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Research Article

Diversification of narrow-clawed crayfish (*Pontastacus leptodactylus* Eschscholtz, 1823) populations from different parts of Turkey

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ABSTRACT

The present study was to evaluate diversification of crayfish populations based on length and weight measurements. Crayfish were collected from natural stock with fyke-net at five different populations of Turkey in the lakes Iznik (IL), Egirdir (EL) and Sera (SL) as well as Hirfanlı Dam Lake (HD) and Keban Dam Lake (KL) from June to July, 2008. The mean length, weight and hepatopancreas moisture content (HM, %) of the individuals sampled from different regions were found in order from largest to smallest as EL>HD>KL>IL>SL, EL>HD>KL>SL>IL and IL>KL>HD>KL>SL, respectively. In this study the median value of b coefficient, describing growth type from length-weight relationship, of five populations was 3.08, and fifty percent of the values were fell between 2.72 and 3.75. The growth types in these populations were determined as isometric, except in Keban Dam Lake (positive allometric). DFA (Discriminant Function Analysis) showed that there were no significant differences among the populations, meanwhile 53.06 % dissimilarities between populations was driven by length and HP with the contribution of 60.18% and 39.82% (based on SIMPER analysis), respectively. Although results from b coefficients, HM values and DF analysis of populations may reveal that their environmental conditions and growth types were similar in a certain extent, many more data taken from at least one-year sampling period is highly recommended for better understanding on factors that influenced the growth of its crayfish structure.

Introduction

Recent research has shown that there are more than 640 species of freshwater crayfish known worldwide (Crandall and De Grave, 2017). Crayfish belong to three taxonomic families: Parastacidae in the Southern Hemisphere with 175 species, and Astacidae and Cambaridae in the Northern Hemisphere wit 39 species and 426 species, respectively (Hobbs, 1989). Freshwater crayfish are favored as model organisms in several disciplines such as ecology, biology, genetics, evolution, physiology and population. Because of their ability to survive in different habitats and climatic conditions, body shape and other morphological characteristics may be quite different in this species (Hossain et al., 2018). Moreover, high adaptability, morphological differences caused by environmental conditions, and hybrid formation of some species make taxonomy of crayfish difficult (Benzie, 2005).

Crayfish has been considered as one of the most valuable fishery sources in Turkey. Until 1984, crayfish has a great demand in Europe market. However, after 1986, natural stocks of crayfish declined rapidly in most lakes and dams as a result of over fishing, habitat loss and disease. Especially crayfish plague (*Aphanomyces*

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astaci) infected in Turkish inland water resources and had been causing large scale mortalities and severe damage on crayfish populations. All of these inevitably have been led to massive economic losses in crayfish harvest industry. Even in some attempts like supplemental stocking of *Pontastacus leptodactylus* only has increased the harvest up to 3%. (Mazlum et al., 2017).

Growth in crayfish is not as continuous as in fish, but is based on the periodic increase in body length. As in all arthropods, crayfish have to change their exoskeleton to grow. For molting, crayfish terminate to feed and decrease activity. In addition, biochemical composition of crayfish changes depending on environmental conditions and reproduction period (Dutra et al., 2008). Food, stress, overcrowding and disease or reproductive cycle can affect morphometric variation of crayfish. Therefore, growth measurement in crustaceans is complex because of molting, sexual dimorphism and the growth increment can vary in different ontogenetic stages in crustaceans (Lindqvist and Lathi, 1983; Aiken and Waddy, 1987).

Total length and weight are the most commonly used measurements to measure growth in crayfish biometric studies (Mazlum et al., 2007). Relationships between these two measurements, commonly called as length-weight relationships, are very suitable for fisheries studies such as stock assessment (Valset et al., 2007; Demirci et al., 2018), growth and development (da Rocha et al., 2015; Vasileva et al., 2017), biomass, condition factor, sexual maturity (Waiho et al., 2016), age structure (Stevenson and Woods, 2006; Demirci et al., 2016), biology and ecology (Mazlum, 2003; Kumar et al., 2018) and life cycle (Goncalves et al., 1996, Froese, 2006; Moutopoulos and Stergiou, 2002; Anastasiadou et al., 2008).

Crayfish store large amounts of energy (lipid) in the hepatopancreas for reproduction (Harrison, 1990; Moore et al., 2000; Rosa and Nunes, 2003), larval development (Rosa et al., 2005) and survival (Eversole and Mazlum, 2002; Mazlum and Eversole, 2004). The relative moisture content of the hepatopancreas has been suggested as an indicator of crayfish physiological conditions (Huner et al., 1985, 1990). Previous studies have shown that there are inversely a linear relationship between moisture and energy content in the hepatopancreas (HM) (Jussila and Mannonen, 1997). In addition, high moisture in the hepatopancreas indicates insufficient food intake in crayfish (McClain, 1995).

In this study, the following measurements were evaluated i) the length-weight relationships of five different *P. leptodactylus* populations, and ii) diversification of these population based on their length and HM values (%).

Materials and Methods

Sample collection

Crayfish *(P. leptodactylus)* were collected from natural stock with fyke-net at five different locations of Turkey; Iznik lake (40°27'06.2"N 29°32'04.3"E), Hırfanlı dam lake (39°12'10.8"N 33°32'48.0"E), Sera lake (40°59'00.1"N 39°36'46.3"E), Keban Dam lake (38°38'14.3"N 39°28'58.7"E) and Egirdir lake (37°59'33.4"N 30°52'03.4"E) on June and July 8, 2008 (Fig. 1). Each location consists of 10 samples for analysis. Crayfish shipped by bus and arrived at University Aquaculture research facilities. The total length (TL) of each crayfish was measured from the tip of the rostrum to the end of the telson to the nearest mm with a measuring board. Whole frozen crayfish were placed on filter paper for several minutes to remove excess water then wet weight (W) was measured to the nearest 0.01 g. The hepatopancreas from each individual was removed and weighed to the nearest 0.01 g after blotting (Hww) and dried in a convection oven (80ºC) to a constant dry weight Hdw). Hepatopancreas moisture content (HM) was calculated using following formula (Eversole and Mazlum, 2002).

$$HM = \frac{(Hww - Hdw)}{Hww} * 100$$

Data analysis

Length-weight relationship

All data were checked out for outliers. Freshwater crayfish has a nonlinear relationship between length and weight, as in fishes, the weight may be considered as the function of length therefore more satisfactory formula for the expression of the relationship is $WT = a\ TL^b$, where, WT=wet weight in g, TL=total length in mm. Parameters a and b were estimated by using the non-linear fitting approach. Confidant limits of the b with 95 % values were calculated to determine the growth type as b = 3 represents an isometric growth, b< 3 represents a negative allometric growth, and b> 3 represents a positive allometric growth.

Discriminant Function Analysis (DFA)

Discriminant Function Analysis was used to determine which combinations of variables (distances) discriminated the best among populations and detected which populations were the most different (Ruiz-Campos et al., 2003). Total weight was not considered in DFA due to having high variable nature. Before conducting DFA, the

variables (total length and HM) were standardized by logarithmic transformation-log(x). In addition to DFA, SIMPER (Similarity Percentage) was used for assessing which variables are primarily responsible for an observed difference between populations (Clarke, 1993). All computations and statistical analyses were carried out by using Microsoft Excel and Past software (V. 3.23) (Hammer et al., 2001).

Results and Discussion

Freshwater crayfish can easily survive in lakes, ponds, dams and rivers that are exposed to various environmental factors. Geographical and environmental factors affect population density, growth and life cycle of species. Temperature, pH and dissolved oxygen values in the life cycle of crayfish known as highly effective environmental factors (Erol et al., 2017). In addition, these factors are affecting the diversity of the population of some species. Systematic and taxonomic studies have shown that crustaceans display a high degree of variation, which includes variability in size and proportion of major body parts.

The descriptive statistics of three variables (total length, total weight and HM %) according to the populations were given in Table 1. The mean length, weight and HM values of the individuals sampled from different regions were given in order from largest to smallest as EL>HD>KL>IL>SL, EL>HD>KL>SL>IL and IL>KL>HD>KL>SL, respectively.

It is well known that the male and female crayfish are different in size have been well documented in the crayfish literature since the work of Huxley (1881). Mazlum et al. (2007) analyzed the weight–length relationship of Procambarus acutus acutus and they were revealed that males gained weight with increasing length faster than female specimens during the growth period, showing the common nature of sexual dimorphism in freshwater crayfish (Balık et al., 2005; Güner, 2006). Having a sexual dimorphism in any animal population requires evaluating of sexes separately. But in this study, sexual dimorphism was not evaluated for each population due to a small number of samples. However, there was also no significant difference between the mean length and HM percent values of the populations ($p > 0.05$). Also, based on the coefficients of variations (CV, %), variation in length (IL: 8.35, HD: 13.02, EL: 11.56, SL: 16.39 and KL: 7.59) and hepotopancreas moisture content (HM, %), (IL: 14.42, HD: 10.98, EL: 12.56, SL: 15.96 and KL: 7.44) were almost similar. Therefore, sexual dimorphism related errors in calculating both length-weight relationships, DA, and simper analysis somehow may be considered as not significant. The variable “crayfish weight” is not as stable as length and hepatopancreas moisture, because of the even the nutrients taken during the day affect the weight of the crayfish

Crayfish store large amount of lipid as an energy in their hepatopancreas for growth, survival and reproduction (Harrison, 1990). For this reason, moisture content of the hepatopancreas has been used to describe the feeding condition of crayfish (Mannonen and Henttonen, 1995; McClain, 1995). Generally, as organic reserves are depleted from the hepatopancreas tissues, moisture level in the tissues increases. In this study, non-significant differences were present among the mean HM of the populations reveals that the factors affecting the condition of the different populations are almost same.

Populations	TL mean ±sd (min-max)	W mean ±sd (min-max)	HM (%) mean ±sd (min-max)
İznik Lake (IL)	9.45±0.79	19.76±4.86	73.54±10.61
	(8.1-10.5)	(11.87-26.21)	(56.01-86.44)
Hirfanlı Dam Lake (HD)	10.06±1.31	31.42±13.11	66.44±7.30
	(7.9-12.1)	(12.95-51.50)	(57.05-82.16)
Eğridir Lake (EL)	11.85±1.37	47.34±16.56	64.29±8.08
	(9.9-14.5)	(28.77-85.48)	(55.88-82.24)
Sera Lake (SL)	8.72±1.43	21.19±10.38	62.37±9.96
	(6.6-10.6)	(8.36-40.12)	(48.93-79.77)
Keban Dam Lake (KL)	9.74 ± 0.74	27.14±8.81	66.99±4.99
	(8.1-10.5)	(12.33-40.26)	(63.08-76.30)

Table 1. Mean (±sd) and range (min-max) of total length (TL), weight (W) and hepatopancreas moisture (HM %) of *P. leptodactylus* used in this study from various populations of Turkey.

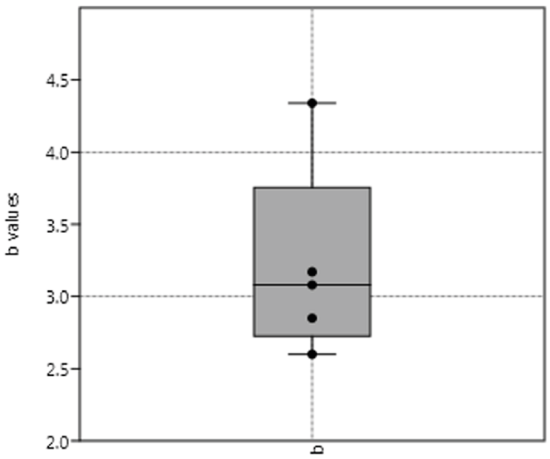
The coefficient b in the length-weight relationships of crayfish individuals obtained from different populations varied between 2.85 and 4.34. The growth types in these populations were determined as isometric, except in Keban Lake (positive allometric) (Table 2).

Figure 1 shows the Box-jitter plot of b coefficient with some descriptive statistics of five populations. In our study the median value of b coefficient of these five populations was 3.08, and fifty percent of the values were fall between 2.72 and 3.75 (Figure 1). The median of b values of reviewed studies was 3.00 and fifty percent of the b values fall between 2.85 and 3.11 (Figure 2). These results showed that our findings were almost consistent with the previous studies. However, our b values tend to be positive allometric growth (Skewness = 1.62) with the range of 1.74 (4.34-2.60), other studies tend to be negative allometric growth (Skewness = -2.26) with the range of 1.12 (3.22-2.10) (Table 3, Figure 2). The two values of b, namely 4.34 (this study) and 2.10 (previous studies) were out of the general tendency.

Population	a	b	R-square	CL of b (95 %)	Growth Type
IL	0.035	2.85	0.79	(1.65-3.95)	I
HD	0.019	3.17	0.91	(2.36-3.98)	I
EL	0.072	2.60	0.89	(1.80-3.40)	I
SL	0.024	3.08	0.96	(2.59-3.36)	I
KL	0.001	4.34	0.93	(3.39-5.28)	(+) A

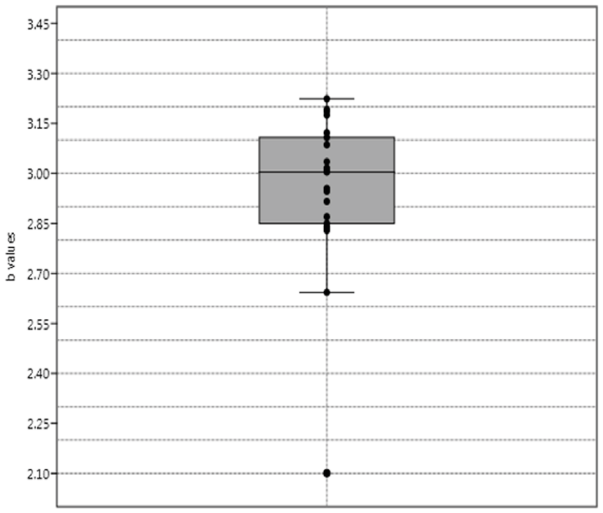
I: isometric growth, (+) A : positive allometric growth

Table 2. Length-weight parameters with R-square and growth type of *P. leptodactylus* from various populations of Turkey.



	b
N	5
Min	2.60
Max	4.34
Mean	3.20
SD	0.67
Median	3.08
25 prcntil	2.72
75 prcntil	3.75
Skewness	1.62
C. var	20.89

Figure 1. Box-jitter plot of b coefficient with some descriptive statistics of *P. leptodactylus* from five populations.



	b
N	23
Min	2.10
Max	3.22
Mean	2.95
SD	0.23
Median	3.00
25 prcntil	2.85
75 prcntil	3.11
Skewness	-2.26
C. var	7.93

Figure 2. Box-jitter plot of b coefficient with some descriptive statistics of *P. leptodactylus* in the reviewed literatures.

Location	b	r^2	Literature
Iznik Lake	3.035	0.95	Aydın et al. (2015)
Sera Lake	3.122	0.97	Erkbay (2004)
Keban Dam Lake	2.643	0.92	Harlıoğlu (1999)
Eğirdir Lake	2.829	0.96	Bolat (2001)
Apolyant Lake	2.955	0.96	Berber and Balık (2009)
Mogan Lake	3.086	-	Tüzün (1987)
Ayrancı Dam Lake	3.015	-	Erdem and Erdem (1994)
İznik Lake	3.004	-	Erdem et al. (2001)
Mogan Lake	2.101	0.99	Benzer et al. (2015)
Thrace region reservoirs	3.224	0.92	Deniz Bök et al. (2010)
Demirköprü Dam Lake	3.1685	0.97	Balık et al. (2005)
Yeniçağa Lake	2.836	0.99	Gencay (2019)
Sapanca Lake	3.186	0.91	Baltacı (2018)
Bafra Lake	2.916	0.91	Uzun (2013)
Keban Dam Lake	2.946	0.83	Yüksel (2007)
Keban Dam Lake	2.946	0.83	Yüksel and Duman (2012)
Mogan Lake	3.086	-	Karabatak and Tüzün (1989)
Aktaş Lake	3.016	0.97	Aksu and Kurt Kaya (2017)
Eğirdir Lake	2.870	0.90	Bolat and Kaya (2016)
Seven inland waters	2.07	0.99	Deniz et al. (2013)
Apolyont Lake	3.008	-	Berber and Balık (2009)
Terkos Lake	2.842	0.91	Güner (2006)
Eğirdir Lake	2.850	0.95	Balık et al. (2005)

Table 3. Literatures on length-weight relationship [log(W) = log a + b log (L)] of *P. leptodactylus* in different populations from Turkey (b : slope of the equation, r-square: coefficient of determination)

Previous study and our knowledge indicate that there are several factors that could contribute to the variations in allometry of decapod crustaceans such as age, sex, differences in diet, foraging behavior, availability and quality of food, environmental conditions with abiotic factors which consist of season, temperature, salinity and rainfall (Taddei et al., 2017). In crustaceans, the body growth occurs by continuous molts dependent upon physiological aspects regulated by abiotic factors, such as photoperiod, temperature, and availability and quality of food (Hartnoll, 1982; Mazlum and Eversole, 2005). In the morphometric relationship of length and weight of crustaceans, study of total length, carapace length and abdominal length in the length-weight relationships are prevalent as these parameters are less variables and more easily measured in the field. As such, the use of these measurements in aquaculture are extremely suggested because there are the most accurate and simple alternative of analyzing growth pattern of animal (Lalrinsanga et al., 2012). In habitat with more food availability and higher temperature, crayfish tend to have higher growth rates (Eversole et al., 2006). The differences in the values of the b parameter between the study species probably results of differences in feeding, sex, breeding behavior and greater capacity of obtaining, converting and storing energy according to living conditions.

Discriminant analysis (DFA) was used to determine the differentiation among the populations. Four functions were identified with DFA, but only first two were explained the 99.51% of total variance as 79.09% and 20.42%, respectively. The specimens were correctly classified into populations with 53.06 % using Jackknife estimation procedure from DFA results (Table 4).

Although DFA showed that there were no significant differences among the populations (Figure 3, Figure 4), SIMPER analyses showed that the 53.06 % dissimilarities between populations was driven by Length and HM with the contribution of 60.18 % and 39.82%, respectively. Recall, precision and F-measure of populations calculated from confusion matrix implied that the diversification for the populations in descending order (based on F-measure) as; EL>SL>IL>KL>HD. So, from that point of view we can conclude that the EL (Eğridir Lake) population with 70 % ratio is much more diverged from then other populations, while HD (Hirfanlı Dam Lake) population is only 20 % (Table 5).

Populations	IL	HD	EL	SL	KL	Total
IL	6	1	0	2	1	10
HD	2	2	3	2	1	10
EL	0	2	7	0	0	9
SL	1	2	0	7	0	10
KL	2	3	0	1	4	10
Total	11	10	10	12	6	49

Table 4. Confusion matrix with Jackknifed classifications from DFA

Populations	Recall (%)	Precision (%)	F-measure
IL	60	54.55	57.14
HD	20	20.00	20.00
EL	70	70.00	70.00
SL	70	58.33	63.64
KL	40	66.67	50.00

Table 5. Recall, precision and F-measure of populations calculated from confusion matrix

The pikeperch is a piscivorous species whose natural role is top predator in a complex of fish species. The lake Egirdir was known for 11 local species until it was stocked in *Stizostedion lucioperca*. However, after 1955, the number of local species decreased by 3-4. Lake Eğirdir nowadays pollution, excessive vegetative development, entering new species and the extinction of natural species is under the pressure of many environmental impacts resulting from changes in the water structure (Campbell, 1992). For this reason, 70 % differentiation is thought to be caused by the pressure of environmental impact of this lake. Hirfanlı is one of Turkey's

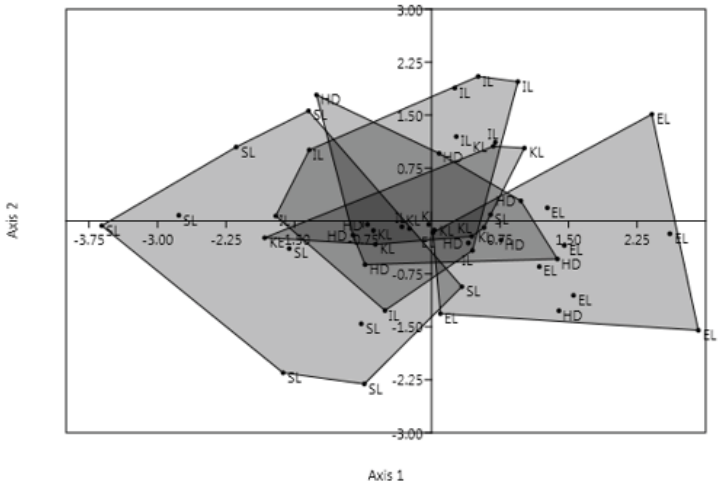


Figure 3. DFA results with convex hulls for *P. leptodactylus*

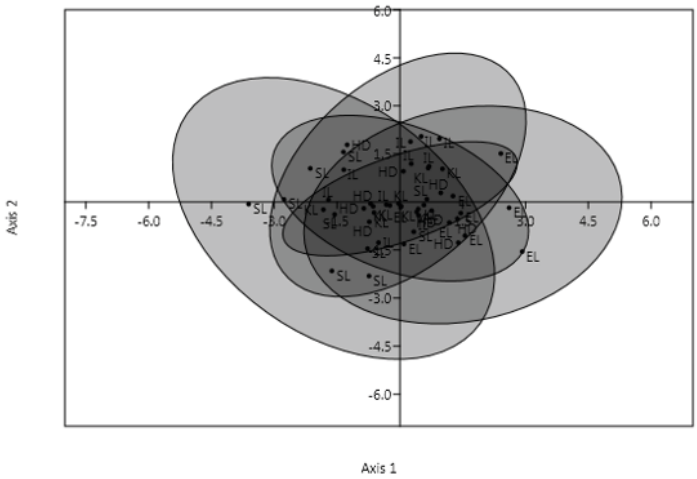


Figure 4. DFA results with 95 % Confidant limits for *P. leptodactylus*.

largest dam. There are many small islands in the reservoir. Tinca tinca was the dominant fish species in Hirfanlı dam lake. The dam, which was built for electricity generation, is also used for irrigation.

In current study, the main reason of why the length and HM did not differ among the populations in terms of parameters (length and HM) it may be due to the low number of samples, and not to evaluating the populations separately as male and female. It may be suggested that, sex discrimination should be made by taking sample data of at least 1 year. Moreover, the generality of our results can be evaluated by investigating the properties of the sample populations including physico-chemical parameters, nutrient status, and competition, etc.

Conclusion

The present study provided some knowledge on length-weight relationships and diversification of five crayfish populations from different regions of Turkey. Although results from b coefficients, HM values and DF analysis of populations may reveal that their environmental conditions and growth types are similar in a certain extent, many more data taken from at least one-year sampling period is highly recommended for better understanding on factors that influenced the growth of its crayfish structure.

Acknowledgement

Authors wish to acknowledge that there is no conflict of interest.

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