Chapter I

Geographical Variation of Morphometric and Meristic Characteristics of *Channa gachua*

The comprehensive study of life history traits identify and define appropriate management of threatened and endangered species. Therefore, a detailed knowledge on the salient biological features of the threatened species will be highly valuable in implementing any programme on conservation of fish genetic resources (Ponniah, 2001). The importance of morphological characters in ichthyotaxonomy needs no mention. The nature of adipose eyelids, its development, extension of maxillae, position of nostrils, nature of operculum, presence of pores around the mouth region and its numbers, type of mouth, the arching of lateral line, naked area of breast region, pigments, bands on the lateral side etc., are to be studied carefully in large number of specimens covering different length groups. Sometimes a morphological character attributed by a taxonomist as valid one for a species at a given length, may prove to be invalid for the highest or lowest length groups (Mann, 1974). Hence taxonomists have to study the morphological characters of all length groups covering large number of specimen. Ichthyotaxonomists should not give more importance to colouration. Morphological characters have been commonly used in fishery biology to measure discreteness and relationships among various taxonomic categories. There are well documented morphometric studies which provide evidence for stock discreteness (Shepherd, 1991).

Fish identification is a process of matching the diagnostics features with the specimen of a species. The fish is identified by morphological characteristics taking into consideration
the variation exhibited by each character. In some instances considerable biological information may be available (on behavior, genetics, physiology and ecology) and could further confirm the identification of fish in one way or the other (Bookstein et al., 1985). The first step in the identification procedure is to sort out the fish collections according to the shape of fish, presence or absence of scales and barbles, position and nature of fins and colour pattern. Secondly, measurements of biometric characters (morphometric and meristic counts) can be easily observed and recorded. To ensure uniformity in the description and comparison of taxa, a set of standard terms and procedures are followed while making measurements. The general nomenclature of the external morphology and the principal measurement of fishes are measured by means of dividers, vernier calipers, ordinary ruler or tape. Measurements are made in millimeters (mm) centimeters (cm) or meters (m) and imply the shortest straight line distance between two points (Haniffa, 2002).

Taxonomic classification and understanding the diversity of biological life were historically based on descriptions of morphological forms (Dean et al., 2003). Morphometric characters represent one of the major keys for determining fish systematics, growth variability, ontogenetic trajectories (Kováč et al., 1999) and other parameters. With the development of morphometric techniques, the morphometric characters of organisms have become the principal indices for taxonomy. In the early phases of morphometry, morphometry was described by mean or coefficient of variation of morphometric measurements like length and width and then analysed by regression analysis of log-transformed measurements. In recent years multivariate statistical analysis has been operated for morphological discrimination of different stocks (Rohlf and Marcus, 1993, Sara et al., 1999, Shea and Vecchione, 2002).
Poor understanding of fish and fishery management can lead to dramatic changes in the biological attributes and productivity of a species (Altukhov, 1981; Ricker, 1981; Smith et al., 1991). Morphological variations between fish populations are influenced by a mixture of environmental factors that include but are not limited to temperature, salinity, radiation, dissolved oxygen, water depth and current flow (Hubbs, 1926; Vladykov, 1934; Smith, 1966; Lindsey, 1988; Turan, 2000). Morphometrics and meristics are the two types of morphologic characters that have been most frequently used to delineate stocks of a variety of exploited fish species (Murta, 2000; Silva, 2003; O’Reilly and Horn, 2004; Turan, 2004). Morphometric characters are continuous characters describing aspects of body shape. Meristic characters are the number of discrete, serially repeated, countable structures that are fixed in embryos or larvae. Studies of morphologic variation between populations continue to have an important role to play in stock identification while stable differences in shape between groups of fish may reveal different growth, mortality or reproductive rates that are relevant to the definition of stocks (Swain and Foote, 1999; Cadrin, 2000).

Multivariate analysis of a set of morphometric and meristic characters has been widely used in stock identification of freshwater and marine fish species (Mamuris et al., 1998; Cadrin, 2000; Murta, 2000; Pakkasmaa & Piironen, 2001; Cabral et al., 2003), and to a lesser extent in marine invertebrates (e.g., Henderson et al., 1990; Kassahn et al., 2003). This method is also regarded as more appropriate than the use of single morphological characters for investigating taxonomic problems in determining relationships between populations or closely related (cryptic) species (e.g., Scapini et al., 1999; Clark et al., 2001; De Grave & Diaz, 2001; Debuse et al., 2001; Doadrio et al., 2002; Lee & Frost, 2002). Moreover, morphometric analysis can also be a tool in assessing habitat-specific differentiation of
populations, such as differentiation related to predation pressures, salinity, temperature, food availability, (Gee, 1988; Scapini et al., 1999; Maltagliati et al., 2003). Differences in morphometric and meristic characters among populations of a species are thought to be the result of genetic differences or environmental factors, or their interactions (Lindsey, 1988; Scheiner & Callahan, 1993; Hoffman & Merila, 1999). Strong genetic differentiation of populations, accompanied by reproductive isolation, can lead to local adaptation. On the other hand, changing environmental conditions can produce phenotypic plasticity in genetically similar populations (Thompson, 1991). Hence, the comparison of the degree of variation in molecular markers with that of morphological characters maybe important in assessing the degree of phenotypic plasticity shown by species (O’Reilly & Horn, 2004).

Morphometric and meristic characters have often been used in discrimination and classification studies by statistical techniques (Pope and Hall, 1972; Jahnson and Barnett, 1975; Agnew, 1988 and Avsar, 1994). Considering the need of an investigation the present study was undertaken to compare the morphometric and meristic discrimination of *Channa gachua* stocks of Tambirabarani, Godavari and Brahmaputra rivers.
Materials and Methods

*Channa gachua* samples were undertaken for the present study of morphological characterization. Fish samples were collected from various water bodies of India such as Tamirabarani river (8.44°N, 77.44°E), Godawari river (18.43°N, 79.15°E) and Brahmaputra river (24.8°N, 89.42°E). Fishes were identified in the field and then preserved in 10% formalin for morphometric studies. Larger specimens were injected with 15% formalin carefully through the vent prior to preservation. Specimens are preserved in Centre for Aquaculture Research and Extension (CARE). Measurements were made point to point using digital caliper. The meristic and morphometric measurements are based on the method by Howes and Teugles (1989).

**Morphometric Study**

The Morphometric measurements were taken using an electronic caliper and TPS software (Rohlf, 1998). The measurements taken were followed by the methodology of Gatz (1979) and Hubbs and Lager (1958). Morphological study was carried out for the three population and a total of 42 morphometric and meristic characters were taken, of these 19 measurements viz: standard length, body depth, head length, head width, head depth, preopercular length, post orbital head, pectoral fin length, pectoral fin length, caudal fin length, dorsal depth, first dorsal ray, anal depth, pelvic anal distance, snout length, eye diameter, inter orbital width, length, lower jaw length, upper jaw length were made using digital caliper (nearest to 0.1 mm) and 8 characters were taken by truss network method (TPS software of Rohlf www.life.bio.sunysb.edu) where each character measures the distance between the selected landmarks or coordinates. The characters are; snout to centre of orbit,
centre of orbit to posterior end of lower jaw, posterior end of lower jaw to dorsal origin, dorsal origin to pectoral origin, pectoral origin to dorsal base, dorsal base to anal base, anal base to origin of upper lobe of caudal fin, origin of upper lobe of caudal fin to origin of lower lobe of caudal fin. The rest of the characters are meristic characters like dorsal fin rays, pectoral fin rays, pelvic fin rays, anal fin rays, caudal fin rays, anal origin on dorsal ray, lateral line curved at dorsal ray, lateral line scales, lateral line curved at scale, number of scales above lateral line, number of scale rows below lateral line, preopercular scales, opercular scales, predorsal scales and circumpeduncular scales (Table 2.1).

All geometric measurements were transferred to an excel spread sheet file and each specimen, the x and y uniform components were computed using TPS (Rohlf, 1993). The uniform component refers as affine expresses the shape variation (Bookstein, 1991). The first uniform component accounts to the stretching along the x axis of the configuration where as the second uniform components indicate dilations or compressions along the y axis. In the present study, the x axis corresponds to the antero-posterior axis, and the y axis corresponds to the dorso - ventral axis of the fish bodies.

**Statistical Analysis**

For the comparison of morphometric characters, the measurements were expressed in the percentage of standard length (SL) and the head length (HL). To find out the differences in the morphological and meristic characters between different species Principal Component Analysis (PCA) was performed. All the statistical analyses were performed using statistical packages PAST (Hammer et al., version 1.89). The percentage morphometric and meristic measurements undertaken in the present study are given tables 2.2- 2.17.
River Tamirabarani populations

Description
Dorsal, caudal and anal fin margins are white; a large ocellus with a light edge on the last five dorsal rays, body black getting lighter ventrally and abdomen creamish. Maxilla and premaxillary processes extending to vertical level of the posterior end of the orbit; one or two large cycloid scales on each side of lower jaw undersurface; cephalic sensory pores single and branchial toothplate count 9. The counts and proportions from 30 specimens showed 63.9 – 115.6 mm standard length. The morphometric characters taken by digital caliper are presented in the proportions of standard length, head length, postorbital head length etc.

The characters presented in the standard length ratio are: body depth 18.29 – 24.23 mm (19.98 ± 1.67); head length 31.50 – 42.25 (34.09 ± 3.04); head width 20.53 – 27.94 (22.61 ± 2.03); head depth 16.45 – 23.44 (18.66 ± 1.90); preopercular length 12.90 – 17.78 (15.09 ± 1.20); post orbital head length 21.55 – 28.87 (23.21 ± 2.09); pectoral fin length 18.52 – 21.43 (20.17 ± 0.93); pelvic fin length 9.24 – 11.12 (10.10 ± 0.63); caudal fin length 20.42 – 28.95 (23.46 ± 2.59); dorsal depth 10.09 – 16.42 (12.48 ± 1.90); first dorsal ray 7.17 – 9.80 (8.53 ± 1.06); anal depth 8.05 – 10.19 (9.11 ± 0.68) and pelvic anal distance 8.07 – 10.48 (9.38 ± 0.88).

The proportionate values of the characters in head length are: head width 64.56 – 68.67 (66.43 ± 1.45); head depth 48.02 – 60.32 (66.43 ± 1.45); preopercular length 39.22 – 48.64 (44.40 ± 3.21); post orbital head length 64.75 – 69.81 (68.08 ± 1.59); snout length 12.85 – 16.29 (14.92 ± 0.89); eye diameter 14.10 – 17.18 (15.88 ± 0.99); inter-orbital width
28.44 – 35.22 (31.58 ± 2.34); lower jaw length 35.10 – 40.89 (38.27 ± 2.16) and upper jaw length 30.18 – 37.65 (34.44 ± 2.29). Eye diameter in inter-orbital length was 43.68 – 69.84 (56.37 ± 10.91); pelvic fin length in pectoral fin length 44.59 – 55.41 (50.16 ± 3.61); pelvic fin length in post orbital head length was 38.53 – 48.27 (43.32 ± 3.40) and pectoral fin length in post orbital head length was 73.85 – 96.73 (87.32 ± 6.21).

Fins without spines, dorsal and anal fins quite elongated, small pelvic fins, prominent pectoral fins and caudal fin rounded. The counts are- dorsal fin rays 33 – 34; pectoral fin rays - 14 – 15; pelvic fin rays 6, anal fin rays 20 – 21; caudal fin rays 14; anal origin on dorsal ray 11 – 12; lateral line curved at dorsal ray 7 – 8; lateral line scales 45 – 47; lateral line curved at scale 13; scales above lateral line 4.5; scales below lateral line 8; preopercular scales 5; opercular scales 3; predorsal scales 12 - 13 and circumpeduncular scales 24 - 25.

**River Godavari populations**

**Description**

The characters presented in the standard length ratio are; body depth 15.51 – 18.65 (16.68 ± 0.96); head length 22.92 – 33.17 (27.60 ± 2.88); head width 15.56 – 22.92 (18.68 ± 2.12); head depth 13.75 – 19.02 (15.74 ± 1.71); preopercular length 10.44 – 15.36 (12.42 ± 1.56); post orbital head length 17.16 – 22.19 (19.46 ± 1.49); pectoral fin length 15.03 – 19.51 (16.35 ± 1.36); pelvic fin length 7.35 – 11.34 (8.51 ± 1.23); caudal fin length 17.42 – 23.78 (19.52 ± 1.76); dorsal depth 9.05 – 12.13 (10.16 ± 0.97); first dorsal ray 6.33 – 7.86 (7.17 ± 0.52); anal depth 7.04 – 9.26 (8.19 ± 0.63) and pelvic anal distance 6.95 – 8.53 (7.84 ± 0.50).

The proportionate values of the characters in head length are; head width 64.84 – 72.64 (67.67 ± 2.37); head depth 49.33 – 61.35 (57.13 ± 3.39); preopercular length 40.96 –
post orbital head length 66.51 – 74.88 (70.71 ± 2.79); snout length 15.87 – 20.46 (17.37 ± 1.56); eye diameter 15.23 – 18.76 (16.48 ± 1.29); inter-orbital width 28.76 – 36.01 (32.17 ± 2.50); lower jaw length 35.24 – 42.10 (39.18 ± 2.32) and upper jaw length 33.48 – 41.87 (37.90 ± 2.32). Eye diameter in inter-orbital length was 44.14 – 69.36 (55.48 ± 9.41); pelvic fin length in pectoral fin length 45.39 – 58.12 (51.92 ± 4.17); pelvic fin length in post orbital head length was 38.69 – 51.09 (43.64 ± 3.73) and pectoral fin length in post orbital head length was 75.44 – 89.24 (84.12 ± 4.33).

Fins without spines, dorsal and anal fins quite elongated, small pelvic fins, prominent pectoral fins and caudal fin rounded. The counts are- dorsal fin rays 33 – 35; pectoral fin rays 14 – 16; pelvic fin rays 5-7; anal fin rays 20 – 21; caudal fin rays 14 – 15; anal origin on dorsal ray 11 – 12; lateral line curved at dorsal ray 7 – 8; lateral line scales 45 – 47; lateral line curved at scale 13 – 14; scales above lateral line 4.5; scales below lateral line 8; preopercular scales 5 – 6; opercular scales 3- 4; predorsal scales 11- 14 and circumpeduncular scales 24 - 26.

**River Brahmaputra populations**

**Description**

The characters presented in the standard length ratio are; body depth 15.45 – 20.0 (17.63 ± 1.34); head length 26.03 – 31.96 (28.83 ± 1.60); head width 16.23 – 26.88 (20.72 ± 3.60); head depth 14.59 – 20.81 (16.96 ± 2.13); preopercular length 10.70 – 18.82 (14.15 ± 2.54); post orbital head length 17.01 – 25.08 (20.61 ± 2.74); pectoral fin length 12.53 – 20.0 (16.74 ± 2.38); pelvic fin length 8.27 – 11.79 (9.77 ± 0.94); caudal fin length 14.84 – 23.93 (19.33 ± 2.75); dorsal depth 8.51 – 12.07 (10.22 ± 1.12); first dorsal ray 7.02 – 11.14 (8.27 ±
0.96); anal depth 7.34–11.14 (9.00 ± 1.03) and pelvic anal distance 7.29–10.49 (8.84 ± 0.98).

The proportionate values of the characters in head length are; head width 58.96–96.55 (70.87 ± 1.07); head depth 51.22–71.92 (59.70 ± 6.47); preopercular length 40.79–60.09 (46.30 ± 5.87); post orbital head length 61.17–85.71 (71.16 ± 7.28); snout length 14.92–25.61 (18.14 ± 2.36); eye diameter 13.55–19.21 (16.10 ± 1.91); inter-orbital width 28.97–36.45 (30.84 ± 2.36); lower jaw length 36.91–47.29 (39.90 ± 3.01) and upper jaw length 32.71–44.82 (37.85 ± 3.69). Eye diameter in inter-orbital length was 52.43–79.03 (66.38 ± 7.90); pelvic fin length in pectoral fin length 51.63–66.01 (58.98 ± 5.21); pelvic fin length in post orbital head length was 41.17–53.42 (47.67 ± 3.43) and pectoral fin length in post orbital head length was 72.53–96.79 (81.22 ± 7.52).

Fins without spines, dorsal and anal fins quite elongated, small pelvic fins, prominent pectoral fins and caudal fin rounded. The counts are- dorsal fin rays 32–35; pectoral fin rays 13–16; pelvic fin rays 5–8; anal fin rays 20–22; caudal fin rays 13–16; anal origin on dorsal ray 10–13; lateral line curved at dorsal ray 6–9; lateral line scales 45–47; lateral line curved at scale 12–16; scales below lateral line 8; preopercular scales 4–8; opercular scales 3–8; predorsal scales 12–15 and circumpeduncular scales 23–27.

**Principal Component Analysis on Morphological Data**

Data collected for the morphological characters of the three different populations of *Channa gachua* viz: Tamirabarani, Godavari and Brahmaputra with 33 characters of which 18 were morphometric and the remaining 15 were of meristic counts were analysed for Principal Component Analysis. Twenty seven characters showed variations and hence were utilized for the PCA ordination (1.8). Fourteen components were extracted and first three
axis showed eigenvalues > 0.7 which showed the variance about 70% of the total variance. Tamirabarani and Godavari populations showed overlap.

**Cluster Analysis**

Morphometric and meristic characters of three different populations of *C.gachua* were analysed for the cluster analysis. Tamirabarani and Godavari populations were found to be closer when compared to Brahamputra population (Fig 1.5). This may be due to the similar environmental condition and water bodies being in closer association. Brahamputra river has a different origin with all together different environmental conditions with more rainfall. There are also chances of different food availability, water pH, tubidity, salinity etc which might have influenced the development and growth of various morphological characters among the three *C. gachua* populations.
Snakeheads belonging to family Channidae are diverse and complex group of air breathing freshwater fishes represented by two genera, *Channa* and *Parachanna* and are generally characterized by elongate body, large plate like scales on their heads, elongated dorsal and anal fins supported only by rays and a round caudal fin. Their peculiar morphological characteristics, color variations, economic importance, ability to survive in oxygen depleted waters and adaptations to changing environmental conditions have made them to receive moderate amount of systematic attention in recent times (Li, *et al* 2006., Munafi *et al* 2007., Vishwanath & Geethakumari, 2009). Information regarding morphological characterization is limited and quite scattered for these fishes. Since 1990, four new species have been described (Zhang *et al* 2006, Musikasinthorn,1998., Musikasinthorn P 2000., Musikasinthorn P, Taki Y 2001).

Morphometric characters of *C.gachua* on three different populations were used for Principal Component Analysis. Among the three populations studied: Brahmaputra population were well distinct whereas the two other populations Tamirabarani and Godavari were overlapping each other. Most of the characters studied were well distinct among the populations. The characteristics which were found to be significant showed higher loadings caudal fin rays, anal origin on dorsal ray, lateral line scales, lateral line curved at scale, scales above lateral line, scales below lateral line, preopercular scales, opercular scales, predorsal scales and circumpeduncular scales. Dorsal fin rays (33 - 34), anal fin rays (20 - 21), lateral line scales (45 - 47), larger mouth, dorsal and anal fins were slightly darker in color and
cephalic pores could not be a single diagnostic character for the identification of species as also reported by previous authors (Vishwanath and Geethakumari 2009).

_Channa gachua_ is often confused with _Channa harcourtbutleri_ and _C. orientalis_ (Vishwanath & Geethakumari, 2009). Hora’s (1921) list of fishes from Chidwin basin, Manipur include _C. harcourtbutleri_ (Annandale). The species was later synonymised with _C. gachua_ (Hora and Mukerji, 1934) and distribution of _Channa harcourtbutleri_ in Mnipur (India) is doubtful. Ng _et al_ (1999) reported the species to be distributed in Inle lake Mynamar only. Sen’s (1985) list of _Channa_ of northeastern India included _C. orientalis_ which is distributed in Sri lanka only. Courtney _et al_ (2004) clearly reported that _C. orientalis_ is endemic to Sri Lanka which has been always confused with _C. gachua_.

The present morphometric analysis of the _C. gachua_ in Indian rivers revealed three considerably distinct populations with varying degrees, though not necessarily with any clear geographic pattern. The Brahmaputra river (24.8°N, 89.42°E) sample was the most divergent from the others. The overlapping of the Tamirabarani (8.44°N, 77.44°E) and Godavari river (18.43°N, 79.15°E) samples in discriminated space may suggest a sufficient degree of intermingling between these rivers to homogenize populations, and there has been not enough time to generate phenotypic differentiation among populations since these rivers have similar environmental conditions.

The present finding showed that there is a need of employment of genetic techniques especially the involvement of molecular markers along with the morphological studies for the better understanding of stock analysis. PCA revealed that morphometric differentiation between samples was largely located in the head of _C. gachua_ (Fig 1.1). In the Tamirabarani population, the dorsal and ventral fins were placed more anteriorly, pectoral spine and head
length were both shorter, and the eyes were closer to each other than that in the other samples. Such differences between the populations maybe related to different habitat characteristics, such as temperature, turbidity, food availability, water depth and flow. Villaluz and MacCrimmon, (1998) reported that the meristic characters are often subjected to early phenotypic modifications by environmental variations (eg. Temperature, salinity and food).

In the present study, eye diameter was greater in the Brahmaputra populations (19.21mm). Viswanath & Geethakumari (2009) was observed C.gachua eye diameter ranged between 15.04 – 17.1mm in northeastern region of India. Cemal et al (2005) reported that in C.gariepinus eye diameter was greater in Aksu and Ceyhan populations, which may be due to differences in turbidity among rivers. Position of eyes in the head was related to vertical habitat preference (Aleev, 1969). Lateral placement was assumed to indicate pelagic habitat, and dorsally increased displacement was assumed to reflect a more sedentary mode of life (Aleev, 1969). Moreover, short head length was correlated with small prey size in stream fishes (Gatz, 1979). In the Godavari sample, the dorsal fin and posterior fontanelle were placed posteriorly, and the head was smaller in comparision to the other samples. Kessler et al., (1995) considered that a benthic sculpin and four benthic darter species had differences in habitat use in streams at high flow that corresponded to differences in morphology, with two species having robust bodies and large pectoral fins, which allowed them to withstand currents on smaller, smoother substrata. On the other hand, in order to support the given hypotheses above, more detailed data on the environmental conditions from each location (river) sampled were needed. Consequently the present analysis suggests high morphologic differentiation among C. gachua populations. The results indicated that Tamirabarani and
Godavari stocks are more or less morphometrically similar. This may be explained in terms of fluctuations in temperature and other environmental factors during spawning and post-spawning periods and related to differences in the growth characteristics influenced by seasonal as well as annual changes in environmental conditions (Ali Ismen, 2001). However, it should be emphasized that application of genetic techniques (Carvalho & Hauser, 1992, Turan et al., 1998, Shaw et al., 1999, Hauser et al., 2001) would be very beneficial to confirm the detected phenotypic differentiation.

The morphometric and meristic analyses showed complete discrimination between the three populations. Recent reports and the present study highlighted that morphological characterization is primary step for the stock structure analysis and species discrimination of the genus *Channa*. Due to the morphological closeness and varying color patterns during different life stages it is important to undertake stock structure analysis and species discrimination by molecular techniques especially by employing molecular genetic markers along with the morphological studies (Li et al 2006, Adamson et al 2010., Benziger et al 2011).