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Effect of some Biotic and Abiotic Factors on Hard and Soft-shell of Crayfish (*Pontastacus leptodactylus* Eschscholtz, 1823): A Case Study from Hirfanlı Dam Lake

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Research Article

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Abstract

In this study, the effect of some biotic (total length, sex) and abiotic (sampling station and month) factors on the shell status (soft or hard) of *Pontastacus leptodactylus* were investigated in a natural crayfish population. The study was carried out in Hirfanlı Dam Lake (Central Anatolia), where one of the most important crayfish harvesting areas of Turkey, with a monthly basis between Jun-2017 and May-2018. Permutational multivariate analysis of variance (*PERMANOVA*) was used for the determination of the differences between nonparametric variables. A total of 5920 crayfish were checked, ultimately determined as 106 (1.79%) individuals with a soft shell and 5814 (98.21%) hard shell. Shell status was shown statistical difference with total length, stations, and sampling month ($p<0.05$), while it was no significant by sex group ($p>0.05$).

Keywords: *PERMANOVA*, Hirfanlı Dam Lake, moulting, narrow-clawed crayfish

Bazı Biyotik ve Abiyotik Faktörlerin Kerevit (*Pontastacus leptodactylus* Eschscholtz, 1823) Kabuğunun Yumuşak veya Sert Olma Durumu Üzerine Etkileri: Hirfanlı Baraj Gölü Örneği

Özet

Çalışmada, doğal bir popülasyonda, bazı biyotik (total boy, eşey) ve abiyotik (örnekleme istasyonu, örnekleme dönemi) faktörlerin kerevit (*Pontastacus leptodactylus)* kabuğunun yumuşak veya sert olması üzerine etkileri araştırılmıştır. Arazi çalışmaları Türkiye'nin en önemli kerevit üretim merkezlerinden bir tanesi olan Hirfanlı Baraj Gölü'nde Haziran-2017 ile Mayıs-2018 döneminde aylık örneklemeye dayalı olarak yürütülmüştür. Paramerik olmayan değişkenlerin istatistiksel olarak değerlendirilmesinde permütasyonel çok değişkenli varyans analizi (*PERMANOVA)* kullanılmıştır. Toplamda 5920 adet kerevit kontrol edilmiş ve sonuçta 106 (%1.79) örneğin kabuğunun yumuşak, 5814 (%98.21) örneğin kabuğununda sert olduğu belirlenmiştir. Kerevit kabuğunun sert veya yumuşak olma durumu total boy, örnekleme dönemi ve örnekleme istasyonuna göre istatistiksel farklılık göstermiş ($p<0.05$), eşeye göre değerlendirildiğinde ise istatistiksel farklılık bulunmamıştır ($p>0.05$).

Anahtar kelimeler: PERMANOVA, Hirfanlı Baraj Gölü, kabuk değişimi, dar kıskaçlı kerevit

INTRODUCTION

As an extremely old group freshwater crayfish (159 million years ago) is very important for the freshwater ecosystem and they carry out some key roles in the environment such as water quality indicators, biodiversity indicators, keystone trophic regulators, and ecological engineering (Reynolds et al., 2013). Besides ecological importance, freshwater crayfish are very importance for economically as well. Although quality value of crayfish meat was also known in medieval times (Gherardi, 2011), as commercial mass harvesting (both fisheries and aquaculture based) started in 20th century according to official records. The narrow-clawed crayfish (*Pontastacus leptodactylus*, Eschscholtz 1823) is the only commercial crayfish in Turkey, almost all of the products are export to the European country. Total annual production occurred as 696 t in 2019 (TurkStat, 2020).

The moulting process is very important for crustaceans because size increasing connected with moult frequency. Crayfish growth is discontinuous and can be resolved into two components: moult increment and intermoult interval (McLay and van den Brink, 2016). Hence, exhibiting the factors that can affect moulting is extremely important for both comprehending species life history and managing of the commercial stock. Crayfish with soft shells are undesirable in Turkey's commercial inland fisheries in that marketing is almost impossible. Discrimination is an improbable soft and hard shell in fishing operations because of the used catching technique, ultimately, all of them are harvested from fyke nets onboard. Finally, crayfish with soft shells are most likely convert to discard. If close season applied in the mass moulting period, this strategy can be directly affected the decrease of discard ratio. Current and similar studies are very important for the management of natural stocks when results using within this scope.

There are very limited studies conducted on the shell status of commercially exploited natural crayfish stock. It is seen that similar research is focus on indoor aquaculture studies (TaugbØL and Skurdal, 1992, Kouba et al., 2010, Kozák et al., 2009, Renai et al., 2007, Hesni et al., 2009, Yu et al., 2020). Turvey and Merrick (1997) investigated relationships of moulting frequency and moult increments with biological or environmental factors for freshwater crayfish *Euastacus spinifer* in Sydney Region, Australia. Coughran (2011) investigated basic biology (including population structure, behaviour, reproduction, growth, and moulting) of Blood Crayfish (*Euastacus gumar*) in the north-eastern New South Wales region (Australia). Walsh and Walsh (2013) researched growth rates and moult frequency of Tasmanian giant freshwater crayfish (*Astacopsis gouldi*) with using marking technic (tailfan clips and Passive Integrated Transponder) in northern Tasmania.

The aim of this study was to investigate the effect of some biotic (total length, sex) and abiotic (sampling station and month) factors on having whether soft or hard-shell in a natural crayfish population.

MATERIAL AND METHODS

The study was conducted in Hirfanlı Dam Lake (Ankara, Aksaray, Kırşehir- Turkey) where one of the most important crayfish harvesting areas of Turkey (Figure 1). The dam was constructed on Kızılırmak river in 1959 and its average surface area 218.81 km^2 and average depth 20.15 m (Gençoğlu and Ekmekçi, 2016). Fishing operations were conducted six stations where determined from the introduction section of the river in the dam toward to dam body. All stations were in a commercial crayfish fishing area. The study was conducted monthly between June-2017 and May-2018. Single-input crayfish fykes equipped with red net with 210d/12 rope thickness and 18mm mesh were used during sampling. 600 fyke nettings were used during sampling, 100 at each site.

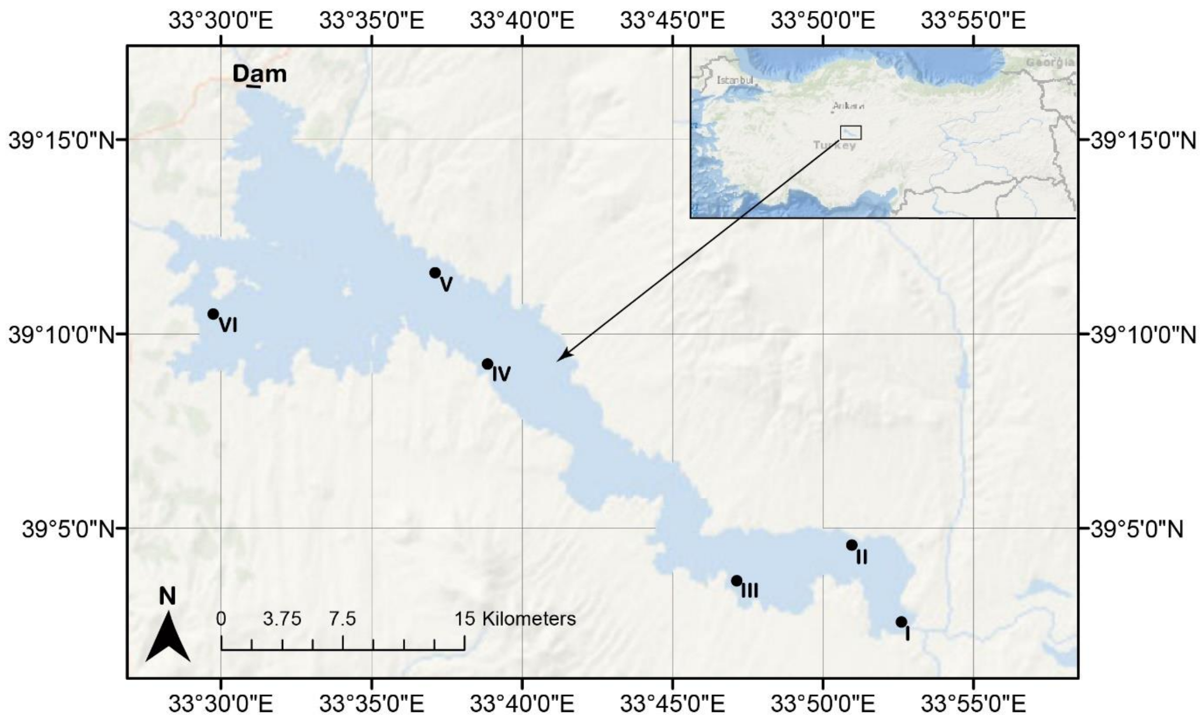

Figure 1. Hirfanlı Dam Lake and sampling stations

The sex of caught crayfish was determined macroscopically and carapace and abdomen length were measured with a digital calliper in 0.01 precision. Total length was obtained with the summing of carapace and abdomen length. The shell status (soft or hard) was determined as organoleptically from the same body part (carapace bilateral area) for each individual.

Determining the effect of some biotic and abiotic factors on crayfish shell status (soft-hard) permutational multivariate analysis of variance (*PERMANOVA*) was used. All of the categorical data were transformed as numerical before statistical analysis. Polychoric Correlation Analysis (Drasgow,1986) was used to determination the correlations between variables through *polycor* (0.7-10) *R* package (Fox, 2019). *vegan* (v 2.5-7) *R* package, *adonis2* function was used for statistical evaluation (Oksanen et al, 2020). *Bray–Curtis* distance matrices with 999 permutations were performed on the function. We utilized from *GGally* (v 2.1.0) *R* package for plotting of the interactions between variables (Schloerke et al, 2020). All statistical process was computed with *R* (v4.0.3) based *RStudio* (v1.3.1093) software.

RESULTS

At the end of the fishing trial, a total of 5920 crayfish were caught consist of 2595 females and 3325 males. Shell status of caught each crayfish was checked, in conclusion, we determined 106 (1.79%) individuals with a soft shell and 5814 (98.21%) hard shell. The status of a shell (soft or hard) was shown remarkable differences by total length class of crayfish. (Table 1). This difference was found significant as statistically ($p<0.05$) (Table 3), on the other hand, correlations between shell status and total length were founded weak correlation (Table 2). The status of the shell is shown in Table 1 by sampling stations. Caught number crayfish were shown differences by stations despite applied equal fishing pressure. The ratio of having soft shells was founded different by stations ($p<0.05$) (Table 2). It can say that moulting frequency is more intense in the middle areas of the dam than the beginning and last part. Sampling month was another important variable effected on the moulting process, in this case, most crayfish with soft shell were sampled in May (Table 1). Despite the founded weak correlation between shell status and sampling month (Table 2), differences shown significant as statistically ($p<0.05$) (Table 3). Crayfish inhabit Hirfanlı Dam Lake have generally chosen spring months for moulting by this result. When evaluating the shell status by sex very similar result was gained (Table 1), no statistical significant was founded between variables ($p>0.05$) (Table 3). All both double and tribble interactions between variables were found to be insignificant ($p>0.05$) (Table 3). The monthly mean water temperatures were measured as 5.8, 7.4, 9.2, 13.8, 16.4, 23.3, 25.2, 26.1, 22.8, 15.2, 10.7, 9.1^{o}C from January to December respectively.

Table 1. Shell status by biotic and abiotic factors

Shell	Total Length (cm)											
	4	5	6	7	8	9	10	11	12	13	14	15
Hard	8	32	218	921	1111	719	1016	1004	571	166	43	5
Soft	0	0	1	14	42	20	12	9	8	0	0	0
	Sampling Location											
	Sarıyahşi (1)	Sıddıklı (2)	Evren (3)	Geçitli (4)	Savcıllı (5)	Şanlıkışla (6)						
Hard	198	656	2203	1194	1321	242						
Soft	13	23	33	29	7	1						
	Sampling Month											
	Jan.(1)	Feb.(2)	Mar.(3)	Apr.(4)	May.(5)	Jun.(6)	Jul.(7)	Aug.(8)	Oct.(9)	Sep.(10)	Now.(11)	Dec.(12)
Hard	505	277	1163	1123	512	420	272	136	172	253	392	589
Soft	0	0	0	3	58	16	6	7	7	9	0	0
	Sex											
	Female (1)	Male (2)										
Hard	2545	3269										
Soft	50	56										

Table 2. Polychoric correlation matrix of variables

Variables	Shell status	Total length	Sampling location	Sampling month
Total length	0.181	-	-	-
Sampling location	-0.223	-0.078	-	-
Sampling month	0.355	0.110	0.065	-
Sex	-0.034	0.081	-0.053	-0.144

Table 3. *PERMANOVA* results on the differences in crayfish soft or hard-shell by other variables

Source of Variation	*df*	Sum of square	R^2	*F*	*p*
tl	1	0.3602	0.6999	45.3870	**0.0039**
ss	1	0.0539	0.1047	6.7910	**0.0336**
sm	1	0.0108	0.0210	22.7190	**0.0012**
sex	1	0.0064	0.0124	0.0807	0.3869
*tl * ss*	1	0.00425	0.00826	0.5357	0.4870
*tl * sm*	1	0.00777	0.01510	0.9791	0.3461
*tl * sex*	1	0.00111	0.00216	0.1399	0.7218
*ss * sex*	1	0.00236	0.00459	0.2974	0.6343
*sm *sex*	1	0.00840	0.01632	1.0582	0.3375
*tl * ss * sex*	1	0.00178	0.00346	0.2241	0.6688
*tl * sm * sex*	1	0.00202	0.00393	0.2547	0.6511
Residual	1	0.05556	0.10795		
Total	18	0.51462	1.00000		

(*tl*: total length, *ss*: sampling station, *sm*: sampling month)

Figure 2. Plot matrix of interaction between variables

DISCUSSION

In our study, which investigated the effects of some biotic and abiotic factors on the hardness or softness of shells of individuals in a natural crayfish population, total length and sex as biotic factors and sampling period and station as abiotic factors were evaluated. Kouba et al. (2010) revived the effect of several abiotic and biotic environmental factors on growth (or moult frequency) of crayfish, by the author, *abiotic factors* are temperature, photoperiod, the composition of the bottom substrate, shelter availability, pH, available calcium and *biotic factors* are age, sex, density, size, feeding frequency, individual growth rate and food source (Abrahamsson 1966, Mason 1979, Price and Payne 1984, Appelberg 1986, Lowery 1988, Gydemo 1989, Gydemo and Westim 1989, Hessen et al. 1991, Ackefors et al. 1992, Skurdal and Taugbol 1994, Jussila and Evans 1996, Savolainen et al. 2003, Ulikowski and Krzywosz 2004, Ahvenharju et al. 2005).

Correlations between variables were found to be quite weak in the study. This situation does not constitute any handicap in terms of the applied statistical method. As a matter of fact, according to Pasin et al. (2016), *PERMANOVA* is insensitive to correlations between dependent variables, and it also uses permutation techniques while calculating artificial (Pseudo) F test statistics and p-value. For this reason, this method does not require any distribution assumption. Therefore, the normal distribution assumption could not be achieved. The use of *PERMANOVA* in areas such as environment, ecology, medicine, and genetics has been increasing in recent years (Killi, 2020; Postnikoff et al., 2020; Kim et al., 2020). In this context, it is believed that *PERMANOVA* method, which we used in the evaluation of non-parametric data, is a current and accurate method.

It was observed that only 1.79% of the samples examined in the study had a softshell. The main reason for this low rate is that it is often difficult to sample crayfish that have entered the moulting cycle. Reynolds et al. (2013) explain this situation as follows: the moulting, or ecdysis, is hazardous to the individual, which must find somewhere to hide until its new, larger exoskeleton has hardened. The soft-shelled crayfish we caught were probably those that shed their shells in the fyke net (shell fragments were observed from time to time in the fyke nets during fishing operations) and those that have entered the shell hardening process. The softness of the shell was accepted as an indication that the sample had recently shed its shell.

Softshell texture was observed to be relatively intense in the 7, 8, and 9 cm length class (Figure 2), and the difference between total length and shell condition was found to be statistically significant (Table 2). This situation is thought to be since the shell change occurred more intensely in young individuals (6, 7, 8 cm total length) in this length class compared to other height classes. A similar result was reported by Wals and Wals (2013) for *A. gouldi*. By the authors, smaller specimens moulted more frequently than other individuals. Similarly, Reynolds (2002) reported that juveniles moult many times at first, larger individuals only once or twice a year in temperate regions. By the Turvey and Merrick (1997), small individuals (20-35 mm carapace length (*CL*)) of *E. spinifer* usually moult three times per year, the medium size class (35-55 mm *CL*) typically moults twice and large specimens (>55 mm *CL*) moult once per year. In addition, the authors reported that there are no differences in annual frequencies of moult related to sex and site.

In the study, it was observed that the softness of the crayfish shell peaked in May and June (Figure 2). It was observed that the sampling period was quite effective on moulting. The main reason for this situation is thought to be the water temperature. Because the process of moulting is inhibited when water temperates are lower than 10 – 11°C (Ackefors et al. 1989; Henttonen et al. 1993). The effect of water temperature on moulting frequency has been revealed more clearly in aquaculture studies conducted under controlled conditions. Kouba et al. (2010) reported that higher temperature influenced growth in juvenile noble crayfish (*Astacus astacus*) by causing a higher moulting frequency. Another reason for the seasonal difference in shell change is based on the reproductive biology of the species. Females with eggs in their pleopods cannot change their shells in the December-May period (Cilbiz, 2020). In the study, the increase in the number of soft-shelled crayfish in May and June is the fact that female crayfish start to moulting immediately after they get rid of their eggs.

The amount of catch and the correspondingly soft or hard shell varied significantly between the sampling stations. This situation is generally thought to be caused by the ecological characteristics of the habitat (depth, vegetation, pollution, ground structure, etc.). Particularly in the 1st station, which represents the region where the river enters the reservoir, unlike the other stations, the floor was covered with mud as a result of the precipitation of suspended solid material over the years. However, crayfish mostly prefer sandy-gravelly ground areas (Alpbaz, 2005). Another issue regarding habitat preference is vegetation. Reynolds et al. (2013) stated that in most open water crayfish, juveniles live away from the adults in vegetation, litter or gravel, usually in shallower, warmer water, and grow relatively rapidly through many moults. This situation may also cause a difference in length between stations. According to France (1993), acidification may have severe impacts on moulting and egg survival (Reynolds et al., 2013), in this context, the difference of water quality criteria in the reservoir depending on the fishing area may have caused the differentiation of the moulting process.

In addition to the increasing pollution, global climate change has caused serious changes especially on the lives of aquatic creatures on a global scale and continues to do so. Recently, plasticity has been among these negative factors. According to Reynold et al. (2013), unsuspected plasticity is affecting crayfish ecology such as physiology, behaviour, and reproductive strategies. In this direction, it causes changes in the life cycles of living things. For example, in Eğirdir Lake (Isparta-Turkey), it has been observed that crayfish with a total length of 11-12 cm, which is generally not expected to change their shells in the 2020 hunting season, have moulted densely. The soft-shelled catch is directly discarded in line with the demands of the traders (unpublished data). This has resulted in increased fishing pressure on the stock, whose sustainability is already severely troubled (more fishing has been required to reach the targeted marketable quota). By clearly demonstrating the seasonal moulting, the discard rate can be significantly reduced by applying the closed season in fishing during these periods. Therefore, it will be useful to carry out similar studies in other fishing areas.

In this study, it is demonstrated that shell status was shown statistical difference with total length, stations and sampling month ($p<0.05$), while it was no significant by sex group ($p>0.05$). Results of the study will contribute both understanding of the biological properties of *P. leptodactylus* and improving the management of its natural stocks.

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