

Study of some morphometric, meristic characters and length-weight relationship in wild and domestic populations of the eastern river prawn, *Macrobrachium nipponense* (De Haan, 1849) (Crustacea: Decapoda: Palaemonidae), in Iranian Basin of the Caspian Sea

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Abstract

Differences of meristics, morphometrics characters and length-weight relationship were used to estimate the variability of wild and cultivated populations of the eastern river prawn, *M. nipponense* in the populations in Iranian Basin of the Caspian Sea. Three samples were collected from the northern and southern parts of the Caspian Sea in Iran. Ten measurements which made for each individual were size-standardized by allometric methods and principal component, and the resulting measurements were analyzed by cluster analysis and discriminant analysis (DA). The results of cluster analysis and DA indicated that the samples were clustered into two groups: the first group included the Caspian Sea sample; the second included the Anzali Lagoon and the third was Aquaculture farm sample. DA tests showed that morphometric, meristic and length-weight relationship differences among the two groups were significant. At least two morphologically populations of this species in the Iranian Basin of the Caspian Sea were determined. This study also suggested that external morphological traits in *M. nipponense* are variable and change markedly when exposed to different environmental conditions regardless of their geographic origin and that extensive variation was present among the populations studied.

Keywords: Caridea, Population variation, Culture, Wild

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Introduction

The oriental river prawn, *Macrobrachium nipponense* (de Haan, 1849), is a species that presents distributional pattern peripheral (Bânârescu, 1990), colonizing freshwater, estuarine and subterranean waters (Bânârescu, 1990; Udekem-D'Acoz, 1999). This species is widely distributed in waters of China, Japan, Korea, Vietnam and Myanmar (Yu and Miyake, 1972; Ma *et al.*, 2012) whilst it has been introduced to Singapore, Philippines, Uzbekistan, Iraq and Iran from the southern and south-eastern parts of the Caspian Sea (De Grave and Ghane, 2006). Due to overfishing for food, the wild stocks of *M. nipponense* are endangered (Feng and Li, 2008). In addition, the species is cultivated in a wide range of systems to the extreme in the cooling reservoirs of several thermal power stations in Russia, Belarus and Moldova (Alekhovich and Kulesh, 2001). In general, it has been established wild populations of this species in basins, reservoirs and estuarine lagoon systems, offsite original distribution.

The species has high aquaculture potential, as it can withstand in low winter temperatures and can grow in freshwater (Kwon and Uno, 1969). Due to the *M. nipponense* habitat fragmentation and its high adaptability, the species seems to be useful for the study of shaping patterns concomitant to varying ecological conditions. In general, it has been found that the genetic diversity populations of *M. nipponense* grown in farms decrease with generations (Ge *et al.*, 2011).

Studies of phenotypic and genotypic aspects have focused on the morphological characteristics, interspecific hybridization, sexually dimorphic expression, phylogeny, or the genetic diversity of the wild populations (Zhao *et al.*, 2006; Shen *et al.*, 2013; Chen *et al.*, 2015a) and in China on the genetic variation of wild and cultured organisms (Ge *et al.*, 2011). However, knowledge of the genetic variability for the domestic populations were cultured with different models is limited. Many differences were found in genetic content and morphometric of oriental river prawn populations in estuarine and freshwater in Japan and Taiwan (Liu *et al.*, 2007). However, there is very limited information on the structure of populations and morphometric characteristic in Iran, where it was recorded since 1998 (De Grave and Ghane, 2006). The aim of this study was to assess to the morphometric, meristic characters and length-weight relationship variations in wild and domestic populations of the eastern river prawn, *M. nipponense* in the populations of Iranian Basin of the Caspian Sea (Anzali Lagoon, southern Caspian Sea and aquaculture farms).

Material and methods

Sample collection

Samples of *M. nipponense* were collected from wild and domestic populations in three regions of Iran: Anzali Lagoon (AL; 37°29'20.70"N; 49°19'44.30"E), Caspian Sea (CS; 37°27'52.72"N; 49°43'2.64"E) and Aquaculture Farms (AF;

37°09'21.45"N; 49°37'36.61"E). The ponds for prawn culture were about 1000 m², and during the harvest period from September to November 2014 samples were collected. One hundred fifty specimens were collected by using 5.0 mm mesh trap nets and 5.5 mm mesh dip nets. The sex was identified and individuals were separated accordingly. All samples were measured in fresh conditions and molted individuals with soft exoskeleton were excluded from analysis. The freshwater prawns were identified to species according to Cai and Ng (2002).

Morphometric relationships

Six hundred specimens were collected at each site. In total, 1,800 specimens have been analyzed using 11

morphometric, metrics and weight characters (Table 1). Morphometric variables were measured according to Konan *et al.* (2008). The measurements of the second pereopods and its joints were made on the major leg for prawns. Where pereopods were of equal size, the measurements were taken on the right leg. All measurements were made using a Vernier caliper (± 0.05 mm). Specimens with missing or regenerating limbs were excluded. In the present study, the carapace length (CL) was used as a reference dimension because it was the easiest, fastest and most reliable.

Table 1: Description of morphometric, meristic and weight parameters of *Macrobrachium nipponense* used in the study.

| No. | Variable | Abbreviation | Measurements and Counts |
|-----|----------------------------------|--------------|---|
| | Morphometric parameters | | |
| 1 | Total length | TL | Distance between rostrum tip and the distal tip of the telson with shrimp stretched out |
| 2 | Carapace length | CL | Distance between the posterior margin of the right orbit and the midpoint of the posterior margin of the carapace |
| 3 | Arm length | AL | Length of the second pair of pereopods |
| | Meristic parameters | | |
| 4 | Number of upper teeth of Rostrum | NUT | Total count of upper teeth of the Rostrum |
| 5 | Number of lower teeth of Rostrum | NLT | Total count of lower teeth of the Rostrum |
| | Size parameters | | |
| 6 | Body weight | BW | Entire weight of prawn |
| 7 | Carapace weight | CW | Only the weight of carapace |
| 8 | Abdomen weight | AW | Weight of the abdomen with carapace |
| 9 | Gut weight | GW | Single weight of digestive tract |
| 10 | Arm weight | ARW | Weight of the arm |

Statistical analysis

Means, standard error, range and coefficient of variation (CV%) of all measurements were recorded for each character. All morphometric variables were standardized by $M_S = M_0(L_S/L_0)^b$ (Konan *et al.*, 2010). M_S is the standardized measurements, M_0 is the length of measured variable, L_S is the arithmetic mean of the standard length for all prawn from all samples in each analysis and L_0 is the standard length of each specimen. The parameter b was estimated for each variable from the observed data by allometric growth equation $M = aL^b$ where M is total weight (g), L total length (cm), a intercept and b slope (Leonart *et al.*, 2000). Principal components analysis was used to standardized data and evaluate morphometric variation among organisms and to identify variables contributing substantially to that variation. A dendrogram of the ten variables was constructed by unweighted pair-group method with arithmetic means by using Square Euclidean distances between population centroids to assess the degree of similarity between the samples. Canonical variate analysis was performed to discriminate among sites. All specimens were randomly assigned to one of three groups. The new dataset was then analyzed by multivariate discriminant analysis. All analyses were

performed using STATISTICA System software Ver. 5.5 1998-2000.

Results

Morphometric, meristic and weight parameters of males, females and offspring specimens *M. nipponense* are presented in Table 2. The coefficients of variation (CV) values were relatively low (CV<30%) for all standardized variables (between 5.08% and 27.98%) of total count of upper teeth of the rostrum (NUT) and total count of lower teeth of the rostrum (NLT; meristic parameters). The lowest value (5.08%) was recorded for NUT in AL while the highest (27.98%) was registered for NUT in males of CS. The coefficients of variation (CV) were found for all variables between 7.07% and 34.46% of TL and CL (morphometric parameters). The lowest value (7.07%) was recorded for total length (TL) in CS while the highest (34.46%) was registered for CL in male of CS. The morphometric variability within populations was high for males groups. The highest CV of all types of organisms within a population were to AF>AL>CS. The weight parameters within populations were lower for CS groups. The greater variability was in weight of the arm (36.66% to 171.22%) and the lowest in weight of carapace (21.54% to 100.02%). In general, the descending order of morphometric, meristics and weight variability were FB>AL> CS.

Growth coefficients prawns are presented in Table 3. These show that most population has a development comprised within the range isometric. There is a tendency in the CS group to

present a negative allometric growth (2.53 to 2.95). In general, populations of AL and AF had a coefficient b closer to the isometry (2.98 to 3.23) and had better weight than CS.

Table 3: Relationships among carapace length (CL), total length (TL), and body wet weight (BW) of *Macrobrachium nipponense* in Caspian Sea, Anzali Lagoon and Aquaculture Farms in Iran. M, male; F, female; TL, total length; CL, carapace length; BW, body weight; r², correlation determination; n, no. of prawn.

| Parameter | TL=a+b(CL) | | BW=a(TL) ^b | | BW=a(CL) ^b | |
|-------------------|---------------------|---------------------|-----------------------|---------------------|-----------------------|---------------------|
| | M | F | M | F | M | F |
| Caspian Sea | | | | | | |
| a | 0.0016 | 7.7078 | 0.00005 | 0.00005 | 11.217 | 0.0002 |
| b | 2.1127 ^a | 2.0942 ^b | 2.53 ^b | 2.9558 ^b | 1.9013 ^b | 2.9458 ^b |
| r ² | 0.9611 | 0.6753 | 89 | 0.8131 | 0.9652 | 0.8122 |
| n | 200 | 370 | 230 | 370 | 200 | 370 |
| Anzali Lagoon | | | | | | |
| a | 4.352 | 2.4907 | 0.000003 | 0.00001 | 0.00009 | 0.00005 |
| b | 2.0723 ^a | 2.4514 ^a | 3.2327 ^a | 2.9827 ^b | 3.0231 ^a | 3.2886 ^a |
| r ² | 0.981 | 0.954 | 0.9661 | 0.9655 | 0.9414 | 0.9223 |
| n | 340 | 370 | 340 | 375 | 340 | 367 |
| Aquaculture farms | | | | | | |
| a | 6.3139 | 4.2534 | 0.000006 | 0.000006 | 0.0001 | 0.0002 |
| b | 2.0187 ^a | 2.1519 ^b | 3.093 ^a | 3.0001 ^a | 2.984 ^a | 2.8129 ^b |
| r ² | 0.935 | 0.9399 | 0.9396 | 0.9332 | 0.933 | 0.9186 |
| n | 378 | 303 | 378 | 316 | 369 | 303 |

Correlation coefficients between characters before and after size effect removal are shown in Table 4. The 47% and 41% of the coefficients of males and females respectively were highly

significant before size effect removal and were considerably reduced after. All data used in cluster, principal component and discriminant analyses are almost free from size effect.

Table 4: Correlation coefficients between variables, before and after the removal of the size effect, are respectively shown below and above the diagonal. a). Male and b). Female. * Significant differences (p<0.05).

| (a) | BW | TL | CL | NUT | NLT | AL | AW | GW | CW | ARW |
|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| BW | 1 | 0.09 | 0.23 | -0.05 | -0.04 | 0.23 | 0.85* | 0.72* | 0.02 | 0.94* |
| TL | 0.93* | 1 | -0.01 | -0.03 | -0.01 | 0.76* | 0.01 | 0.10 | 0.17* | 0.12 |
| CL | 0.88* | 0.95* | 1 | 0.00 | -0.02 | -0.01 | 0.14 | 0.14 | 0.82* | 0.24 |
| NUT | -0.02 | 0.00 | 0.00 | 1 | 0.10 | -0.02 | -0.05 | 0.02 | -0.03 | -0.05 |
| NLT | -0.02 | -0.03 | -0.02 | 0.10 | 1 | 0.03 | -0.02 | 0.02 | -0.01 | -0.02 |
| AL | 0.94* | 0.93* | 0.90* | -0.02 | -0.01 | 1 | 0.25 | 0.22 | 0.16 | 0.21 |
| AW | 0.94* | 0.81* | 0.76* | -0.03 | -0.01 | 0.89* | 1 | 0.67* | 0.00 | 0.76* |
| GW | 0.85* | 0.84* | 0.80* | 0.03 | 0.01 | 0.83* | 0.75* | 1 | -0.03 | 0.68* |
| CW | 0.92* | 0.87* | 0.82* | -0.03 | -0.01 | 0.86* | 0.85* | 0.77* | 1 | -0.00 |
| ARW | 0.98* | 0.94* | 0.89* | -0.02 | -0.02 | 0.93* | 0.90* | 0.85* | 0.91* | 1 |
| (b) | BW | TL | CL | NUT | NLT | AL | AW | GW | CW | ARW |
| BW | 1 | 0.09 | 0.23 | -0.05 | -0.04 | 0.23 | 0.85* | 0.72* | 0.02 | 0.94* |
| TL | 0.93* | 1 | -0.01 | -0.03 | -0.01 | 0.76* | 0.01 | 0.10 | 0.17* | 0.12 |
| CL | 0.88* | 0.95* | 1 | 0.00 | -0.02 | -0.01 | 0.14 | 0.14 | 0.82* | 0.24 |
| NUT | -0.02 | 0.00 | 0.00 | 1 | 0.10 | -0.02 | -0.05 | 0.02 | -0.03 | -0.05 |
| NLT | -0.02 | -0.03 | -0.02 | 0.10 | 1 | 0.03 | -0.02 | 0.02 | -0.01 | -0.02 |
| AL | 0.94* | 0.93* | 0.90* | -0.02 | -0.01 | 1 | 0.25 | 0.22 | 0.16 | 0.21 |
| AW | 0.94* | 0.81* | 0.76* | -0.03 | -0.01 | 0.89* | 1 | 0.67* | 0.00 | 0.76* |
| GW | 0.85* | 0.84* | 0.80* | 0.03 | 0.01 | 0.83* | 0.75* | 1 | -0.03 | 0.68* |
| CW | 0.92* | 0.87* | 0.82* | -0.03 | -0.01 | 0.86* | 0.85* | 0.77* | 1 | -0.00 |
| ARW | 0.98* | 0.94* | 0.89* | -0.02 | -0.02 | 0.93* | 0.90* | 0.85* | 0.91* | 1 |

The square Euclidean distances tree of ten variables is shown in Fig. 1b. The ten variables were clustered into two groups. The first group included variables that relate to weight and the second with length. The first group might be further divided into two subgroups. The first subgroup included BW and the second CW mainly, and second group included TL and CL mainly, at least two clusters were identified among the ten variables. When included only the morphometric and meristic variables in the cluster analysis (Fig. 1a), similar result was found that when the weight variables are included (Fig. 1b).

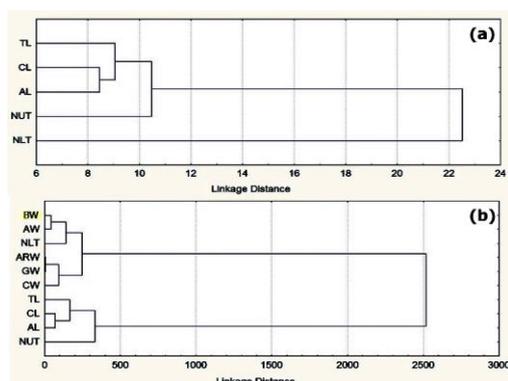


Figure 1: Dendrogram for characters for *Macrobrachium nipponense* data set. a). Morphometric and meristic variables, and b). All variables.

The stepwise discriminant analysis was in two forms. First, single morphometric and meristic variables, and second, the variables related to body weight. In the first case the analysis showed that the classification of organisms in different environments was defined by the morphometric and meristic variables in canonical factor I and canonical factor II respectively and in the second case it was defined by

weight and morphometric characteristics in canonical factor I and canonical factor II respectively. The percentage of correct classification of individuals in the analysis with morphometric and meristic characteristics was 89.49% and when weight measurements were included in, the analysis increased to 92.2%. Therefore, the stepwise discriminant analysis retained ten variables that most discriminate the different populations. These characters of primary importance in distinguishing between the three populations where total weight ($\lambda=0.36$; $F=174$; $p<0.001$), total length ($\lambda=0.79$; $F=157.58$; $p<0.001$) and carapace length ($\lambda=0.95$; $F=32.54$; $p<0.001$). The most well-defined populations were from aquaculture farm and Caspian Sea with classified individual percentage of 100.0% and 89.01% respectively, followed by Anzali Lagoon (26.72%). The number of misclassified individuals from Anzali Lagoon assigned aquaculture farm (85) was higher than Caspian Sea. The confusion matrix indicated that 73.28% of population from Anzali Lagoon has morphometric characters similar with aquaculture farm population. This resulted in an overall rate of correct classification of 92.2%. This analysis revealed that the Linkage distances between the different groups were significant at 0.1%. The cross-validation procedures show that 2,436 of 1,465 prawns (60.14%) were correctly classified in their respective group. The best proportion classification (100%) was obtained in aquaculture farm populations. A higher proportion (58.46%) of misclassified

individuals from Anzali Lagoon was allocated to aquaculture farm. The ordination of organism-site on the canonical factor I \times canonical factor II showed that Caspian Sea population was separated to the other populations on the canonical factor I (Fig. 2a). In contrast, the canonical factor II does not

discriminate prawns from aquaculture farm and Anzali Lagoon. When weight measurements were included in the discriminant analysis, the two groups were separated by maintaining the same trend as in the previous analysis (Fig. 2b).

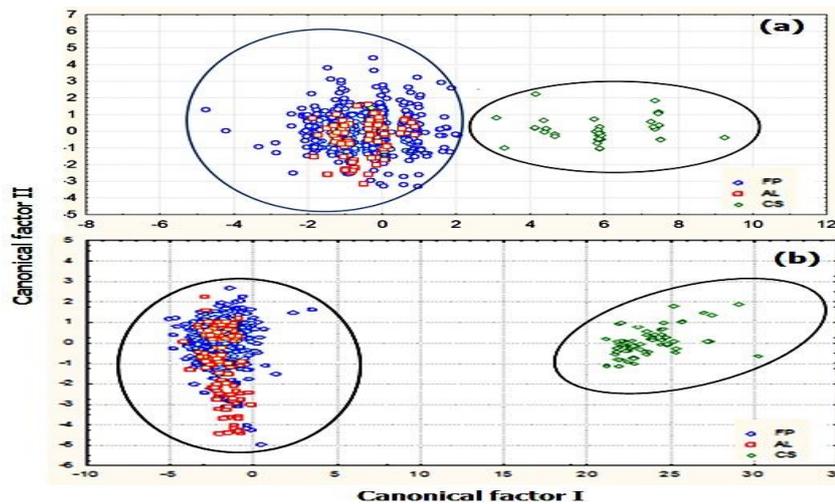


Figure 2: Plot of 95% confidence ellipses around sample from individuals (CS, AF, AL) and group means of first two canonical scores, a). Morphometric and meristic variables, and b). All variables.

Discussion

Morphological and meristics characteristics of prawn are affected by genetic and environmental factors, mainly and social variables in a lower proportion. Thus, variation can reflect genetic differences between stock and/or environmental differences between localities (Tzeng, 2004). In *M. nipponense* it was found that genetic differentiation existed among the populations in different geographical locations, mainly (Ge *et al.*, 2011). It has demonstrated that the difference of lines *M. nipponense* populations in freshwater and estuarine ecosystem due to genetic variations and morphometric in their areas of natural distribution as in Japan (Mashiko and Numachi, 2000), Myanmar (Cai and Ng, 2002) and

Taiwan (Chen *et al.*, 2015b). But in countries where it has introduced little has been studied about the structure of populations (Mirabdullaev and Niyazov, 2005; Cai and Shokita, 2006; De Grave and Ghane, 2006; Salman *et al.*, 2006). The present study demonstrated that *M. nipponense* population in southern coast of the Caspian Sea exhibit extensive morphometric variability. Intra-population variation in morphometric, meristics and weight parameters was found to be high for populations from Caspian Sea with AF-AL. Lower values were found of CV (<20%) for all variables and suggested that Caspian Sea population consisted of a phenotypically homogeneous group (Konan *et al.*, 2010). Low values of

coefficient of variation may indicate high inheritability and limited influence of environmental variation on morphological variability (Soulé and Couzin-Roudy, 1982).

The results of growth coefficients (b) denoted that prawn collected from AF-AL habitat have a weight increase faster than length, showing that AL provides abundant food supply in comparison to Caspian Sea habitat. Bandani and Shokri (2012) reported that in Ajigol wetland Golestan Province, the length-weight ratio of *M. nipponense* is 2.96 to 3.10 (b) similar to what we found in this work for AL-AF (b; 2.98 to 3.23), suggesting that there is a greater availability of feed in areas of greater freshwater influence on the coastal plain of northern Iran. However, the growth coefficients also change due to physiological growth condition such as feed availability and gonads development for the population (Mazlan *et al.*, 2012).

The morphometric variability among these populations was mainly due to the variation of characters related to the length and weight. This variation was attributable to environmental variability of different systems and management of populations. It has already been reported that when *Macrobrachium* populations are identified environmental factors may be more determinant than genetic ones to differentiate the morphological characters (Dimmock *et al.*, 2004). It has also been shown that when exposed to a controlled environment, offspring of wild stocks have little morphological

characteristics of their parental (Dimmock *et al.*, 2004).

In morphometric multivariate measures, variability is usually derived from the size (Chen *et al.*, 2015b). Thus, shape analysis should be free from the effect of size to avoid misinterpretation of the results (Strauss, 1985). In this work standardization equation (Tzeng, 2004) regression and principal component analysis to remove the effect of size were used (Leonart *et al.*, 2000). This showed a clear separation of the two groups obtained (CS and AF-AL) and allometric techniques used achieve size and shape separation and meet statistical assumptions (Reist, 1985). Once defined the difference in groups, improved the classification of individuals from each site to include the size.

It has been found differences in populations of *M. nipponense* which inhabit freshwater and estuarine conditions due to genetic content and morphometric traits (Mashiko and Numachi, 2000), as in the case of populations of CS and AF-AL. It lives from brackish to fully freshwater, and can adapt to a change to fully freshwater in three generations (Wong and McAndrew, 1994).

In general, it was found that the morphometric, meristic and weight parameter used allowed discrimination between Caspian Sea and Anzali Lagoon-Aquaculture farm populations studied. These results showed the possibility to use the selected parameters and this corroborates the use of that meristic measures are less

efficient discriminators than morphometric ones (Waldman *et al.*, 1988; Hurlbut and Clay, 1998; Konan *et al.*, 2010). It's also, demonstrates the utility of multivariate morphometric and weight characteristics for defining stocks of prawn, and two separate stocks have been identified in Iran (southern coast of the Caspian Sea). Thus, to ensure resource sustainability and maintenance, prawns in the Caspian Sea strait and aquaculture farms in Iran should be treated as two separate stock groups to be managed separately. However, in China by comparing wild populations and domestics it was found that farmed oriental river prawn show a decrease in genetic diversity after several generations (Ge *et al.*, 2011). Causes for these differences include inbreeding, bottleneck effect and random drift (Chen *et al.*, 2009). This study also suggested that external morphological traits in *M. nipponense* are variable and change markedly when exposed to different environmental conditions regardless of their geographic origin and that extensive variation was present among the populations studied. The information generated in this work is important for decision making in genetic management programs of *M. nipponense* that are grown in the region and can be used for selective breeding, biotechnology and the molecular genetics of this species.

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