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Sexual Dimorphism in *Artemia urmiana* Günther, 1899 (Anostraca: Artemiidae) from the Urmia Lake, West Azerbaijan, Iran

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Abstract: Sexual dimorphism is an important subject in biosystematic and evolutionary studies of the animal kingdom. In this survey, 4 geographical stations have been chosen from the Urmia Lake (west Azerbaijan, Iran). Males and females of *Artemia urmiana* were examined in order to study sexual dimorphism. The t-Test was used to find out significant differences between means. For classified the male and female samples in each station, Principal Components Analysis (PCA) and Discriminant Function Analysis (DA) were used. PCA analysis let us to separate male and female groups in each station. Also by using DA we can find out that %100 of original groups were correctly classified. This research shows that *A. urmiana* in each 4 different stations is a sexually dimorphic. Also size difference between male and female can be interpreted as mating advantage; according to *Artemia* breeding mechanism, the female carries male during copulation process then large size of female is necessary for this breeding system and can prove mating process.

Key words: Sexual dimorphism, *Artemia urmiana*, body size, mating advantage, breeding mechanism

INTRODUCTION

The genus *Artemia* (Crustacea: Anostraca) is a complex of bisexual and parthenogenetic species, which has wide distribution in the 5 continents which include salt lakes, coastal lagoons and solar salt-works (Van Stappen, 2002) and also the Urmia Lake is the second large lake of Iran (by the total average surface of 5000 km^2), which situated in West Azerbaijan Province. It is the habitat of the endemic *Artemia urmiana* Günther (1899). This species was confirmed by Clark and Bowen (1976) when its reproductive isolation with other bisexual species was confirmed.

Brine shrimp *Artemia* is an economical taxon which is the main and important usage of it has been shown in aquaculture industry (Bengtson *et al*., 1991).

Sexual Dimorphism (S.D.) between adult male and female sex in the same species is a different pattern. These differences in pattern include: size, color and the present or absent a part of the body. This character is a phenomenon which occurs among a variety of animal taxa. In the most vertebrates, the male is the largest sex. Dimorphism has been attributed to sexual selection for larger male and the competitive advantages which confers during competition for females (Anderson, 1994). Although in some taxa, reversed size dimorphism has been regarded as a problem (Figuerola, 1999). For example in *Artemia*; female is larger than male. But up to now we couldn't see any research or critique about S.D. in *Artemia* genus. In this essay, we discuss sexual dimorphism of *A. urmiana* from the Urmia Lake.

MATERIALS AND METHODS

Field and morphological study: Four stations were chosen from the middle, northern and southern parts of the Urmia Lake (Fig. 1) and 4 primary ecological factors for each station were measured (Table 1):

- Salinity (0.5 m from surface and 0.5 m from depth).
- PH (0.5 m from surface and 0.5 m from depth).
- Depth.
- Transparency.

Also 30 male and female specimens of *Artemia urmiana* were randomly collected from each station. Twelve morphometric characters were measured which

Table 1: The primary ecological factors for each sampling station

Station	Salinity (s)*	Salinity (d)**	PH (s)	PH (d)	Depth	Transparency
N2	260 ppt	268 ppt	7.34	7.34	2.2 m	1.8 m
M3-2	260 ppt	260 ppt	7.26	7.3	4.1 m	2.5 m
M1-2	274 ppt	240 ppt	7.23	7.3	2.7 m	0.5 m
S2	245 ppt	256 ppt	7.3	7.35	2.7 m	0.5 m

* 0.5m from surface; ** 0.5m from depth

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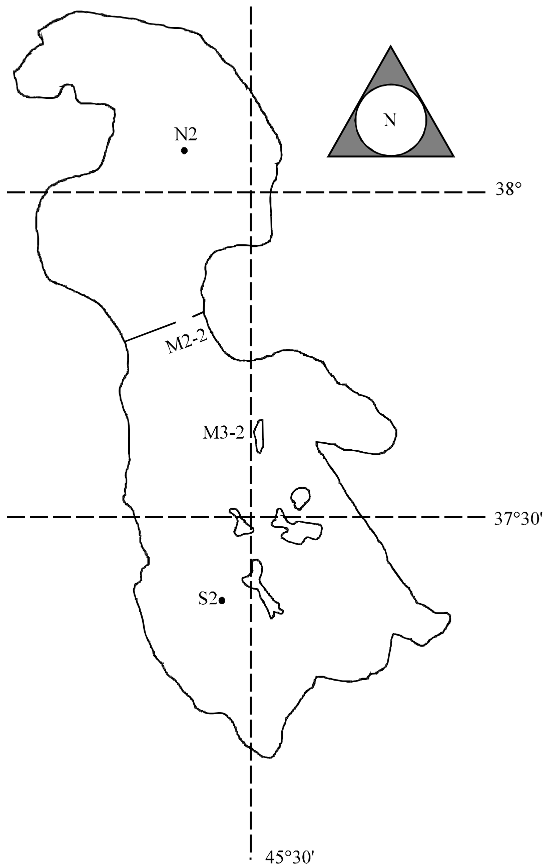

Fig. 1: Geographical location of the studied area, Lake Urmia-Iran

include: total length, abdominal length, head width, distance between compound eyes, diameter of left eye, diameter of right eye, abdominal width, distance between 3rd abdominal segment to 8th abdominal segment, length of telson, length of furca, length of right antenna, length of left antenna and the number of setae per furca (left and right branch) was counted (Hontoria and Amat, 1992; Pilla and Beardmore, 1994; Triantaphyllidis *et al*., 1997b; Cohen *et al*., 1999; Zhou *et al*., 2003; Camargo *et al*., 2003; Amat *et al*., 2005). Morphometric characters have been shown in Table 2.

Statistical analysis: Sexual dimorphism, between male and female individuals of *A. urmiana* was investigated by t-test ($p<0.05$) from four different geographical locations in the Urmia Lake, also all of these analyzed via Principal Components Analysis (PCA) and Discriminant function Analysis (DA). Testing has been done by SPSS computer program.

Table 2: The morphometric and meristic character used in this study

Characters	Definition
tl	total length
al	abdominal length
hw	head width
de	distance between compound eyes
ed_le	eye diameter (left)
ed_ri	eye diameter (right)
aw	abdominal width
das3_8	distance between 3rd abdominal segment to 8th abdominal segment
lte	length of telson
lf	length of furca
la_ri	length of antenna (right)
la_le	length of antenna (left)
sf_ri	number of setae per furca (right branch)
sf_le	number of setae per furca (left branch)

RESULTS

The values for the morphometric and meristic characters as well as the statistical comparison of the results are summarized in Table 3.

Station N2: As we can see in Table 3, in the first station, N2, the means of HW, AW and number of setae on left branch of the furca do not have significant differences ($p<0.05$). Based on the PCA, male and female groups were separated in the factor1, but these 2 groups don't show any separation in the factor 2 (Fig. 2a). In companent1, ed_le, ed_ri, la_ri, la_le, ed, al and tl are the most important characters causes to exist the sexual dimorphism in station N2 (Table 4). The first two components in orderly 61.32 and 21.10% show of the total variation (Table 4). Also, according to the DA, 100% of the main groups of male and female were correctly classified (Table 5).

Station M1-2: According to Table 3 the length of LTE and number of setae on right branch of the furca do not show significant differences between both sexes ($p<0.05$) in station M1-2. In the PCA, male and female groups with regard to factor 1 were relatively divided, but these 2 groups are not separated in factor 2 (Fig. 2b). In the first component, la_le, ed_le, la_ri, ed_ri and LF orderly have the most important role in sexual dimorphism in station M1-2 (Table 4). Percentages of first and second components are 56.49 and 28.48%, respectively and 2 components show 84.97% of variation in total the (Table 4). 100% of the original male and female groups were correctly recognized in the DA (Table 5).

Station M3-2: With regard to Table 3, the length of hw, aw and the number of setae on right and left branch of the furca don't show any significant differences ($p<0.05$)

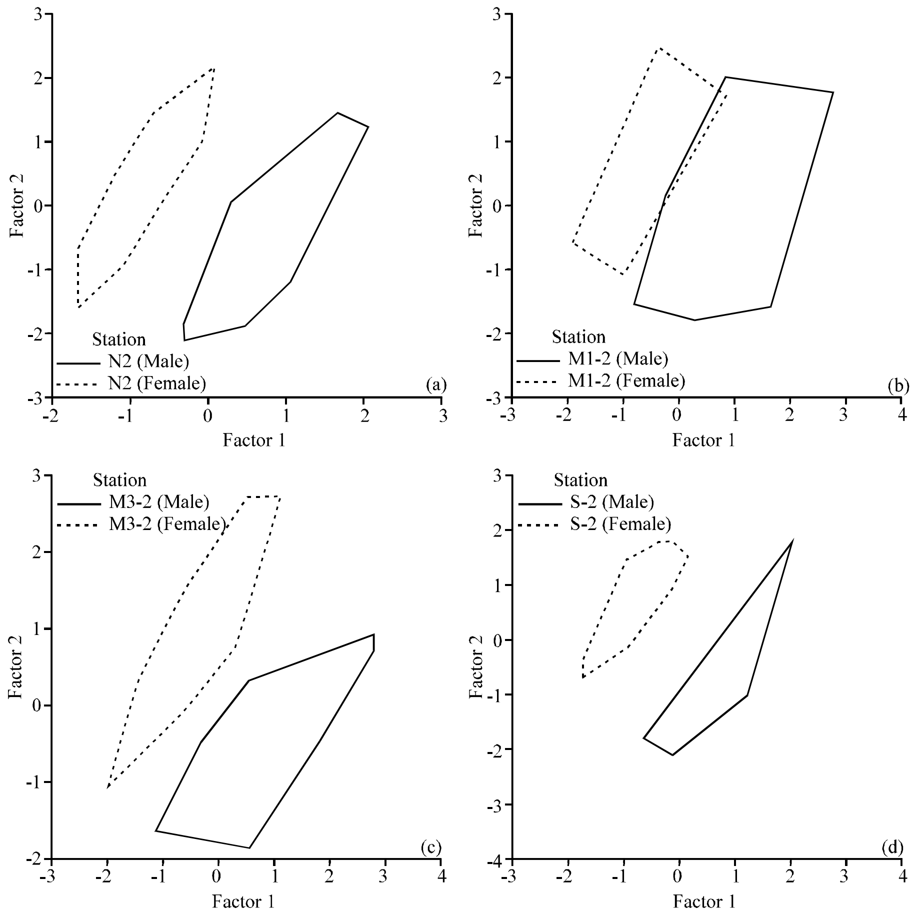

Fig. 2: Scatterplot of aggregation of males and females from station N2 (a), M1-2 (b), M3-2 (c) and S2 (d) by principal component analysis

Table 3: Mean (mm)±S.D. of morphometric and meristic characters for male and female samples from four stations (symbol "a" shows non-significant differences between means of male and female in each row of any station)

	N2		M1-2		M3-2		S2	
	Male	Female	Male	Female	Male	Female	Male	Female
tl	10.25±1.01a	12.72±0.91a	13.20±0.93a	15.24±0.95a	8.96±0.88a	12.63±1.10a	9.62±0.81a	12.71±0.76a
al	5.98±0.59a	8.01±0.57a	7.70±0.54a	9.59±0.59a	5.23±0.51a	7.95±0.69a	5.61±0.47a	8.00±0.48a
hw	0.65±0.059	0.65±0.07	0.66±0.057a	0.76±0.09a	0.67±0.051	0.69±0.07	0.71±0.048a	0.66±0.06a
de	1.84±0.13a	1.32±0.13a	1.81±0.14a	1.53±0.14a	1.70±0.14a	1.33±0.12a	1.74±0.12a	1.33±0.09a
ed_le	0.42±0.041a	0.26±0.025a	0.41±0.043a	0.31±0.032a	0.39±0.033a	0.28±0.026a	0.39±0.026a	0.26±0.020a
ed_ri	0.43±0.041a	0.26±0.023a	0.41±0.043a	0.30±0.037a	0.39±0.037a	0.28±0.024a	0.39±0.025a	0.26±0.021a
aw	0.36±0.037	0.36±0.039	0.34±0.040a	0.40±0.050a	0.34±0.047	0.38±0.057	0.33±0.035a	0.35±0.034a
das3_8	4.40±0.61a	5.52±0.46a	5.27±0.58a	6.35±0.59a	3.35±0.79a	5.41±0.75a	3.98±0.58a	5.37±0.50a
lte	1.53±0.15a	1.65±0.11a	2.00±0.14	1.97±0.12	1.36±0.13a	1.64±0.15a	1.46±0.12a	1.63±0.10a
lf	0.16±0.016a	0.13±0.009a	0.21±0.015a	0.15±0.011a	0.14±0.014a	0.13±0.011a	0.15±0.013a	0.13±0.007a
la_ri	1.50±0.14a	0.91±0.08a	1.45±0.15a	1.07±0.12a	1.38±0.13a	1.01±0.08a	1.39±0.08a	0.93±0.07a
la_le	1.50±0.14a	0.92±0.08a	1.42±0.15a	1.08±0.11a	1.38±0.11a	1.00±0.99a	1.37±0.09a	0.92±0.06a
sf_ri	2.69±0.89a	2.01±0.34a	2.73±0.94	2.43±0.67	2.83±1.11	2.46±0.62	2.80±0.96a	2.30±0.53a
sf_le	2.76±1.04	2.36±0.61	2.70±0.91a	2.26±0.58a	2.70±1.11	2.33±0.54	2.83±1.05a	2.33±0.71a

in station M3-2. male and female groups were divided relatively with regard to the first factor in PCA, but in the second factor they were not separated (Fig. 2c). In the component1, la_le, ed_le, la_ri, ed_ri, al and tl are important in sexual dimorphism in station M3-2 (Table 4).

The first and second components, show 61.71 and 17.64% of the total variation orderly, 79.36% of variation is presented in the first two components in the whole (Table 4). 100% of the original male and female groups were correctly classified in the DA (Table 5).

Table 4: Portion of each trait and component in sexual dimorphism for any one of stations according PCA

	N2		M1-2		M3-2		S2	
	Component		Component		Component		Component	
traits	1	2	1	2	1	2	1	2
TL	-0.828	0.544	-0.656	0.733	-0.879	0.431	-0.909	0.376
AL	-0.892	0.430	-0.782	0.601	-0.906	0.370	-0.938	0.306
HW	0.016	0.486	-0.420	0.645	-0.140	0.489	0.445	0.400
DE	0.914	0.321	0.809	0.414	0.872	0.328	0.926	0.222
ED_LE	0.960	0.244	0.930	0.293	0.943	0.262	0.965	0.173
ED_RI	0.960	0.235	0.922	0.329	0.939	0.278	0.964	0.197
AW	0.071	0.323	-0.436	0.583	-0.317	0.226	-0.225	0.425
DAS_3_8	-0.779	0.556	-0.661	0.587	-0.832	0.340	-0.839	0.368
LTE	-0.476	0.846	0.191	0.897	-0.726	0.638	-0.638	0.726
LF	0.718	0.590	0.892	0.243	0.395	0.761	0.682	0.585
LA_RI	0.959	0.235	0.923	0.326	0.941	0.273	0.960	0.192
LA_LF	0.959	0.246	0.933	0.281	0.944	0.263	0.965	0.179
	Total variance explained							
	N2		M1-2		M3-2		S2	
Eigenvalues	7.35	2.53	6.77	3.41	7.40	2.11	8.11	1.77
% of Variance	61.32	21.10	56.49	28.48	61.71	17.64	67.59	14.76
Cummulative %	61.32	82.43	56.49	84.97	61.71	79.35	67.59	82.35

Extraction method: Principal component analysis

Table 5: The predicted group membership for males and females of each station according DA

Classification results

			Predicted group membership		
		Station	N2 (Female)	N2 (Male)	Total
Original	Count	N2 (Female)	30	0	30
		N2 (Male)	0	30	30
	%	N2 (Female)	100	0	100
		N2 (Male)	0	100	100
			M1-2 (Female)	M1-2 (Male)	
Original	Count	M1-2 (Female)	30	0	30
		M1-2 (Male)	0	30	30
	%	M1-2 (Female)	100	0	100
		M1-2 (Male)	0	100	100
			M3-2 (Female)	M3-2 (Male)	
Original	Count	M3-2 (Female)	30	0	30
		M3-2 (Male)	0	30	30
	%	M3-2 (Female)	100	0	100
		M3-2 (Male)	0	100	100
			S2 (Female)	S2 (Male)	
Original	Count	S2 (Female)	30	0	30
		S2 (Male)	0	30	30
	%	S2 (Female)	100	0	100
		S-2 (Male)	0	100	100

100.0% of the total original grouped cases correctly classified

Station S2: As shown in Table 3, all morphometric and meristic characters show significant differences ($p<0.05$) in station S2. In the PCA, male and female groups were divided with regard to factor 1, but factor 2 did not show considerable separation (Fig. 2-d). In the first component, al_le, ed_le, ed_ri, la_ri, al, de and tl are most important in sexual dimorphism (Table 4). The first and second components show 67.59 and 14.76% of the total variation respectively; in total the two components show 82.35% of variation (Table 4). As in the other stations, 100% of the original male and female groups were correctly classified in the DA (Table 5).

DISCUSSION

Sexual dimorphism is the phenotypic difference between the two sexes of a species (Mayr and Ashlock, 1991; Anderson, 1994). This concept is an important subject in biosystematic and evolutionary studies. According to biosystematic and taxonomic concepts, if species show sexual dimorphism, the separate morphological analyses for male and female data must be performed; otherwise, male and female samples must be combined (Manley, 1994; Fowler *et al*., 1998).

Pilla and Beardmore (1994), Triantaphyllidis *et al*. (1997b), Zhou *et al*. (2003), Camargo *et al*. (2003) and Amat *et al*. (2005) separately analyzed male and female samples of different *Artemia* populations, but they did this before they survey the sexual dimorphism between male and female samples.

Hontoria and Amat (1992) and Cohen *et al*. (1999) studied morphometric characteristics of different bisexual populations of *A. franciscana* and *A. persimilis*. In these surveys, only female samples were used by Discriminant Function Analysis and male samples were not analyzed. But Gajardo *et al*. (1998) employed a different method for the study of morphological differentiation of *Artemia* populations. They analyzed the data combining both males and females for the individuals grouped by each population to which they belonged.

Zhou *et al*. (2003) have shown that the overall percentage of correctly classified cases of females was a little higher than the male in *A. sinica* and *A. tibetiana* from China. The result of Camargo *et al*. (2003) show that the classification based on male characters provides better group membership than females of *A. franciscana* populations from the Colombian Caribbean. The recent studies on *A. urmiana* shows that morphological differentiation between male samples is higher than female samples (Asem, 2005).

Now according to the upon topics account, two important questions poses:

- If males show more morphological differentiation than females in some populations of *Artemia*, Why are male samples removed from the analysis? However, using of male samples can show clear biosystematical results.
- If male and female samples have morphological differentiation; why are the data of males and females combined with together? This method presents ambiguous results. For example, *A. urmiana* males have high morphological differentiation than females (Asem, 2005), therefore, if these 2 groups are mixed with together then the variation will decrease because female's low variation lessening total morphological differentiation.

The results of this study have shown that *A. urmiana* is a sexually dimorphic taxon in all the 4 different stations from the Urmia Lake and for each morphological study, male and female samples must be analyzed separately. This method should be considered for other bisexual population and species of *Artemia*.

Sexual Dimorphism (S.D.) is a pervasive phenomenon. Researchers have considered patterns of sexual dimorphism and have provided theories which explaining its existence (Lappin and Swinney, 1999). And also S.D. is related to mating systems in many vertebrate taxa (Lovich and Gibbons, 1992; Promislow, 1992; Stamps, 1993).

Several theories can be applied to define the origin and maintenance of sexual dimorphism in animals. Sexual selection can be explained as directional selection of certain characteristic, large body (size) associated with an increase in reproductive fitness. It can occur by two different mechanisms: Intersexual selection and intrasexual selection (Darwin, 1874). Intersexual selection occurs via female choice of mates with particular characteristics. Intrasexual selection occurs when a morphological charecter increases an individual's ability to get and keep mates or territories. For example, male-male combat for territories during the breeding season, it can be selected for enlarged or otherwise modified characteristics in males. Males try to hold the largest territories possible which females are present in this place typically. Protecting for a large territory which has overlaps with several female home-ranges increases access to breeding privileges and the probability of successful copulations. For example, in mammals S.D. has an important function in the mating system; so the males which are larger than females try to find many mates in this system (Heske and Ostfeld, 1990). Similarly, the birds with the lek mating systems rather than birds without lek system often show more sexually size dimorphic (Oakes, 1992). In snakes (Shine, 1978) and amphibians (Shine, 1979), S.D. is a function of male-male competition. Snelson (1972) proved that in North American cyprinids (minnows), males are usually larger than females in species that males keep territories. If natural selection for female be less than sexual selection, males should be larger than females in mating systems with male-male contest and female choice for larger male size. This assumes that any effects of increased size on male fitness are greater than comparable effects on female fitness.

In lizards, having a larger body and head size has been shown that it can give the advantageous during male-male combat for keeping breeding territories (Carothers, 1984; Cooper and Vitt, 1989). This is most likely the mechanism responsible for the cranial sexual dimorphism observed in *Gambelia sila* (Tollestrup, 1982, 1983). A lack of selection for raised male size could be related to the loss of territoriality (Lappin and Swinney, 1999). Loss of territoriality may define why males are not larger than females, but it does not make clear why females are larger than males (Tinkle *et al*., 1970). Selection for intensified fecundity, therefore, may select for large female body size (Tollestrup, 1983).

Body size comparison of male and female belong to each bisexual *Artemia* species generally show a size sexually dimorphic which female individuals have larger body than males (Triantaphyllidis, 1997a). Brine shrimp, *Artemia* doesn't show a complex sexual behavior and elaborate mating system, then size difference between sexes can be interpreted as mating advantage because with regards to *Artemia* breeding system, female carries male during copulation process therefore large body of female is necessary for this breeding mechanism and can prove mating process. This phenomenon portrays an evolutionary relation among mating system of *Artemia* and its size sexually dimorphic.

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