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Diseases of cultured Thai *Koi* in Mymensingh region

Md. Abdullah Al Mahamud[1]*, Kamruzzaman[2], Md. Shahadat Hossen[3], Md. Ariful Islam[4], Gias Uddin Ahmed[5]

[1]*Agriculture, Fisheries and Livestock Development Program, HEED Bangladesh, Moulvibazar, Bangladesh*
[2]*Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh*
[3]*Upozila Fisheries Officer, Department of Fisheries, Kamalgonj, Moulovibazar, Bangladesh*
[4]*Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh*
[5]*Department of Aquaculture, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh*

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Corresponding Author

MAA Mahamud
Email:
mduzzalprodhan@gmail.com

ABSTRACT

A study was conducted to investigate of diseases and culture of Thai *Koi Anabas testudineus* over a period of 165 days from 29 September 2010 to 13 March 2011 in nine earthen ponds located in the northern side of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. Three stocking densities such as 500/decimal (T_1), 750/decimal (T_2) and 1000/decimal (T_3) were tested with the three replications. The water quality parameters were monitored at fortnightly interval. The mean value of water temperature in T_1, T_2 and T_3 was 23.25 ± 1.95°C, dissolved oxygen content were 5.0 ± 1.33 mg/L, 4.95 ± 1.32 mglL and 5.05 ± 1.11, pH were 7.50 ± 0.20, 7.62 ± 0.28 and 7.70 ± 0.36 and alkalinity were 171.00 ± 19.12, 168.00 ± 18.14 and 164.00 ± 25.47 respectively. The survival rates were 95.08%, 95.49% and 88.00% in T_1, T_2 and T_3 respectively. The health and diseases status of Thai *Koi* were investigated for seven months during the September 2010 to March 2011. Clinically, it was observed that fish were affected with scale loss, rough skin discoloration and dermal ulcer during the winter season. Histopathalogically, in the months of December and January, necrosis and vacuums were observed in skin and muscle. Gill of the Thai *Koi* were affected with loss of primary and secondary gill lamellae, necrosis and hemorrhage in the months of December and January. In case of liver, necrosis, pyknosis, vacuums and hemorrhages were observed in the months of December and January. However, increased clinical and pathological sign were observed in T_2 and T_3 in December and January. Dig the focus group discussion, farmers also mentioned that diseases like EUS and tail and fin rot were found in the months of December and January.

Introduction

The climbing perch locally known as *Koi*, *Anabas testudineus* (Bloch 1792) is a teleost belonging to the family Anabantidae and order Perciformes. This species is naturally distributed in Bangladesh, and highly culture due to fast growth and higher production. For this, farmer cultured the species in largely in the country.

With the increase of intensification, culture fish were become stressed, infected and diseased. During the culture period, Thai *Koi* become stressed due to unfavorable environmental condition and over feeding and farmers are facing problems with diseases of Thai *Koi* in Bangladesh under farming condition (Ahmed et.al. 2007).

Disease has become a major problem in fish production in culture system and wild condition in Bangladesh (Rahman and Chowdhury, 1996). One of the important constraints in Thai *Koi* farming in Bangladesh is the occurrence of diseases especially in the juvenile stage (Dhar, 2007) Thus, minimization of disease problem has become essential task for increase production and good health maintenance. Clinical and histopathological procedures are important for the diagnosis of disease. The present study, attempted to evaluate the health condition of Thai *Koi* at on farm management under different stocking densities.

Materials and Methods

Study area

The study was conducted for a period of 165 days from 29 September-2010 to 13 March 2011 in 9 experimental ponds each of 0.60 decimal, located in the northern side of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. Water depth was maintained to a maximum level of 1.5 m and all the ponds were similar in size, depth and configuration.

Pond preparation

To eradicate all undesirable fish, insect and other aquatic organism ponds were drained out completely. Aquatic weeds were removed and embankments were repaired manually. Lime was applied at the rate of 1 Kg/decimal. After one week of lime application, the ponds were filled with water from a deep tube-well supply.

Experimental design

The ponds were selected randomly to accommodate the relevant treatments. The experimental layout is shown in Table 1.

Table 1
Experimental layout of Thai *Koi* culture.

Treatment	Replication (pond no)	Pond size (d)	Stocking density	Total Stocked	Stocking size (g)
T_1	R-1 (1)	0.60	500	325	1.53
	R-2 (2)	0.60	500	325	1.53
	R-3 (3)	0.60	500	325	1.53
T_2	R-1 (4)	0.60	750	488	1.53
	R-2 (5)	0.60	750	488	1.53
	R-3 (6)	0.60	750	488	1.53
T_3	R-1 (7)	0.60	1000	650	1.53
	R-2 (8)	0.60	1000	650	1.53
	R-3 (9)	0.60	1000	650	1.53

Collection and release of fry

The fry of Thai *Koi* of one month aged were collected from the Brahmaputra Hatchery, Shambhugonj, Mymensingh and transported to the experimental ponds with polythene bag having oxygen. Then the fry were acclimatized with experimental pond water in polythene bag and then stocked at 5 pm.

Water quality parameters

The water quality parameters were recorded throughout the experimental period. The physico-chemical parameters like temperature (°C), dissolved oxygen (mg/l), alkalinity (mg/l), Nitrate (mg/l) and pH were measured fortnightly on the day of sampling. Water quality measurements and sample collection were made between 9.00 am to 10.00 am on each sampling day (Table 3).

Sampling of fish

Sampling of fish was done at 15 days intervals in the morning at around 7am to 8 am. During each sampling fish were caught by cast net and weight was taken by correctness weighing balance (Accuracy up to 0.1 g).

Growth performance

The weight gain (g), percent weight gain, specific growth rate (SGR), survival rate (%) and production (Kg/ha/165days) performance were measured to evaluate the growth of the fishes according to the standard procedure described by Mondal et al. (2016).

Clinical and histological observation

During the experimental period, clinical changes were recorded fortnightly. From each pond, fishes were examined by naked eyes to observe the external signs and color changes, injury, infection in fin and muscle, appendage damage and other abnormalities. Monthly sample was collected from various organs such as skin, muscle, gill, liver and kidney by a sharp scalpel and forceps for histopathological study from T1, T2 and T3 of experimental ponds and proceed for histological observation.

Results and Discussion

Physicochemical parameters of the pond water

The results of the water quality parameters such as temperature (°C), pH, dissolved oxygen (mg/L), nitrite (mg/L), ammonium (mg/L) and alkalinity (mg/L) during the experimental period.

Water temperature (°C)

Temperature

During the study period, the water temperature varied from 33.0 to 13.0°C in T_1, 33.0 to 13.0°C in T_2 33.0 to 13.0 oc in T_3. The mean values of water temperature in treatments T_1, T_2 and T_3 were 23.25±1.95 °C and 23.25±1.95°C and 23.25±1.95 respectively. Alim (2005) measured water temperature to range from 17.30 to 33.50°C that was more or less similar. Masud *et al.* (1996) were measured water temperature to range (24.7 to 30°C). The temperature as observed in this study appeared to be suitable for fish culture.

Dissolved oxygen (mg/L)

During the study period, the dissolved oxygen content of the water was varying from 3.50 to 8.50 mg/L in T_1, 3.0 to 8.0 mg/L in T_2 and 4.0 to 7.5 mg in T_3. The mean values of dissolved oxygen content of the water in T_1, T_2 and T_3 were 5.0±1.33 mg/L, 4.95±1.32 mg/L and 5.05± 1.11 respectively. Alim (2005) measured dissolved oxygen ranged from 1.2 to 8.5 mg/l and Banerjee (1967) considered 5 to 7 mg/l of dissolved oxygen content of water is fair in respect of productivity.

pH

During the study period, the range of pH values recorded in T_1, T_2 and T_3 were varying between 7.4 to 8.0, 7.4 to 8.3 and 7.4 to 8.3 respectively. The mean values of pH were 7.50±0.20, 7.62±0.28 and 7.70±0.36 recorded in T_1, T_2 and T_3 respectively. There was no significant variation of pH values under different treatments.

Most natural water has pH values of 6.5 to 9 (Boyd, 1982). The present finding was within acceptable range required for fish culture 6.5 to 8.5 (DOF, 1996).

Alkalinity (mg/L)

Alkalinity (mg/l) of the experimental ponds was varied from 120 to 180 mg/L, 120 to 190 mg/L and 100 to 190 mg/L in T_1, T_2 and T_3 respectively. The mean

values of alkalinity were 171.00±19.12, 168.00±18.14 and 164.00± 25.47 recorded in T_1, T_2 and T_3 respectively. There was no significant variation of alkalinity among different treatments.

Raihan (2010) funded alkalinity on his experiment was 114 to 180 that was more or less similar. Rahman (2000) found alkalinity varied from 37 to 151 mg/l. Uddin (2002) conducted an experiment in earthen ponds Field Laboratory of Bangladesh Agricultural University, Mymensingh observed that alkalinity varied from 45 to 180 mg/l.

Nitrite (mg/L)

During the experiment there was a little amount nitrite (<0.03mg/L) found in experimental ponds which is negligible. Nitrite is the least toxic of the major inorganic nitrogen compounds.

Ammonium (mg/L)

A little amount of ammonium (0 to 0.02,0 to 0.03and 0 to 0.03 mg/L in respectively T_1, T_2 and T_3) was found in experimental ponds and it is insignificant.

Table 3. Average (Mean ± SD) values of water quality parameters under different treatments during the study period

Treatment	Temperature (˚C)	Dissolved oxygen (mg/L)	Alkalinity (mg/L)	pH	Nitrite (mg/L)	Ammonium (mg/L)
T_1	23.25±6.17	5.0±1.33	171.00±19.12	7.50±0.20	<0.03	0 to 0.02
T_2	23.25±6.17	4.95±1.32	168.00±18.14	7.62±0.28	<0.03	0 to 0.03
T_3	23.25±6.17	5.05± 1.11	164.00± 25.47	7.70±0.36	<0.03	0 to 0.3

Growth performance of fish

In terms of weight (g) gain, percent weight (g) gain, specific growth rate (SGR% per day), survival (%) and production (Kg/0.6dec/165days) were calculated in Table 4 for the evaluation of growth performance of fish in different treatments.

The stocking density of 500/d, 750/d and 1000/d were used in the experiment. Kohinoor *et al.* (2007) tested 300/d, 400/d and 500/d in three treatments which is lower than present experiment. Farmers of Mymensingh region were practiceed stocking densities 1000/d (2011) and 3500-4000/d (2011, personal communication) which was higher than the present experiment.

Weight gain

There were no significant (P<0.05) difference in initial weight of fish under different treatments. The mean weight gains of fish at the end of the experiment were followed by 45.22 g, 38.22 gm and 37.89g in T_1, T_2 and T_3 respectively. Weight gains in all the treatments were significantly different with each other.

Mustafa *et al.* (2010) observed the initial average weight of *Anabas testudineus* fry increased from 0.9g to 3.5g, 1.0g to 7.3g, 0.95g to 5.82g and 0.92g to 5.4g in the treatments T_1, T_2, T_3 and T_4 respectively. The highest and lowest average final length was found in T_2 and T_1 respectively. But the present study is closely related to Khan (2008) who observed that the average highest weight of individual Thai *Koi* was 91.0g and lowest 68.5g after four months culture and Kohinoor *et al.* (2007) observed highest weight gain was 88.89g and lowest weight gain was 67.83g in four months culture operation.

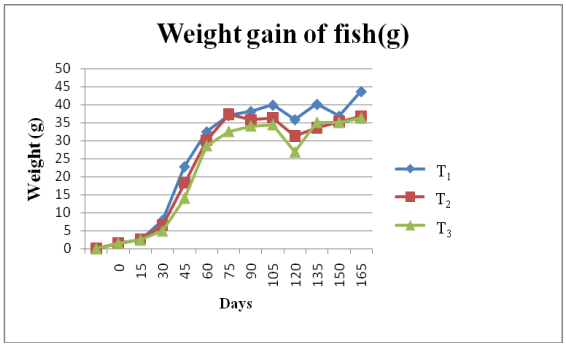

Figure 1
During the study period weight gain of fishes at different treatments.

Table 4. Growth parameters of Thai *Koi* (*Anabas testudineus*) observed in different treatments during the study period.

Growth parameters	Treatments		
	T_1	T_2	T_3
Initial weight(g)	1.53±0.05	1.53±0.05	1.53 ±0.05
Final weight(g)	45.22 ±4.14^a	38.22±3.81^b	37.89±3.82^b
Weight gain (g)	43.69±0.11^a	36.69±1.90^b	36.36±0.29^b
% Weight gain	2855.56±7.19^a	2398.26±124.07^b	2376.25±19.23^b
SGR (%/day)	0.89±0.23^a	0.85±0.05^b	0.84±0.45^b
Survival %	95.08±.64^a	95.49±1.14^a	88.00±1.69^b
Fish production (Kg/0.6 dec/165days	13.50±0.07^a	17.11±1.04^b	20.79±0.23^c

Percent weight gain

The % weight gains of Thai *Koi* in different treatments were 2855.56 %, 2398.26 %, 2376.25 % in respectively T_1, T_2 and T_3. The significantly ($P<0.05$) highest % weight gain values (2855.56) was recorded in T_1 while the lowest (2376.25) was obtained in T_3.

Survival (%)

The survivals (%) in different treatments were 95.08%, 95.49%, and 88% in T_1, T_2 and T_3 respectively. The survival rates were significantly different among the treatments. The highest survival was obtained in T_2 (95.49%) and the lowest was in T_3 (88%) due to higher stocking density.

This result does not agree with Akhteruzzaman (1988) who reported that the survival rate of *Anabas testudineus* varied from 60 to 80. But these results were closed to the present study with Kohinoor *et al.* (2007), who observed that the survival rate of Thai *Koi* varied from 79% to 92%. Mustafa *et al.* (2010) also observed the survival rates were recorded 74, 85, 81 & 79 % in the treatments T_1, T_2, T_3 and T_4 respectively. However, these differences were not significant ($P>0.05$) among treatments of each experiment. Mookerjee and Mazumdar (1946) in a study with climbing perch observed survival rate ranging from75%-89%. This observation is within our observed value of survival rate of *A. testudineus*. Hasan *et al.* (2010) reported that the survival rate of Thai *Koi* were 73%, 76% and 83% in T_1, T_2 and T_3 respectively during the period of experiment.

Clinical observation

Clinical observation of the T_1

Fish showed normal in appearance in the months of September, October and November, but in December and January moderate rough skin was observed. However skin was almost normal in February and March.

Clinical observation of the T_2

In the month of September and October fish showed normal in appearance. But in months of November, December and January moderate rough skin and scale loss was observed. Injured skin and scale was recovered in February and March.

Clinical observation of the T_3

In the month of September and October fish showed normal in appearance. But in month of November mild rough skin and scale loss were observed. Mild lesions on body surface and small red spots of fish were observed in December and January. All clinical signs were started to recover in February and March.

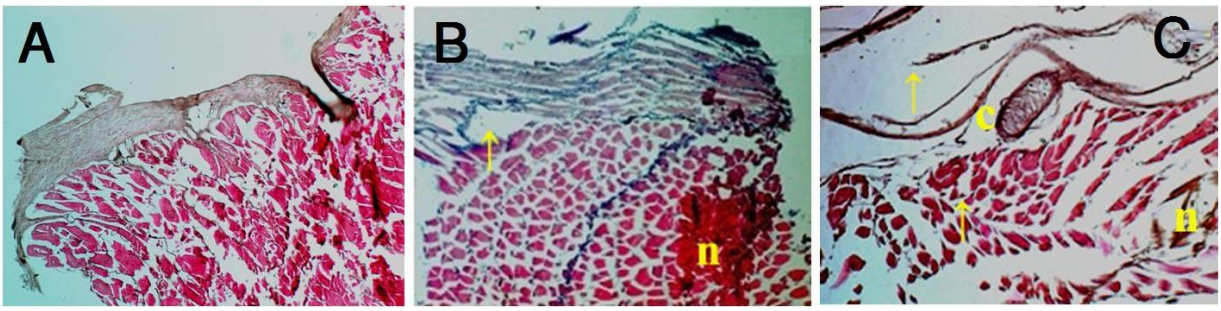

Figure 2. Histological section of skin and muscle of Thai *Koi* (H & E × 220). *A.* T_1- Normal skin and muscle, B. T_2- Normal muscle with parts of dermal missing (↗) and necrotic muscle (n) and C. T_3-Epidermal loss (↗), dermal necrosis (n), sloughed off (↗), cysts (c), muscle necrosis (n).

Histopathological observation in different treatments

During the study period, skin and muscle, gill, liver and kidney were affected from November to February in different treatments. In November had almost normal structure of skin (Fig. 2A) and muscle but portions of epidermis was still missing and sloughing off (↗) (Fig. 2B). Severely affected skin and muscle with epidermal loss (↗), dermal necrosis (n), sloughed off (↗), cysts, muscle necrosis (n) were found in December and January (Fig. 2C). Affected primary and secondary gill lamellae having lamellar missing (↗), necrosis (n), hyperplasia (hp) were found in December and January (Fig. 3A), gill had normal structure but primary gill lamellae spitted (↗) in January and February (Figs. 2C and 3C). In case of liver severe necrosis (n), vacuums (v), melanomacrophages (mm) and hemorrhage (h) were observed in December and January (Fig. 4B). Severely affected kidney with necrosis (n), vacuums (v), pyknosis (p) and deep hemorrhage (h) were found in December and January (Fig. 4C). Pathological healing were evidenced but mild vacuums (v), hemorrhage (h) and necrosis (n) were still seen in February (Fig. 5C).

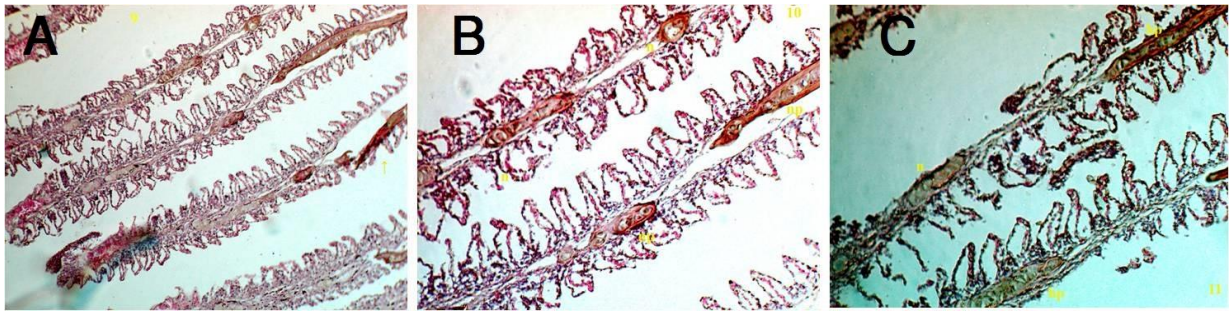

Figure 3. Section of gill of Thai *Koi* (H & E × 220). A. T_1- Gill structure was normal except primary gill lamellae spitted in few places (↗), B. T_2 -Severely affected primary and secondary gill lamellae having lamellar missing (↗), necrosis (n) and hyperplasia (hp) and C. T_3- Secondary gill lamellae were partly lost (↗), necrosis (n) and hyperplasia (hp).

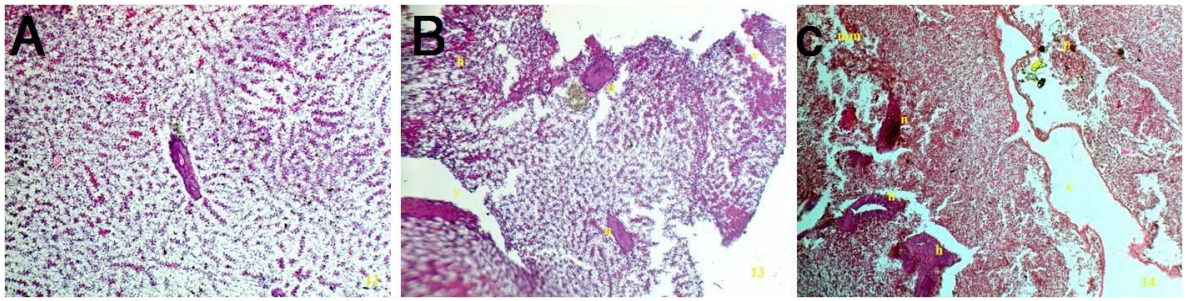

Figure 4. Section of liver of Thai *Koi (*H & E × 220). A. T_1 -normal liver, B. T_2- Mild vacuums (v), hemorrhage (h) and necrosis (n), and C. T_3-Vacuums (v), hemorrhage (h), necrosis (n) and pyknosis (p).

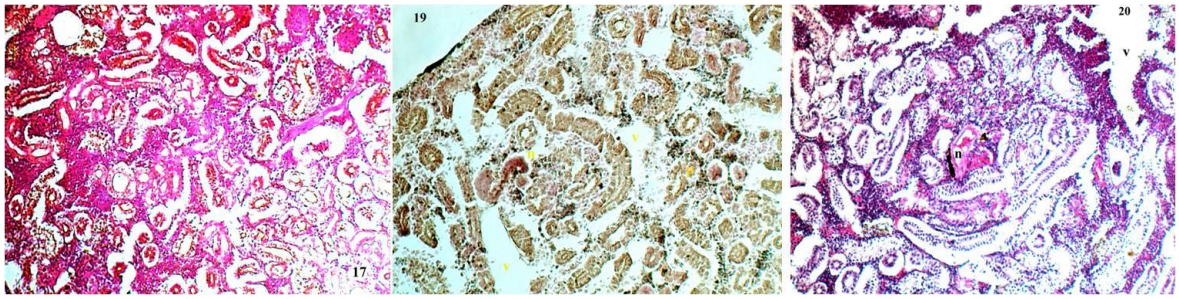

Figure 5. Section of kidney of Thai *Koi.*(H & E × 220). A. T_1- Almost normal, B. T_2- Severely affected kidney having marked necrosis (n), vacuums (v), pyknosis (p) and deep hemorrhage (h) and C. T_3- Mild necrosis (n). and vacuums (v).

Diseases in Thak *Koi* fish were the major problem in the Mymensingh region, Bangladesh. Common diseases were tail and fin rot, red spot and EUS. They also mentioned that, fish diseases were recorded in winter season especially in the month of December and January. Most farmers and farm owners used preventive measures like lime, salt, geolite and gasonex for water treatment. The farmers also used different types of antibiotics along with lime and salt when disease outbreak was severe.

Investigation of the diseases and production of Thai *Koi* was the main objectives on my present experiment. Clinically, it was observed that Thai *Koi* was apparently healthy and normal in appearance during the month of September and October in all the treatments. In November mild clinical signs like rough skin, scale loss and minor body discoloration were observed especially in T_2 and T_3. In the month of December and January minor clinical signs like rough skin and scale loss was observed in T_1, whereas, various clinical signs changes like scale loss, mild ulcer, rough skin and small red spots in T_2 and T_3. The result of the present experiment also agreed with the works of Patwary *et al.* (2008), Ahmed *et al.* (2005), Ahmed *et al.* (2004), Ahmed *et al.* (2000), Chinabut (1994) and Rodgers and Burke (1981).

During the study period, histopathologically, it was observed that, skin and muscle, gill, liver and kidney were almost normal in months of September, October and March in all the treatments. However, reduced pathological changes like mild necrosis and hemorrhage were observed in skin and muscle in T_2

and T_3 in December and January whereas, marked pathological symptoms like epidermal and dermal missing, sloughed off were found in T_3 in December and January..

Pathological healing was started in February in all the treatments. Ahmed *et al*. (2007) observed that skin and muscle normal in August and September. Akter *et al.* (2009) observed that several gill pathologies such as pyknosis, hypertrophy, hyperplasia and hemorrhage were found in winter season i.e., in December and January.

In T_1 and T_2, liver affected with mild necrosis, pyknosis, small vacuums, and hemorrhage whereas, marked pathological signs like hemorrhage, large vacuums, necrosis and pyknosis were found in T_3 in December and January. However, pathological healing was started in February in all the treatments. Ahmed *et al*. (2009) observed that liver affected with mild necrosis, hemorrhage and vacuoles in November. Large vacuoles with hepatic necrosis, fungal granuloma and pyknotic cells were observed in December and January.

In case of kidney mild necrosis, vacuums, and hemorrhage were found in T_1 and T_2 in December and January whereas, deep hemorrhage with large vacuums, necrosis and pyknosis were found in T_3 in December and January. It was thus observed that clinically and histopathologically most of the organs especially inT_2 and T_3 were affected in December and January. The result of the present experiment also agreed with the works of Raihan (2010), Ahmed *et al*. (2009), Patwary *et al.* (2008), Ahmed *et al.* (2005), Chinabut (1994) and Rodgers and Burke (1981).

The results of the present investigation coincided with the farmer's opinion during focus group discussion in the sense that clinically and histopathologiclly fishes were more affected during the similar season i.e., in December and January.

Conclusion

Clinically, fishes were normal in appearance in the months of September, Octotter, February and March. Fish had scale loss, rough skin; small red spot and body discoloration were found in the months of November, December and January. In the higher stocking density treatments more clinical sign were recorded than the lower stocking density treatments.

Histopathologically, skin and muscle, gill, liver and kidney had normal structure in the months of September, October, and March. Mild pathological sign like lamellar missing, necrosis, pyknosis, and hemorrhage were observed in the month of November. Marked pathological sign like epidermal and dermal missing, sloughed off, lamellar missing, necrosis, pyknosis, large vacuums, and hemorrhage were observed in the months of December and January. Again higher stocking density treatments had more pathological signs than the lower stocking density.

The farmers mentioned that disease was the major problem in the area. EUS is the main diseases that were mentioned by the farmer during the focus group discussion. Generally disease occurs in the moths of December and January. Most farmers and farm owners used preventive measures like lime and salt Geolite Gold, Gasonex for treatment. They also used different types of antibiotics along with lime and salt when disease outbreak was severe.

From the above discussion it can be concluded that Thai Koi culture with 1000/d stocking density may be conducive to get high production and maximum benefit. Diseases and pathology in Thai Koi was dominant in December and January. Thus special attention should be taken during winter season, to prevent occurrence of diseases in fish.

Introduction of pathogen through carriers like other animals, utensils, nets should be prevented. Entry of pollutants and other chemicals should also be taken care of so that fishes would get reduced stress especially during the winter season. Lime and salt shouldd be applied before stocking as preventive measures.

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