SOME BIOLOGICAL ASPECTS OF LOCAL AND THAI KOI, *Anabas testudineus* (BLOCH), THEIR ARTIFICIAL HYBRIDIZATION AND EVIDENCE OF NATURAL HYBRIDIZATION

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Approved as to style and content
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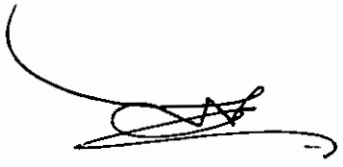

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Declaration

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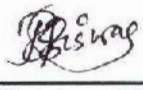
The results presented in this thesis with the above mentioned title are entirely of the candidate's own investigations and no part of the results has been accepted for any degree nor is it being concurrently submitted for any degree.



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Dedicated To

All the Physically Challenged Persons

Who

Fight and Dream for Success

Acknowledgement

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Abstract

The different strains of the climbing perch, *Anabas testudineus* are found in different countries. Being different in origin, Thai koi possesses some biological differences from the native koi of Bangladesh. A comparative study of some biological aspects was carried out from July to December, 2008. All the fishes were reared and collected from Batiaghata, Khulna. The total length and body weight of Thai koi broods were higher than that of the local koi broods with a significant difference ($p<0.05$). The length-weight relationship of both strains was positive with r=0.95 and 0.76 for Thai and local respectively. The fecundity of Thai koi found 765245±105027 after PG hormone administration (8 mg kg^{-1}) was higher than that of local koi (345508±114502). Managing by the supplementary feed, a high GSI was found in both Thai and local koi over three months period having higher value in Thai than that in the local females. Three nutritional compositions (moisture, protein and lipid) were not significantly different between the Thai and local koi muscle; however, a little higher value was found in the local koi than Thai ones. The juvenile of local koi surfaced out for air gulping more frequently than that of Thai koi ($p<0.05$) though the adult of the two strains did not show any significant difference ($p>0.05$). From most of the morphometric, meristic and qualitative characteristics the local koi varied from the Thai koi. In case of artificial hybridization pure local and Thai produced more fertilization and hatching rates than the reciprocal cross-breds though the survival rate of hybrids in cisterns was higher than the two single strains. During checking out the natural hybridization of koi in local *beels*, the incidence of escaping of Thai koi from culture ponds was found and average 17.40% of the *beel* catches were found with Thai and/or putative hybrids with body spots; among the sampled spotted koi, 66.7% were identified as hybrids based on morphometric, meristic and qualitative characters.

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List of Abbreviations & non-English words

ANOVA	Analysis of Variance
Beel	A saucer like depression which generally retains water througout the year
Deshi koi	The endemic climbing perch in Bangladesh
L_T	Total length
L_S	Standard length
Local (M)/L_m	Male of local koi
Local (F)/L_f	Female of local koi
Thai (M)/T_m	Male of Thai koi
Thai (F)/T_f	Female of Thai koi
W_B	Body weight

Chapter One

INTRODUCTION

Chapter 1

INTRODUCTION

The climbing perch (*Anabas testudineus*), locally known as koi, is a prominent fresh water endemic species of Bangladesh. Among the 260 fresh water species 150 are Small Indigenous Species (SIS) (Hossain, 2007). In Bangladesh, a total of 114,161 ha *beel* area (natural depression) is the main home of the SIS. Koi is one of the important SIS contributing an amount of catch every year (721 metric ton in 2006-07, 0.96% of total *beel* catch) though it is not satisfactory comparing with other fishes (Fisheries Statistical Yearbook, 2006-07). Climbing perch is considered as an important commercially valuable species due to its nutritional value, survivability even in adverse environmental conditions, ability to breed and spawn in closed water body, feeding habit as it feeds on every trophic level of the water body (Shiddique and Chowdhury, 1996; Smith, 1945). The live fishes have very good market demand and they command higher and more lucrative price than the Indian major carps and other table fishes. Having nutritious value and being suitable for marketing live, the market price of koi never goes down. The popular belief is that there is a special nutritive and medicinal quality in this fish, which is good for patients and convalescents (Chandi, 1970).

The species climbing perch is scientifically recognized by Bloch (1792) and termed it *Anabas testudineus* based on its classification is as follows:

Phylum: Chordata
Subphylum: Vertebrata
Series: Pisces
Class: Osteichthyes
Subclass: Actinopterygii
Order: Perciformes
Suborder: Anabantoidei
Family: Anabantidae
Genus: *Anabas*
Species: *Anabas testudineus* (Bloch, 1792)

Etymology: *Anabas* (L.); the climber, *testudineus* (L.); turtle like

Synonyms: *Anabas scandens, Amphiprion scansor, Amphiprion testudineus, Anabas elongatus, Anabas macrocephalus, Anabas microcephalus, Anabas spinosus, Anabas trifoliatus, Anabas variegatus, Anthias testudineus, Cojus cobujius, Lutjanus scandens,*

Lutjanus testudo, Perca scandens, Sparus scandens, Sparus testudineu (Riehl and Baensch, 1991).

Climbing perch usually feeds on macrophytic vegetation, shrimps and fish fry (Pethiyagoda, 1991). Possessing an accessory air-breathing organ it is able to survive for several days or weeks out of water if the air breathing organs can be kept moist (Rahman, 2005). With the aid of spiny operculum and fin spines the species is famous for walking. During the dry season, the climbing perch stays in pools associated with submerged woods and shrubs (Sokheng *et al.*, 1999) and remains buried under the mud during complete dry condition (Rahman, 2005). *A. testudineus* is distributed throughout the Asia, mainly India to Wallace line including China. More specifically, it is found as different strains in Bangladesh, India, Thailand, Myanmar, Sri Lanka, Nepal and other Asian countries (Axelrod *et al.*, 1971; Jayaram, 1981; Talwar and Jhingran, 1991). Most of the perciform fishes are predominantly marine but a vast majority of them enter estuaries and rivers of Bangladesh. Besides, members of many perciform families are largely restricted to freshwater habitat and koi (*A. testudineus*) is the major one among them (Rahman, 2005).

It had been a common practice in most Asian countries to introduce suitable species of fish from one country to another having similar environmental conditions (Rahman, 2005). There is no authentic report on the time first introduction of Thai koi in Bangladesh, however, it was gathered that Thai koi was first introduced around 2002 (BFRI, 2006) in Bangladesh and seed production of this strain was first started in 2003 in one hatchery named Brahmaputra Hatchery in Mymensingh (M.N. Huq, Personal communication). Thai koi is recognized as a variety of *A. testudineus* although Bleeker (1855) described it as *A. oligolepis* based on a higher body form and absence of spot at the base of the caudal fin (Axelrod, 1974). However, the name *A. oligolepis* is no longer used and *A. testudineus* is widely used for both the varieties.

At present, Thai koi is cultured in Bangladesh along with the local strain (known as desi koi). The Thai koi is seemed to have adapted to the country at present. However, having different regional origins they possess some biological differences that are important to know for the well being of the potential culture practice in the country.

Morphometric study especially length, weight and their relationship, breeding biology especially Gonado-somatic index (GSI), annual realized fecundity, fertilizing rate, hatching rate, hatchery parameters etc., nutritional composition, rate of surfacing out for air gulping and taxonomic characteristics, can provide significant information towards the population study, conservation, culture management and production of *A. testudineus*. The length-weight relationship is used in fishery to investigate environmental adaptability, racial discrimination as well as to estimate growth of a given species (Hile, 1936). Reproductive biology such as gonado-somatic index (GSI) is important to know the maturity and exact time of breeding which is essential for better understanding of spawning (Lagler, 1956). The knowledge of nutritional compositions of fish especially at the harvesting period is very important for the well being of consumption. Taxonomic characteristics can be used as the key for distinguishing two species or two strains of the same species (Rao, 1968). Therefore, the comparison of some biological aspects between the two strains of koi would provide significant and pertinent information as to the status of the populations.

In addition, the two strains can be hybridized to produce hybrid vigor in which the dominant gene can contribute the potential performance in terms of survivability, taste, nutritious and fast growth (Tave, 1993). The environmental differences of *A. testudineus* originating in Bangladesh and Thailand may have the role to make different phenotypic characters by the interaction variance components between the genotype and environment ($V_{G\text{-}E}$). Besides, only environmental variance (V_E) can change the phenotype of fish regardless the genetic variance (Tave, 1993). Thai koi is larger, able to take supplementary feed properly and therefore faster growth is noticeable. However, Thai koi is comparatively less demanded in market for its deep body color, more body spots and tastes. Alternatively, the local koi is very much demanded in the market for its taste though it does not grow as fast as Thai koi and has not so interest on supplementary feeds. The two strains, therefore, can be crossed to produce hybrid vigor that may pick up the constituents of both strains.

On the other hand, the Thai strain can mate with the local strain and can produce the hybrids or crossed offspring in natural water bodies. There are the records of the incidence of interspecies and intra-species natural hybridization of various fishes (Hauser *et al.*, 2006; Leary *et al.*, 1983 and Strauss, 1986) Therefore, the Thai and local koi being

the same species, their hybridization in natural waters of the country may not be unexpected. The local strains are likely to be endangered if Thai koi comes in natural waters escaping from the cultured pond or anywhere. Over the past 10 years large-scale introductions of exotic species have been made into the wild through floodplain stocking and through inadvertent escapes from aquaculture (Rahman, 2005). Therefore, it is time demanded to find out the scenario of the particular species composition in the natural water bodies whether the endemic species is being affected by the exotic strains.

Comparative study of some biological aspects of the two strains and artificial hybridization were carried out from July to December, 2008 collecting samples from a hatchery, Batighata, Khulna. Putative hybridization of Thai and local koi was checked in the natural water bodies of Batiaghata, Khulna area throughout the four months of the study.

The two strains were found biologically different with respect to different aspects. The hybrid produced artificially survived more than the either strain. The taxonomic study showed that the two strains are available in natural waters of Khulna area and assessment of the *beel* catches showed that the two strains can mate to produce hybrid offspring in open waters in the area.

Objectives of the study

In the present study, several objectives were set to obtain some information and to deliver a message for the concern of *A. testudineus*. The objectives are as follows:

- To make a comparison of some aspects of biology of two different strains of *A. testudineus* available in Bangladesh.
- To make a taxonomic comparison of the two strains in Khulna region.
- To produce hybrid of the two strains and to check survival rate of larvae in cisterns.
- To find out the incidence of mixing Thai and local strains and the consequent hybridization in natural water bodies in Khulna district.

Chapter Two

LITERATURE REVIEW

CHAPTER 2

LITERATURE REVIEW

2.1. Biological aspects of *A. testudineus*

Anabas testudineus (locally called 'deshi koi') is endemic species in Bangladesh. On the other hand, another variety of this species locally called 'Thai koi' has been introduced in Bangladesh for aquaculture. Any introduced species has a risk to be spread out into the natural water bodies (Rahman, 2005). Therefore, the biological aspects should have to be studied.

Some aspects of this economic fish species have been studied in neighboring countries and also in this country. However, comparative study of biology of two strains of *A. testudineus* is very scanty. Mookerjee and Mazumdar (1946) and Khan and Mukhopadhyay (1972) gave some detailed accounts of the life-history, breeding and fecundity of this species from Indian waters. Rahman (1975) studied the development and life-history of the species from Bangladesh water. Shafi and Mustafa (1976) studied length-weight relationship, food and feeding habit, sex ratio, fecundity and egg size of *A. testudineus* (deshi koi) collected from a pond in Dhaka.

Later, Nargis and Hossain (1992) studied the reproductive periodicity, gonado-somatic index (GSI) and fecundity of climbing perch *A. testudineus* collected from the natural water bodies in Khantakhali area of Rajshahi district. Herre (1935) and Davidson (1975) reported the taste and quality of palatable parts of this fish in Philippine and Laos respectively. Nargis (2006) studied on the changes of nutritional composition (Moisture, protein, lipid, Carbohydrate and ash) of *A. testudineus* (deshi koi collected from Rajshahi) over the year round.

Rahman (2005) reported a brief of *A. testudineus* including some morphometric, meristic and qualitative characteristics though he mentioned no specific information on any strain rather reported a range of value of characteristics of the species *A. testudineus*. The distinguishing morphometric characteristics of *Anabas* have also been provided by Sterba (1973) and Kottelat *et al.* (1993). Rao (1968) showed two distinct species and gave the name *A. oligolepis* to the second one from Indian waters. It is notable that no one has worked on the biology of climbing perch with a view to compare the two strains of the

species in greater Khulna region except the study on taxonomic accounts of local *testudineus* by Hassan (2004) and Hassan *et al.* (2005).

Basavaraju (2007) and Burnhill (2007) described the habitats and ecology of *A. testudineus* in Indian and Thailand water bodies respectively. Nghia *et al.* (2008) reported the age, size and weight of climbing perch (Thai strain) at maturity stage.

As *A. testudineus* is an air breathing fish it must need the surface air along with the dissolved oxygen in water (Anon, 2004). Several works have been done on this aspect with various views. Hughes and Singh (1970) studied on oxygen uptake and carbon dioxide release into air and water and mentioned that *Anabas* usually surfaces for air at intervals of 3-15 min. but the intervals may be as short as 30 sec. Datta Munshi (1986) studied morphology and vascular anatomy of the accessory respiratory organs. Nargis and Hossain (1995) carried out an experiment on longevity of *A. testudineus* without taking surface air. However, nobody reported the rate of air gulping with respects to feeding.

2.2. Artificial breeding and hybridization of *A. testudineus*

Artificial breeding of *A. testudineus* is a common practice in Thailand, Vietnam, Philippine, India and other countries. Induced breeding of local koi was done by Kohinoor *et al.* (1991) and Mahmood (2006) in Bangladesh. In our country, the induced breeding of Thai kio is being practiced in some hatcheries (Sarder, 2007). However, local koi has yet to be encouraged for induced breeding due to the lack of aquaculture demand. The record of exact year when the induced breeding of Thai koi was started in Bangladesh is ambiguous. From an interview by the researcher (2008) it was found that the seed production of Thai strain was first started in Bangladesh in 2003 by a hatchery owner named Md. Nurul Huq in his hatchery called Brahmaputra Hatchery, Mymensingh. Later Kohinoor and Zaher (2006) worked on induced breeding of Thai koi in Bangladesh.

The eggs of *Anabas* have the floating nature (Axelrod *et al.*, 1971). Hypophysation is very successfully used to produce large-scale fry of koi (Banerji and Prasad, 1974). Moitra *et al.* (1979) described the spawning behaviour and larval development of koi and found 28.5±1 °C to be a vital factor in inducing spawning of the fish. The hatchlings are small and tender and require careful rearing to achieve high survival rate. Some

information on the spawning behavior, light intensity, temperature and fecundity of the species *A. testudineus* were reported by Anon (2004).

Nghia *et al.* (2008) reported the hatching parameters, fertilization and hatching rate of *A. testudineus* with different hormones (PG, $LRHa_3$ and PG) with different doses in Vietnam. Sarkar *et al.* (2005) used synthetic hormone Wova-FH with the dose level of 0.1, 0.2 and 0.3 ml kg^{-1} of body weight respectively for the induced breeding of *A. testudineus* from Indian water bodies.

Kohinoor *et al.* (1991) worked on the induced breeding of local strain of *A. testudineus* and reported the fertilization rate 86±4.0%, hatching rate 78±2.0% and interval time from the injection to spawning 7.39±.30 hrs at water temperature 27-30 °C during the middle of the June.

Unlike the artificial propagation of fry of Thai koi, local koi is not widely produced for commercial hatching. However, it was reported that recently some small and medium hatcheries were taking initiatives for artificial reproduction of wildly-bred indigenous fish such as pabda, koi, shing, magur, chital in the captivity (Sarder, 2007).

The artificial hybridization between two strains of *A. testudineus* was not so performed. However, Richter (1988) reported enhanced production of yellow pigmentation in *A. testudineus* hybrid through mutation. Artificial hybridization of Thai and local koi and the fertilization, hatching survival and growth rate have yet to be checked perfectly in Bangladesh condition.

2.3. Incidence of natural hybridization of *A. testudineus*

In Bangladesh, 11 fish species have been introduced so far (FishBase, 2007). However, *A. testudineus* introduced from Thailand is not included in the popular fish documentary site 'www.FishBase.org'. The lack of proper information on status and accounts of introduced fish in the country is enlightened by the statement of Rahman (2005) as he reported "records of fish introductions in the country have not been systematically maintained and as such it is not possible to provide a comprehensive account of such introductions."

Leary *et al.* (1983) and Strauss (1986) reported the incidence of inter-species natural hybridization of *Cottus* spp and trout respectively. However, Hauser *et al.* (2006) reported

the incidence of inter-strain hybridization of *Salmo trutta* in UK. Over the past 10 years large-scale introductions of exotic species have been made into the wild through floodplain stocking and through inadvertent escapes from aquaculture (Rahman, 2005). Therefore, the escape of Thai koi is a matter of fact and the ultimate consequence is the hybridization in natural water bodies. There has not been any attempt known on the risk of escaping of Thai koi into natural water bodies especially to the concern on the conservation of endemic *A. testudineus*.

Hybrids and close related strains of fish can be distinguished by several methods. Morphometric, meristic, qualitative traits analysis and electrophoretic study are noticeable. Welsh and Cincotta (2004) used morphological techniques to find out the natural hybrids of *Noturus flavus* and *Noturus insignis*, from the Monongahela River Drainage, West Virginia. Ramaseshaiah and Dutt (1984) used electrophoresis technique to distinguish *Anabas testudineus* and *A. oligolepis* (Osteichthyes: Anabantidae) (Climbing perch) from Lake Kolleru, Andhra Pradesh, India. Strauss (1986) used both morphological and electrophoretic techniques to distinguish hybrids of freshwater sculpins *Cottus bairdi* and *Cottus cognatus*.

Chapter Three

MATERIALS & METHODS

CHAPTER 3

MATERIALS AND METHODS

Collecting the specimen from a pond located in Batiaghata of Khulna district, comparative study of some biological aspects were carried out from July to December, 2008 in different laboratories of Fisheries and Marine Resource Technology Discipline, Khulna University. Artificial hybridization was carried out in a hatchery, Batiaghata, Khulna and the incidence of natural hybridization was checked out from two local *beels* in Khulna.

3.1. Biological Aspects of *Anabas testudineus*

3.1.1. Length-weight relationship of brood fish of *A. testudineus*

60 broods of both strains of *A. testudineus* (40 Thai and 20 Local) were collected in July, 2008 from the pond in the hatchery complex, Batiaghata, Khulna. Total length and standard length were measured with normal centimeter scale and body weight was measured with 1 kilogram normal weighing machine (Miskai, Japan). Length-weight relationship, regression line of logarithm value of the data and correlation coefficient were determined using SPSS 12.0 for Windows software. The log to log transformation of the length and weight relationship was followed by the following equation (Le Cren, 1951):

$\log Y = \log a + b \log X$; Where, b=regression coefficient and log a=intercept of the line with the y-axis.

3.1.2. Annual realized fecundity

The total number of eggs spawned annually by a fish is termed as annual realized fecundity (Hunter *et al.*, 1992). Six gravid females of each strain (local and Thai) were selected for inducing on July 24, 2008. The female fish were spawned by PG hormone at the dose 8 mg kg^{-1}. The eggs were counted by volumetric method (Simpson, 1951).

Student's t-test was performed to compare the annual realized fecundity between the females of local and Thai strains.

3.1.3. Gonado-somatic Index (GSI)

The Gonado-somatic index (GSI) was calculated for collected females. For the GSI study, 15 females of *A. testudineus* of each strain were collected from the rearing tank (cemented, measuring 6′× 4′× 2′). The GSI was recorded for three times during the period of the study (October-December) on 5 females at a time. The fishes were dissected and the ovaries were removed very carefully. Prior to this the fish was weighed by a sensitive digital balance (Model No. B-303S). The blood and other visceral substances were removed carefully and wet weighed was taken by the digital balance. Then the GSI was calculated according to the proposed formula by Parameswarn *et al* (1974) and Hossain (1976):

$$GSI = \frac{W_1}{W_2} \times 100$$; Where, W_1 = Wet weight of gonad and W_2 = Total wet weight of fish

Student's t-test was performed to compare the GSI between the females of local and Thai strains.

3.1.4. Nutritional Composition

Laboratory Analysis: Nutritional composition of moisture, crude protein, and crude lipid were determined in the Fish Nutrition Laboratory of Fisheries and Marine Resource Technology Discipline, Khulna University, Bangladesh. Live mature specimens of local and Thai strain were subjected for the experiment. The nutritional composition was determined sex wise over three months *viz.*, October, November and December. Sampled fishes were collected from the rearing tank (cemented tank measuring 6′× 4′× 2′) in which the fishes (both local and Thai) were treated under keeping same conditions and feeding. Only fish muscles were taken from the dorsal and tail portion of the fish for this experiment. No replication was kept in each month.

Moisture: Moisture was determined by keeping collected flesh of *A. testudineus* in a thermostat oven at 105°C for 24 hours. Thereafter samples were kept in dessicator for about one hour. The dry weight of each sample was taken by a sensitive electric balance (Model No. B-303S).

Crude protein: Total nitrogen was estimated by following the standard Kjeldahl procedure (Pearson, 1976). Crude protein content was obtained by multiplying the total nitrogen value by the conventional factor 6.25 (Pearson, 1976). The results were reported as percentage (%) of dry weight. About 0.2-0.5 g sample was taken in a Kjeldahl flask and about 2 g resolvent (potassium sulfate and mercuric oxide in the ratio of 10:0.7 preserved in an amber glass bottle) was added into the flask. About 5 ml of concentrated sulfuric acid was added in to the flask slowly. Then the flask was kept on to a digestion chamber until a clear color of the digestion mixture appeared. After digestion, the flask was removed from the digestion plant and kept at room temperature until cooling. After cooling the digestion mixture, 0.5g of sandy zinc and 60 ml distilled water were added to the digested mixture. About 25 ml of 40% sodium hydroxide (NaOH) solution (containing 1% potassium sulfide) was poured slowly along the flask's inner wall and washed down the remaining solution on the mouth. The flask was then connected to the distillation apparatus and switched on. Before connecting the sample containing flask with the distillation plant, 15 ml of 2% boric acid was taken in another Erlenmeyer flask (conical flask) and 2 drops of Tashiro's indicator was added into it. Then the distillation tube was poured into the Erlenmeyer flask so that the end of the glass tube was immersed in the boric acid solution. Distillation procedure was continued for about 30 minutes to obtain about 30 ml distilled solution. After completion of distillation, the solution was titrated against 0.1 N hydrochloric acid (HCl). The protein percentage found was calculated as dry weight of the sample.

$$\%\textit{Total Nitrogen}\ (\%N) = \frac{\text{Titrant (ml)} \times \text{Strength of titrant} \times 0.014}{\text{Weight of sample (g)}}$$

Then, % Crude protein = % N × 6.25 (Pearson, 1976)

Crude lipid: About 3 mg of fish tissue was taken in a homogenizer containing 100 ml chloroform-methanol mixture and the contents were homogenized. The contents are then transferred to a beaker which was allowed to stand for sometime. Afterwards, the contents were filtered into a conical flask and a 50 ml of distilled water added to the filtrate. By this process, the non-liquid contaminants are removed and the bound acidic lipids released. The upper phase (distilled water wash solution) was then removed and the lower phase transferred to a pre-weighed beaker, which was dried for few minutes in oven at 105°C until the liquid parts disappeared and then few hours in a vacuum desiccator. After

thorough drying, the container was weighed again. The difference between the two values gives the lipid extracted. The percentage of lipid present in the tissue was calculated as follows:

$$\% \ Crude\ lipid = \frac{\text{Weight of lipid}}{\text{Weight of sample}} \times 100$$ (Bligh and Dyer, 1959)

t-test was performed to compare the nutritional contents between the two strains using SPSS 12.0 for Windows.

3.1.5. Rate of air gulping

Total 16 live specimens (local 8 and Thai 8) were selected for the experiment carried out in the physiology laboratory, Fisheries and Marine Resource Technology Discipline, Khulna University. Among 8 of each strain, 4 were mature (2 male and 2 female) and the rest were juvenile. Each individual was observed under two treatments *viz.*, at starvation and in full stomach. Each individual was taken in a single glass tank (size 2′×1′×1′, water depth 7′′). After the first surfacing, the air gulping interval was counted up to four successive surfacing. The interval time was counted in seconds.

t-test, One-way ANOVA and two-way ANOVA (in case of two factor viz., strains and stomach contents) were performed to compare the rate of air gulping of local and Thai strains of *A. testudineus*.

3.1.6. Taxonomic comparison

Collection of sample: Samples of local strain of *A. testudineus* were collected for four months from the local fishermen who fish in the local *beels* (natural depression) of Khulna district in Bangladesh but the samples of Thai strain were collected from the nearby aqua farm located in Batiaghata upazila of Khulna. It is notable that the Thai strain was produced in the local hatchery maintaining the purity of the parents. Specimens were collected during the months of October to December, 2008. A total of 30 mature and 12 juvenile specimens were collected from each strain. After collecting the specimens, the fishes were brought to the Fish Biology Laboratory of Fisheries and Marine Resources Technology Discipline, Khulna University.

Preservation of sample: Along with the morphometric and meristic data, some qualitative traits such as presence of body color, spot and the color of ventral and pelvic fin base were assessed. Therefore, the samples were analyzed in live condition and without using any preservative. However, after taking the qualitative data some samples were preserved if necessary in 5% formalin for further study.

Morphometric and meristic data measurement (Lagler *et al.*, 1977)*:* Data on both morphometric and meristic characters were recorded using normal centimeter scale, divider, forceps and needle recorded to the nearest centimeter (cm). The morphometric characters *viz.*, total length, standard length, head length, depth of body, pre-dorsal length, post-orbital length, pre-orbital length, eye diameter, length of base of 1st dorsal fin, length of base of 2nd dorsal fin, least depth of caudal peduncle, length of caudal peduncle, length of base of 1st and 2nd anal fin, length of chest, length of upper jaw and length of pelvic spine were recorded separately. The total length was measured from the tip of the snout to the longest ray of the caudal fin and the standard length was measured from the tip of the snout to the base of the caudal fin (Fig. 1). These measurements were taken with the help of a centimeter scale placed on measurement board. Meristic characters with respect to fin rays were counted excluding the smallest anterior rudiments. The countable characteristics such as number of dorsal spine or 1st dorsal fin rays, number of soft or 2nd dorsal fin rays, number of pectoral fin rays, number of anal spine or 1st anal fin rays, number of soft or 2nd anal fin rays, caudal fin rays (excluding rudiments), number of pelvic fin rays (excluding the spine), number of scale on lateral line (it is notable that lateral line of *A. testudineus* is broken (Rahman, 2005), therefore, in the present study, the lateral line is termed as upper lateral line and lower lateral line and the sum of the scale of the two lines is the number of scale on lateral line), number of scale above lateral line and number of scale below lateral line were recorded separately.

Comparison of various morphometric and meristic data of the two strains was carried out by t-test. However, to compare the qualitative characteristics frequency test was performed by using SPSS 12.0 for Windows.

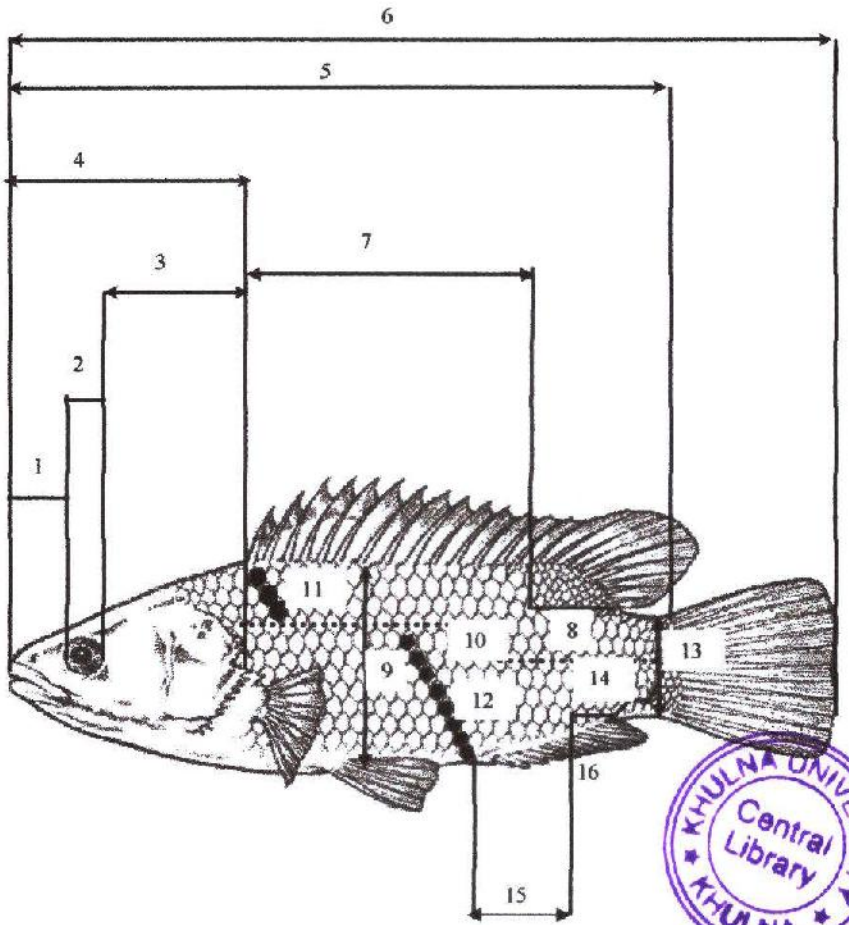


Fig. 1. A typical *A. testudineus* illustrating the parts and methods of counting and measuring: 1. Snout length; 2.Eye length; 3. Post orbital length; 4. Head length; 5. Standard length; 6. Total length; 7. Length of spinous or first dorsal fin base; 8. Length of soft or second dorsal fin base; 9. Body depth; 10. Scale on lateral line; 11. Scale above the lateral line; 12. Scale below the lateral line; 13. Least depth of caudal peduncle; 14. Length of caudal peduncle; 15. Length of spinous or first anal fin base; 16. Length of soft or second anal fin base (Source: Trautman, 1957).

3.2. Artificial breeding and hybridization of *A. testudineus*

To produce hybrids and to compare with pure strains (local or Thai) of koi induced breeding was carried out in Gain Agro-Fishery Hatchery, Batiaghata on July 24, 2008.

Brood collection: The broods of both local and Thai strains of *A. testudineus* were collected by cast net from the brood ponds of a hatchery complex. The broods of both the strains were being reared in the separate ponds over the year. The purity of strains was ensured by keeping the records as well as by observing the body spot and colour of the broods.

Selection of broods: The female of *A. testudineus* is larger in size than the male (particularly when in spawning condition) (Marshall, 2001). However, for the breeding purpose, the females were identified by the swollen abdomen and the males with their flat abdomen. Moreover, a gentle hand pressing on the abdomen extrudes white milt from the male. Weight of Thai koi female and male ranged from 75 to 120 g and 30 to 90 g respectively and the same for local koi ranged from 20 to 30 g and 35 to 50 g respectively.

Breeding Set and Sex ratio: For producing hybrid by crossing the local and Thai strains of koi and making a comparison with pure breeding within Thai and local strains, four breeding sets were used *viz.,* Thai (M)-Thai (F), Local (M)-Local (F), Thai (M)-Local (F) and Local (M)-Thai (F). The sex ratio of female and male was kept at 1:2 in every breeding set to ensure the sufficient milt in case of the smaller size male of local koi bred with comparatively large female of Thai koi.

Conditioning of the broods: Prior to hypophysation, the males and females were kept in two separate plastic drums for 12 hours with water shower. The plastic drums fitted with outlet pipe were filled with deep tube well water up to 20 cm.

Hormone administration: The broods were taken out from the tank, weighed and amount of hormone required was calculated out. The alcohol preserved PGs were first dried with tissue paper, weighed and homogenized with half the quantity of the distilled water. After that it was diluted with rest of the distilled water and centrifuged. The supernatant solution was then drawn out in a 3 ml syringe for injection. The injection was administered in the muscle of the base of pectoral fin. A single dose of PG hormone (8 mg kg^{-1} for female and half the quantity of female was for male) was injected (Hoq, 2006 and Kohinoor *et al.*, 1991).

Spawning: Male-Female (2:1) of each breeding plan was performed in four separated hapas especially made of plastic bucket with outlet net under constant showering. After complete spawning the eggs were collected with soft cloths and transferred into special hatching trays made of fine nylon net with bamboo slats. Six hours after the ovulation the fertilization rate was reckoned.

Fertilization rate and hatching rate: For determining fertilization rate a sample of eggs was taken in a Petri dish and the total number of eggs and the number of fertilized eggs were carefully counted. Fertilized and unfertilized eggs were identified by the sight of

transparent and opaque state of eggs respectively. Six random samples were drawn from the incubation hapa of each breeding set. Thus, the percentage of fertilization of each treatment was determined using the formula:

$$\text{Percentage of fertilization} = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs in the sample}} \times 100$$

Approximately 200 fertilized eggs were transferred to another four incubation tray (specially made of bucket and net) with same water and showering. Hatchlings came out through a range of time. Hatching rate was calculated after a successive completion of hatching of each breeding set. Randomized and repeated sampling was done from the secondary hapa for counting hatching rate by the following formula:

$$\text{Percentage of hatching} = \frac{\text{Number of hatchlings}}{\text{Total number of fertilized eggs in the sample}} \times 100$$

Survival rate: As most of the new hatchlings of *A. testudineus* remain attached with the cistern wall, the counting of hatchlings is very difficult (Moe, 2003). However, in the present study the survival rate was measured after 3 days of hatching out when most of the hatchlings move freely in the cistern water. 15 randomized samples were drawn to pick up the hatchlings in a jar. Prior to the sampling the force of water shower was increased so that the larvae could move and scatter freely. Therefore, the survival rate was determined by the equation as follows:

$$\%\ \text{Survival Rate} = \frac{\text{Total number of larvae observed in the cistern by sampling}}{\text{Total number of larvae released in the cistern}} \times 100,$$

where the observed number of larvae was counted from the calculation:

$$\text{Number of larvae observed} = \frac{\text{Number of larvae}}{\text{Volume of jar water}} \times \text{Volume of cistern water}$$

To make a comparison of fertilization and hatching rate of four breeding sets, ANOVA test was applied by using SPSS 12.0 for Windows.

3.3. Incidence of natural hybridization of *A. testudineus*

The incidence of natural hybridization between local and Thai koi in the *beels* Pipremari and Putimari, Khulna (Fig. 2) was checked with respect to several considerations. First, the Thai koi farms located in the selected regions of Khulna district were visited and surveyed in view to obtain the information about the incidence of escaping of Thai koi from the pond into *beel* or river. Second, an interview (open discussion) was conducted among 8 local fishermen who fish koi, especially by hook and line, net and bamboo traps, etc. Finally, 70 *beel* catches were analyzed over four months (September-December) to assess the variety composition. Subsequently, the hybrids were identified by studying their morphometric, meristic and qualitative characteristics with pure local and Thai koi.

To compare the morphological characters among the pure local, Thai and putative hybrids, one-way ANOVA and frequency test was carried out.

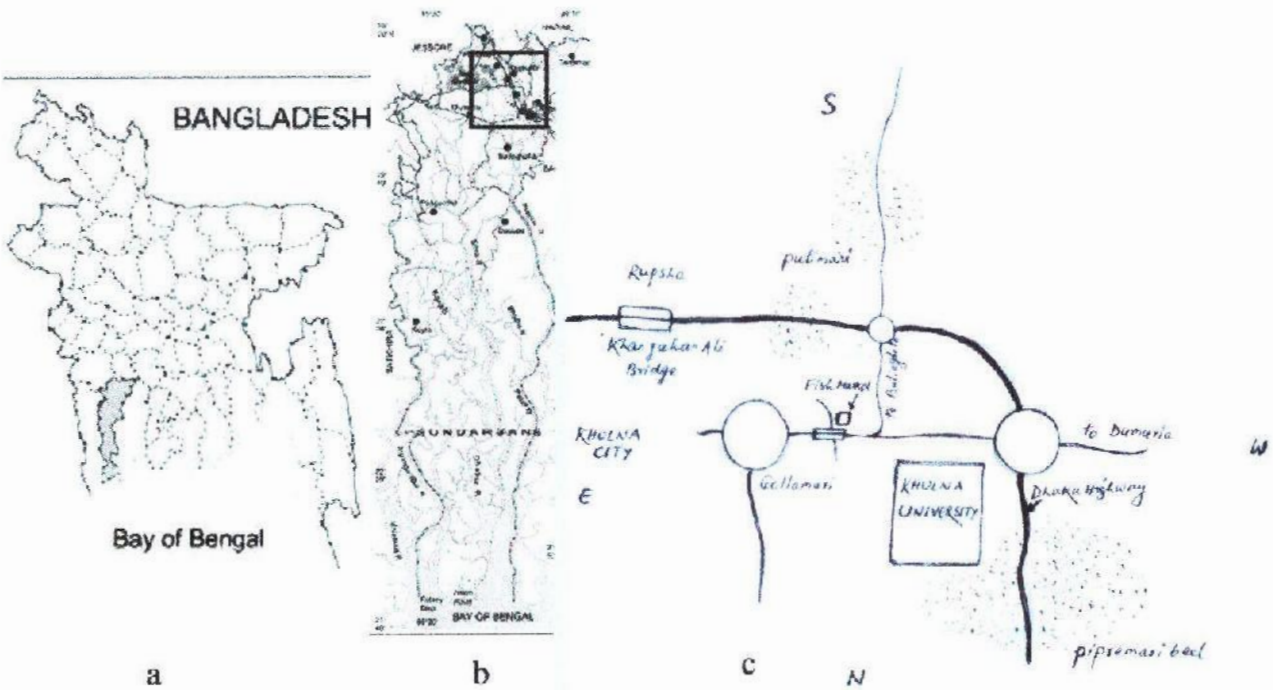


Fig. 2. The locations of two *beels* for studying the natural hybridization in Khulna, Bangladesh (a=Bangladesh, b=Khulna, c=*beel* in cluster dots)

Chapter Four

RESULTS

Chapter 4

RESULTS

Throughout six months period different biological aspects *viz.*, length-weight of brood fish, annual realized fecundity, gonado-somatic index, nutritional compositions, rate of air gulping, taxonomic characteristics as well as artificial and natural hybridization of koi were studied in Khulna.

4.1. Some biological aspects of *A. testudineus*

4.1.1. Length-weight of brood fish

The total length (L_T) of local koi was found 11.86±1.10 cm ranging from 10.0 to 14.20 cm in which the L_T of male was 11.10±0.62 cm ranging from 10.0 to 12.0 cm and that for female was 12.63±0.946 cm ranging from 11.50 to 14.20 cm. On the other hand, L_T of Thai koi was 14.99±1.95 cm ranging from 11.0 to 18.40 cm in which 13.45±1.44 cm (min 11.0 and max 15.70) cm and 16.52±0.88 cm (min 15.0 and max 18.40) cm for male and female respectively. The standard length (L_S) of local strain varied from 8.20 to 12.0 cm (9.89±1.10 cm) having differences between the male (9.08±0.52 cm) and female (10.71±0.90 cm). On the other hand, L_S of Thai koi varied from 9.0 to 15.70 cm (12.39±1.69 cm) having 11.03±1.19 cm for male and 13.75±0.76 cm for female. Body weight (W_B) of local koi was found 30.80±10.47 g with a wide range from 20.0 to 52.5 g in which the W_B of female varied widely (25.0 to 52.5 g) than that of male (20.0 to 30.0 g). In contrast, W_B of Thai koi was found comparatively high (76.0±11.22 g) ranged from 58.0 g to 100.0 g in which 38.65±11.51 g and 76.0±11.22 g for male and female respectively.

The Thai koi was higher in total length, standard length and body weight with a significant difference from the local koi at 5% level. The statistical analysis of the comparative study of these morphometric characteristics of brood fishes are shown in Table 1.

Table 1: The t-test value of length and weight of broods of two strains of *A. testudineus.*

	t value (Equal variances not assumed)	df	Status of p value
Total length	-7.899	56.978	P<0.05
Standard length	-6.865	53.899	P<0.05
Body weight	-6.327	57.937	P<0.05

The correlation between L_T and W_B as well as L_S and W_B was found positive for the local broods (r=0.76 and 0.83); for Thai broods, the correlation coefficient (r) was found 0.95 and 0.95 respectively. In both the cases, the correlations were significant at 1% level. The regression of logarithm value of L_T and W_B & L_S and W_B was found positive for both the strains (Fig. 3).

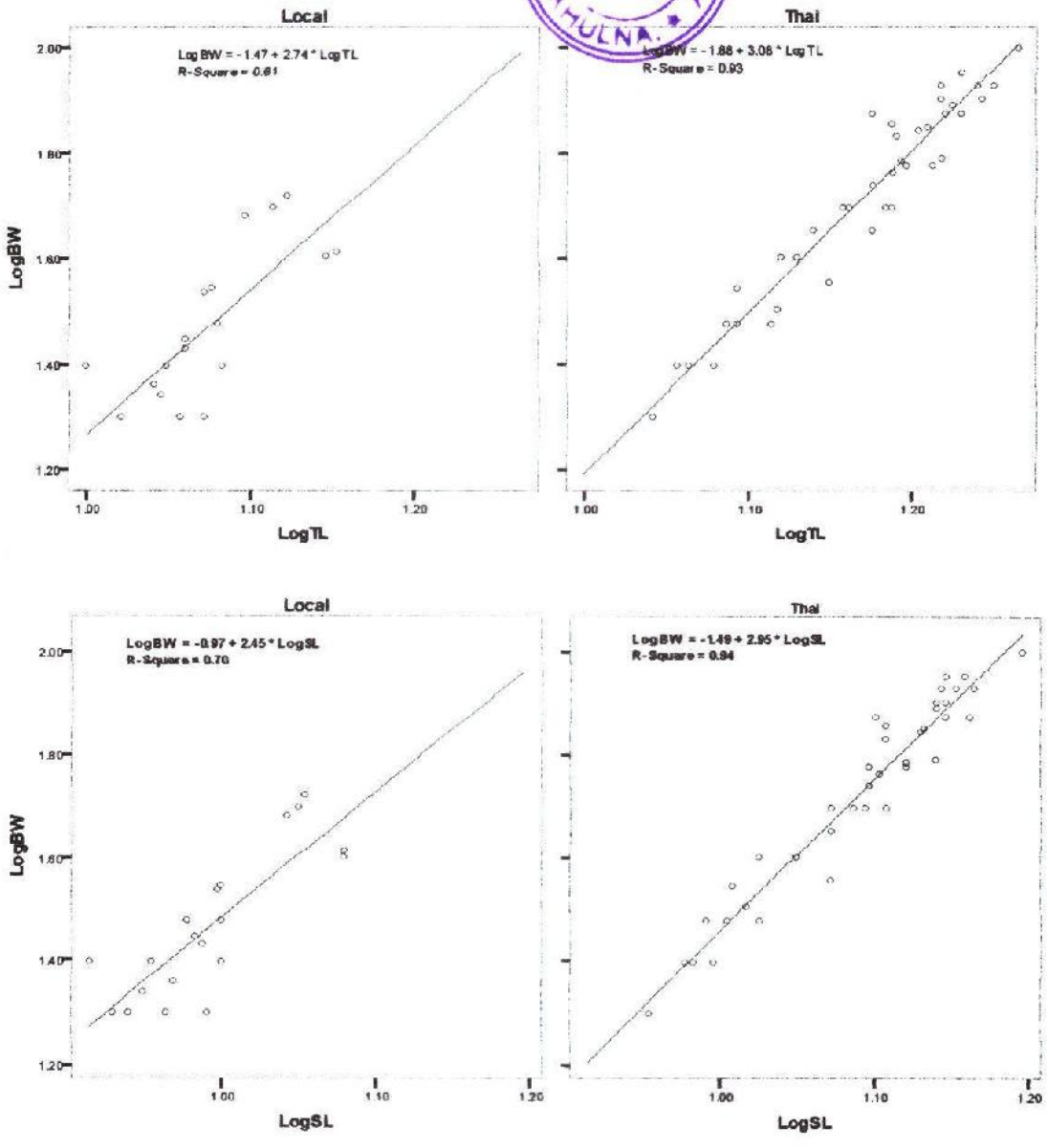


Fig. 3. Regression line of logarithm of L_T and W_B & L_S and W_B of local and Thai koi

4.1.2. Annual realized fecundity

The females of the local koi released eggs ranging from 4211 (L_T 12.1 cm and W_B 25 g) to 23025 (L_T 13 cm and W_B 50 g). The mean estimated fecundity (eggs/kg body weight of fish) was 345508±114502. The Thai koi released eggs ranging from 47462 (L_T 15.5 cm and W_B 76 g) to 109801 (L_T 17.8 cm and W_B 120 g) resulting the estimated mean fecundity of (eggs/kg of fish) 765245±105027. In comparison, the Thai koi had significantly higher fecundity ($t = -6.617, df = 10, p < 0.05$) than the local females (Fig. 4). The fecundity and the body weight of fish of both the strains was found correlated significantly (r=0.90 for local and r=0.92 for Thai at 5% level)

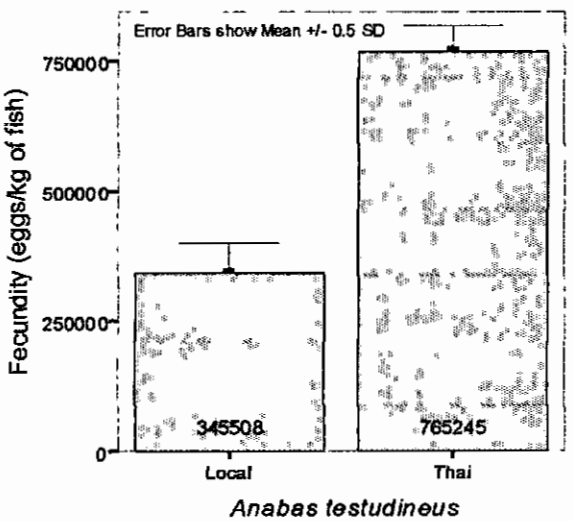


Fig. 4. The fecundity (eggs/kg of fish) of the two strains of koi.

4.1.3. Gonado-somatic Index (GSI)

The female gonad of koi was found fused at one near end of the two lobes (Plate 4). The Gonado-somatic index (GSI) of local koi was found 1.23±0.65 (L_T 13.51±0.61 cm) in the middle of October having a range of GSI from 0.56 to 2.04. The GSI value then stepped up and reached to 6.62±0.98 (L_T 14.3±0.86 cm) in the middle of December having a range from 5.39 to 7.92. The GSI of local koi was varied significantly over three months period ($F = 10.33, df = [2,12], p < 0.05$) though in between November and December the GSI was not found significantly different ($p>0.05$). On the other hand, Thai females showed a far difference from the local ones (Fig. 5) producing GSI 8.81±4.01 (L_T 15.81±0.79 cm)

in the middle of October with a wide range of variability from 4.56 to 14.34. Like the local ones the GSI of Thai females increased up to 14.68±3.99 (L_T 14.14±1.11 cm) with the minimum 10.96 and maximum of 21.31. However, there were no significant differences in GSI of the samples collected over the three months period (October-December) ($F = 2.31, df = [2,12], p > 0.05$).

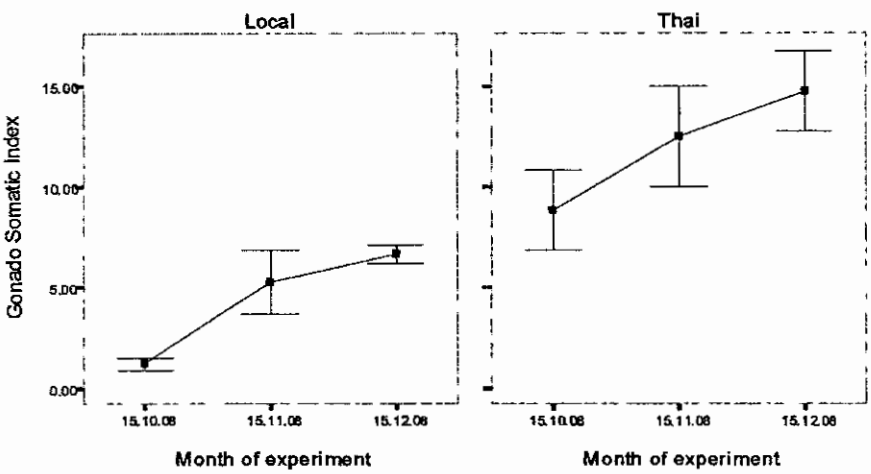


Fig. 5. Change of GSI of two strains of *A. testudineus* over the experiment period.

4.1.4. Nutritional composition

The collected local koi (from the natural water bodies, *beel*) had been reared and fed with supplementary feed (min. crude protein 26%, lipid 6%, fiber 10% and moisture 11%) in a cemented tank together with the Thai strain since one month before the start of the experiment.

Moisture: The moisture contents were found averagely higher in the muscle of the local koi (79.13±3.51%) than Thai (78.88±2.34%). However, no significant differences were found in two strains ($t = 0.145, df = 8.704, p > 0.05$). In case of month-wise estimation the change patterns of the moisture contents of Thai koi were found in a definite direction such as it decreased over the three months period (October-December) from 81.04±0.40% to 76.64±2.73% but that in the local koi there had been abrupt ups and downs; it October-November it decreased from 82.65±2.61% to 75.72±1.36% and in December it increased up to 79.03±2.26% (Fig. 6a). In each month, the moisture contents were not same between two sexes (Fig. 7a). In case of sex, no significant differences were found

between the sexes of either local or Thai koi regardless the period of sampling (p>0.05). Similarly, the males as well as females of the two strains showed no significant difference in moisture contents (p>0.05). The t-test showed the results as follows:

$t = 0.533$ (Equal variances not assumed), $df = 2.77$, $p > 0.05$ (Sexes of local)
$t = 0.579$ (Equal variances not assumed), $df = 2.96$, $p > 0.05$ (Sexes of Thai)
$t = 0.162$ (Equal variances not assumed), $df = 2.42$, $p > 0.05$ (Males of two strains)
$t = 0.007$ (Equal variances not assumed), $df = 3.56$, $p > 0.05$ (Females of two strains)

Protein: The protein (dry weight basis) was found averagely higher in the muscle of the local koi (81.26±11.94%) than Thai (71.07±5.22%) though significant difference was not found ($t = 1.606, df = 9.29, p > 0.05$). Protein of Thai koi increased over October to December period from 71.07±5.29% to 85.99±11.945%. However, that in the local koi, the change patterns were same as moisture contents such as 83.49±18.56% (October) 71.90±7.25% (November) and 88.41±5.30% (December) (Fig. 6b). In each month, the protein was not same between two sexes (Fig. 7a). In case of sex, no significant differences were found between the sexes of either the local or Thai koi regardless the period of sampling (p>0.05). Similarly, the males as well as the females of two strains showed no significant difference in protein (p>0.05). The t-test showed the results as follows:

$t = -0.262$ (Equal variances not assumed), $df = 3.69$, $p > 0.05$ (Sexes of local)
$t = 0.898$ (Equal variances not assumed), $df = 3.64$, $p > 0.05$ (Sexes of Thai)
$t = -0.294$ (Equal variances not assumed), $df = 3.97$, $p > 0.05$ (Males of the two strains)
$t = 0.716$ (Equal variances not assumed), $df = 3.96$, $p > 0.05$ (Females of the two strains)

Lipid: The lipid (dry weight basis) was found averagely higher in the muscle of the local koi (9.33±5.01%) than Thai (4.60±5.19%) though significant difference was not found ($t = 0.357, df = 8.704, p > 0.05$). Like the protein content, lipid of Thai koi increased over October to December period from 1.94±0.06% to 8.86±8.88%. However, in case of lipid of the local koi, the change patterns were abruptly ups and downs (5.27±3.56% in October, 14.99±0.16% in November and 7.73±3.29% in December) (Fig. 6c). In each month, the lipid content was not same between two sexes (Fig. 7c). In case of sex, no significant differences were found between the sexes of either the local or Thai koi for lipid regardless the period of sampling (p>0.05). Similarly, the males as well as the females of two strains showed no significant difference in lipid contents (p>0.05). The t-test showed the results as follows:

$t = 0.044$(Equal variances not assumed), $df = 3.87$, $p > 0.05$ (Sexes of local)

$t = -1.007$ (Equal variances assumed), $df = 4$, $p > 0.05$ (Sexes of Thai)

$t = 2.372$ (Equal variances assumed), $df = 4$, $p > 0.05$ (Males of the two strains)

$t = 0.454$ (Equal variances not assumed), $df = 3.88$, $p > 0.05$ (Females of the two strains)

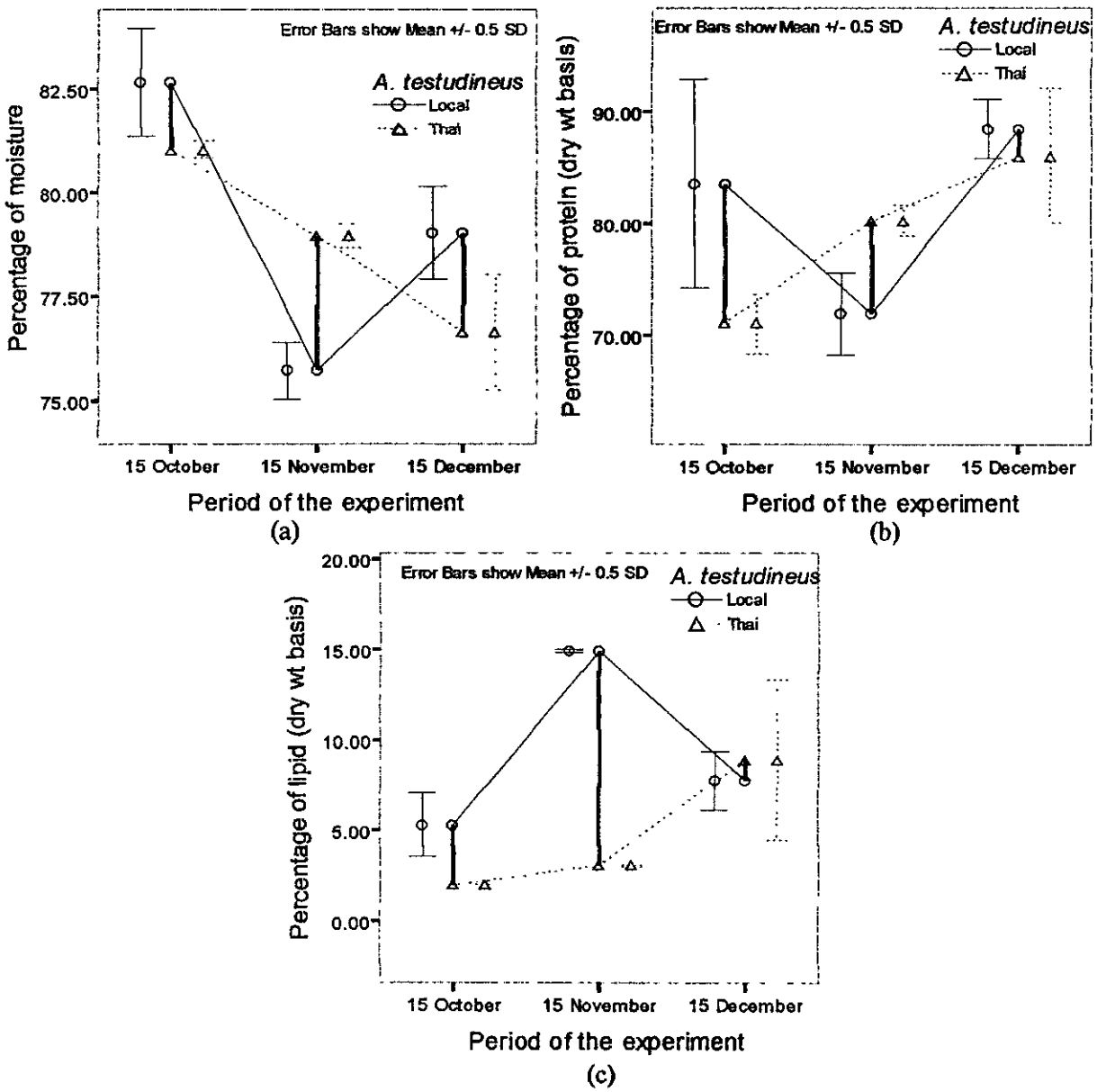
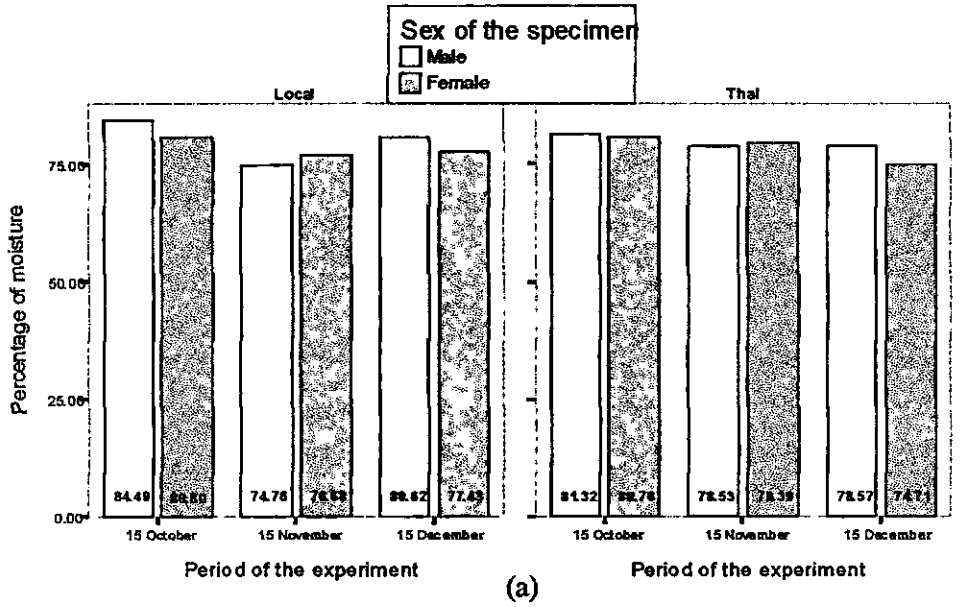
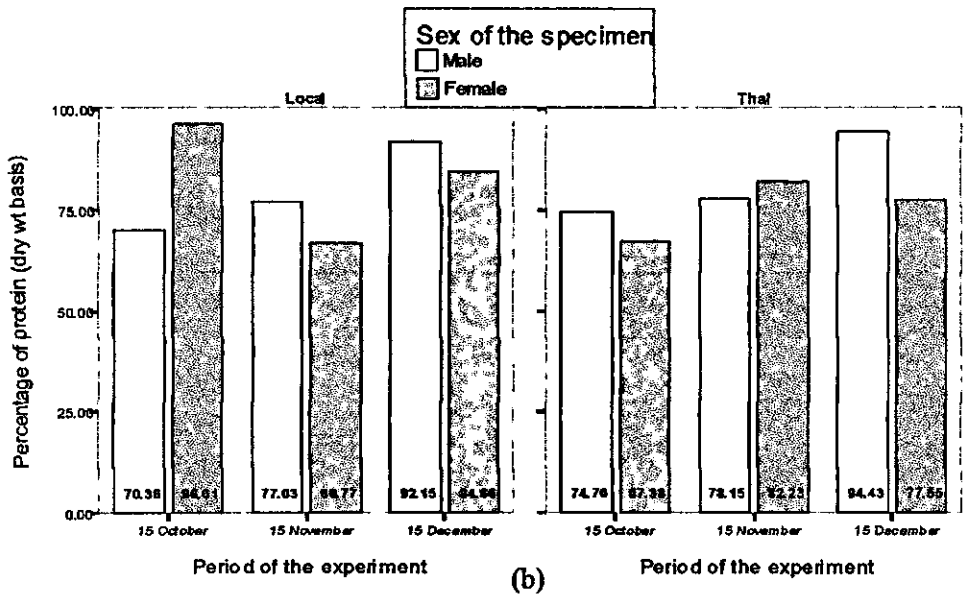


Fig. 6. Changes of moisture, protein and lipid content of local and Thai koi over the three months period; the thicker line shows the drops between the two strains.





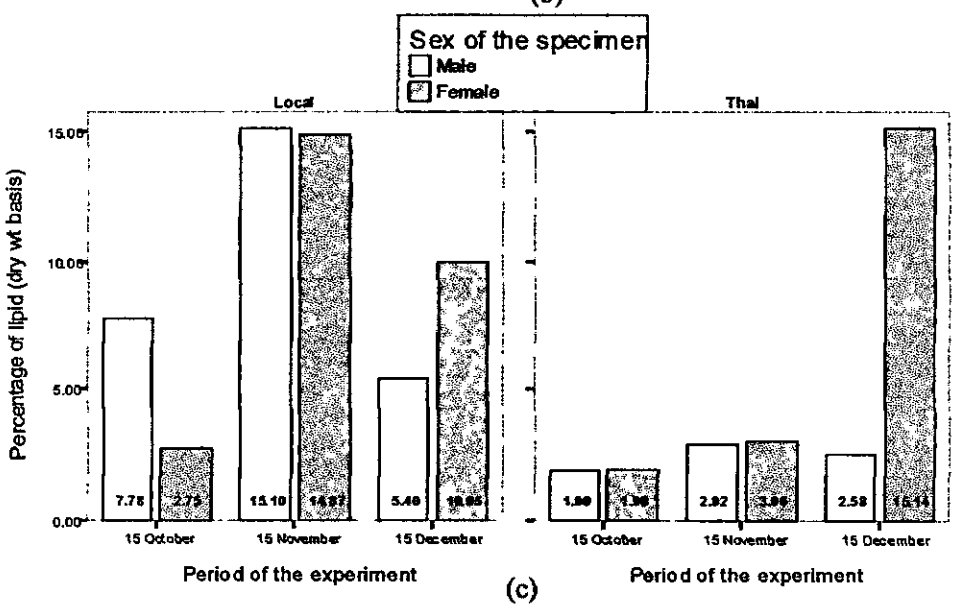


Fig. 7. Monthly changes of moisture, protein and lipid content of the two strains of koi with respect to sex.

4.1.5. Rate of air gulping

***Juvenile*:** The Juveniles (L_T limit 6.00-8.50 cm) of the two strains of koi differed significantly in their interval time of air gulping (local koi 119.34±54.35 sec and Thai koi 286.56±143.01 sec, $p<0.05$). There was no significant difference of interval time of surfacing by the fishes with respect to stomach contents i.e. either they were full or starved condition ($p>0.05$), though there was a tendency to have long interval time of air gulping when the stomach was full in both the strains (Fig. 8a). It is notable that the interaction of strains and stomach contents for surfacing time was not significant ($p>0.05$).

Adult: The adult (L_T limit 11.80-17.00 cm) of the two strains of koi did not differ significantly in their interval time of air gulping (local koi 157.22±72.71 sec and Thai koi 254.16±126.61 sec, $p>0.05$). Similarly, there was no significant difference of interval time of air gulping being affected by stomach contents i.e. either they were full or starved condition ($p>0.05$) though there was a tendency to have long interval time of surfacing when the stomach was empty for both the strains (Fig. 8b). It is notable that the interaction of strains and stomach contents on air gulping time was not significant ($p>0.05$).

On the other hand, the local koi did not differ in their air gulping time significantly with neither of their age (adult or juvenile) nor the stomach contents (full or empty) ($p>0.05$) though the adults took more time (157.22±72.71) than the juvenile (119.34±54.35 sec) and the interval was more at starvation (148.22±78.31 sec) than the full stomach condition (123.66±47.99 sec). The same scenario was found in Thai koi for their age and stomach contents ($p>0.05$) having 286.56±143.01 sec in the juveniles and 254.16±126.61 sec in the adults & 282.13±138.91 sec at starved and 258.59±132.17 sec in full stomach condition. The dissolve oxygen and water temperature were recorded at 3 mg l^{-1} and 25±1 °C respectively from the experimental glass tank.

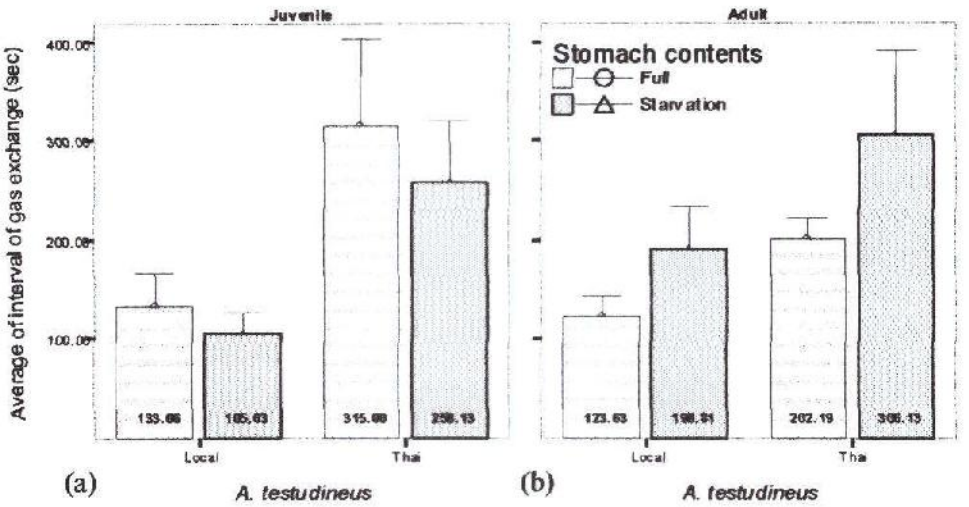


Fig. 8. Interval time of air gulping of the two strains of *A. testudineus* (juvenile and adult) under fed and starved condition

4.1.6. Taxonomic comparison

From September to December the koi was found to have a wide range of variation in size. Therefore, for the convenience of morphometric studies, mature (above 9.5 cm total length was considered in the present study) specimens of both the strains were collected and studied.

Average length (L_T) of local koi was found 11.48±1.64 cm (min. 9.60 cm and max. 14.70 cm) whereas the L_T of Thai koi was found 13.65±1.83 cm (min. 11.0 cm and max. 17.20 cm). The difference in L_T of the two strains was significant at 5% level (Table 2). Standard length (L_S) was also found different significantly at the same level (9.14±1.33 cm in local and 11.13±1.48 cm in Thai).

Among the other morphometric characteristics, body depth, snout length and length of spinous dorsal fin base of the two strains were not different significantly at 5% level though in every case the metric values were higher in Thai koi than the local (body depth 3.43±0.57 cm in Thai, 3.22±0.34 cm in local; snout length 0.73±0.10 cm in Thai and 0.71±0.09 cm in local; length of 1st dorsal fin base 4.97±0.51 cm in Thai and 4.43±0.67 cm in local) (Table 2).

Most of the morphometric characteristics differed significantly between the two strains having greater value for Thai koi. Among them, pre-dorsal length, postorbital length, least peduncle depth, length of caudal peduncle, length of 2nd (soft) dorsal fin base, length of 1st (spinous) and 2nd anal fin base, length of chest, length of upper jaw and length of pelvic spine were noticed (Table 2).

There was significant difference between the proportion of standard length and 1st dorsal fin base length in the two strains ($p<0.05$). Like this, proportion of standard length and body depth, head length and eye diameter, head length and snout length, head length and postorbital length were found significantly different between the local and Thai koi. However, proportion of standard length and head length did not differ significantly ($p>0.05$) (Table 3).

Three non-parametric (qualitative) characteristics (body spot, spot on the caudal fin base and colour of pelvic fin base region) were studied in the present experiment. The local koi had no body spots whereas Thai koi possessed body spots all over the body surface (92.3%) and in some cases limited spots were found (7.7%). Spot on the base of caudal fin was found in various patterns and some had no spots in both the strains. In most of cases, black spot was found on both side of the peduncle (63.33% for local and 46.15% for Thai koi). Another distinguishing feature was observed in the two strains and was that the colour of pelvic fin base region was yellowish (63.33%) and whitish to yellowish (36.67%) were in the local koi. In contrast, yellowish pelvic fin base was not found in the present study in Thai koi but had whitish to yellowish (53.85%) and whitish (46.15%) pelvic fin base (Fig. 9).

Table 2: Average morphometric and meristic characteristics of local and Thai koi in samples from Khulna district

	Strains of *A. testudineus*	
	Local	Thai
	(Mean±SD cm)	
Morphometric Characteristics		
Total length	11.48±1.64[a]	13.65±1.83[b]
Standard length	9.14±1.33[a]	11.13±1.48[b]
Body depth	3.22±0.34[a]	3.43±0.57[a]
Pre-dorsal length	3.10±0.36[a]	3.52±0.43[b]
Snout length	0.71±0.09[a]	0.73±0.10[a]
Postorbital length	1.76±0.21[a]	2.24±0.49[b]
Eye diameter	0.72±0.04[a]	0.74±0.07[a]
Head length	3.00±0.31[a]	3.58±0.55[b]
Least peduncle depth	1.49±0.22[a]	1.67±0.21[b]
Length of caudal peduncle	1.09±0.18[a]	1.37±0.26[b]
Length of spinous dorsal fin base	4.43±0.67[a]	4.97±0.51[a]
Length of soft dorsal fin base	1.07±0.16[a]	1.60±0.26[b]
Length of spinous anal fin base	2.41±0.39[a]	2.68±0.38[b]
Length of soft anal fin base	1.26±0.19[a]	1.68±0.21[b]
Length of chest	2.00±0.31[a]	2.38±0.46[b]
Length of upper jaw	1.14±0.13[a]	1.26±0.17[b]
Length of pelvic fin spine	0.92±0.12[a]	1.19±0.18[b]
Meristic Characteristics		
Dorsal spine	16.90±0.61[a]	17.08±0.48[a]
Anal spine	9.97±0.32[a]	9.08±0.39[b]
Dorsal fin ray	8.77±0.43[a]	9.69±0.55[b]
Anal fin ray	10.63±0.49[a]	10.31±0.68[b]
Pectoral fin ray	15.50±0.57[a]	15.65±0.89[a]
Caudal fin ray	16.40±0.56[a]	16.54±0.71[a]
Pelvic fin ray (without one spine)	5.03±0.18[a]	5.00±0.00[a]
Scale on the upper lateral line	17.20±1.06[a]	16.08±0.98[b]
Scale on the lower lateral line	10.60±0.93[a]	11.15±1.35[a]
Scale on lateral line	27.80±1.19[a]	27.23±1.73[a]
Scale above lateral line	4.00±0.26[a]	3.88±0.33[a]
Scale below lateral line	10.80±0.76[a]	9.73±0.67[b]

(Differences in superscript letter denote significant variation ($p<0.05$) in means)

Table 3: Different morphometric proportion of the two strains of koi from Khulna region

		SL: HL	SL: BD	HL: ED	HL: Snout	HL: Postorbital	SL:1st dorsal fin base
Strains of koi	Local	3.03±0.16[a]	2.84±0.24[a]	4.19±0.40[a]	4.26±0.35[a]	1.71±0.07[a]	2.07±0.09[a]
	Thai	3.12±0.19[a]	3.27±0.32[b]	4.83±0.68[b]	4.93±0.68[b]	1.62±0.12[b]	2.24±0.21[b]

(Differences in superscript letter denote significant variation ($p<0.05$) in means)

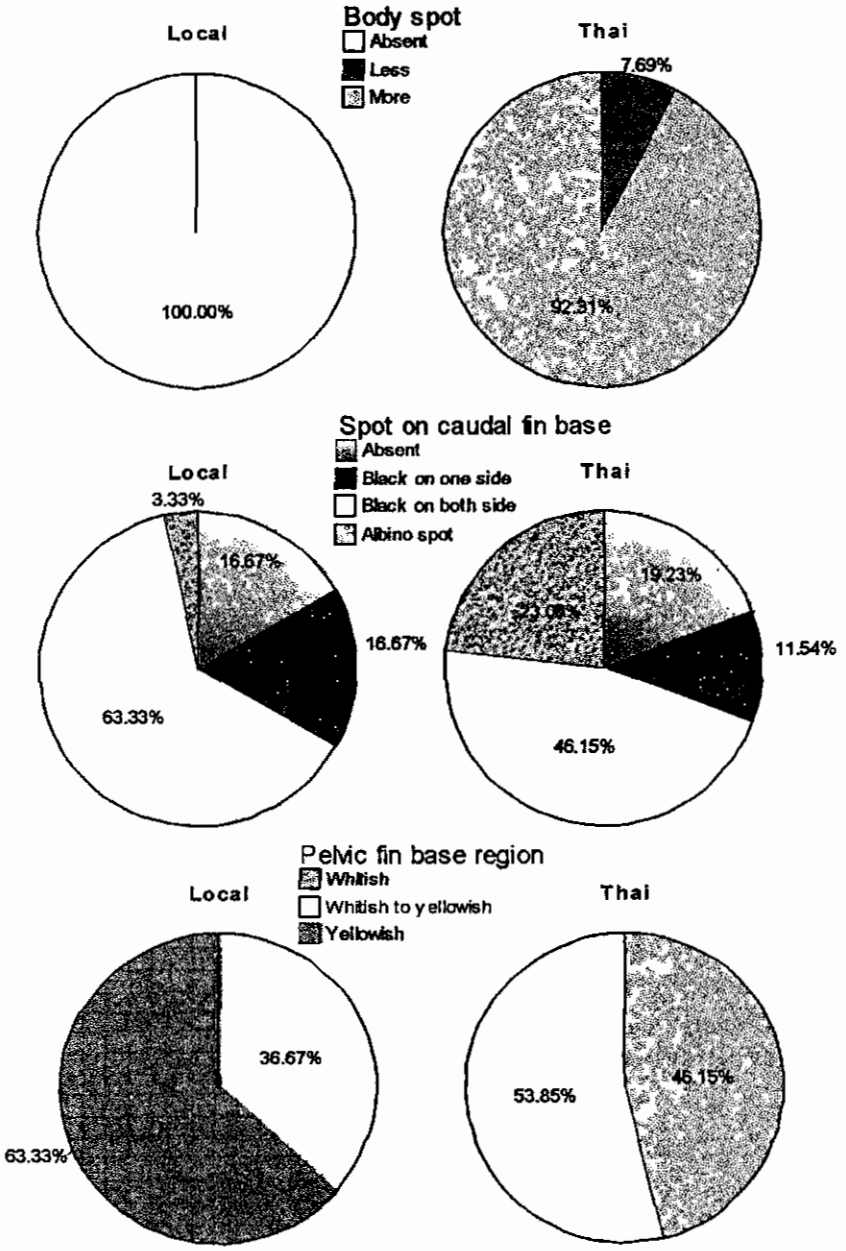


Fig. 9. Percentage of occurrence of non-parametric characteristics *viz.*, body spot, spot on caudal fin base and colour of pelvic fin base of *A. testudineus*

4.2. Artificial breeding and hybridization in hatchery

Induced breeding and hybridization: The Pituitary Gland (PG) hormone was injected at 8.45 P.M on 24th July, 2008 at the rate of 8 mg kg^{-1} body weight to the female of koi and half of the quantity to male. Complete spawning was observed after 9 hours of hormone administration. However, female of L_m-T_f breeding set was still releasing eggs on the water column of the spawning tank. Therefore, the complete spawning of this set was

recorded as 10 hours. The percentage of fertilization rate was significantly different at 5% level among the four breeding set ($F = 18.004, df = [3, 20], p < 0.05$). The fertilization rate was found maximum (98.36±2.67) for T_m-T_f and minimum (65±6.17) for L_m-T_f. Complete hatching took place at 17-18 hrs after fertilization in T_m-T_f, L_m-L_f and T_m-L_f breeding sets of koi whereas 19-20 hrs in L_m-T_f at 26-27 °C water temperature, 6 mg l^{-1} dissolved oxygen (DO) and 7.5 water pH. Among the four treatments, the hatching rate was significantly different ($F = 50.20, df = [3, 20], p < 0.05$) producing maximum (94.12±5.56) for L_m-L_f and minimum (48.73±8.12) for L_m-T_f (Table 4).

Table 4: Fertilization and hatching rate of the four breeding sets with the water quality parameters.

Breeding set	PG dose (mg kg^{-1} fish)		Fertilization rate (%) (Mean±SD)	Hatching rate (%)	Water quality recorded		
	Male	Female			Temperature (°C)	DO (mgl^{-1})	pH
T_m-T_f	4	8	98.36±2.67[a]	92.60±6.73[a]	26-27	6	7.5
L_m-L_f			85.50±12.33[b]	94.12±5.56[a]			
T_m-L_f			89.67±7.34[b]	73.96±8.49[b]			
L_m-T_f			65.91±6.17[c]	48.73±8.12[c]			

(Differences in superscript letter denote significant variation (p<0.05) in means)

Survival rate: The hatchlings were transferred to four separate cisterns (in the hatchery after 36 hours of complete hatching out. Prior to this, methyl blue (1g/5000 litter water) was mixed with the water of the hatching tray. Oxymax (calcium peroxide 80% at the dose of 1g/1000 litter water) was mixed with the cistern water just before the introduction of hatchlings into the cisterns. For required dissolved oxygen the artificial shower was on with a control over the rate of showering. The stocking density was 550/m^2 at 0.2 m water depth. The total water volume was estimated at 850 liter in each tank. The yolk sac was checked randomly and first feeding (one chicken boiled yolk/50000 larvae) was started after few minutes of larvae introduction into the cistern water. The feeding frequency was maintained two times daily after close observation of the stomach contents of the larvae. The first sample was drawn at 36 hour after releasing of the larvae. Survival rate was observed at above 90% for all categories of offspring (91.63%, 86.81%, 91.63% and 96.45% for T_m-T_f, L_m-L_f, T_m-L_f and L_m-T_f respectively). This rate was almost satisfactory for all up to 60 hrs. However, it dropped very rapidly to around 50% in around 108 hrs (Fig. 10). It is notable that, the tanks could not be maintained properly and algal bloom was produced at 60-70 hrs after the introduction of the hatchlings due to the lack of

proper water exchange, water shower being maintained. With this adverse condition, the local and Thai fry died after 132-140 hrs but 2.13% and 3.40% fry of the reciprocal hybrids (T_m-L_f and L_m-T_f respectively) were alive. After 108 hrs, it was not possible to sample out the live fry; at 132 hrs careful observation was made on the survivability of the larvae in the cisterns and it was surprising that the reciprocal hybrids were still survived at 0.51% (T_m-L_f) and 0.8% (L_m-T_f) respectively though the cistern were at adverse water quality, algal bloom and without any supplementary feed at that time on the 19th day of the experiment.

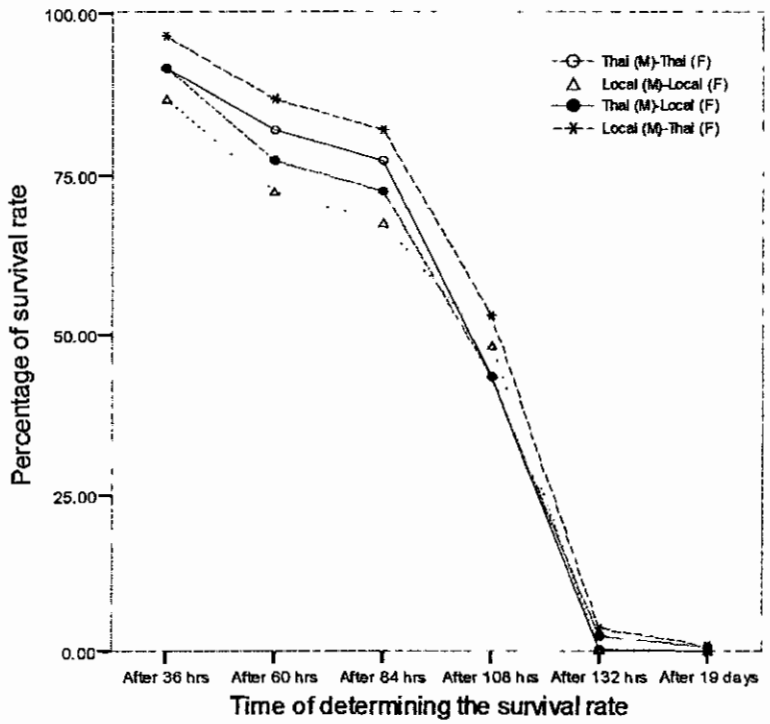


Fig. 10. Survival rate of the four categories of offspring in the cisterns

4.3. Incidence of natural hybridization of *A. testudineus*

As the two strains of koi can mate and produce offspring, it is possible to have the hybrids or crossed offspring in natural water bodies if both strains available there.

Risk of escaping Thai koi: Most of the culture ponds of Thai koi were located in vulnerable locations for escapement to the natural water bodies. 53.85% and 38.46% of the surveyed ponds were adjacent to the nearby ***beel*** and river respectively. It is notable that only 7.69% were in closed ponds. Among the surveyed ponds, 46.15% had no barrier

to protect the escaping of Thai koi and 53.85% had netting but poorly managed by the farmer (Fig. 11).

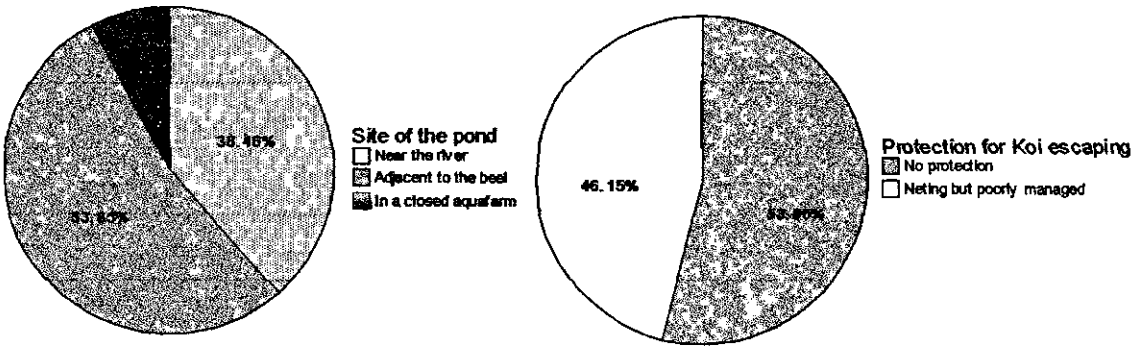


Fig. 11. The location of Thai koi culture pond and the status of protection from escaping in the local areas of Khulna

Beel catch composition: In case of investigating incidences of escapement of Thai koi into the local *beel*, some local fishermen were interviewed. They mentioned that 25.38±7.99% of their daily total catch were consisted of spotted koi (looks like Thai koi which have body spot). From the daily catch analysis the spotted koi was found to consist of 17.40±19.20% having a wide range of standard deviation (Fig. 12).

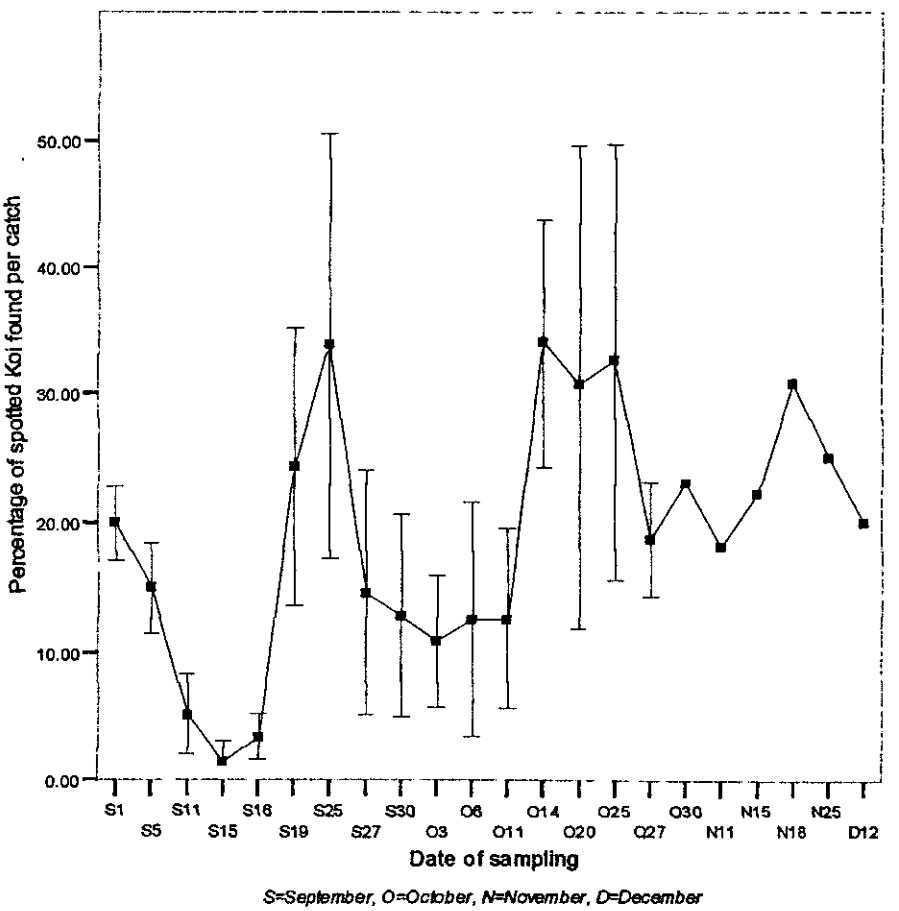


Fig. 12. The percentage of catch of spotted koi presumed to be Thai or hybrid in the local *beels*

Morphological analysis of spotted koi from beel catch: From the analysis of meristic and non-parametric characteristics of the two strains of mature koi it was found that the local koi had no body spots, but yellowish or whitish to yellowish colour at pelvic fin base. On the other hand, Thai koi had body spots densely (most of the cases) or partially with whitish or whitish to yellowish colour at pelvic fin base. Another significant distinguishing feature was the number of anal spine; in most of the cases 10 spines (9.97±0.32 averagely) were found in the local koi but 9 (9.08±0.39 averagely) in the Thai koi. In the present study, only one local specimen had 9 anal spines among 42 samples (2.38%). On the other hand, above 9 anal spines were found in only two Thai specimens among 38 samples (5.26%). Therefore, the specimens which had body spots and more than 9 anal spines were recognized primarily as hybrids in the *beel* catch. After this identification, another trait was compared with the pure local and Thai koi. It was notable that, no dense spots were found on body surface in the primary selected specimens but had black lines in two specimens (10%). Colour at pelvic fin base region was also mixed such as yellowish (25%), whitish to yellowish (55%) and whitish (20%) in the selected specimen. It is mentionable that the Thai koi did not have yellowish colour at ventral region even in mature stage in the study period. Some nonparametric characteristics are showed in Table 5.

Table 5: Comparison of nonparametric traits among the local, Thai and hybrid koi

Strains	**Body spot (%)**				**Spot on caudal fin base (%)**				**Colour at pelvic fin (%)**		
	Absent	Less	More	B. lines	Absent	B1	B2	A	W	W-Y	Y
Local	100	0.0	0.0	0.0	16.7	16.7	63.3	3.3	0.0	36.7	63.3
Thai	0.0	7.7	92.3	0.0	19.2	11.5	46.2	23.1	46.2	53.8	0.0
Hybrids	0.0	90.0	0.0	10.0	10.0	10.0	70.0	10.0	20.0	55.0	25.0

(B: Black, B1: Black spot at one side, B2: Black spot on both side, A: Albino, W: Whitish, Y: Yellowish)

As the analysis of *beel* catch was conducted throughout the four months (September-December) some specimens were small in size (L_T 4.0-9.0 cm). Therefore, the small size fishes (juvenile) of the local and Thai koi were studied to avoid any bias to observe whether the meristic (especially anal spine) and non-parametric characteristics were different in different age classes. It is noticeable that, the number of anal spines and the status of body spots in juvenile of the two strains were found almost the same in the present study (10.17±0.39 anal spine for the local koi and 9.08±0.29 for Thai; body spot 100% absent in the local koi and 8.3% limited spots, 83.3% dense spots and 8.3% black lines in Thai of the juveniles).

In the present study, analyzing the meristic and nonparametric characteristics, 66.7% of the collected spotted koi from the natural water bodies (*beel*) were adjudged to be hybrids produced by natural mating of the local and Thai koi.

Chapter Five

DISCUSSION

Chapter 5

DISCUSSION

5.1. Biological aspects of *A. testudineus*

5.1.1. Length-weight of brood fish

Thai strain of *A. testudineus* was mainly introduced in Bangladesh for commercial aquaculture in the year 2002. At present the local strain of the species is being considered for valuable endemic species for aquaculture. The total length (L_T) of Thai koi (14.99±1.95 cm) was found higher than that of the local koi (11.86±1.10 cm) during the breeding season in July 2008. Nghia *et al.* (2008) reported 12.1±0.1 cm and 14.9±0.2 cm L_T of male and female of Thai koi respectively at the mature stage that were very close to the findings of the present study (13.45±1.44 cm for male and 16.52±0.88 for female). Body weight (W_B) of brood of Thai koi found in the present study (38.65±11.51 g for male and 76.0±11.22 g for female) was deviated from a mean. Nghia *et al.* (2008) reported it as 63.9±1.2 g and 81.2±1.1 g for Thai male and female koi respectively. However, some variation may occur depending on the pond management, feed ingredients, and the environmental parameters. It is notable that Nghia *et al.* (2008) used the feed containing minimum protein level at 40% in fish pond in Vietnam; in the present study the protein level was the minimum 25%. On the other hand, L_T and W_B of female of the local koi (12.63±0.95 cm and 38.4±9.54 g) were almost the same as to the observation of Nargis and Hossain (1988).

Thai koi was higher in respect of total length, standard length and body weight with a significant difference from the local koi at 5% level ($p<0.05$). The correlation between L_T and W_B as well as L_S and W_B was found positive for local broods (r=0.76 and 0.83); for Thai broods, the correlation coefficient (r) was found at 0.95 and 0.95 respectively. In both the cases, the correlations were significant at 1% level.

5.1.2. Annual realized fecundity

Annual realized fecundity is normally estimated as the number of eggs per kg body weight of fish, released annually. In the present study, the females of koi were induced for spawning by the dose (8 mg/kg fish) of PG hormone (Hoq, 2006). The mean estimated

fecundity (eggs/kg body weight of fish) was 345508±114502 for local koi and 765245±105027 for Thai koi, whereas Trieu and Long (2008) found 822416 for Thai koi using 8 mg kg^{-1} PG hormone. On the other hand, Nghia *et al.* (2008) reported 379,349 egg/kg fish for Thai koi. The present result was comparatively close to the observation of Trieu and Long (2008). However, fluctuation in the number of eggs spawned per individual derived the high standard deviation in the mean value. For the local koi, Shafi and Mustafa (1976) reported 20872 (average number of eggs) with average body weight 68.96 g, therefore the calculated fecundity was 302668 egg/kg of fish, and Nargis and Hossain (1988) reported it as 668936±346313. It is notable that both of the studies had observed the fecundity on the basis of complete count and not the basis of egg release/spawning. Therefore, the variation could be found from the present study.

In comparison, the fecundity of Thai koi was significantly higher than the local females. The fecundity and the body weight of fish (both strains) was found correlated significantly (r=0.90 for local and r=0.92 for Thai at 5% level of significance) that were similar to the work reported by Shafi and Mustafa (1976) for local koi (r=0.93) but higher than the result obtained by Nargis and Hossain (1988) (r=0.72).

5.1.3. Gonado-somatic Index (GSI)

GSI of local koi varied significantly during the present study from October to December ($p<0.05$). However, no significant differences were found in the GSI of Thai koi, though an increase was observed (Fig. 4). GSI indicates the peak breeding season and frequency of breeding throughout the year. Therefore, GSI should be determined throughout the year (Venkataramanujam and Ramanathan, 1994). However, the present study had a limitation and assessments were made for only three months in a view to find out the gonadal development of local and Thai koi reared in a water tank with proper feeding. Nargis and Hossain (1992) reported that GSI of local koi collected from natural waters was averaged at 0.1 in September, 0.82 in December and peak at 8.8 in May. However, from the present study, the GSI of local koi was found 1.23 in October and 6.62 in December. This result greatly differs from Nargis and Hossain (1992). It was noticeable that no immature fishes were taken in the present study unlike the study of Nargis and Hossain (1992). The circumstantial conditions which affect and influence the nature and pattern of reproduction and gonad development in fishes are the day length, temperature,

salinity, turbidity, availability and nature of food, interspecific and intraspecific social relations (Waterman, 1961). Supplementary feed (min. 26% protein and 6% lipid) was used in the present study that influenced the gonadal development. As immature and mature eggs comprise the gonads, the proper feeding and management can easily change gonadal materials. Comparatively, Thai koi possessed a higher GSI than the local females in the three months study period producing 8.81 in October and 14.68 in December. It was not clear why Thai koi had such a high GSI, however, it was known that many hatcheries in the country operate and manage the Thai koi seed production in the later part of the breeding season during October to December. Nurul Huq (Brahmaputra Hatchery, Mymensingh) and Mrinal Gain (Gain Agro-Fishery, Batiaghata, Khulna) agreed with the present findings and stated that they had produced hatchlings around December though the mortality rate of the larvae was high due to low temperature.

5.1.4. Nutritional composition

The average moisture in local koi throughout the three months study was 79.96±4.90% in males and 78.30±2.19% in females that were very close to the observation of Nargis (2006). However, Nargis (2006) worked throughout the year in Rajshahi whereas the present investigation was limited for three months (October to December) in Khulna. Nargis (2006) reported the gradual decrease pattern of moisture from October to December in males and almost static pattern in females whereas the same patterns were observed in the present study except an abrupt decrease in November. The average moisture in Thai males and females was 79.47±1.60% and 78.29±3.17% respectively that were not significantly different with local males and females (p>0.05).

In the present investigation the protein and lipid contents were determined in dry weight basis. The change patterns in local male was the same to the observation made by Nargis (2006) and that was protein contents in the fish muscle increased from October to December and in female it decreased in November then increased again in December. The average protein contents were found 79.85±11.16% in local male and 82.68±15.02% in female in dry weight basis but Nargis (2006) found 10.24 ±0.79% for males and 11.07 ±0.31% for females in wet weight basis. On the other hand, Thai male possessed more protein (82.45±10.52%) than female (75.72±7.59%) on an average throughout the study period.

The lipid contents of *A. testudineus* fluctuate more vigorously than the other composition. Nargis (2006) reported the same patterns for both the sexes of local koi. Average 9.43±5.06% lipid was found in local male and 9.22±6.10% in female whereas Nargis (2006) reported 5.54 ± 0.25 % in males and 5.45 ± 0.26 % in females in wet weight basis. On the other hand, 2.47±0.52% lipid contents were found in Thai male and 6.73±7.31% in female during the study period. The abrupt increase of lipid content (15.14% in December where only 3.06% in November) in a female of Thai strain makes the higher standard deviation.

In case of all the compositions studied, the local strain was higher in content than Thai though significant difference was not found ($p>0.05$). The Figure 5 shows clear change patterns of moisture, protein and lipid in Thai koi. From October to December the moisture decreased whereas the protein and lipid increased. The onset of winter lowers the moisture contents. The percentage of lipid depends on reproductive cycle and food ingredients. Similar results were found in Gobi fish and *Macrognathus aculeatus* (Ahmed *et al.*, 1977 and Nabi and Hossain, 1989). The same change patterns were found in local koi but an abrupt change in November. This was probably due to the difference in food intake competition, size, age and health conditions of the fish. Khuda *et al.* (1954), Nargis (2006) and Shewan (1951) reported some of the cases in various fish species. In general, body composition of fishes seems to depend on age, sex, season, diet and habitat (Graves, 1970 and Phillips *et al.*, 1966).

5.1.5. Rate of air gulping

A. testudineus is an air breathing fish and it must need the atmospheric air for breathing along with the dissolved oxygen in water (Anon, 2004). Therefore, in the present study, attempt was made to assess the frequency of air gulping by the koi in the tank. The interval time was significantly higher in Thai juvenile than the local ($p<0.05$) but the adult did not show the difference between the strains. However, the juvenile or adult of both the strains did not show any significant difference in their surfacing depending on stomach contents though comparatively quick surfacing were observed in the adult when they had full stomach at 3 mg l^{-1} DO and 25±1 °C water temperature (Fig. 8b). The exact cause behind this phenomenon may be the metabolic activity i.e. when the stomach is full with feeds the fish require more supply of oxygen for maintaining their metabolic activity

(Lawson, 1995). In juveniles of both the strains, the quicker surfacing was observed at starved condition that was an opposite phenomenon to the adult. The causes behind this phenomenon may be the greedy nature and fear of the juvenile being alone in the tank. The surfacing times varied from 30 sec (local juvenile) to 1010 sec (16 min 50 sec) (Thai juvenile and adult) in this study that agrees with Hughes and Singh (1970). On the other hand, Nargis and Hossain (1995) reported 55 minutes to 385 minutes longevity of local koi without taking atmospheric air until death.

5.1.6. Taxonomic comparison

The local and Thai koi were collected and studied for morphometric, meristic and nonparametric traits to compare between the strains. Both the juvenile and adult fishes were collected but only investigation on the adult has been presented in the present dissertation. Hassan (2004) and later Hassan *et al.* (2005) worked on local koi. In the present study, among the morphometric characteristics, standard length, head length, body depth and eye diameter were found very similar to the observation by Hassan (2004) and Hassan *et al.* (2005). Total length slightly differed from Hassan *et al.* (2005) but was same to the result of Hassan (2004). Some meristic characteristics of local koi were found in this investigation very close to the results of Hassan (2004) and Hassan *et al.* (2005); however, number of dorsal spines was widely different from both of the findings as they reported 9.56±0.65 and 9±0.74 respectively whereas 16.90±0.61 in the present study. The present result was strongly supported by FishBase (2007), Rahman (2005) and Talwar and Jhingran (1991). The lateral line of *A. testudineus* is broken (Rahman, 2005), therefore, number of scale on both lateral lines were studied separately and the result agreed with Rahman (2005). The results of proportion of some morphometric characteristics match with the observation of Hassan *et al.* (2005).

Thai koi was also studied and a significant difference was found ($p<0.05$) from morphometric, meristic, qualitative characteristics and for some proportions between Thai and local strains of *A. testudineus* (Table 2 & 3). Thai koi is recognized as a variety of *A. testudineus* although Bleeker (1855) described it as *A. oligolepis* based on a higher body form and absence of spot at the base of the caudal fin (Axelrod, 1974). Rao (1968) also showed that there are two distinct species koi and gave the name *A. oligolepis* to the second species. The morphometric variations may come from age, food and physiological

conditions of fish but the meristic ones need to be justified for the actual taxonomic identity of a species. Qualitative traits can also be important keys in this case. In this investigation significant variation was found between the anal spines, dorsal soft fin ray, scale on upper lateral line and scale below lateral line. In addition, Thai koi had body spots either all over the body surface (92.3%) or partially (7.7%) and local koi had the yellowish color at the base of pelvic fins but Thai has either whitish (46.15%) or whitish to yellowish (53.85%) even at maturity stage.

5.2. Artificial breeding and hybridization of *A. testudineus*

Recently some small and medium hatcheries are taking initiatives for artificial reproduction of wildly-bred indigenous fish species such as pubda, koi, shing, magur, chital in the captivity (Sarder, 2007). In the present study, along with the induced breeding of local and Thai koi, hybridization was carried out to produce hybrid vigor. The spawning time was 9 hrs for local and Thai breeders that was a slight longer time than that found by Kohinoor *et al.* (1991) for local koi, Nghia *et al.* (2008) Trieu and Long (2008) for Thai koi. However, Sarder (2007) reported the complete ovulation time for *A. testudineus* to be 9-12 hrs after the PG hormone injection. The spawning time depends on the PG doses and other supporting conditions such as showering, darkness and free from disturbance (Anon, 2004). The fertilization rate found in this study (85.50%) for local koi was supported by Kohinoor *et al.* (1991) and Mahmood (2006) and for Thai koi it was 98.36% that was little higher than that reported by Trieu and Long (2008). However, Kohinoor and Zaher (2006) found 50% fertilization rate of Thai koi in Bangladesh.

Kohinoor *et al.* (1991) kept the sex ratio at 1:1 in single spawning tank but the ratio was 2:1 (Male: Female) in the present experiment that was similar to Sarker *et al.* (2005). The hatching rate of local koi was higher as much as 94.12% than the available report (Kohinoor *et al.*, 1991) but that of Thai koi (92.60%) is a little lower than the available records (Trieu and Long, 2008; Nghia *et al.*, 2008) but higher than Kohinoor and Zaher (2006).

The scenario in the hybridization, the fertilization and hatching rate were satisfactory (89.67% and 73.96% respectively) in the cross breeding between Thai male and local female of *A. testudineus*. However, the results were not satisfactory (65.91% and 48.73% respectively) for the local male and Thai female breeding set. Some factors can provide

insight in this case such as lack of synchronous of eggs-sperm release, problems in intraspecific social relations (Waterman, 1961) and other physiological conditions. Further research should be conducted on the nature of sperm of local koi and eggs of Thai female to find out if there is any barrier in the fertilization process. The complete hatching took 18±1 hrs at 26-27 °C for local, Thai and T_m-L_f breeding sets and 19±1 hrs for L_m-T_f that agreed with Amornsakun *et al.*, (2005) and Rahman (2005) but Kohinoor *et al.* (1991) reported more time than the present one. The hybridization is carried out mainly to produce hybrid vigor from two strains (Tave, 1993). Richter (1988) reported the hybridization of *A. testudineus* to produce xanthorous form (excess yellow pigmentation). The exploitation of genetic-environmental interactions may produce better yields for growth, food consumption, disease resistance, parasite load, heat, cold and salinity tolerance, body conformation, brood size etc. (Kirpichnikov *et al.*, 1967; Smitherman *et al.*, 1974 and Wales and Berrian, 1937). In the present study, no growth and other performance were checked. However, survival rate was estimated in the present study and higher survival rate and more tolerability to environmental odds were observed in the hybrids than in the pure local and Thai fry. Though fry counting was difficult as the fry were used to get attached to the cistern wall, however, the desirability of volumetric method of counting was reported by some authors (Begenal and Nellen, 1980; Delventhal, 2000). Stressful handling can reduce the survival rate of fish larvae (Moitra *et al.*, 1979). However, the main difficulties faced in the present study were the algal bloom due to the poor outlet system of the tanks. This adverse conditions, though were the same for all the categories of hatchlings (pure local, Thai and two hybrids); the hybrids lived longer days than the pure ones.

5.3. Incidence of natural hybridization of *A. testudineus*

In 2002, private entrepreneurs had imported the first growing koi strain seed from Thailand. Presently, this strain is getting more popularity than the native strain as the growth rate of Thai koi is much higher (BFRI, 2006). However, there should have concerns about the impact on endemic fish and their conservation. In the present study, Thai koi ponds were found to be located mostly near the open water bodies among surveyed ponds (53.85% near *beel* and 38.46% near river). In addition, 46.15% of surveyed ponds had no barrier to protect the escaping of Thai koi and 53.85% had netting but poorly managed by the farmers. As the climbing perch can easily walk over land and

Chapter Six

CONCLUSION

Chapter 6

CONCLUSION

Anabas testudineus are found in different countries in different strains. Thai koi has been introduced in Bangladesh as a strain. In the present investigation, some differences were found between Thai and local strains of *A. testudineus*. Length and weight and their relationship of brood koi showed differences between the two strains. Annual realized fecundity (induced with equal amount of PG hormone at 8 mg kg^{-1} of fish) was observed and significant variation was found in the number of eggs spawned per kg fish. Gonado-somatic index (GSI) was studied after couple of months of peak breeding season and proportionate gonadal development was not found with the same feeding rather Thai koi showed higher GSI. Though the nutritional components (moisture, protein and lipid) were higher in the muscle of local koi, the significant variation was not found. The juvenile of local koi surfaced out for air gulping more frequently than that of Thai koi though the adult of two strains did not show any significant difference for surfacing out. Quicker surfacing was observed just after feeding than at starvation in mature koi of both strains. From most of the morphometric, meristic and qualitative characteristics the local koi varied from the Thai koi. In case of induced breeding pure local and Thai produced more fertilization and hatching rates than the reciprocal cross-breds (hybrid). The survival rate of hybrids in cisterns was found higher than the pure local and Thai. However, to check the viability of hybridization, growth, food consumption, suitability in aquaculture and consumption preference further studies should be made. On the other hand, Thai koi is being cultured in a number of ponds in Khulna but the protection from escaping was very poor. Therefore, incidence of escaping of Thai koi from ponds was found alarming to the conservation of endemic koi. The present investigation revealed that a significant percentage of *beel* catch consists of Thai koi and hybrids along with local strain of *A. testudineus*.

Chapter Seven

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APPENDIX

Table 6: t-test for Annual realized fecundity of two strains of *A. testudineus*

	Strain	Mean±SD	df	t
Fecundity (egg/kg of fish)	Local	345508±114502	10	-6.617*
	Thai	765245±105027		

Table 7: ANOVA for GSI of Local strain of *A. testudineus* over three months period

	SS	df	MS	F
Between Groups	78.709	2	39.355	10.328*
Within Groups	45.726	12	3.810	
Total	124.435	14		

Table 8: ANOVA for GSI of Thai strain of *A. testudineus* over three months period

	SS	df	MS	F
Between Groups	87.966	2	43.983	2.310
Within Groups	228.532	12	19.044	
Total	316.498	14		

Table 9: t-test for nutritional composition of two strains of *A. testudineus* during the study period

	Strain	Mean±SD	df	t
% moisture	Local	79.1300±3.51363	10	0.145
	Thai	78.8800±2.33904		
% lipid (dry wt basis)	Local	9.3250±5.01291	10	1.606
	Thai	4.5967±5.18691		
% lipid (dry wt basis)	Local	9.3250±5.01291	10	1.606
	Thai	4.5967±5.18691		

Table 10: Two-way ANOVA of surfacing interval of two strains of *A. testudineus* (juvenile) with stomach contents

	SS	df	MS	F
Strains	111848.441	1	111848.441	8.611*
Stomach	7108.598	1	7108.598	.547
Strains × Stomach	866.566	1	866.566	.067
Error	155860.672	12	12988.389	
Total	934723.813	16		

* Significant at 5% level; SS=Sum of Square; MS=Mean of Sum of Square; df=Degree of Freedom

Table 11: Two-way ANOVA of surfacing interval of two strains of *A. testudineus* (Adult) with stomach contents

	SS	df	MS	F
Strains	37587.516	1	37587.516	3.804
Stomach	29283.766	1	29283.766	2.964
Strains × Stomach	1350.563	1	1350.563	0.137
Error	118575.844	12	9881.320	
Total	863715.250	16		

Table 12: t-test for surfacing interval of juvenile and adult of local koi

	Age	Mean±SD	df	t
Average surfacing interval (sec)	Juvenile	119.3438±54.34561	14	-1.180
	Adult	157.2188±72.70874		

Table 13: t-test for surfacing interval of juvenile and adult of Thai koi

	Age	Mean±SD	df	t
Average surfacing interval (sec)	Juvenile	286.5625±143.00935	14	0.480
	Adult	254.1563±126.60639		

Table 14: Percentage of Fertilization of four breeding set of *A. testudineus*

	Sample No.	Total eggs	Fertilized eggs	Percentage of fertilization
Thai (M)-Thai (F)	1	48	45	93.75
	2	61	61	100.00
	3	22	22	100.00
	4	56	54	96.43
	5	9	9	100.00
	6	2	2	100.00
Local (M)-Local (F)	1	7	6	85.71
	2	11	9	81.82
	3	11	8	72.73
	4	7	7	100.00
	5	4	4	100.00
	6	11	8	72.73
Thai (M)-Local (F)	1	8	7	87.50
	2	9	7	77.78
	3	14	13	92.86
	4	17	15	88.24
	5	5	5	100.00
	6	12	11	91.67
Local (M)-Thai (F)	1	41	27	65.85
	2	36	22	61.11
	3	81	57	70.37
	4	90	60	66.67
	5	35	26	74.29
	6	7	4	57.14

* Significant at 5% level; SS=Sum of Square; MS=Mean of Sum of Square; df=Degree of Freedom

Table 15: Percentage of hatching rate of four breeding set of *A. testudineus*

	Sample No.	Fertilized eggs	Hatchlings	Percentage of hatching
Thai (M)-Thai (F)	1	50	47	94.00
	2	45	38	84.44
	3	40	40	100.00
	4	35	32	91.43
	5	15	15	100.00
	6	14	12	85.71
Local (M)-Local (F)	1	35	35	100.00
	2	42	37	88.10
	3	32	31	96.88
	4	47	43	91.49
	5	17	15	88.24
	6	9	9	100.00
Thai (M)-Local (F)	1	52	43	82.69
	2	43	32	74.42
	3	32	20	62.50
	4	45	32	71.11
	5	19	13	68.42
	6	13	11	84.62
Local (M)-Thai (F)	1	50	26	52.00
	2	22	11	50.00
	3	48	22	45.83
	4	11	4	36.36
	5	36	22	61.11
	6	17	8	47.06

Table 16: Morphometric characteristics of local Koi collected from Khulna

No.	Total length	Standard length	Body depth	Peduncle width	Peduncle length	Predorsal length	Snout length	Postorbital length	Eye length	Head length	Length of 1st dorsal fin base	Length of 2nd dorsal fin base	Length of 1st anal fin base	Length of 2nd anal fin base	Chest length	Length of upper jaw	Pelvic spine length
1	10.90	8.70	3.15	1.40	1.10	3.00	0.60	1.75	0.70	2.90	4.40	1.00	2.50	1.20	1.90	1.20	.90
2	10.80	8.75	3.38	1.40	1.10	2.90	0.60	1.75	0.80	2.90	4.40	0.90	2.40	1.30	2.05	1.10	.80
3	10.80	8.50	3.30	1.40	1.10	3.00	0.70	1.70	0.70	2.90	4.10	1.00	2.30	1.30	1.80	1.15	.90
4	10.60	8.60	3.30	1.35	1.00	2.80	0.60	1.70	0.70	2.90	4.10	1.00	2.35	1.25	1.80	1.10	.90
5	10.00	7.80	2.95	1.25	0.90	2.75	0.65	1.52	0.70	2.70	3.80	0.90	2.10	1.15	1.80	1.10	.90
6	11.10	8.90	3.10	1.40	1.10	3.00	0.65	1.70	0.70	2.90	4.00	1.00	2.60	1.30	1.80	1.25	.90
7	10.60	8.40	3.10	1.30	1.00	2.90	0.70	1.65	0.70	2.75	4.20	1.10	2.15	1.30	1.80	1.20	.90
8	10.00	7.80	2.80	1.25	0.85	2.60	0.65	1.65	0.72	2.70	3.90	1.15	1.80	1.20	1.70	1.15	.80
9	9.70	7.60	2.80	1.20	0.85	2.60	0.60	1.40	0.70	2.52	3.70	0.80	1.95	1.15	1.50	.90	.75
10	10.10	8.00	2.80	1.30	0.90	2.80	0.80	1.60	0.75	2.80	3.90	1.15	2.00	1.10	1.60	1.10	.80
11	10.40	8.20	3.00	1.40	1.05	2.90	0.70	1.60	0.72	2.75	3.80	1.10	2.10	1.20	1.90	1.05	.90
12	10.50	8.40	3.12	1.40	1.00	2.80	0.65	1.60	0.70	2.80	4.20	1.00	2.35	1.20	1.90	1.00	.80
13	11.20	8.90	3.20	1.45	1.05	3.10	0.70	1.60	0.70	2.80	4.00	1.05	2.40	1.10	1.90	1.10	.90
14	11.10	8.80	3.30	1.50	1.10	3.25	0.70	1.70	0.71	2.95	4.20	1.00	2.30	1.10	1.95	1.10	.90
15	10.10	8.10	2.90	1.30	1.00	2.80	0.68	1.50	0.70	2.70	4.08	0.98	2.09	1.09	1.80	1.10	.80
16	12.70	10.00	3.75	1.70	1.30	3.20	0.80	1.80	0.70	3.35	5.00	1.20	2.80	1.50	2.20	1.40	1.00
17	11.30	9.10	3.40	1.40	0.95	3.00	0.70	1.80	0.70	3.10	5.00	0.98	2.20	1.20	2.00	1.20	.98
18	13.80	11.10	3.60	1.80	1.30	3.60	0.80	2.00	0.80	3.50	5.10	1.35	3.00	1.65	2.50	1.25	1.00
19	14.00	11.20	3.50	1.70	1.40	3.80	0.90	2.10	0.70	3.45	5.50	1.15	3.00	1.40	2.40	1.25	1.10
20	12.00	9.50	3.40	1.60	1.20	3.30	0.70	1.80	0.70	3.15	4.70	1.00	2.40	1.25	2.00	1.15	1.00
21	14.70	11.80	4.10	2.00	1.40	3.60	0.80	2.20	0.80	3.50	5.90	1.38	3.10	1.50	2.85	1.35	1.20
22	12.50	9.80	3.30	1.70	1.20	3.45	0.70	1.80	0.73	3.10	4.60	1.10	2.50	1.50	2.15	1.10	1.00
23	14.60	10.80	3.60	1.70	1.30	3.60	0.70	2.00	0.75	3.30	5.10	1.20	2.90	1.45	2.45	1.25	1.10
24	14.20	11.20	3.85	1.80	1.40	3.80	0.80	2.00	0.75	3.40	5.70	1.45	3.15	1.60	2.45	1.30	1.10
25	11.70	9.40	2.83	1.45	1.10	3.30	0.90	1.90	0.70	3.10	4.30	1.15	2.40	1.20	1.90	1.00	.90
26	14.70	12.50	3.35	2.00	1.40	3.70	0.90	2.25	0.70	3.80	5.90	1.40	3.10	1.60	2.60	1.40	1.00
27	10.70	8.50	3.00	1.40	1.00	2.95	0.70	1.90	0.80	3.05	4.10	1.10	2.40	1.25	1.75	1.15	1.00
28	9.60	7.90	3.00	1.30	0.85	2.80	0.60	1.60	0.65	2.60	3.65	0.80	1.90	0.90	1.85	1.00	.70
29	10.00	7.90	2.90	1.30	0.90	2.90	0.60	1.60	0.70	2.90	3.75	0.95	2.00	1.00	1.90	.90	.80
30	10.00	8.00	2.70	1.40	0.85	2.80	0.70	1.60	0.65	2.80	3.75	0.90	2.05	1.00	1.85	1.00	.80
Mean	11.4800	9.1383	3.2160	1.4850	1.0883	3.1000	0.7093	1.7590	0.7177	3.0023	4.4277	1.0747	2.4097	1.2647	2.0017	1.1433	.9177
SD	1.63652	1.32884	0.34092	0.21781	0.18037	0.35767	.09055	0.20682	.03936	0.30949	0.67331	0.16483	0.38900	0.18884	.31472	.12847	.11738

Table 17: Morphometric characteristics of Thai Koi collected from Khulna

No.	Total length	Standard length	Body depth	Peduncle width	Peduncle length	Predorsal length	Snout length	Postorbital length	Eye length	Head length	Length of 1st dorsal fin base	Length of 2nd dorsal fin base	Length of 1st anal fin base	Length of 2nd anal fin base	Chest length	Length of upper jaw	Pelvic spine length
1	12.50	10.10	2.80	1.40	0.90	3.20	0.70	1.90	0.70	3.10	4.80	1.40	2.50	1.50	2.00	1.20	1.10
2	12.80	10.20	3.10	1.55	1.30	3.10	0.70	1.90	0.70	3.20	5.10	1.50	2.70	1.50	2.00	1.00	1.10
3	11.60	9.60	2.80	1.40	1.35	3.10	0.65	1.80	0.70	3.10	5.00	1.25	2.20	1.50	2.00	1.10	.95
4	11.00	9.10	2.70	1.40	1.20	2.85	0.70	1.80	0.70	3.15	4.00	1.35	2.30	1.90	1.80	1.10	1.00
5	12.10	9.90	3.40	1.50	1.20	3.10	0.80	1.80	0.70	3.50	4.80	1.70	2.50	1.40	2.00	1.10	0.95
6	15.90	12.80	4.50	1.95	1.75	3.65	1.00	2.50	0.80	4.00	6.10	1.95	3.30	2.00	2.65	1.50	1.25
7	12.45	10.20	3.25	1.60	1.30	3.40	0.71	2.00	0.70	3.25	3.95	1.30	2.25	1.65	2.05	1.15	1.18
8	14.40	11.50	3.55	1.70	1.45	3.59	0.70	2.28	0.71	3.69	4.60	1.68	2.60	1.57	2.50	1.22	1.30
9	13.80	11.20	3.90	1.70	1.40	3.45	0.70	2.30	0.61	3.80	5.15	1.50	2.65	1.75	2.40	1.20	1.10
10	12.50	10.90	3.90	1.75	1.40	3.30	0.60	2.15	0.80	3.50	5.00	1.60	2.70	1.60	2.30	1.15	1.28
11	15.17	13.20	4.80	2.00	1.80	4.10	0.80	2.80	0.90	4.00	5.90	2.20	2.95	2.00	2.90	1.35	1.15
12	12.50	10.40	3.10	1.50	1.10	3.20	0.60	1.90	0.80	3.05	4.75	1.40	2.40	1.60	1.95	1.20	1.20
13	14.40	11.50	4.00	1.70	1.35	3.85	0.70	2.35	0.80	3.65	4.70	1.60	2.85	1.80	2.35	1.25	1.25
14	11.80	9.90	3.10	1.50	1.15	3.30	0.60	1.90	0.80	3.00	4.65	1.40	2.25	1.40	2.04	1.50	1.00
15	16.00	12.90	3.40	1.95	1.80	4.00	0.90	2.80	0.80	4.20	4.90	1.70	3.00	1.60	2.80	1.45	1.60
16	16.20	13.30	3.65	1.90	1.70	4.20	0.80	2.60	0.90	4.10	5.50	1.90	3.15	1.79	2.79	1.30	1.40
17	13.50	10.69	3.32	1.70	1.25	3.40	0.70	2.00	0.75	3.33	5.20	1.70	2.60	1.76	1.95	1.20	1.10
18	17.20	14.30	3.90	2.00	1.80	4.40	0.85	3.90	0.80	5.50	5.70	2.00	3.40	2.10	3.00	1.60	1.30
19	17.00	13.70	4.20	2.10	1.80	3.80	0.60	2.90	0.80	4.25	5.60	2.00	3.30	1.90	3.30	1.60	1.30
20	15.00	12.00	3.34	1.80	1.25	3.80	0.80	2.60	0.65	3.60	5.00	1.70	2.95	2.10	2.80	1.40	1.50
21	15.40	12.20	3.35	1.80	1.30	4.30	0.80	2.40	0.70	3.80	4.90	1.75	3.10	1.60	2.90	1.30	1.55
22	11.50	9.20	2.62	1.60	1.35	3.30	0.70	2.00	0.70	3.40	4.30	1.30	2.00	1.45	2.00	1.15	1.00
23	13.40	10.80	3.85	1.59	1.25	3.70	0.85	2.00	0.73	3.60	4.80	1.55	2.50	1.60	3.30	1.40	1.10
24	12.40	10.10	2.80	1.40	0.90	3.20	0.70	1.90	0.70	3.10	4.80	1.40	2.50	1.50	2.00	1.20	1.10
25	12.80	10.20	3.10	1.55	1.30	3.10	0.70	1.90	0.70	3.20	5.10	1.50	2.70	1.50	2.00	1.00	1.10
26	11.60	9.60	2.80	1.40	1.35	3.10	0.65	1.80	0.70	3.10	5.00	1.25	2.20	1.50	2.00	1.10	0.95
Mean	13.6508	11.1342	3.4319	1.6708	1.3731	3.5188	0.7312	2.2377	0.7438	3.5835	4.9731	1.5992	2.6750	1.6758	2.3762	1.2585	1.1850
SD	1.82899	1.48199	.56972	.21470	0.25970	0.42656	0.09897	0.48557	0.07070	0.54723	0.50561	0.25623	0.38059	0.21362	0.46128	0.17047	0.18204

Table 18: Meristic characteristics of local Koi collected from Khulna

No.	Dorsal spine	Dorsal fin ray	Anal spine	Anal fin ray	Pelvic fin ray (without Spine)	Pectoral fin ray	Caudal fin ray	Scale on the upper lateral line	Scale on the lower lateral line	Total Scale on lateral line	Scale above lateral line	Scale below lateral line
1	17	9	10	11	5	15	16	17	13	30	4	11
2	18	8	11	11	5	15	16	17	11	28	4	10
3	17	9	10	11	5	15	17	17	10	27	4	10
4	17	9	10	11	5	15	16	18	10	28	4	11
5	17	9	10	11	5	16	16	17	9	26	4	11
6	17	9	10	11	5	16	16	19	10	29	4	11
7	17	9	9	11	5	15	17	18	10	28	4	10
8	16	9	9	11	5	15	18	18	11	29	4	10
9	17	8	10	11	5	15	17	17	11	28	4	11
10	16	9	10	11	5	15	16	17	10	27	4	12
11	17	9	10	10	5	15	16	17	11	28	4	11
12	17	9	10	11	5	16	16	16	11	27	5	12
13	17	8	10	10	5	15	16	17	12	29	4	11
14	17	9	10	10	5	16	16	16	10	26	4	12
15	16	9	10	11	5	15	16	16	10	26	4	10
16	18	9	10	11	5	16	16	18	12	30	4	10
17	18	8	10	11	5	16	17	16	11	27	4	11
18	16	9	10	10	5	15	16	17	10	27	4	10
19	17	8	10	10	5	15	16	17	11	28	4	10
20	17	8	10	10	5	15	16	16	11	27	4	11
21	17	9	10	10	5	16	16	18	9	27	4	10
22	16	9	10	10	5	16	16	21	9	30	4	11
23	17	8	10	10	6	17	17	18	11	29	3	12
24	17	9	10	11	5	15	16	17	10	27	4	10
25	16	9	10	11	5	16	17	16	12	28	4	11
26	16	9	10	11	5	16	16	17	11	28	4	11
27	18	9	10	11	5	16	17	18	11	29	4	10
28	17	9	10	11	5	16	17	16	10	26	4	10
29	17	9	10	10	5	15	17	17	10	27	4	12
30	17	9	10	10	5	16	17	17	11	28	4	12
Mean	16.90	8.77	9.97	10.63	5.03	15.50	16.40	17.20	10.60	27.80	4.00	10.80
SD	0.607	0.430	.320	0.490	0.183	0.572	0.563	1.064	0.932	1.186	0.263	0.761

Table 19: Meristic characteristics of Thai Koi collected from Khulna

No.	Dorsal spine	Dorsal fin ray	Anal spine	Anal fin ray	Pelvic fin ray (without Spine)	Pectoral fin ray	Caudal fin ray	Scale on the upper lateral line	Scale on the lower lateral line	Total Scale on lateral line	Scale above lateral line	Scale below lateral line
1	17	10	9	10	5	15	16	16	9	25	4	10
2	17	10	9	10	5	17	17	16	10	26	4	9
3	18	10	9	11	5	17	16	15	11	26	3	10
4	17	9	9	11	5	15	17	15	12	27	4	9
5	16	10	9	10	5	16	16	16	12	28	4	10
6	17	10	9	11	5	16	16	17	10	27	4	11
7	17	10	9	11	5	16	18	17	10	27	4	9
8	17	10	9	11	5	16	16	18	12	30	4	10
9	17	10	9	11	5	15	16	15	12	27	4	9
10	16	10	9	10	5	15	16	15	12	27	3	10
11	17	9	9	10	5	16	17	15	13	28	4	10
12	18	9	9	10	5	17	18	15	11	26	4	8
13	17	10	9	11	5	15	17	17	13	30	4	10
14	17	10	11	9	5	17	18	16	12	28	4	10
15	18	9	9	9	5	15	16	17	11	28	4	9
16	17	10	9	10	5	14	16	15	9	24	4	10
17	17	8	9	10	5	16	16	15	11	26	4	10
18	17	10	9	11	5	15	16	17	10	27	4	10
19	17	10	9	10	5	15	16	17	14	31	4	9
20	17	9	9	10	5	15	17	18	11	29	4	10
21	17	10	9	11	5	15	17	17	13	30	4	10
22	17	9	9	9	5	15	17	16	12	28	4	11
23	17	10	9	11	5	15	16	16	10	26	4	10
24	17	10	9	10	5	15	16	16	9	25	4	10
25	17	10	9	10	5	17	17	16	10	26	4	9
26	18	10	9	11	5	17	16	15	11	26	3	10
Mean	17.08	9.69	9.08	10.31	5.00	15.65	16.54	16.08	11.15	27.23	3.88	9.73
SD	0.484	0.549	0.392	0.679	0.000	0.892	0.706	0.977	1.347	1.728	0.326	0.667

PHOTO GALLERY

Plate 1: Rearing tank of *A. testudineus* (both local and Thai) for studying some biological aspects in Batiaghata, Khulna.

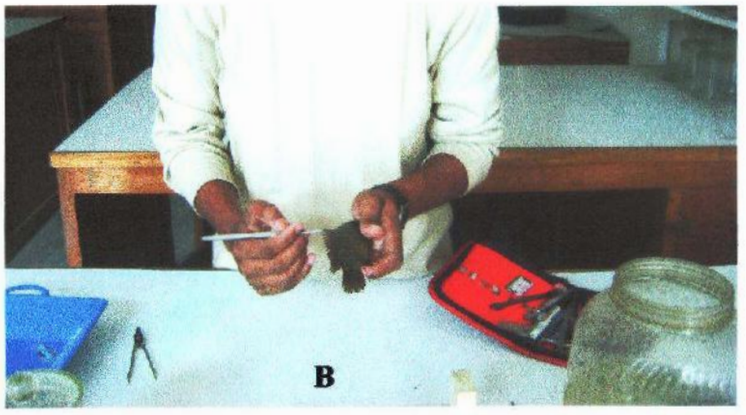

Plate 2: Total length measurement (A) and dorsal fin rays count (B) of *A. testudineus* in Fish Biology Lab.

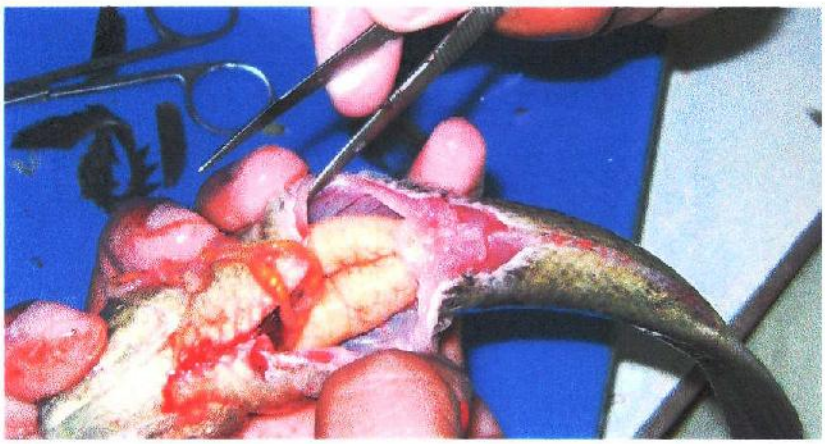

Plate 3: The gonad of Thai koi filled with eggs in the month of December

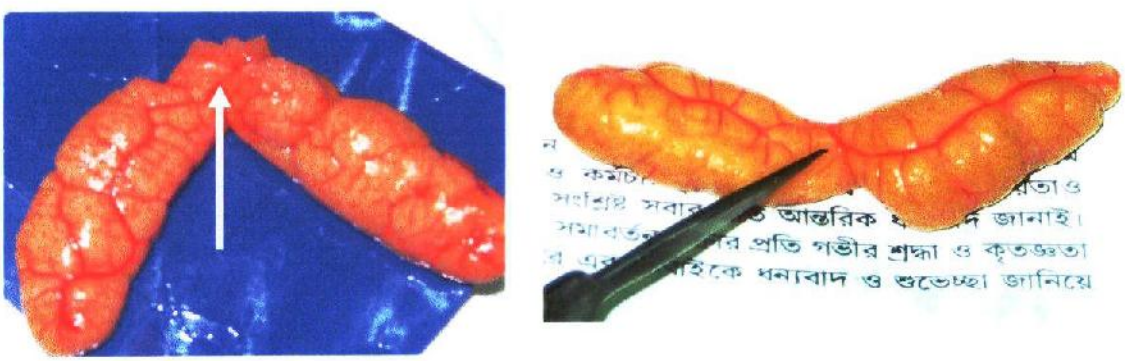

Plate 4: The female gonad of Thai koi being fused in the near end of the lobes

Plate 5: The air gulping of *A. testudineus* from water surface in a glass tank

Plate 6: Two apparatus of Kjeldal method of protein determination from the muscle of *A. testudineus*

Plate 7: Administration of PG hormone at the base of pectoral fin of *A. testudineus*

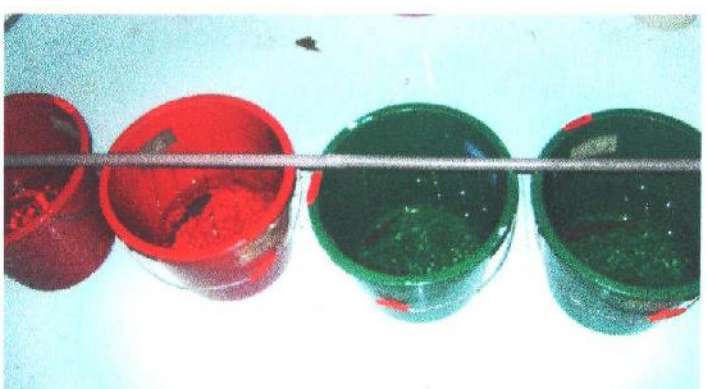

Plate 8: Spawning buckets for *A. testudineus* in the hatchery

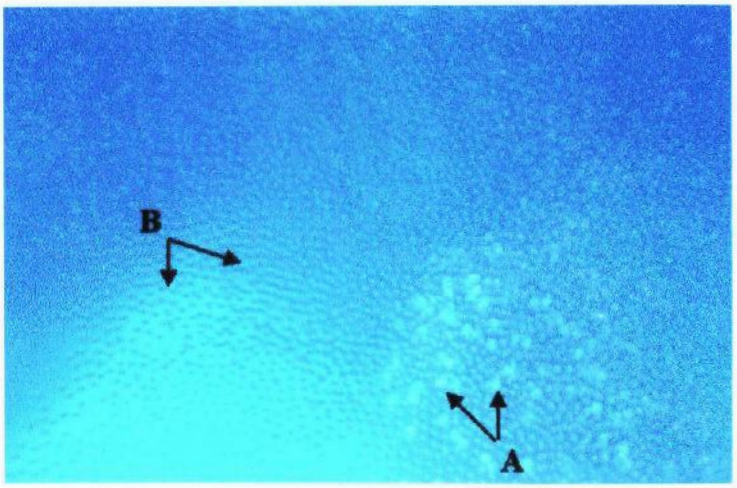

Plate 9: The state of fertilized and unfertilized eggs of koi after six hours of spawning; unfertilized eggs are opaque (A) while the fertilized ones are transparent (B)

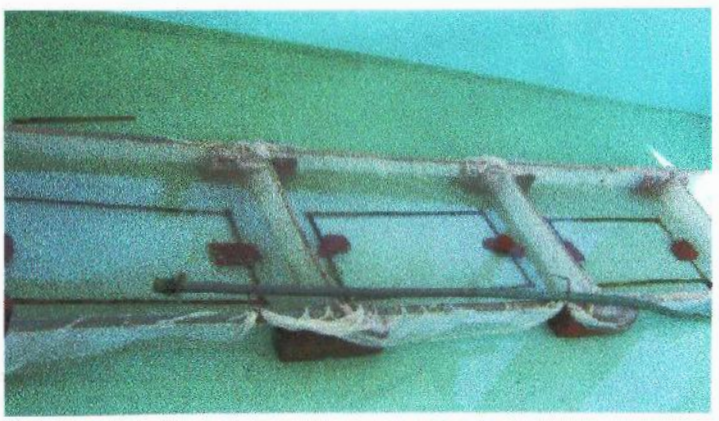

Plate 10: The hand made hatching tray made of nylon net, bamboo slats and ropes.

Plate 11: Black spots on body surface of Thai koi and no spots on the body of local koi; A, Adult and juvenile Thai; B, Juvenile Thai; C, local koi

Plate 12: The spotted koi (in red circle) and local koi (in yellow circle) in a *beel* catch

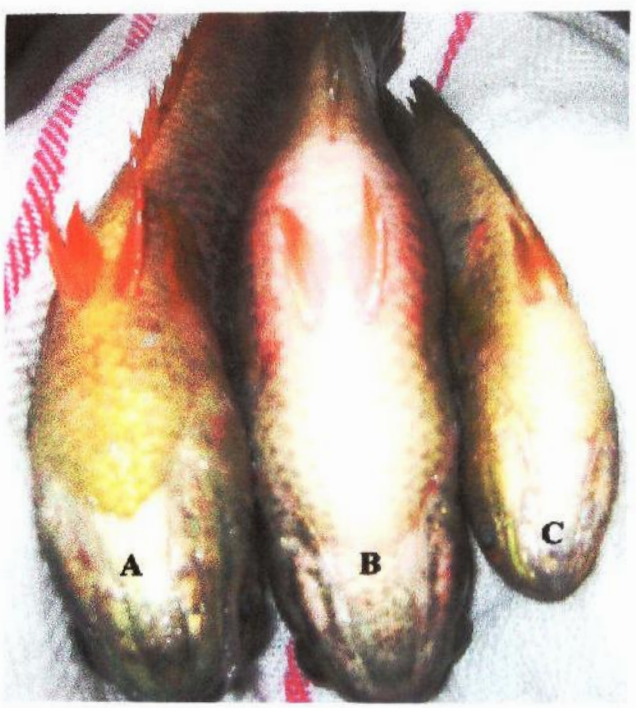

Plate 13: Three distinguished pelvic fin base color of local (A), Thai (B) and hybrid koi (C)

A

B

C

Plate 14: The ponds of Thai koi being cultured without netting near the *beel* (A) and river (B & C)