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Domestication and breeding of native ornamental fish species in Nepal

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Abstract
Fish diversification is one way expected to support the growing aquaculture production which requires the domestication of new species. Domestication of new species is the adaptation of an animal from wild to new environment and it is a long, difficult and expensive process. The main goal of this study was to domesticate and propagate the native ornamental fish species collected from Begnas Lake to introduce new candidates in ornamental fish of Nepal. Four small size attractive fish species *Puntius conchonius*, *Puntius sophore*, *Danio devario* and *Danio rerio* were collected from Begnas Lake and irrigation Canal of Begnas Lake from February to September 2020 for domestication in captive condition. The results showed that all collected fish species were domesticated and accepted formulated feed with higher survival (62.4-94.6%). We try to explore the self-bred condition of domesticated ornamental fish species by providing semi-natural environments. Two fish species *Danio devario* and *Danio rerio* were successfully self-bred in the semi-natural condition. Further research needed to find out farmer's friendly breeding technology of ornamental fish species and also domesticate the new more attractive native fish species.

Keywords: Domestication, ornamental fish, propagation, native fish, Begnas Lake

1. Introduction
Domestication can be defined as the adaptation of an animal to the human environment and its constraints. The five steps of domestication process includes: capture and transport (step 1), acclimatization (step 2), obtaining the first generation of captive fish from wild fish (step 3), adaptation and phenotypic changes (step 4), and effect of the controlled artificial selection (step 5) [1]. The advantages of domestication are production, reproduction of organisms, and their costs can be controlled [2]. Fish diversification is one way expected to support the growing aquaculture production [3]. However, it requires the domestication of new species, and domestication of a new species is a long, difficult and expensive process [4].
Ornamental fish production is a multibillion dollar industry [5]. Ornamental fish keeping is becoming popular as stress relieving hobbies [6, 7]. It is estimated that over 6,000 aquarium fish species are now traded internationally each year among which three quarters (ca. 4,000) live in freshwaters [8, 9]. One study had found that 86 countries (48 exporting and 38 importing) were involved in freshwater and marine ornamental fish trade [10]. Thirty freshwater fish species are dominate in the global market of ornamental fish [11] and Asia is the biggest exporter of ornamental fish [7]. Ornamental fish farming has the potential to become an important source of employment and income, especially for empowering women financially [6]. Ornamental fish sale of 27 exotic fish species in Nepal have been imported from foreign country and it is in increasing trend [12].
Danio devario is common ornamental fish with silvery greenish body. It is omnivorous, feeding on fish, zooplankton, dipteran larvae and plant matter. Inhabits in streams, riverine pools, canals and lakes. The zebra fish (*Danio rerio*) is a tropical fresh water teleostei, belonging to the Cyprinidae family. It is omnivorous of fish and Inhabits in streams, riverine pools, well-vegetated pools and rice paddies adjacent to slow moving streams, canals and lakes [13-15]. *Puntius sophore* is a small cyprinid (minnow) found in rivers, streams and ponds of South-east Asian countries. It commonly known as 'pool barb'. This species is surface-pelagic, shoaling and herbivore in nature [14, 16]. *Puntius conchonius* which is commonly known as "rosy barb" is a freshwater species of cyprinidae family.

It is commonly found in lakes, river streams, ponds, ditches, inundated water bodies [13, 15].

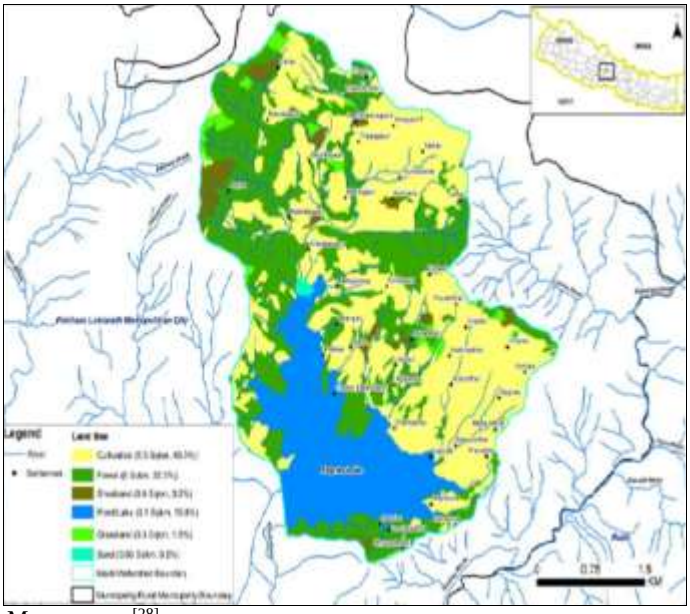
Total of 230 native fish species belonging to 104 genera, 34 family and 11 order are found in Nepal [17]. Fifteen native fish species of Nepal possess attractive color and attributes to be a potential candidates for ornamental fish [12]. Twenty native fish species have been reported in the Pokhara valley lakes [18]. Previously, breeding and nursing technology of six exotic fish species and one native fish species have been developed at Fishery Research Station (FRS), Pokhara, Nepal. The up-scaling of successfully developed breeding and nursing technologies of some ornamental fish species and domestication of new potential ornamental fish species will ensure to develop ornamental fish agribusiness in Nepal [12].

The domestication of ornamental fish, among others, has increased in recent decades worldwide [19]. The attractive native fish species should be domesticated and its breeding and nursing technologies should be developed. If these species are promoted, the import of ornamental fish could be lessen in the near future [11]. The *Danio rerio* is most studied fish species for breeding, culture, care and maintenance and behavioral aspects [20-25] and major model organism used in biomedical studies [26]. We don't find any literature on the studies of *Danio devario* for domestication and breeding aspects. Furthermore, this is the first attempts to domesticate and bred the Danionins species and *Puntius* species in Nepal. Therefore, the aim of this study to firstly domesticate the native fish species, and secondly develop farmer's friendly breeding technology to reduce the import of ornamental fish from abroad.

2. Materials and Methods

2.1 Study area

This present study was conducted at Fishery Research Station, Begnas, Pokhara, Nepal. The fish samples were collected from Begnas Lake and adjacent irrigation canal. Begnas Lake is the second biggest lake (328 ha) which is located in the eastern part of Pokhara Valley, Kaski districts in central part of Nepal at altitude at 28°10'26.2″N and 84°05'50.4″E, 650 m above mean sea level (Fig. 1). It is fed by a perennial stream with a catchment area of 19 km^2 and an average depth of 6.6 m. This lake fluctuates between oligotrophic and meso-trophic in different seasons [27].



Map source: [28]

Fig 1: Location map of Begnas Lake

2.2 Collection and identification of native ornamental fish

Attractive, small to medium size native ornamental fish species were collected from Begnas Lake and irrigation canal which flow from Begnas Lake by using cast net in the months starting from February to June 2020. The collected fish were identified species level by using the keys and photos from Shrestha 2008 [15] and, Husen and Sherpa [29]. Initial length and weight of these fishes were taken. All collected fish species were brought to Fishery Research Station, Begnas, Pokhara, Kaski, Nepal. The possible candidates for the ornamental fish species of Begnas Lake was also explored based on illustrations of fish species in the fish catalogue [29] and field survey.

2.3 Domestication of native ornamental fish

The collected native fish species were kept into the circular tank (500 liter capacity) for 7 days to acclimatize in the new environment. Then these fishes were separately kept species wise in the glass aquaria (40 liter capacity) with proper aeration by air pump with air stone having filter system. Automatic water heater was arranged in the winter months in the aquarium. The water of aquaria was exchanged in every week. Fishes were fed three times a day ad-libitum with formulated micro feed (Table 1) containing 34.85% CP (Table 5).

2.4 Feed formulation and nutrients analysis

Feed formulation for ornamental fish and their proximate and minerals analysis were done in the laboratory. The ingredients for feed formulations were collected from the local market. The compositions of selected feed ingredients are presented in the Table 1. The sample were dried in the sun and soya whole was lightly fried, and kept to the room temperature for few hours. The required amounts of samples were finely ground by a small mortar and kept it in the airtight container. The weight of samples were taken before packing and packed in to

zipper plastic bag. The samples of feed were sent to National Food Research Center, Khumaltar, Kathmandu, Nepal for analysis. The proximate and minerals composition of formulated ornamental feeds were analyzed according to standard procedure given in Association of Official Analytical Chemists (AOAC) [30].

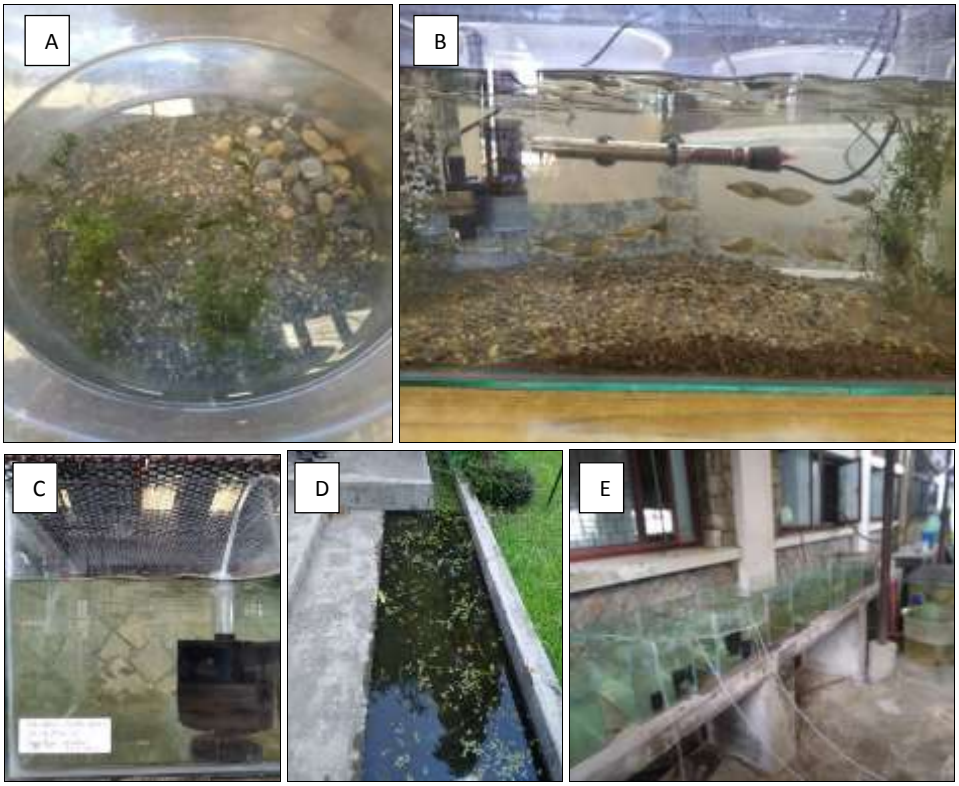


Fig 2: Rearing, breeding and nursing environment for ornamental fish (A-E): Circular plastic tank with pebbles, stone and aquatic grasses (A); Glass aquaria with pebbles, stone and aquatic grasses (B); Glass aquaria without any substrates and (C); Canal pool with pebbles, stone and aquatic grasses (D); Series of aquarium for growth study (E).

Table 1: Feed formulations for ornamental fish

Ingredients	% Composition
Shrimp	40
Mustard oil cake	15
Soya whole	36
wheat	4
Sunflower oil	3
Mineral	1
Vitamins	1

2.5 Setting up of semi-natural breeding environments

The collected fish species after rearing in circular tank and aquarium were tested in several ways to bred in the captive condition. The fish species were exposed to four different environmental condition: aquarium with heater and aerator (Environment A) in shed house; circular transparent plastic tank (500 liter capacity) having direct sunlight in open shed with aerator (Environment B); circular semi-transparent plastic tank (500 liter capacity) tank having continuous water flow without direct sunlight inside shed house (Environment C) and artificial canal pool (10 m Length, 0.75 m wide and 30 cm depth) getting direct sunlight, and open with continuous water supply by pipes (Environment D) to assess the suitable environment for breeding. The natural bedding for breeding grounds was prepared in each environment with gravels, pebbles and submerged aquatic plants.

2.6 Breeding of fish species

No hormone was used in this study. 3 pairs (female and male sex ratio of 1:3), of each fish were exposed these four environment except *Danio rerio* which only kept in environment (A and C). The fish species were left in these environments for natural spawn. After spawning, brood were separated and hatched larvae were nursed for few 7 days in that environments. Then hatchlings were collected with smooth scoop net.

2.7 Larval nursing and rearing management

Hatchlings of fish species were nursed and reared in 40 liter aquarium. The fish larvae were fed with formulated micro feed of 34.85% CP at ad-libitum thrice a day (Table 1 and Table 5). The water of each environment were repealed to maintain water quality parameters.

2.8 Calculation of gonad somatic index (GSI)

The GSI is the percentage of gonad weight to the total weight of the fish. The GSI of the collected fish species (*Danio devario* and *Puntius sophore*) was calculated for each of the female separately and using by the following formula:

$$GSI = \frac{\text{Weight of ovary}}{\text{Weight of fish}} \times 100$$

2.9 Estimation of fecundity

We follow the gravimetric method described by [31]. In this method, the ovaries were dissected out by a pair of scissors. The external connective tissues were removed from the surface of each pair of ovaries. The moisture of the ovaries was removed with the help of a blotting paper. The weight of the ovaries of each fish was measured with the help of an electronic balance. Then 20 mg of each ovary was taken

separately from anterior, middle and posterior portions of each lobe accurately. The number of mature and maturing eggs from each portion was found out separately by actual counting. The mean number of eggs in 20 mg was determined and then multiplied by the total weight of the ovary, which gave the total number of eggs i.e., the fecundity of respective fish.

This value was proportional to the total ovary weight; the number of eggs for the subsample was estimated by using the following equation:

$$\text{Fecundity} = \frac{\text{No. of eggs in sub sample} \times \text{Gonad weight}}{\text{Weight of sub sample}}$$

2.10 Water quality measurements

Temperature, pH and dissolved oxygen were measured in weekly interval in the all environments for domestication, breeding and nursing of larvae. Temperature was measured with mercury thermometer, pH by portable Hanna pH meter and dissolved oxygen by Winklers method [32].

2.11 Data analysis

All data were summarized and analyzed using the Microsoft excel 2013 var. and SPSS var. 20. The data were analyzed to find mean and standard deviations of fish length and weight, % survival of fish, GSI, fecundity and nutrients in micro feed. The survival rate of the fishes as calculated by the following equation.

$$\text{Percentage survival} = \frac{\textit{numbers recovered}}{\textit{numbers stocked}} \times 100$$

3. Results

Thirteen native fish species which found in the Begnas lake and irrigation canal (Table 2) could be included for ornamentals fish.

Table 2: Small size and attractive native fish species found in the Begnas Lake and adjacent irrgation canals

S. N.	Scientific name	Local name
1	*Barilius barna*	Lam Fageta
2	*Barilius vagra*	Fageta
3	*Barilius bendelisis*	Fageta
4	*Puntius sophore*	Bhitte/Bhitta
5	*Puntius conchonius*	Bhitte/Bhitta
6	*Puntius ticto*	Bhitte/Bhitta
7	*Danio devario*	Serra Bhitte
8	*Danio rerio*	Chelawa/zebra fish
9	*Mastacembelus armatus*	Chuche Bam
10	*Xenentodon cancila*	Dhunge Bam
11	*Mystus bleekeri*	Junge
12	*Channa orientalis/Channa gachua*	Bhoti
13	*Channa punctatus*	Bhoti

Among thirteen fish species, the attractive four native fish species were collected from the Begnas Lake and adjacent irrigation canals in the winter and summer months are presented in the Table 3 and Fig 2. These all four species belongs to order cypriniformes and family cyprinidae. *Puntius conchonius* and *Danio rerio* were not collected in the winter season.

Table 3: Length and weight of native ornamental fish species collected from Begnas Lake

S. N.	Fish species	Local name	Length (cm) Mean ± Sd.	Weight (g) Mean ± Sd.
1.	*Puntius conchonius*	Rato pothi /Bhitte	5.7 ± 0.9	3.0 ± 1.4
2.	*Puntius sophore*	Pothi machha /Bhitte	6.3 ± 0.5	3.1 ± 0.7
3.	*Danio devario*	Sera bhitte	3.3 ± 0.4	0.4 ± 0.2
4	*Danio rerio*	Chelawa/zebra fish	4.7 ± 0.3	2.1 ± 0.2

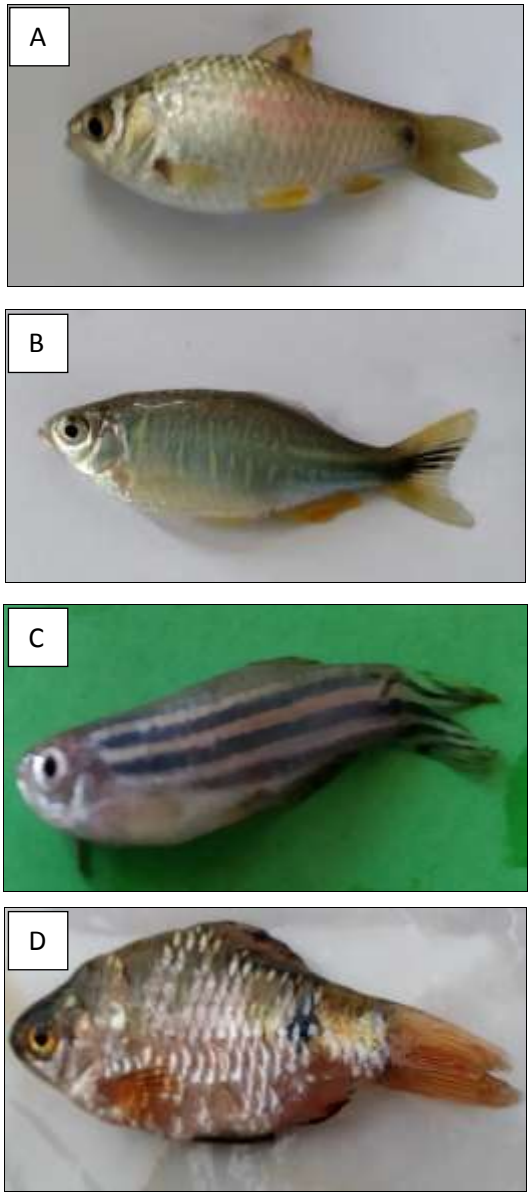


Fig 3: Native ornamental fish species domesticated at FRS Pokhara; *Puntius sophore* (A), *Danio devario* (B) *Danio rerio* (C), *Puntius conchonius* (D)

Domestication of all fish species were found successful in the provided environment with higher survival rate. The survival for the *Puntius sophore* (86.66%) and *Danio devario* (94.59%) were higher in the months of summer than the winter months (Table 4). These fish species were also accepted the feed formulations for the ornamental fish species. The proximate analysis showed that feed formulations for ornamental fish have high crude protein content 34.8% with higher level of minerals (Table 5).

Table 4: Seasonal effects on domestication of native ornamental fish species

S. N.	Scientific name	Winter month's survival (%)	Summer month's survival (%)
1.	*Puntius conchonius*	66.6	-
2.	*Puntius sophore*	82.8	86.66
3.	*Danio devario*	62.4	94.6
4.	*Danio rerio*	-	84

Table 5: Proximate and minerals analysis of formulated ornamental micro feed

Nutrients parameters	Ornamental micro feed (Mean ± SD)
Moisture (%)	7.02 ± 0.13
Crude fat (%)	16.49 ± 0.19
Crude protein (%)	34.85 ± 0.33
Total ash (%)	20.14 ± 0.18
Crude fiber (%)	12.43 ± 0.23
Calcium (mg/100g)	2045.07 ± 12.3
Phosphorus (mg/100g)	875.17 ± 2.25
Iron (mg/100g)	33.13 ± 0.29
Carbohydrate (%)	21.5 ± 0.25
Energy (Kg cal/100g)	373.81 ± 7.14

Table 6: Mean and standard deviation (Mean ± SD) of Gonado somatic index (GSI) and fecundity of native ornamental fish species found in the months of July

S. N.	Scientific name	Length (cm)	Weight (g) (Mean ± SD)	GSI (%) (Mean ± SD)	Fecundity (Mean ± SD)
1.	*Puntius sophore*	7.4 ± 1.3	5.6 ± 3.5	17.58 ± 0.27	4384.08 ± 1786
2.	*Danio devario*	6.9 ± 0.7	5.5 ± 0.93	10.65 ± 0.7	1283.33 ± 225.46

Gonado somatic index (GSI) and fecundity of *Puntius sophore* were found 17.58 ± 0.27% and 4384.08 ± 1786 respectively. Similarly, GSI and fecundity of *Danio devario were* 10.65 ± 0.7% and 1283.33 ± 225.46 respectively (Table 6). We do not perform fecundity of two species (*Puntius conchonius* and *Danio rerio*) due to low number of collections.

The water quality parameters recorded during the periods of study are presented in the table (Table 7). The water quality were found within acceptable range for fish species [33].

Table 7: Water quality parameters recorded during the domestication and breeding seasons of ornamental fish species

Water quality parameters	Domestication period	Breeding season, July to August, 2020 (Mean ± SE)			
	Range	Environment (A)	Environment (B)	Environment (C)	Environment (D)
Temperature (°C)	17.7-29.5	29.2 ± 0.16	28.0 ± 0.14	27.9 ± 0.19	27.4 ± 0.14
Dissolved oxygen (mg/L)	5.7-6.4	5.3 ± 0.09	6.3 ± 0.16	5.6 ± 0.07	5.9 ± 0.03
pH	7.4-7.5	7.4 ± 0.06	7.6 ± 0.04	7.5 ± 0.08	7.7 ± 0.03

Successful breeding of two fish species *Danio devario* and *Danio rerio* were observed in the provided environment. The results showed that *Danio devario* were bred in the artificial canal pool (10 m Length, 0.75 m wide and 30 cm) depth getting direct sunlight, and open with continuous water supply by pipes and natural bedding for breeding with gravels, pebbles and submerged aquatic plants (Environment D). *Danio devario* were bred in last week of August in the rainy days at temperature 27.44 ± 0.14 °C. The present results showed that *Danio devario* prefer natural environment than the aquaria environment for the self-breeding. The survival of fry (Fig. 3 A) was found 64%.

Danio rerio were bred successfully in circular semi-transparent plastic tank having continuous water flow without direct sunlight inside shed (Environment C) in the month of August, at temperature 27.8 ± 0.19 °C and hatchlings were collected with scoop net. The survival of fry (Fig. 3B) was found 58%. The other two fish species: *Puntius conchonius* and *Puntius sophore* were not bred this year. The rearing of these fish is continued to breed in the next year.

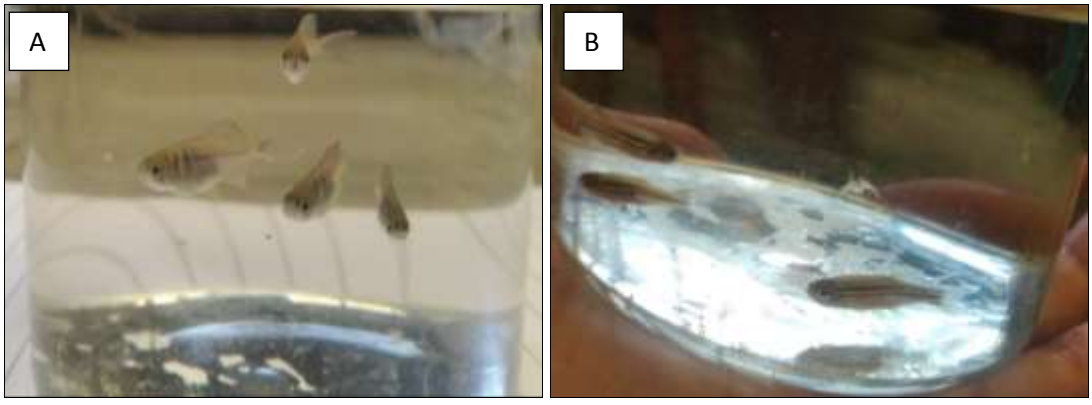


Fig 4: Fry of *Danio devario* (A) and *Danio rerio* (B)

4. Discussions

Globally, Ornamental fish production is emerging as a big industry [5]. More species of ornamental fish have been increased due to advancements in breeding, transport, and aquarium technology in recent year. In the global ornamental fish industry, moreover 2500 fish species are included among which 60% are of freshwater origin. Thirty freshwater fish species dominate in the global market of ornamental fish [11]. Generally aquarium fish are small sized and colored having attractive bands on the body [13].

Feed and feeding management decides the sustainability, profitability, and well-being of an aquaculture system and is essential for the growth, health and reproduction of ornamental fishes [34]. Acceptance of formulated feed is the major challenge for the domestication of fish species. In the present study, small native fish species under domestication were accepted the formulated feed and it was found very suitable for ornamental fish species. The formulated feed in the present study contains crude protein (34.8%), fat (16.49%) and carbohydrate 21.5% and energy 373.81 ± 7.14 (Kg cal/100g) (Table 5). Normally, ornamental fish require 30-45 % protein, 4-8 % lipid and 30-50 % carbohydrate in their regular diet [34]. Protein, lipid and energy levels for optimum growth and nutrient utilization varied between 30-40%, 6-10% and 3.5-4.0 kcal/g, respectively for ornamental fish species [35].

In the present study, Gonado somatic index (GSI) of *Puntius sophore* was found 17.58 ± 0.27% (Mean ± SD) of mean length 7.4 ± 1.3 cm and weight 5.6 ± 3.5g (Table 6). The findings of Hossain *et al*. 2012 [36] that the size at first sexual maturity to be 5.0 cm and individuals with a GSI ≥ 4.2% could be categorized to mature *P. sophore* females is supported to present findings that the fish were found > 5.0 cm with mean GSI > 4.2%. The present findings of GSI in *P. sophore* (17.58 ± 0.27%) in July is higher than the findings of Hasan *et al*. 2018 [37] that GSI in *P. sophore* was found 15.43 ± 2.20% in the month of April and 15.60 ± 1.74% in June while it is lower than the average GSI reported in female *P. sophore* populations during July month (20.88 ± 4.55%) [38]. Most of the females *P. sophore* with >6.00 cm total length were recorded with a calculated GSI of >6.0 [36].

Mean fecundity of *Puntius sophore* was found 4384.08 ± 1786 of mean length 7.4 ± 1.3 cm and weight 5.6 ± 3.5g in the present study. Wide variations of fecundity were reported in the earlier studies. The value of fecundity may vary due to various reasons including habitat, environmental factors, and food availability [37]. It was reported that the mean total fecundity was 5300 ± 2700 in *Puntius sophore* [36]. Another study was reported that the highest fecundity of *Puntius sophore* was found in April (5053 ± 878.265) for the sample (length 10.06 cm and body weight 13 g) collected from Gazipur while it was found in July (4032 ± 622.68) for the sample (length 9.93 cm and a body weight 10.32g) collected from Jessore [37]. Likewise, Tareque *et al*. 2009 [40] have reported that mean number of eggs in *Puntius sophore* was 2294 ± 700 for a fish with a mean total length of 8.31 ± 0.92 cm and a mean weight of 9.16 ± 2.72 g while Bithy *et al*. 2012 [41] was estimated 7951 eggs for a body weight of 10.12 g. Fecundity of *Puntius sophore* ranged from 1824-7004 [39].

Similarly, GSI and fecundity of *Danio devario were* 10.65 ± 0.7% and 1283.33 ± 225.46 respectively in the present study. Literature on fecundity of *Danio devario* have been not found so far. It is the first attempt to determine the fecundity of *Danio devario*. We found that *Danio devario* at the length of 6.9 ± 0.7 cm and weight 5.5 ± 0.93g was mature in July month. More work should be continued with seasonal as well on first maturity.

Domestication of collected fish species in the present study was found successful with higher survival rate (62.4-94.6%) (Table 4). For the suitability for aquarium trade, survival should be good enough over 80% during the domestication [42]. After domestication the next steps is the obtaining the first generation of captive fish from wild fish [1]. The process for obtaining the first generation from domesticated fish species *Danio devario* and *Danio rerio*, in semi-natural condition was the first attempt for breeding in Nepal, and it was found successful with an easy practical methods to obtain fish seed. Breeding of *Danio devario* was observed in the last week of August in the rainy days at temperature 27.44 ± 0.14 °C without hormones. The results of this study could be comparable to the previous study on the breeding of *D. aequipinnatus* which was found successful in the captivity without use of any hormones from July to August at water temperature 27 ± 0.5 °C [43].

Danio rerio were bred successfully in circular plastic tank having continuous water flow without direct sunlight inside shed and natural bedding for breeding with gravels, pebbles and submerged aquatic plants in the month of August, at temperature 27.8 ± 0.19 °C. Our results is in agreement with previous study that zebra fish bred at the temperature of the breeding set-up between 27 and 28 °C [23, 25, 44]. The semi-natural breeding environment maintained for breeding of *Danio rerio* in present study is supported by previous studies that this fish prefer floating plants, submerged plants [22], prefer the substrate, gravel over sand and prefer dark environments [19, 20], release eggs and sperm in a cloud over the substrate [45], choosy with respect to sites for oviposition [46], and preferring a gravel substrate to silt [47]. *Danio rerio* bred in the months July-September [13] is agreement to our study.

It was reported that annual import of ornamental fish was 1233.0 metric ton with its value NRs.153.4 million in 2017 [11]. To reduce the annual import of ornamental fish, the domestication and propagations of attractive native fish species suitable for aquarium rearing should be continued. The present study is preliminary study which attempts to domesticate and propagate the ornamental fish species with simple way as every farmer could adopt in Nepal. More species should be domesticated and effort to breed in captivity, and also standardize the domestication and breeding techniques as farmers friendly should be continued to adopt quickly by the farmers.

5. Conclusion

Addition of fish species in the culture by domestication from wild will be a milestone success for species diversification in aquaculture. Among the four domesticated ornamental fish species, two fish species (*Danio devario* and *Danio rerio*) were bred successfully in the semi-natural condition. The success of breeding of these fish species will ensure for the conservation and addition of new candidate for ornamental fish in Nepal. Further studies required to recommend for commercial production.

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