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Investigation of Diseases of Thai koi, *Anabas testudineus* (BLOCH) from Farming Conditions in Winter

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Investigation on health conditions of Thai koi (*Anabas testudineus*) were carried out through clinical and histopathological observations from different farms of Mymensingh district for seven months during August 2006 to February 2007. Fish sampling and water quality parameters (temperature, dissolved oxygen and pH) were monitored on a monthly basis. Clinical examination of fishes was also carried out for any kind of abnormalities at monthly intervals. Samples of skin, muscle, gill, liver and kidney were observed by histological technique. Among the water quality parameters the values of water temperature, dissolved oxygen were found to be at unfavorable level for fish during the colder months in the farms. Clinically it was observed that fishes were more affected from December to January and almost normal in appearance during August to September and February. Different clinical symptoms like scale loss, dermal lesion, ulcer and loss of caudal fin were noticed in December and January. In histopathological study, structures of fish organs were normal from August to September. In the months of October and November, minor pathologies were found to be started. Marked pathological changes like necrosis, pyknosis, inflammation, hemorrhage, hypertrophy, hyperplasia, missing of primary and secondary gill lamellae were observed in the months of December and January. Whereas, in the month of February the pathological condition of fish gradually reduced. Again when considered individual fish farm, fishes of Reliance Aqua Farm were more affected than Sotota Matshya Hatchery. The study showed that severity of clinical and pathological changes were increased in December and January. During the period EUS and protozoan diseases were noticed in *A. testudineus* of the investigated farms.

Key words – Thai koi, *Anabas testudineus*, fish diseases, pathological investigation

Introduction

Climbing perch or koi are native to Asia. Among the small indigenous fishes, the climbing perch (*Anabas testudineus*) or koi is an important favorite small indigenous fish of Bangladesh. For its special nutritive and medicinal qualities, ailing patients use it as diet for recovering from illness [9]. *Anabas testudineus* contains high amount of iron and copper, which are essentially needed for hemoglobin synthesis. Considering its culture potentials very recently, a sharp increasing trend has observed on Thai koi (*A. testudineus*) culture in some selected areas of Bangladesh, particularly in several upazillas of Mymensingh district. In 2002, a private entrepreneurs, Aqua Farm Ltd. Dhanmondi, Dhaka, Bangladesh imported Thai koi strain seed from Thailand. Presently, this strain is getting more popular than our native strain due to its increased growth rate. This fish attains 80-100 g within 3-4 months. The fish culture system in Bangladesh is mostly extensive type and with the introduction of intensive system, fish farmers are encouraged due to increased fish production. Intensive culture system with very high stocking densities, poor water qualities is encountered with much of the disease problems. Disease among culture fish can cause death, poor growth and food conversion which increased production costs and interrupted production schedule [6]. With the outbreak of epizootic ulcerative syndrome (EUS) in 1988, *Channa* spp., *Puntius* spp., *Anabas* spp. and other indigenous species of fish are seriously affected [7]. Disease has become a major problem in fish production in culture system and wild condition in Bangladesh [21]. From research findings of Akter it was observed that in *M. tengara*, ill health, scale loss were observed in December and serious dermal lesion, loss of caudal fin and ulcer were noticed in December and January [4].

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Clinical and pathological procedures are important tools used to diagnose diseases in fish. Histopathological technique is one of the most important procedures for disease diagnosis in fish. It has been successfully used throughout the world. Several histopathological studies have been done on major carps, though only few works have been done on freshwater small indigenous fishes. So the present work was undertaken in order to diagnose diseases of Thai koi (*Anabas testudineus*) from fish farms of Mymensingh district, Bangladesh.

Materials and Methods

The study was conducted for a period of seven months from August 2006 to February 2007. The investigated area included two different farms of two upazillas of Mymensingh district, Bangladesh. One farm is Reliance Aqua Farm, situated in Trishal upazilla and another farm is Satata Matshya Hatchery, situated in Tarakanda upazilla of Mymensingh district. These were selected because *A. testudineus* are mainly cultured in the farms. Water quality parameters like temperature, pH and dissolved oxygen were determined at monthly intervals by using HACH's kit (Model FF-1A) from the two mentioned farms. Five live fishes from both the farms were collected and immediately carried to the Fish Disease Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. The sampled fish were clinically examined by naked eye and magnifying glass. The fishes were dissected and organs like skins, muscle, gill, liver and kidney were collected with the help of sharp scalpel and forceps and fixed in 10% buffered formalin for histopathological study. Then the samples were trimmed and placed in an automatic tissue processor (SHANDON CITADEL 1000) for dehydration, clearing and infiltration. The samples were then embedded, sectioned (thickness, 5 micrometers) and stained with Haematoxylin and Eosin. The sections were then examined under a compound microscope (Olympus) and photomicrographs were taken by using a photomicroscope (Olympus Microscope Model-CH_2, Photomicrographic attachment). Record of structural variations and pathologies were done from the slides and photographs. Comparisons of pathologies and diseases in fish were thus made between the farms and among the various months of the investigated fish.

Results and Discussion

The values of water temperature in both selected farms were almost nearer during the experimental period that ranged from 19.5-29.5°C, while in December and January it was 19.5°C in both the farms. Akter observed that in Ailee beel of Mymensingh, *M. cuchia* and *M. aculeatus* were more affected (clinically and histologically) in December and January when water temperature were at minimum level (18°C) [4]. In the investigated farms, the values of dissolve oxygen ranged from 4.2-5.2 mg/l recorded during the experimental period. Hossain and Kohinoor recorded dissolved oxygen values of fish ponds ranging from 3.8 to 6.9 mg/l and 2.04 to 7.5 mg/l respectively at Mymensingh district [14,15]. The pH values were ranged from 7.0 to 7.5 among the selected farms during experimental period. Gosh observed that in private fish farm of Mymensingh, Thai-sharpunti and silver carp were more affected (clinically and histologically) in December and January when pH were at minimum level (6.5) [11].

In the present study clinically affected Thai koi were first noticed during the August and September in Reliance Aqua Farm. This result was also similar with the findings of Gosh [11]. In contrast, *A. testudineus* was recorded to have minor abrasions such as red spots, scale loss, weak body during October to November. Roy observed that in *N. nandus* rough skin and scale loss was found in October and November [22]. Clinically *A. testudineus* was found to be more affected with certain distinguishing symptoms during the winter months (December and January) in Bangladesh. In *A. testudineus* rough skin, scale loss and mild hemorrhagewere observed in December, whereas, hemorrhage with reddish bright spots, fin loss and deep ulcers were evidenced at dorsoventral region of skin during January (Fig. 1). According to Akter *M. aculeatus* and *M. cuchia* of Ailee beelin Bangladeh had rough skin, weak body, red spots and deep ulcer in December to January [5]. Hoque *et al.* were of the opinion that large deep and whitish ulcers were recorded in the lateral region (near dorsal fin) and caudal region where part of fins, scales and muscles were lost in most of the EUS affected fish [13]. However, in February clinical signs were almost reduced to normal level as seen in Thai koi of Satata Matshya Hatchery (Fig. 2).

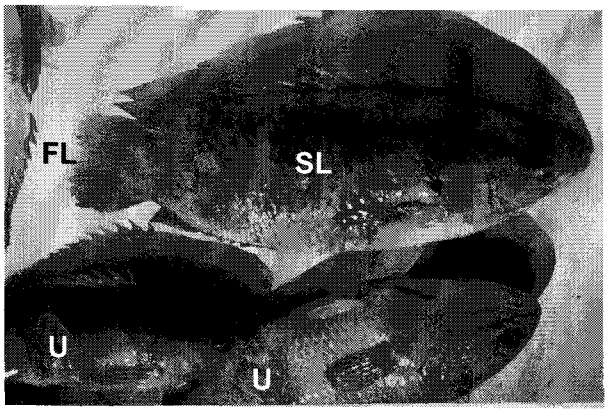

Fig. 1. Photograph of *Anabas testudineus* from Reliance Aqua Farm in January. Scale loss(SL), ulcer(U) & fin loss(FL) were seen.

Fig. 2. Photograph of *Anabas testudineus* from Satota Matshya Hatchery in February with almost reduced or no clinical sign.

Histopathologically, it was observed that, skin and muscle of *A. testudineus* were normal in August and September of both the farms. Roy observed that skin and muscle had almost normal structure in *Puntius sophore* during August and September [22]. Conversely, in the present experiment, less pathological changes such as loss of epidermis and necrotic muscles were observed in October and November. This result was also similar with the findings of Akter who observed that necrotic muscles and epidermal loss in *M. cuchia* and *M. aculeatus* during October and November [3]. Histopathologically, epidermis and dermis were totally lost and necrotic, whereas, muscles had fungal granuloma in *A. testudineus* of both the farms during December and January (Fig. 3, 4). Presence of fungal granuloma in the muscle of investigated fish indicated that the fish were affected by Epizootic Ulcerative Syndrome (EUS). Fungal granuloma as an indicator of EUS had been proved by scientists Ahmed *et al.* and Moniruzzaman [2,19]. Akter *et al.*

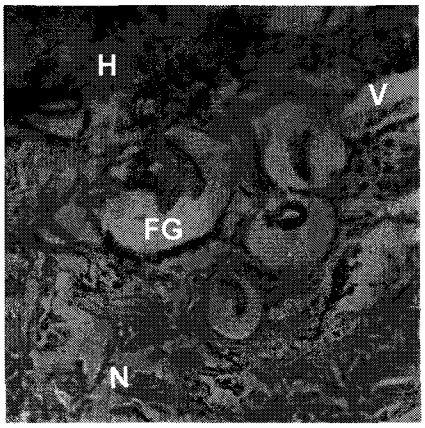

Fig. 3. Section of skin and muscle of *A. testudineus* in December & January from Sotota Matshya Hatchery. Severe necrosis(N), vacuums(V), fungal granuloma (FG) & hemorrhage(H) were seen in muscle (×420).

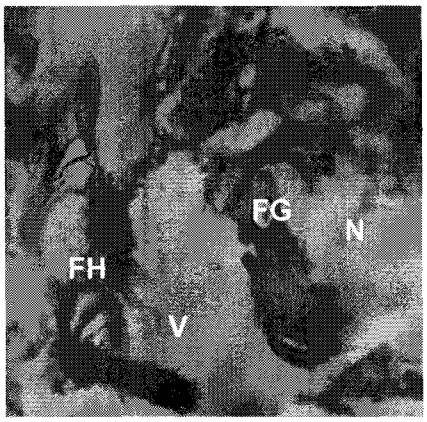

Fig. 4. Section of skin and muscle of *A. testudineus* in December & January from Reliance Aqua Farm. Fungal granuloma(FG), fungal hyphae(FH), necrosis (N) & vacuums(V) were seen in muscle (×420).

reported that loss of epidermis and dermis, necrotic muscle cells and hemorrhage were found in EUS affected *C. punctatus, M. tengara* and *H. fossilis* during the colder months (December and January) [3]. Mohan and Sankar described that in EUS affected fishes of fresh and brackish water, numerous granulomas were found as a result of chronic inflammatory response with fungal hyphae [18]. Hatai *et al.* also reported fungal hyphae and many granulomas in the internal organs and musculature of EUS affected *Colisa lalia* in Japan [12].

In August and September, gill structures were normal in *A. testudineus* of both the farms. Conversely, less pathological changes such as hypertrophy in primary gill lamellae and sloughed off cells were observed during October and November. The result coincided with the

findings of Gosh who observed that sloughed off cells and hypertrophy in primary gill lamellae in Thai sharpunti and silver carp in the months of October and November [11]. However, marked hypertrophy and hyperplasia in primary gill lamellae, protozoan cysts and necrosis in secondary gill lamellae were observed in *A. testudineus* during December and January (Fig. 5). Roy *et al.* also reported that cysts, hyperplasia, lamellar clubbing and hypertrophy were observed in the gills of EUS affected *C. punctatus, M. tengara* and *H. fossilis* [23]. Gerundo *et al.* were of the opinion that joining apical end of secondary gill lamellae of rainbow trout was due to the repeated doses of malachite green [10]. Almost similar gill pathology was also found by Meguid and Eure, Ahmed and Hoque and Moniruzzaman [1,17,19].

Structures of liver were normal in *A. testudineus* during August and September of both the farms. In October and November only rupture of hepatocytes and hemorrhage were found in liver of *A. testudineus*. This result was also similar with the findings of Roy who observed that hemorrhageand ruptured hepatocytes in *N. nandus* and *P. sophore* during October and November in Ailee beel of Bangladesh [22]. However, in the present experiment, highly necrotic hepatocytes, pyknotic cells, hemorrhage, melanomacrophage centre, vacuoles were seen in December and January (Fig. 6). Akter *et al.* also observed necrotic hepatocytes, fungal granuloma, pyknotic cells in EUS affected *C. punctatus, M. tengara* and *H. fossilis* during December and January [5]. Moniruzzaman and Chakma also observed fungal granuloma and vacuolation in the liver of *A. testudineus* from Mymensingh district [8,19].

Like other internal organs, kidney had normal structure during August and September in fish of both the farms. But in October and November, pathologies in kidney were found to be started with mild necrosis, empty spaces, and pyknosis in *A. testudineus*. Gosh observed that mild hemorrhage, necrosis and pyknosis were found in silver carp and Thai sharpunti in October and November [11].

Kidneys were severely affected in December and January, with pathological changes such as swollen kidney tubules, ruptured and lost having pyknosis and hemorrhage in many places in *A. testudineus* of Reliance Aqua Farm (Fig. 7). Akter observed that in *M. aculeatus* and *M. cuchia* kidney tubules had necrosis, fat droplets and hemorrhage during the months of December and January [3]. Sahoo *et al.* found shrinkage in both glomeruli and

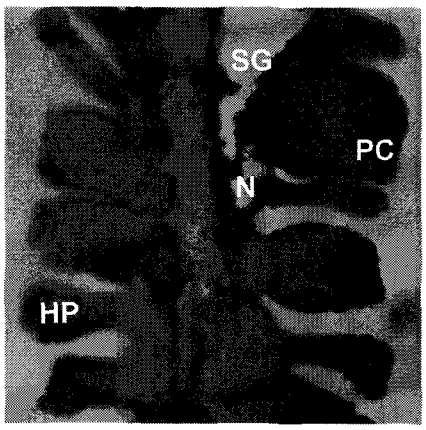

Fig. 5. Section of gill of *A. testudineus* in December & January. Protozoan cysts(PC) were found in both gill lamellae, necrosis (N), hypertrophy (HP), & separation of gill lamellae(SG) (×420).

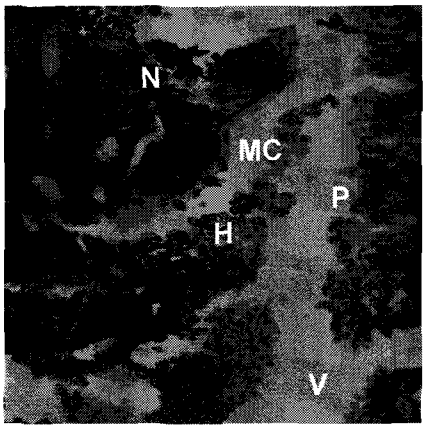

Fig. 6. Section of liver of *A. testudineus* in December & January. Haemorrhage(H), necrosis(N), melanomacrophage centre(MC), vacuums(V) & pyknotic cell(P) were seen(×420).

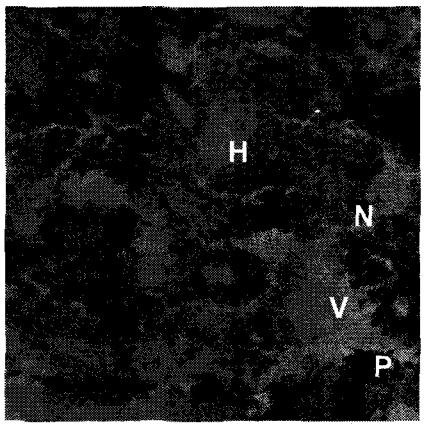

Fig. 7. Section of kidney of *A. testudineus* in December & January. Haemorrhage(H), necrosis (N), vacuums(V) and pyknotic cells(P) were seen(×420).

epithelial cells, deformation of renal tubules and mild necrosis in kidney [24]. Moniruzzaman and Chakma found degenerative changes like necrosis in kidney tubules and hematopoietic cells in freshwater fishes [8,19]. These findings partially agreed with the result of the present study. Results of the present study indicated that, apparently normal appearance were observed in August, September and February whereas, reduced level of pathological changes were found during October and November and severe pathological changes were recoded during December and January. Abnormal water quality in winter season might be the reasons of increased pathologies in fishes of both the farms. Low or rapidly changing water temperature, rapid or prolonged depression of pH, low alkalinity and low dissolved oxygen were seasonal aggressions of fish diseases [16]. So it could be mentioned that, prevalence of pathologies and diseases in the exotic fish like *A. testudineus* of Bangladesh might be related to seasonal variations and environmental factors. It could be mentioned here that fungal pathogen was responsible for the occurrence of fungal granuloma in the skin of Thai koi in December and January. Noga and Dykstra were of the opinion that marked granulomatus and inflammatory response were shown by fish infected with a fungus, *Aphanomyces* sp. [20].

Clinically and pathologically, it was mentioned that during December and January, the fishes of Reliance Aqua Farm were more affected than the fishes of Sotota Matshya Hatchery. The evidence of more clinical and pathological symptoms in the fish of Reliance Aqua Farmwas due to fact that fry imported from Thailand were probably not properly quarantined and provided poor management practices. Moreover, the farm authorities used different varieties of chemicals as a means of treatment for the fry of *A. testudineus*.

Thus, it could be suggested that more precautionary measures would necessary to be carried out in both the fish farms to prevent and control diseases in order to obtain healthy fish. Proper quarantine measures should be carried for imported fish. Fry should be properly conditioned and treated before stocking. Indiscriminate use of medicines should be stopped during disease outbreak and in this regard opinion from experts should be desirable (if necessary). The supplied feed should be appropriate for the species and routine water quality parameters should be checked. Incase of disease outbreak, either facility for disease diagnosis should be introduced or affected fish should be brought to the disease diagnostic laboratory. Success of the implementation of various fisheries development program depends to the extend, on the intensification of fish disease research, as the improvement of fish yield can be achieved from healthy fish stock. It is thus expected that from such attempts, fish production from potential close water bodies of Bangladesh would be increased to a great extent and therefore, national economy would be uplifted to a standard platform.

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초록 : 겨울철 양식장 환경에서 Tai koi, *Anabas testudineus* (BLOCH)의 임상병리학적 특징

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방글라데시의 양식어류 생산에서 가장 큰 문제점은 어병이며, 어병을 진단하는 위해서는 병변부의 조직병리학적 진단이 필수적이다. 방글라데시의 주요 잉어과에 속하는 몇몇 어류에 대해서는 조직병리학적 연구가 진행되었지만, 고유종(indigenous species)이면서 담수 소형어류인 어종에 대해서는 거의 연구가 이루어지지 않은 상태이다. 그러므로 본 연구는 보양식으로 경제적 가치가 높은 Thai koi (*Anabas testudineus*)에 대한 조직병리학적 특성을 규명하고자 하였다. 2006년 8월부터 2007년 2월까지 7개월간 방글라데시 미멘싱(Mymensingh)지역의 두 양어장에서 임상학적 그리고 조직병리학적 관찰을 통해 Thai koi(*A. testudineus*)의 건강상태를 조사하였다. 어류개체의 홍반, 출혈, 외상, 원생동물 피낭(cyst)과 같은 비정상적 증상에 대한 임상학적 진단을 월별로 조사하였다. 동절기 양어장의 수질 요인 중에서 수온, 용존산소량과 같은 변수는 어류에게 적합하지 않은 것으로 나타났다. 임상학적으로는 12월부터 1월 사이에 병에 걸린 어류가 더 많았고, 8월과 9월 사이 그리고 2월에는 외형상으로는 거의 정상에 가까웠다. 12월과 1월에 피부탈락, 피부외상, 궤양과 꼬리지느러미의 손실과 같은 여러 가지의 임상학적 증상이 나타났다. 조직병리학적 측면에서 8월과 9월 기관의 구조는 정상으로 나타났다. 10월과 11월 사이에는 미미한 병적 증상들이 발견되었고, 12월과 1월에는 조직괴사, 핵응축(pyknosis), 염증, 출혈, 이상비대(hypertrophy), 세포과형성(hyperplasia), 1 · 2차 새판(gill lamellae)의 손상과 같은 현저한 조직병리학적 변화가 발견되었다. 반면에 2월에는 어류의 병적증상이 점차적으로 감소하였다. 개개의 어류양식장을 고찰해 보았을 때, Relance Aqua Farm의 어류가 Sotota Mastshya Hatchery의 어류보다 더 감염률이 높았다. 본 연구 결과는, Thai koi의 월별 임상학적 · 조직병리학적 진단을 통해, 어병 발생 시기를 예측하고, 예방하기 위한 기초자료로 활용될 수 있다.