ANOVA). Differences between the means $\pm$ SD of three replicates of each treatment group were regarded as statistically significant when $P < 0.05$ using Tukey's multiple range test as a post-doc test. Statistics were performed using the SPSS software (SPSS version 20, SPSS Inc., Chicago, IL, USA).

Results

Growth performance of pangas in polyculture

The result showed that the growth performance of pangas was significantly influenced by the polyculture system. The final weight value of pangas in T3 was significantly higher than those

in T1, T2, and control ($P < 0.05$). The net weight gain and %weight gain values in T3 and T1 were significantly higher than in T2 and control ($P < 0.05$). The % SGR value in T1 and T3 was significantly higher than in T2 and control ($P < 0.05$). The FCR value in T1 and T3 were significantly lower than those in T2 and the control ($P < 0.05$) (Table 3).

Physico-chemical parameters of pond water

Physico-chemical parameters such as temperature, dissolved oxygen, pH, electric conductivity (EC), total dissolved solids (TDS), salinity, and light penetration showed non-significant differences except nitrates, where significant variation was observed among all treatments ($P < 0.05$) (Table 4).

Proximate analysis

The result revealed that the moisture contents (%) of pangas did not differ among treatments ($P > 0.05$). On the other hand, protein showed significant differences among treatments. It was highest in T3 ($69.21 \pm 1.23\%$) and lowest in the control group ($59.97 \pm 0.41\%$) ($P < 0.05$). The fat content also showed a nonsignificant difference among treatments ($P > 0.05$). Whereas the ash contents of pangas in T1 (4.90 ± 0.07) showed significant differences ($P < 0.05$) among treatments (T2, 3.10 ± 0.78), T3, 2.29 ± 0.07), and control group (2.70 ± 0.07) (Table 5).

Gut contents analysis

Gut contents analysis of pangas was performed, and observed 34 genera of plankton species (Phytoplankton+zooplankton) which consisted of 12 genera of zooplankton and 22 genera of phytoplankton. Further examination showed that 12 genera of zooplankton belong to 4 diverse phyla, including Rotifera, Crustacea, Arthropoda, and Annelida (Table 6). Whereas, 22 genera of phytoplankton belong to 8 phyla; Euglenophyta, Bacillariophyta, Cyanophyta, Charophyta, Mxyophyta, Ochrophyta, Dinoflagellata, and Chlorophyta (Table 7). Among the phytoplankton and zooplankton, the phylums Chlorophyta and Rotifera were higher in the gut content of pangas in T1 than in other treatments ($P < 0.05$; Tables 6 and 7).

Stress responses

Glucose and cortisol levels were analyzed to detect the stress levels, and results indicated that these both parameters were not significantly affected after rearing *P. hypophthalmus* in polyculture with Indian carps (T1), Chinese carps (T2), and Indian and Chinese carps (T3) ($P > 0.05$; Table 8).

Discussion

Growth performance of pangas in polyculture

The growth performance of *P. hypophthalmus* in polyculture was investigated in this study. We observed that the final weight, % weight gain, % net weight, FCR, and %SGR of *P.*

Table 3 Growth performance of *Pangasius hypophthalmus* under polyculture

Treatments	Species	Initial weight (g)	Final weight (g)	Net weight gain (g)	%Weight gain	Final length (cm)	FCR	%SGR
T ₁	Pangasius	35.75 ± 2.8	318.50 ± 79.20 ^b	239.35 ± 17.88 ^{ab}	32.20 ± 0.14 ^{ab}	29.72 ± 0.86 ^a	1.9 ± 0.02 ^b	1.92 ± 0.54 ^{ab}
	Rohu	35.8 ± 3.18	282.55 ± 6.57	163.35 ± 6.15	15.56 ± 1.65	27.80 ± 1.27	2.54 ± 0.26	1.10 ± 0.10
	Mori	34.96 ± 2.21	221.95 ± 7.84	143.35 ± 14.35	16.01 ± 2.17	25.80 ± 1.69	2.47 ± 0.21	1.14 ± 0.11
T ₂	Pangasius	35.31 ± 4.61	312.75 ± 51.54 ^b	206.80 ± 33.09 ^b	27.88 ± 0.28 ^b	29.20 ± 0.70 ^{ab}	2.09 ± 0.05 ^a	1.51 ± 0.26 ^c
	Silver carp	33.05 ± 3.85	240.65 ± 42.2	176.55 ± 26.7	24.90 ± 1.40	27.25 ± 0.91	1.61 ± 0.04	1.69 ± 0.09
	Grass carp	32.93 ± 3.32	284.50 ± 17.11	207.70 ± 23.76	17.10 ± 1.24	25.68 ± 2.09	2.3 ± 0.1	1.21 ± 0.08
T ₃	Common carp	36.34 ± 4.33	226.06 ± 6.68	153.00 ± 0.88	16.095 ± 1.03	25.19 ± 3.62	2.65 ± 0.15	1.05 ± 0.05
	Pangasius	39.57 ± 4.46	385.34 ± 26.24 ^a	385.34 ± 26.24 ^a	39.2 ± 5.45 ^a	28.35 ± 2.47 ^{ab}	1.30 ± 0.28 ^c	2.43 ± 0.15 ^a
	Silver carp	36.05 ± 4.07	230.55 ± 30.47	158.45 ± 7.14	25.78 ± 0.88	29.25 ± 3.04	1.63 ± 0.22	1.74 ± 0.07
Control	Grass carp	40.61 ± 1.96	207.66 ± 18.06	167.034 ± 16.07	29.58 ± 4.94	25.37 ± 1.30	1.49 ± 0.1	2.16 ± 0.53
	Common carp	40.35 ± 29.06	317.05 ± 67.95	276.70 ± 97.01	49.25 ± 29.35	24.37 ± 1.94	1.34 ± 0.25	2.78 ± 1.25
	Rohu	39.95 ± 33.87	181.48 ± 32.07	141.53 ± 3.20	23.37 ± 1.85	24.30 ± 0.42	1.79 ± 0.30	1.60 ± 0.12
Control	Mori	38.08 ± 2.48	247.81 ± 41.27	196.68 ± 0.79	24.92 ± 2.11	22.66 ± 3.45	1.71 ± 0.34	1.71 ± 0.12
	Pangasius	37.00 ± 1.69	201.05 ± 3.18 ^c	169.05 ± 1.48 ^c	23.88 ± 1.52 ^c	25.25 ± 0.63 ^b	2.01 ± 0.12 ^a	1.38 ± 0.001 ^d

Added values are mean ± SD ($n = 3$); values in the each row with different superscripts are significantly different ($P < 0.05$). SGR specific growth rate (% day⁻¹) = $100 \times (\ln \text{Wt}_{t\text{-th}} - \ln \text{Wt}_{0\text{-th}}) / t$

FCR feed conversion ratio = feed intake (g, dry weight)/weight gain (g, wet weight)

Table 4 Water quality parameters of pond water

Parameter	T ₁	T ₂	T ₃	Control
Temperature (°C)	32.03 ± 0.07	31.50 ± 0.25	31.83 ± 0.53 ^a	32.02 ± 0.12 ^a
DO (mg/L)	5.34 ± 0.05	5.36 ± 0.08	5.41 ± 0.02 ^a	5.58 ± 0.30 ^a
pH	7.35 ± 0.04	7.46 ± 0.05 ^a	7.54 ± 0.04 ^a	7.56 ± 0.21 ^a
EC (µS/cm)	2439.28 ± 12.70	2462.75 ± 119.28 ^a	2264.55 ± 138.15 ^a	2391.07 ± 7.4 ^a
TDS (mg/L)	1363.02 ± 97.86 ^a	1281.57 ± 63.07 ^a	1215.66 ± 43.24 ^a	1295.54 ± 37.75 ^a
Salinity	1.0 ± 0 ^a	1.0 ± 0 ^a	1.0 ± 0 ^a	1.0 ± 0 ^a
Nitrates (mg/L)	1.30 ± 0.16 ^a	1.06 ± 0.26 ^a	1.52 ± 0.06 ^b	1.08 ± 0.05 ^a
Light penetration (cm)	17.64 ± 0.72 ^a	17.54 ± 1.04 ^a	18.5 ± 0.05 ^a	19.37 ± 0.86 ^a

Values showing are mean ± SD ($n = 3$); values in each row with different superscripts are significantly different ($P < 0.05$). DO dissolved oxygen, EC electric conductivity, TDS total dissolved solids

Table 5 Proximate composition of various fishes under polyculture (% wet weight basis)

Treatment	Species	Moisture %	Crude protein %	Crude fat %	Ash %
T1	Pangasius	81.16 ± 0.58 ^a	69.20 ± 0.65 ^a	13.34 ± 1.75 ^a	4.90 ± 0.07 ^a
	Rohu	74.40 ± 0.09	61.62 ± 2.28	6.44 ± 0.15	2.22 ± 0.14
	Mori	71.68 ± 0.61	55.97 ± 2.34	4.34 ± 0.52	2.62 ± 0.16
T2	Pangasius	79.67 ± 0.45 ^a	65.35 ± 2.00 ^b	12.84 ± 3.38 ^a	3.10 ± 0.78 ^b
	Silver crap	75.54 ± 1.63	56.08 ± 0.34	7.76 ± 0.71	4.59 ± 0.49
	Common cap	65.86 ± 0.32	55.80 ± 0.61	8.44 ± 0.14	3.19 ± 0.02
	Grass carp	75.19 ± 0.07	54.80 ± 1.27	6.89 ± 1.34	2.89 ± 0.01
T3	Pangasius	69.33 ± 8.56 ^a	69.21 ± 1.23 ^a	13.76 ± 4.98 ^a	2.29 ± 0.07 ^d
	Rohu	73.12 ± 0.30	55.80 ± 1.27	4.88 ± 0.07	1.99 ± 0.23
	Mori	70.55 ± 0.29	55.03 ± 2.27	4.42 ± 0.46	2.49 ± 0.65
	Silver cap	76.10 ± 0.60	56.52 ± 2.58	7.63 ± 0.71	3.47 ± 0.11
	Common carp	64.36 ± 0.88	57.72 ± 0.68	7.77 ± 0.67	3.54 ± 0.42
	Grass carp	74.59 ± 0.3	57.45 ± 0.10	7.49 ± 0.21	2.74 ± 0.01
Control	Pangasius	73.91 ± 1.32 ^a	59.97 ± 0.41 ^c	13.11 ± 1.23 ^a	2.70 ± 0.07 ^c

Values showing are mean ± SD ($n = 3$); values in each row with different superscripts are significantly different ($P < 0.05$)

hypophthalmus showed significant variations among treatments. Overall, the combination of *P. hypophthalmus* with Indian and Chinese carp (T3) showed a higher growth trend followed by a T1 (with Indian carps), T2 (with Chinese carps), and control. This shows that the polyculture of pangas with Indian and Chinese carp is beneficial for the growth of all fishes because they compensate for each other's effect and make the environment beneficial for their growth. This result corroborates the previous studies (Azad et al. 2004; Rehman et al. 2008; Shafiullah et al. 2019). It has also been reported that pangas can be cultured with prawns when sufficient supplementary feed is supplied (Islam et al. 2008). In our study, the optimum growth of pangas was achieved when fed with 30% crude protein at the rate of 3% of their body weight in polyculture with Indian major carps and Indian and Chinese carps (mixed group). Our study, supported by previous observations where a 27.96% (at the rate of 8% of their body weight) feed was found to be optimum, showed

Table 6 Average percentage (%) composition of phytoplankton /10mL gut contents of *Pangasius hypophthalmus*

Phylum	Genus	T1	T2	T3	Control
Chlorophyta	<i>Coelostrum</i>	10.23 ^a	8.76 ^b	9.18 ^a	8.16 ^b
	<i>Cladophora</i>	11.9 ^b	0.00	20.32 ^a	0.00
	<i>Oocystis</i>	7.14 ^a	8.54 ^a	5.55 ^a	0.00
	<i>Chlorella</i>	6.94 ^a	7.34 ^a	6.5 ^a	4.08 ^{ab}
	<i>Zygnema</i>	11.11 ^a	9.77 ^a	0.00	0.00
	<i>Chlamadomonas</i>	10.25 ^a	4.7 ^{ab}	4.34 ^{ab}	0.00
	<i>Eudorina</i>	0.00	0.00	5.43 ^a	0.00
	<i>Ulothrix</i>	7.69 ^a	0.00	6.65 ^a	4.56 ^b
	<i>Oedogonium</i>	7.14 ^a	3.27 ^b	7.69 ^a	1.23 ^b
	<i>Planktospheria</i>	3.42 ^b	9.87 ^a	13.88 ^a	2.45 ^b
	<i>Pediastrum</i>	7.68 ^a	4.56 ^a	2.77 ^b	0.00
Euglenophyta	<i>Euglena</i>	30.45 ^a	44.20 ^a	13.45 ^b	23.45 ^a
	<i>Phacus</i>	13.5 ^a	10.89 ^a	7.68 ^a	5.45 ^b
Cyanophyta	<i>Aphanotheca</i>	3.125 ^a	4.91 ^a	0.00	1.23 ^{ab}
	<i>Lynbya</i>	7.25 ^a	0.00	3.42 ^b	5.67 ^a
	<i>Aphanocapsa</i>	10.89 ^a	11.67 ^a	8.69 ^a	6.78 ^a
	<i>Nostoc</i>	10.89 ^a	10.25 ^a	9.87 ^a	3.45 ^b
Bacillariophyta	<i>Fragilaria</i>	10.43 ^a	15.67 ^a	44.68 ^a	8.79 ^b
	<i>Nitzschia</i>	22.34 ^a	14.35 ^a	8.67 ^{ab}	9.78 ^{ab}
	<i>Naviculla</i>	13.45 ^a	10.87 ^a	8.54 ^a	5.55 ^b
	<i>Cyclotella</i>	0.00	8.92 ^a	4.35 ^a	1.34 ^b
	<i>Synedra</i>	34.25 ^a	0.00	0.00	0.00
Charophyta	<i>Cosmarium</i>	15.625 ^a	0.00	13.23 ^{ab}	8.76 ^{bc}
Mxyophyta	<i>Anabaena</i>	20.79 ^a	13.65 ^a	8.79 ^a	4.56 ^b
Ochrophyta	<i>Chromulina</i>	14.53 ^a	11.89 ^a	0.00	1.34 ^b
Dinoflegallata	<i>Ceratium</i>	9.53 ^a	8.67 ^a	3.45 ^b	1.25 ^{bc}

Values showing are mean $\pm$ SD ($n=3$); values in each row with different superscripts are significantly different ($P < 0.05$)

Table 7 Average percentage (%) composition of zooplankton/10 mL gut contents of *Pangasius hypophthalmus*

Phylum	Genus	T1	T2	T3	Control
Rotifera	<i>Rotaria</i>	21.45 ^a	18.95 ^{ab}	16.75 ^b	12.34 ^c
	<i>Branchionus</i>	36.75 ^{ab}	40.22 ^a	16.24 ^c	5.89 ^d
	<i>Keratella</i>	50.89 ^a	34.20 ^b	6.75 ^c	0.00
Crustacea	<i>Cyclops</i>	14.54 ^a	8.98 ^b	6.54 ^b	0.00
	<i>Diaptomus</i>	25.64 ^a	22.34 ^{ab}	18.96 ^b	0.00
	<i>Daphnia</i>	28.67 ^a	24.54 ^a	22.34 ^b	18.97 ^b
Artropoda	<i>Tanytus</i>	8.33 ^a	6.54 ^a	4.34 ^b	0.00
Annelida	<i>Tubifex</i>	12.34 ^a	10.45 ^a	0.00	1.23 ^b

Values showing are mean $\pm$ SD ($n = 3$); values in each row with the different superscripts are significantly different ($P < 0.05$)

Table 8 Plasma Glucose (g/L) and Cortisol level (ng/mL) in *P. hypophthalmus*

Parameters	T1	T2	T3	Control
Glucose (g/L)	114.82 ± 1.55 ^a	128.93 ± 7.91 ^a	116.815 ± 11.80 ^a	119.56 ± 3.93 ^a
Cortisol (ng/mL)	1.64 ± 0.38 ^a	2.00 ± 0.22 ^a	1.64 ± 0.38 ^a	1.61 ± 0.36 ^a

Values showing are mean ± SD ($n = 3$); Values in each row with same superscripts are not significantly different ($P > 0.05$)

that 30% protein is required for the best growth of Thai pangas (Huq et al. 2004; Shafiullah et al. 2019).

Physico-chemical parameters

Environmental parameters play an important role in fish production. In the present study, the inclusion of pangas in polyculture altered the water quality. With the exceptions of temperature, transparency, pH, and salinity were similar in all treatments and within the range suitable for fish production. Whereas nitrates showed significant changes among treatments (highest in T3). The increase in nitrate with time may be due to the increased feeding rate of pangas in T3 (Tucker et al. 2008; Sarkar et al. 2008).

Proximate analysis

Fish body composition is affected by different pond ecosystems, fertilization, feed ingredients, diet composition, feeding rates, and probiotics (Krishna et al. 2009, 2015). In the present study, moisture contents of pangas were higher (though it is non-significant) when cultured with Indian carp (T1) compared to the other groups, which were in line with the previous study (Babu et al. 2013) which reported optimum moisture content values of 75–79% for efficient muscle growth in pangas. Contrary to our study, Krishna et al. (2015) studied the body composition of four species (*C. catla*, *L. rohita*, *C. mrigala*, and *P. hypophthalmus*) and found maximum (79.24%) moisture contents in mrigala and minimum (75.19%) in pangas.

The amount of crude protein depends on natural and supplementary feed contents. In the present study, protein contents showed a significant difference ($P < 0.05$) among groups. The same results can be referenced by Krishna et al. (2015), where maximum ($68.86 \pm 0.64\%$) protein contents were recorded in pangas. Similarly, Krishna et al. (2015) studied body composition in the polyculture of *P. hypophthalmus* and major carps and found significant ($P < 0.05$) differences in the protein contents among the species. Another study by Panchakshari et al. (2016) observed feed and fertilizer effects on the composition of *Channa striata* and *P. hypophthalmus* and found a significant difference ($P < 0.05$) between the proximate compositions of these fish species. Our results showed that crude protein contents differed among treatment groups compared to the control group. The possible reason might be higher fish weight in treated groups (T3) compared to the control.

Furthermore, the fat content values in our study were found non significantly different among treatments. However, maximum fat contents were recorded in pangas, and our values of fat contents are much higher than Babu et al. (2013). Ash values of pangas were found to be approximately double in treatment T1 and slightly higher in T2 compared to

the control. However, in T3, it was slightly lower than the control. These results are consistent with final growth and fish production (lower ash content is better for efficient productivity). The values of ash contents in our results were similar to those reported previously by Keshavanath and Shivanna, 2006

Gut content analysis

During the current study, higher gut contents of pangas were observed in T1 when cultured with Indian carp, followed by other treatments (T2, T3, and the control group). It is indicated that the pangas have an omnivorous feeding habit because it mainly feeds on zooplankton and phytoplankton species. Similarly, gut contents analysis reported by Sengupta and Homechaudhuri (2011) in their work on *Pangas* demonstrated that adult *P. pangasius* mostly feeds on Molluscas because of its bottom-feeding habit. Among the phytoplankton and zooplankton, the phylums Chlorophyta and Rotifera were higher in the gut content of pangas in T1 than in others treatments. Similar results were observed by Abu et al., 2019 In addition, the most abundant phytoplanktons were observed in T1, followed by T2 and T3. This might be due to the presence of silver carp in T2 and T3, a planktivorous fish that utilized phytoplankton in T2 and T3. As a result, a lower amount of phytoplankton was found in the gut of pangas (Sarkar et al. 2008).

Stress response

Naturally, fish experience stress due to climate, stocking density, and temperature change, as well as during transportation and collection, which can challenge their defense mechanism. Plasma cortisol levels may effectively indicate primary stress response in fish. The fish reared under high density presented significantly higher cortisol levels on day 120, implying that high density produced a stress response. This effect has been described in different species (Costas et al., 2008). Furthermore, glucose is a significant stress factor, which might be up-regulated with the increase in the cortisol level. In the present study, both cortisol and glucose were found non-significantly different ($P > 0.05$) among groups, showing no stress indication throughout the study period. Our results agree with the previous study showed that glucose level was not affected by stocking density in sea bass (*Dicentrarchus labrax*) (Di Marco et al., 2008).

Conclusion

In summary, our study demonstrated that the polyculture of *Pangasius* with Indian and Chinese carp (mixed) is beneficial for weight gain and its production. The highest final weight (385.34 g) and % weight gain (39.2%) of pangas were obtained with comparatively better FCR and SGR% when the feed was supplied at 3% of its body weight. In addition, the polyculture of the pangas influenced the nitrate level, which was increased in the high-density groups compared to the low-density groups and control. At the end of the experiments, we observed that the cortisol and glucose levels showed non-significant variations among treatments, confirming no stress on *Pangasius* in the polyculture system. Due to positive effects on growth parameters, T3 (*P. hypophthalmus*: *L. rohita*: *C. mrigala*: *H. molitrix*: *C. idella*: *C. carpio* = 1:1:0.5:0.5:1:0.5) is an appropriate species combination for Indian carp,

Chinese carp and pangas polyculture. Hence, it should be used to enhance the economic benefits in Pakistan.

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Data availability Not applicable.

Code availability Not applicable.

Declarations

Ethics approval All the experimental procedures, including animal experimentation, were approved by the institutional ethical committee of the Department of Fisheries and Aquaculture, Uvas, Ravi campus, Patokki.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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