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Relative growth and the onset maturity size analysis of *Austruca iranica* (Ocypodidae) along the two coastal regions of Sandspit, Karachi, Pakistan

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Abstract. Morphometric analysis of *Austruca iranica* population from the two different sites of Sandspit (Karachi, Pakistan) mangrove area was examined. Total seven growth related traits: carapace length (CL), carapace width (CW), enlarged chela length (En.Ch.L), enlarged chela width (En.Ch.W), abdominal length (Ab.L), abdominal width (Ab.W) and pleopod length (Pl.L= in male) examined for the relative growth analysis. Carapace width (CW) used as independentt variable to observe relationship between all the stated growth related traits. A total of 1,244 crabs were collected from both sites; among them 566 males and 628 females. Descript analysis showed (CW) ranged from (male = 3.5 mm to 18 mm) and (female = 2.5 mm to 16 mm) from station 1 and (male = 4.00 mm to 18.5 mm) and (female = 3.5 mm to 18 mm) from station 2. The overall sex ratio ($p < 0.05$) skewed in favor of females (0.91: 1.0) during South West Monsoon (SWM) season. Relationship between carapace length and carapace width showed positive allometric relation (R^2=92.1%) in males. The females showed positive allometric relationship from carapace length and abdominal width (R^2= 89.6 %). Overall significant difference observed in both males and females through size at sexual maturity from both sites. The logistic equation indicated that individuals reach at sexual maturity when they attain (male: CW_{50}=6.65mm; female: CW_{50}=6.20mm) from station 1 and (male: CW_{50}=8.20mm; female: CW_{50}=6.30mm) from station 2.

Key words: Fiddler crab, body measurement, allometric relationship, sexual maturity.

Introduction

The fiddlers crabs are intertidal benthic organisms possess burrowing ability for shelter and livelihood in mangrove ecosystem (Saher 2008). Commonly the fiddler crabs of genus *Austruca* greatly influence on nutrient recycling, plant materials and litter processing of mangrove vegetation and also good accumulator of hydrocarbons, pesticides and other heavy metals (Saher & Qureshi 2014, Saher & Siddiqui 2016) along the coastal areas of Pakistan.

The growth of animal is natural process which results through the various biochemical processes and can be influenced by environmental factors (Talapatra et al. 2014).

The relation between different body parts of fiddler crabs show great difference and therefore, fiddler crabs body parts are noticeably allometric (Frith & Brunenmeister 1983). The relationship between different growth parameters (such as length, width, height) can be analysed statistically by growth curve (Saher 2008). Such analysis has great importance in population structure and life cycle of species. To understand population structure and reproductive biology, somatic growth patterns and maturation size can be used as comprehensive characteristics (Saher & Qureshi 2014). Discontinuous somatic growth rate can be observed in crustaceans with respect to non-crustacean species. The growth rate is different in each body part of organism, few body parts increase as size increases, few body parts don't change even growth rate increases and sex may also be factor in growth of organism (Hartnoll 1978). Such growth changes (cheliped length, abdomen breadth and pleopod length) can be observed in the male/female of same individual (Castiglioni & Negreiros-Fransozo 2004).

The fiddler crabs are a well known group of intertidal crabs, characterized by strong sexual dimorphism and asymmetrical enlarged male chela and a minor chela (Saher 2008). The enlarged cheliped in fiddler crabs is associated with defense, reproduction and crushing of shells. Their appendage has been used for the fight of territory resistance, agonistic behaviour, displays and/or courtship and to protect females during copulation and spawning (Crane 1975, Micheli et al. 1991, Branco 1993, Mariappan et al. 2000, Masunari & Dissenha 2005, Costa & Soares-Gomes 2009, Masunari et al. 2015) whereas the minor cheliped is used for feeding and cleaning parts of the body (Levinton et al. 1995) females have a pair of small claws which resemble the males' minor claw; another sexually dimorphic character is broader abdomen width in female (Ahmed & Khan 1978, Weissburg 1991). When species reaches at sexual maturity at certain size represents an important parameter to understand biology of brachyuran crabs. (Hartnoll & Gould 1988). Therefore, it is important to have knowledge on the allometric growth of a species for assessing geographical variation in widespread species. The relative growth analysis in family Ocypodidae was first studied by Huxley (1924a,b, 1927) and Cori (1929), then after subsequently Barnes 1968, Haley 1969 and 1973, Frith & Brunenmeister 1983, Von Hagen 1987, Rosenberg 2001, Negreiros-Fransozo et al. 2003, Saher 2008, Saher & Qureshi (2010, 2011, 2012) than various authors conducted research on species-specific sex ratios of selected appendages and body parts dimensions (Barnes 1968, Miller 1973). The relative growth analysis and morphological sexual maturity on have extensively been described from other coasts of world such as: Brazil (Benetti & Negreiros-Fransozo 2004, Castiglioni & Negreiros-Fransozo 2004, Castiglioni & Negreiros-Fransozo 2006, Pralon & Negreiros-Fransozo 2008, Hirose et al. 2013, Silva et al. 2016)

Previous study described about the biology, ecology and diversity of *A. iranica* from the coastal regions of Pakistan and Iran (Saher 2008, Mokhtari et al. 2008, Naderloo et al. 2010, Mokhlesi et al. 2011, Qureshi & Saher 2012, Saher & Qureshi 2014, Mokhtari et al. 2015). Curttent study was based on information regarding biology of *A. iranica* from two different stations of Sandspit. Both stations varied from each other one is heavily polluted and under an impact by

anthropogenic effect, waste from Kakapeer village and other is is closest to Manora Channel having less impact of pollution as well as less affected by human activities. Therefore, these stations were selected to compare the major difference in relative growth analysis and onset sexual maturity in *A. iranica* (two sub-populations).

Austruca iranica is one of the most abundant species of genus *Austruca*, which is mostly found in muddy and sandy coasts of Karachi, Pakistan (Saher 2008). Due to greater abundance from the coastal waters of Pakistan, they have important role in nutrient cycling and food web. This species can be found in Iran, Oman, UAE (Naderloo et al. 2010) and throughout the coastal belt of Pakistan. This species show variety of adaptations by constructing variety of burrows (Qureshi & Saher 2012) associated with different types of sediment structure such as sandy, sandy-cum and muddy waters of coastal beaches of Pakistan and shows much variety in body size, cheliped length and chela waving behavior (personal observations: Sahir).

Therefore, The present study is aimed to analyze the relative growth analysis, onset size at sexual maturity and relationship between size and age of *A. iranica* from two different sites of Sandspit mangrove area, Karachi, Pakistan to understand the population dynamics of *A. iranica*.

Materials and Methods

Study Area

Coast of Sandspit backwaters is situated on the southern part of North Arabian Sea and about 18 km SW of Karachi, Pakistan. The area contains a complex system of coastal wetlands, shallow tidal lagoons, mudflats and sandy beach. Mangrove forest is dominated by *Avicennia marina* (Forssk.) Vierh. provides habitat for diversified crab species (Tirmizi & Ghani 1996). A study was carried out on two different stations of Sandspit Station1 and Station2. The Station1 is heavily polluted and under an impact by anthropogenic effect, waste from Kakapeer village. Station2 is closest to Manora Channel and less affected by human activities.

Collection methodology

The study was conducted along two different sites of Sandspit (station S1 and S2) once in a month from March 2001 to February 2002. The crabs were collected through transect and quadrate (0.25 m^2) method from both stations, two replicate quadrates (10 meters apart) were collected from each tidal level (Level 1 *low tide*, Level 2 *mid tide* and Level 3 *high tide*). The quadrate was digged down maximum up to 30 cm following Saher et al. (2015). The crabs were recovered from excavated sediment from the quadrate using 1 mm mesh sized sieve and placed in labelled polythene bags and brought to the laboratory.

Identification and Morphometric Measurement

In the laboratory, crabs were identified, sexed, and sorted into five categories (adult males, juvenile males, adult females, juvenile females and ovigerous females) according to the examination of secondary sexual characters (pleopod morphology, free abdomen and distinct cheliped development, and the presence of eggs on the female pleopods). The weight (gm) for each individual were noted along with following morphometric measurement with a vernier caliper to the nearest 0.01 mm: carapace width (CW) carapace length (CL), enlarged chela length (En.Ch. L) for male, chela length (Ch. L) for female, abdomen width (AW) of the fifth somite for each sex and as well as pleopod length (Pl.L) for males.

Statistical Analysis

Size variation between male and female crab along each station and between male and females of two stations were compared with Student's *t* test (Student 1908) ($P<0.05$). Frequency of crabs per class at each season was analysed.

A regression model was analyzed between all the parameters keeping carapace width (CW) as the independent variable. All the variables were log transform and subjected to regression analysis. All the statistical analysis were carried out by using the Minitab (ver. 17). The length weight relationship was studied using the power function $y = axb$ (Huxley 1950) for allometry for each sex, and was linearized ($logy = loga + b\ logx$), where b indicated the slope and a, the Y-intercept, and positive allometry when $b > 3$, negative allometry $b < 3$ and isometry $b = 3$ (Hartnoll 1982, Saher & Qureshi 2011b). The best fit linear regression equation were calculated using least square method to determine the relationship between morphometric variables (Hartnoll 1982) and growth was considered isometric when $0.9 < b < 1.1$ (Castiglioni & Negreiros-Fransozo 2004).

In order to carried out analysis based on functional maturity, size classes of 3 mm carapace width (CW) were made. Functional maturity in crabs was determined based on claw dimensions of adult specimens previously established criteria reported by (Saher & Qureshi 2011; Saher et al. 2016). The relative frequency of mature adults (%) in each size class was plotted and fitted in a sigmoid curve following the result of the logarithmic equation. The size (carapace width CW) at which 50% of the individuals attain maturity by considering relationship between size and percentage of mature individual is estimated.

$$P = 1\frac{1}{1 + er\ (CW - Cw50)}$$

where, P is the percentage of mature crabs, CW50 is the carapace width at which 50% of the individuals attain sexual maturity, and r was the slope of the curve. The adjusted equation was fitted by the least squares regression method (Vazzoler 1996, Bertini et al. 2007, Saher & Qureshi 2011b).

Results

A total of 1,244 individuals of *A. iranica* collected from both stations (Station1 and Station2) of Sandspit back waters each month throughout the year. Current study shows that the Station2 showed highest number (788) of crabs as compared with Station 1 (566) (Table 1). The body size of of male crabs ranged from 3.50 mm to 18 mm (mean=10.63 ± 3.412 mm) and female crab range between 2.5 mm to 16 mm (mean=9.177±3.272 mm) from Station 1 and from Station 2 the body size of male crab started from 4.0 mm to 18 mm (mean=10.63±2.565 mm) and female crabs body size range from 3.5 mm to 18 mm (mean= 9.745±2.619 mm). Current study showed specimen from Station 2 slightly larger from Station 1 for this sample t-test employed to see difference between the body size result showed that female from both stations vary significantly ($P<0.05$) on there body size (Table 1).

Table 1. Total numbers of individuals obtained from two stations by both sexes and their comparative sample t-test value by comparing body size between the two populations of *A. iranica* from two different stations (Station 1 and Station 2).

	Total Number		Sample *t-test* (CW)	
Sex	Station 1	Station 2	T- test	P value
Males	276	368	0.92	0.358
Females	290	420	-2.24	0.025 *

Size class variation observed in adult male, female and Juvenile male female crabs from both stations during all four seasons: North-East Monsoon (NEM), Pre-Monsoon (Pre-M),

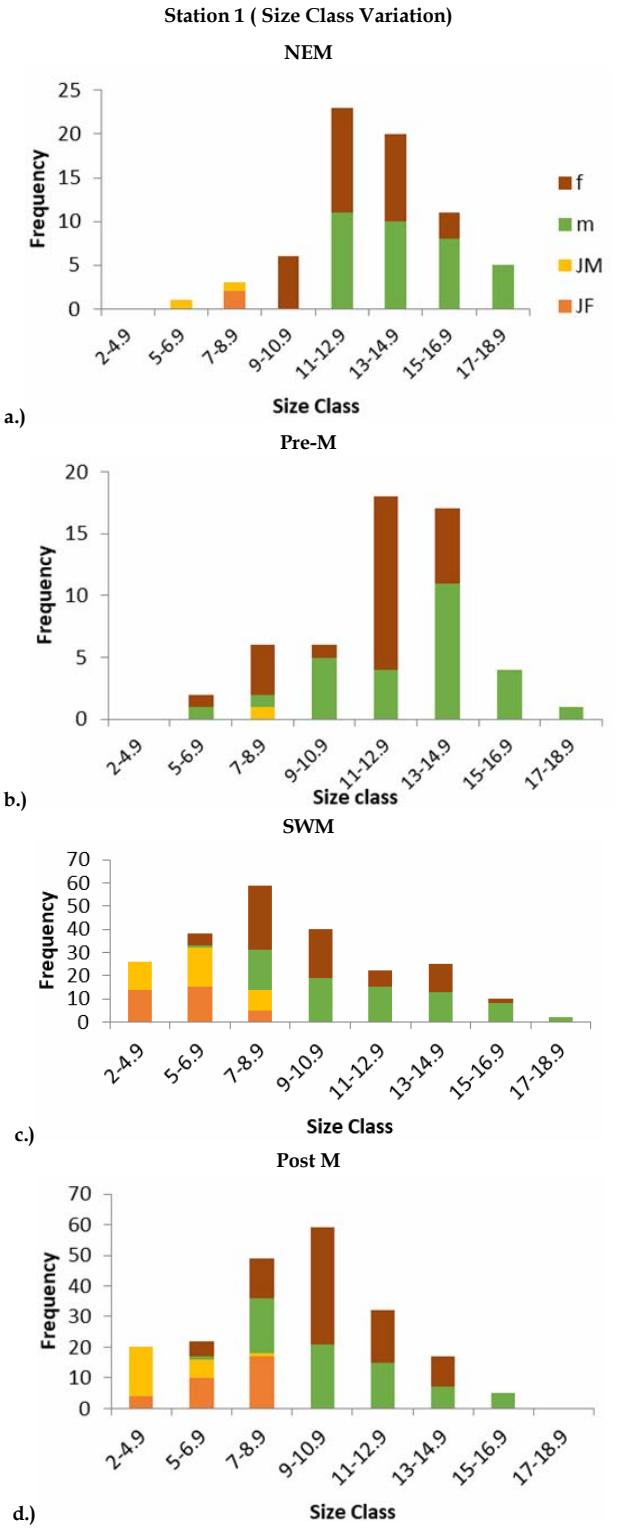


Figure 1.a-d. Results showing the seasonal difference of size class variation among Juvenile and adults of *A iranica* from station 1 along the Sandspit Pakistan (NEM= North East Monsson, Pre-M= Pre-Monsoon, SWM= South West Monsoon, Post-M= Post Monsoon).

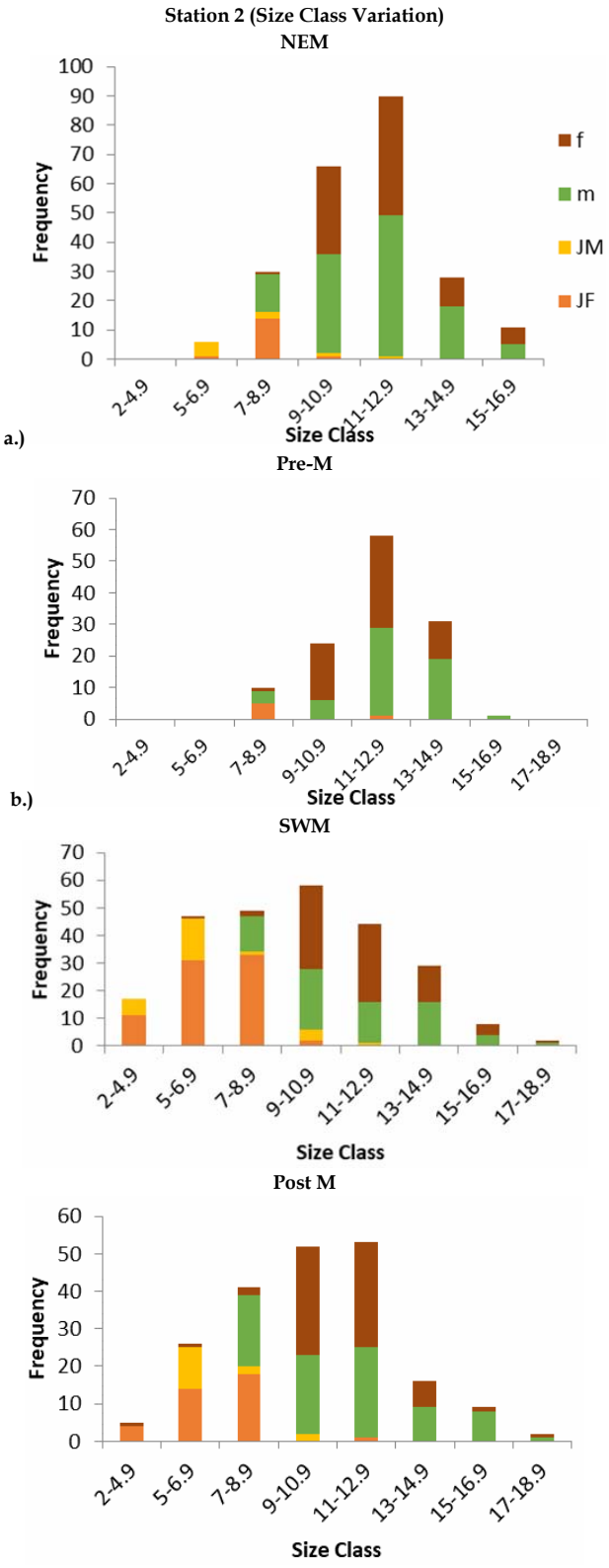


Figure 2 a-d. Results showing the seasonal difference of size class variation among Juvenile and adults of *A iranica* from station 2 along the Sandspit Pakistan (NEM= North East Monsson, Pre-M= Pre-Monsoon, SWM= South West Monsoon, Post-M= Post Monsoon).

South-West Monsoon (SWM) and Post Monsoon (Post M) (Fig. 1.a-h). Figure 1 shows that NEM and Pre-M, possess highest frequency of crabs at the size class range (11 -12.9 mm) from both stations but differs in SWM and Pre-M from Station 1 (7-8.9 mm and 9-10.9 mm) and station 2 (9-10.9 mm and 11-12.9 mm) respectively.

The results of the logistic equation indicate that 50% male crabs at Station1 were sexually mature (CW_{50}) at 6.65

Table 2. Result of the regressions parameters and equations, and the state of allometry corresponding to each regression for the *A. iranica* population from sandspit station Station 1 and Station 2 (M= male; F= female; Ab.L= abdominal length, Ab.W, abdominal width; En.Chl. L= enlarged cheliped length; CL= carapace length; CW= carapace width; Pl.L= pleopod length; N= number of crabs; x= independent variable; y= dependent variable; O= isometry; -ve= negative allometry; +ve= positive allometry; * Linear regression equation; **= coeficient of determinance.

Site	Relationship	Sex	N	LogY= loga+b logX*	(R^2)**	State of allometry
Station 1	CW vs Wet Weight	M	226	Y= - 0.798 + 0.124	71.1	-ve
		F	253	Y = - 6.31 + 1.773	93.2	+ve
Station 2		M	340	Y = - 0.690 + 0.106	68.8	-ve
		F	375	Y = - 11.98 + 2.715	93.4	+ve
Station 1	CW vs CL	M	226	Y = - 0.723 + 1.39	94.15	+ve
		F	253	Y = - 0.3013 + 0.96	32.6	O
Station 2		M	340	Y =- 0.923 + 1.367	88.68	+ve
		F	375	Y = - 0.1794 + 0.999	27.3	O
Station 1	CW vs Ab. L.	M	226	Y = - 0.2599 + 1.021	71.0	O
		F	253	Y = - 0.2723 + 1.033	84.0	O
Station 2		M	340	Y = - 0.2892 + 1.041	74.1	O
		F	375	Y = - 0.3399 + 1.088	41.7	O
Station 1	CW vs Ab. W	M	226	Y = - 0.4982 + 0.883	94.5	-ve
		F	253	Y = - 0.7132 + 1.392	91.1	+ve
Station 2		M	340	Y = - 0.6044 + 0.895	78.1	-ve
		F	375	Y = - 0.7476 + 1.407	41.0	+ve
Station 1	En.Chl. L vs CW	M	226	Y = - 8.634 + 2.117	85.49	+ve
		F	253	Y = - 0.7132 + 0.910	77.6	O
Station 2		M	340	Y = - 9.407 + 2.119	86.5	+ve
		F	375	Y = - 0.2381 + 0.9044	81.3	O
Station 1	CW vs Pl.L	M	226	Y = - 0.5591 + 1.133	78.0	+ve
Station 2		M	340	Y = - 1.094 + 1.631	60.9	+ve

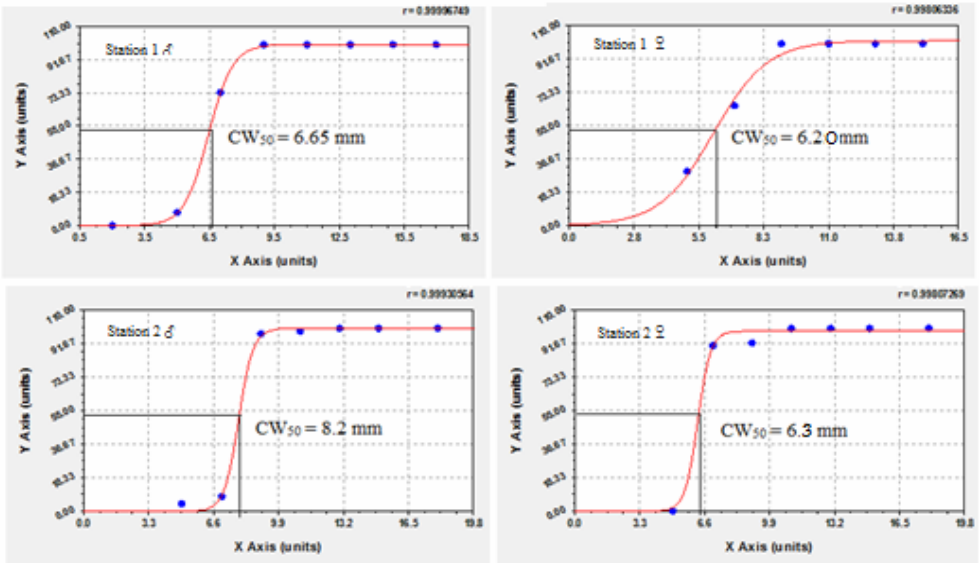
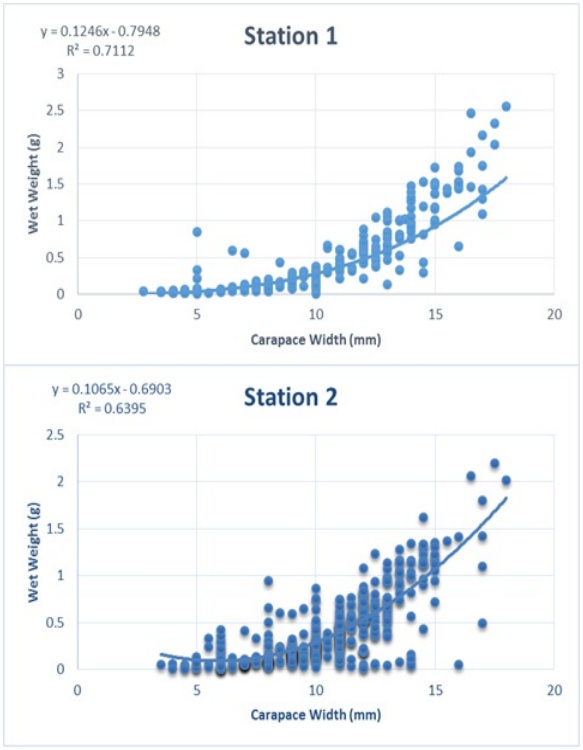


Figure 3. The logistic sigmoid curve showing percent adult males (♂) and females (♀) of *A iranica* reach at sexual maturity at Station and Station 2 of Sandspit.From left to right male (♂) and female (♂) Station 1 and Station 2 respectively.

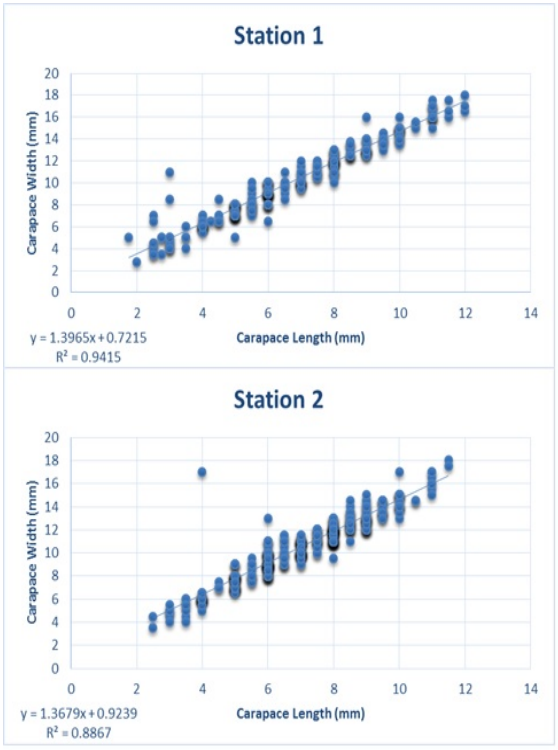
mm and female at 6.20 mm. At Station 2 of Sandspit sexual maturity attended at 8.2 mm and 6.3 mm in male and female respectively. Thus, the estimated size at onset of sexual maturity was different for males and females at each site and males were found to mature at a large size than females for both Stations of Sandspit back waters (Fig. 2.).

The relative growth analysis observed from the both stations by comparing the six morphological traits with one independent variable carapace width (CW) in *A. iranica* presented in Table 2.

Result showed the positive allometric relationship between body size and wet weight in male while female crabs showed negative growth relationship from both stations (Station 1 and Station 2). Which can be confirmed from the higher value of slop of 'b' from Station 2 as compared with Station 1 (Table 2 and Fig. 3a). When carapace length and carapace width compared by keeping CL as independent variable, positive growth relationship male specimen while isometric growth relationship observed in female specimen from both stations (Fig. 3b). Because of the strong sexual

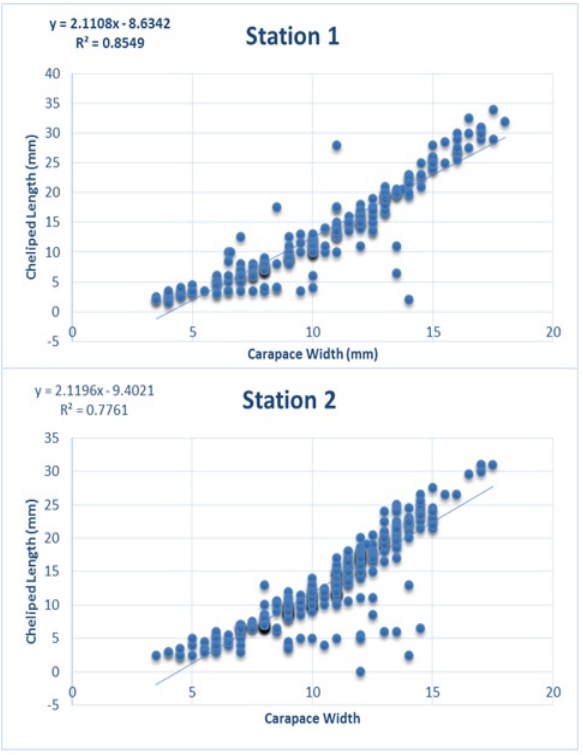


a.)



b.)

Figure 4 (a-c). *Austruca iranica* Morphometric relation between (a) Carapace width vs Wet Weight (b) Carapace Length vs Carapace Width (c) Carapace length vs Enlarged Chela Length.



c.)

dimorphism the male crab showed negative growth relationship between abdominal width and carapace width from both stations while female expressed positive correlation between same abdominal width and carapace width from both stations because female crab possess broader abomen size to keep her eggs inside the abdomen. Like wise, the male crabs possess one enlarged cheliped which showed positive growth relationship with carapace width from both stations as compared to the female which showed isometric growth pattern from both stations because of the small, equal sized chelipeds (Fig. 3c). In male from both stations 1st gonopod structure (Pleopod) showed positive growth relationship with carapace width (Table 2).

Discussion

The present study investigated for the relative growth and onset size at sexual maturity of *A. iranica* from the two different stations of Karachi coast (Sandspit). The relative growth analysis provided the sufficient knowledge on reproductive biology of brachyuran crabs (Pinheiro & Fransozo 1993) about different body parts that grow during developmental stages and stop at certain age limit in the life history of living organisms. During developmental stage, brachyuran crabs pass by many physiological changes, at the sexual maturity they present a remarkable change (Saher2008) these changes are generally caused by endocrine system which is also responsible for the sexual maturity (Teissier 1960, Hartnoll 1982, Perry et al. 2009). The differences observed in relative growth of various body parts taking place when specimen acheive sexual maturity are demonstrated by carapace shape. Such results also obtained from various parts of world by other scientists working on brachyuran species (Haley 1972, Huber 1985, Negreiros-Fransozo et al. 2002, 2003).

The relation between different body parts of fiddler crabs show great difference and therefore, fiddler crabs body parts are noticeably allometric. (Frith & Brunenmeister 1983). During study the carapace width was used as an independent variable for the relative growth analysis such selection was conformed from the previous study by Castiglioni and

Negreiros-Fransozo (2004) stated that carpace width represents the physiological changes that occur throughout the life span of crab. Likewise, cheliped measurements has also been evaluated in many species as an indicator of relative growth and morphological sexual maturity (Williner et al. 2014). During current study *A. iranica* showed positive allometry in carapace width and enlarged cheliped structure. Same results have been confirmed from previous studies on fiddler crabs (Cran 1975, Christy 1978, 1983, Christy & Salmon 1984, Zucker 1986, Murai et al., 1987, Salmon 1987, Goshima et al. 1996, Latruffe et al. 1999, Backwell et al. 2000, Pope 2000, Benetti & Negreiros-Fransozo 2004). In female likewise, abdominal width showed positive allometry with carapace width where abdomen attains the wider size at the certain age which is typically used for storage of eggs for incubation for new generation (Hartnoll 1982, Webb & Lewis 1977, Haefner 1985, Pinheiro & Fransozo 1993).

Abdomen showed the marked dimorphism between male and female crab, as female gets mature it starts investing a significant amount of energy in abdominal growth, since this body part is used as an incubating chamber for eggs and newly hatched crabs (Hartnoll 1974, Vogt 2013). In males, there is no need for the abdomen to grow faster than the carapace, since it only has the reproductive function of protecting the gonopods (Daniels et al., 2001, Castiglioni & Negreiros-Franzoso 2004). Such results can be confimed from previous studies (Hartnoll 1974) on relative growth analysis in female crabs divert energy to abdominal growth, while males prioritize cheliped growth, as discussed above. Among all variables investigated, the length of the major cheliped in adult males and the fifth abdominal segment in adult females showed the highest coefficient of allometric growth as reported previously in relative growth analysis of *Uca rapax (=Minuca rapax*: Shih et al. 2016), whereas secondary sexual characters such as enlarged cheliped and abdominal morphology showed positive relative growth (Benetti & Negreiros-Fransozo 2004).

The overall mean of body size achieved by adult crab may be the result of environmental effect (Campbell & Eagles 1983). The growth rate difference between male and female crabs as sexual dimorphism confirmed in the present study with males reaching larger sizes than females crabs, mostly brachyuran crabs are sexually dimorphic during adult size, males attained larger size than females, this may be the cause of growth rate difference between two sexes (Benetti & Negreiros-Fransozo 2004). The low growth rate is practiced in previous studies by (Warner 1969, Christy & Salmon 1984, Conan 1985) describing that there is difference in energy expenditure between two sexes that may be related to the allocation of energy resources between males and females crabs as male crab direct their energy for growth only while female invest their energy for gonadal development as well. (Hartnoll 1982, 1985).

In crustaceans presence of ovigerous females, and morphometric data are used to calculate the mean size at the onset of maturity. In the present study, the mean size at the onset of morphometric maturity calculated and data set showed that sexual maturity differs between two selected populations of *A. iranica.* Current study revealed that the individuals from station 2 matured earlier when compared with the station 1. This pattern fits the prediction from the previous studies that the individuals maturing at larger size may reach at the larger mean body sizes than individuals maturing at smaller sizes (Atkinson 1994, Angilletta et al. 2004b), which is often related to a prolonged, slower growth (Angilletta et al. 2004a). This delay in size at maturity is likely to be favored because it often leads to an increase in fecundity owed to larger bodies, yet this benefit must compensate for a reduced survival rate until maturity is attained (Angilletta et al. 2004a). The different environmental conditions and sediment properties under the influence of human activities may also effect the growth size at sexual maturity in populations of *A. iranica* at both stations such as salinity of burrow water and back shore area, temperature of burrows and outside the burrow, organic matter content and also granulometric composition of soil (Qureshi & Saher, 2013; Saher et al. 2015). All these parameters can influence directly or indirectly in the population of crab. Like wise, waterborne pollutants may also be responsible for delay in body size growth, can be confirmed from the previous reseach on sendiment and the heavy metal analysis on fiddler crabs of Sandspit backwaters (Saher & Siddiqui, 2016) which may be reason because of the settlement of a larger human population and the activities of a recreational harbour. The sexual maturity in males achieved at higher sizes than females from both stations, same results observed by different investigators (Hartnoll 1985, Mantelatto & Fransozo 1996) probably due to the lower energy investment by females at the growth as mentioned earlier. Thus male crab reach higher size than females with the same food resource such result can also be observed in others species of fiddler crab: *Uca leptodactyla* (= *Leptuca leptodactyla*) (Masunari & Swiech-Ayoub 2003; Shih et al. 2016) and *Uca burgersi* (= *M. burgersi*) (Benetti & Negreiros-Fransozo 2004, Shih et al. 2016).

This study was based on the investigation of relative growth analysis, onset sexual maturity and sexual dimorphism of *A. iranica* populations on the two stations of Sandspit backwaters of Karachi, Pakistan. There is need of further studies on reproductive biology, population structure based on biochemical and molecular biology data. More data is required on relative growth by using multivariate analysis and comparing the species with environmental parameters (Physico-chemical) from different habitats along coastal areas of Pakistan to better understand the biology of this species.

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