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RESEARCH ARTICLE

Temporary Timing of Reproductive Traits with Respect to Environmental Variables in Turkish Crayfish in Yenice Reservoir [1]

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Summary

This study was carried out to determine the relationships between physicochemical parameters and morphometric and reproduction features of the crayfishes *(Astacus leptodactylus)* in Yenice Central Irrigation Pond. Results of MDS analysis revealed that the relationships between physicochemical parameters and morphometric features and reproduction features in crayfishes could be explained at different rates. Reproduction features such as fecundity and sperm production, were within a close relationship with temperature, magnesium, conductivity, dissolved oxygen, and the abdomen weight, carapace weight, abdominal width, carapace width, abdomen length, carapace length from morphometric features were within a close relationship especially with dissolved oxygen, magnesium, calcium, and temperature. These relationships could play an important role while considering the selection of maternal females for cultural environments and for the management of productive population, and for the selection of water sources that can be effective for populations that is generated by vaccination.

Keywords: *Morphology, Reproduction, Traits, Physicochemical, Astacus leptodactylus, Multidimensional Scaling Technique (MDS)*

Yenice Rezervuarında Tatlısu Istakozunun Üreme Özelliklerine Çevresel Faktörlerin Zamansal Etkileri

Özet

Bu çalışma, Yenice Merkez Sulama Göleti'nde bulunan tatlısu istakozlarının *(Astacus leptodactylus)* bazı morfometrik ve üreme özellikleri ile su kaynağının bazı fiziko-kimyasal parametreleri arasındaki ilişkilerin belirlenebilmesi amacıyla yapılmıştır. Bu doğrultuda yapılan MDS analizleri sonucunda elde edilen stres ve korelasyon katsayıları, kullanılan modelin uygunluğunun ölçütleridir. Söz konusu katsayılar incelendiğinde tatlısu istakozlarında, morfometrik özellikler, üreme özellikleri ile su kalitesi parametreleri arasındaki ilişkilerin farklı oranlarda açıklanabildiği tespit edilmiştir. Bu manada araştırma sonuçlarına göre fekondite ve sperm üretimi gibi üreme özelliklerinin, su kalitesi parametrelerinden sıcaklık, magnezyum, kondaktivite, çözünmüş oksijen ile morfometrik özelliklerden de abdomen ağırlığı, karapaks ağırlığı, abdomen genişliği, karapaks genişliği, abdomen boyu ve karapaks boyu'nun özellikle çözünmüş oksijen, magnezyum, kalsiyum ve sıcaklık ile yakin ilişki içerisinde oldukları belirlenmiştir. Tespit edilen ilişkilerin gerek kültür ortamlarında anaç seçimi ve verimli popülasyon yönetimlerinde gerekse aşılama suretiyle oluşturulan popülasyonlar için verimli olabilecek su kaynaklarının seçiminde göz önünde bulundurulduğunda oldukça önemli rol oynayabilecekleri düşünülmektedir.

Anahtar sözcükler: *Morfoloji, Üreme, Özellikler, Fiziko-Kimyasal, Astacus leptodactylus, Çok Boyutlu Ölçekleme Analizi (MDS)*

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INTRODUCTION

The crayfishes are the largest representative of decapod crustaceans in freshwaters. They have over 640 species and sub-species that are mostly distributed in America and Australia [1]. Two crayfish species, the Turkish crayfish, *Astacus leptodactylus,* and the stone crayfish, *Austropotamobius torrentium*, have been determined in approximately 100 water sources including natural lakes, dam lakes, ponds, and rivers in Turkey [2-4]. Although the Turkish crayfish is native for Turkey, the origin of stone crayfish is unknown [5,6].

The Turkish crayfish is economically important species for Turkey because of its countrywide disrubution. The crayfish production was 270 tons in 1965 and increased to approximately 8.000 tons in 1984. However a gradual decrease was evident from this year on and declined to 783 tons in 2008. The most plausible reason responsible for the decline of the crayfish production was the crayfish plague disease; overfishing and deterioration in water quality parameters were also speculated for the cause of the decline [7-10]. Although numerous researches on overfishing and disease effects on population decrease have been conducted, quite few studies exist on the water quality effects on crayfish recruitment.

Biotic (e.g. food, disease, and competition) and abiotic factors (e.g. temperature, dissolved oxygen, pH, light, conductivity, calcium and magnesium levels) affect the density growth, and life cycle of crayfish populations [11-13]. The most important factors that affect mortality, reproduction, and distribution of crayfishes are reported to be temperature, dissolved oxygen, pH, and calcium levels.

The fecundity in crayfishes can be calculated either as the number of eggs in the ovaries or as the number of eggs in the pleopods. Biotic factors such as disease, food quality, physicochemical properties, female and egg sizes are among the factors that affect the fecundity [14-19]. In previous studies, the relationships between environmental factors and morphometric and reproduction features were evaluated separately. In other words, the studies aimed to reveal either the relationships between morphometric features and environmental factors [20,21] or the effects of environmental factors and fecundity [22-24]. The aim of this study was to determine the relationships between physicochemical parameters and morphometric and reproduction features of the Turkish crayfish in Yenice irrigation pond, Çanakkale-Turkey.

MATERIAL and METHODS

Study Area and Sampling

Yenice irrigation pond is built on the Kuru Dere river and has an irrigation area of 1330 ha, a water volume of 3.730.000 m^3, and an area of 0.328 km^2. High fluctuation in water level exists due to irrigation activities and therefore no vegetation exists in and around the pond. In the benthic zone of the pond, allochthonous and silt materials coming with the stream are present. The fish fauna of the pond is composed of common carp, *Cyprinus carpio*, tench, *Tinca tinca*, european chub, *Squalius cephalus*, and gudgeon, *Gobio gobio*.

Crayfish were sampled monthly using fyke nets of 34 mm mesh size from July 2007 to June 2008. The fyke nets were left to different points about 2-3 m depths in the way of characterizing the lake. The nets were set in the late afternoon and were visited early in the following morning, because Turkish crayfish is active at night and often hides in shelters during the day.

Physicochemical Measurements

Water temperature, pH, EC and DO were measured in situ. Conductivity and dissolved oxygen were measured with a hydrolab recorder (YSI 85D, YSI Incorporated, Yellow Springs, Ohio 45387 USA) and pH with a YSI 100 pH-meter. Two replicates of water samples were taken with 1l plastic bottles and transported to the laboratory in coolers. Water samples were then frozen to -30°C before using standard methods for the analysis. The contents of calcium (Ca^{+2}) and magnesium (Mg^{+2}) were analyzed as inductive in coupled plasma-atomic emission spectrometer in Çanakkale Onsekiz Mart University, Science and Technology Application and Research Center.

Morphometric and Reproductive Measurements

All morphological measurements and reproductive characteristics followed the methods of Harlıoğlu [3] and Rhodes and Holdich [25] *(Fig. 1)*. Carapace length (CL), carapace width (CWi), abdomen length (AL), and abdomen width (AWi), were measured using a digital caliper with 0.01 mm accuracy. Carapace weight (CW), abdomen weight (AW), pleopodal egg weight (PEW), ovarian eggs weight (OEW), and reproductive system weight (the total of testis and vasa deferentia weight; RSW) were measured with a digital balance (EK 610i, A&D Company, Ltd. Tokyo, Japan) to the nearest 0.01 g sensitivity. Individual pleopodal egg weights (PINEW) were weighted using a digital balance (Scaltec SBB31, Scaltec Instruments, Germany) to the nearest 0.0001 g sensitivity after the eggs were wiped with a paper towel. The PINEW represent the mean number of individually weighted 15 eggs obtained from a single female.

Subsamples of 10 pleopod eggs were removed from each ovigerous female and placed on moist filter paper in a Petri dish, and individual egg diameters were measured under a dissection microscope equipped with a calibrated ocular micrometer. The pleopodal egg volume (PEVOL), estimated from averaging the volumes of 10 eggs subsampled from a single female, was calculated as [26]:

$$V = \pi LW^2/6;$$

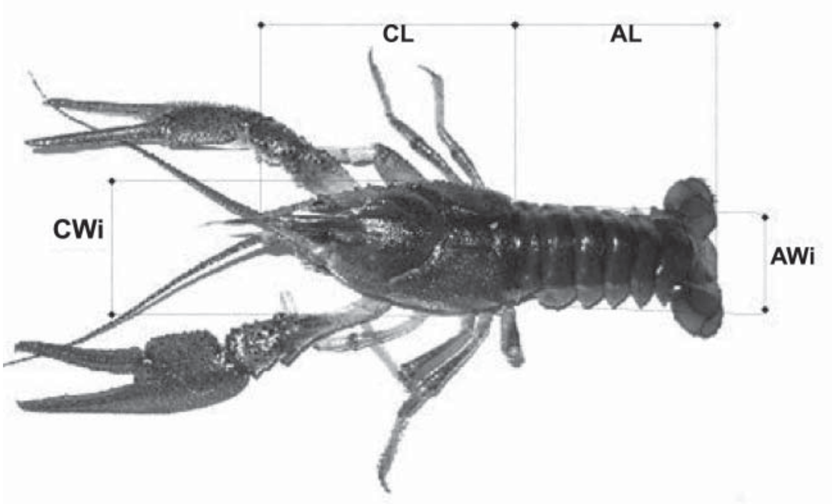

Fig 1. Dorsal view of the Turkish crayfish and locations of which the measurements were taken

Şekil 1. Tatlısu ıstakozu'nun dorsalden görünüşü ve uzunluk ölçümlerinin alındığı bölgeler

Where V = egg volume (mm^3), L = egg lengths (mm) and W = egg width (mm). In addition, to estimate the fecundity, the pleopodal egg numbers (PEN) and the ovarian egg numbers (OEN) were visually counted in a petri dish.

Females were divided into two groups: females that were bearing eggs within the ovaries (non-reproductive period) and females with pleopodal eggs (reproductive period). Males were also divided into two groups: the first group, the mating period, represented the period in which the sperm production started to increase (e.g. males had a RSW value greater than 0.01 g); the second group, non-mating period, represented the period in which the sperm production was inclining which corresponded to the period in which females were bearing pleopodal eggs.

Statistical Analysis

Metric Multidimensional Scaling Analysis (MDS) was used to investigate the relationships between the morphological and reproductive properties of crayfish and physico-chemical parameters of the Yenice irrigation pond water. Multidimensional scaling is an exploratory technique used to visualize proximities (a proximity is a number that indicates how similar or how different two objects or variables) in a low dimensional space. MDS provides a researcher to uncover the hidden structure or relations among the variables. Each object is represented by a point in a multidimensional space. Two similar objects are represented by two points that are close to each other, while two different or dissimilar objects are represented by two points that are apart from each other [27].

Stress coefficient is used as goodness-of-fir criteria in MDS with the formula:

$$\text{Stress=;} \sqrt{\frac{\sum\sum\left(f\,(x_{ij})-d_{ij}\right)^2}{\sum\sum d_{ij}^2}}$$

Where f (x_{ij})= x_{ij} and d_{ij} refer to the Euclidean distance.

The smaller the stress, the better is the representation. When the MDS map perfectly reproduces the input data, f (x_{ij})- d_{ij} is for all $_i$ and $_j$ [28].

Statistical analysis on the reproductive and morphometric measurements were conducted for each sex separate as well as for both sexes combined. In addition, these analyses were conducted for both the reproduction and the non-reproduction periods.

RESULTS

Physicochemical Measurements

Water temperature was the highest, 26.8°C, in August 2007 and the lowest, 5.7°C, in December 2007 *(Table 1)*. Dissolved oxygen (DO) reached its minimum value as 7.6 mg/l on October 2007 and its maximum value as 12.85 mg/l on March 2008. Conductivity (EC) ranged between 229.1 and 387.1 µS.

Morphometric and Reproductive Measurements

A total of 546 Turkish crayfish were sampled between July 2007 and June 2008. During those 12 months, 255 males and 291 females were captured *(Table 2)*.

Sperm production was evident in 153 males *(Fig. 2)*. The RSW started to increase in October and reached its peak value in December which was assumed as the mating period. In this period a total of 62 males were sampled. After the peak value, the RSW declined until May, which was assumed as the non-mating period in which a total of 91 males were sampled. After May the RSW value was less than 0.01 g.

Female non-reproduction period was estimated between September 2007 and December 2007. Out of the 291 females sampled during the study, 115 had ovarian eggs. The reproduction period for the females was estimated between January 2008, start of spawning, and May 2008,

Table 1. *Physicochemical measurements of Yenice irrigation pond*
Tablo 1. *Yenice Sulama Göleti'nin fiziko-kimyasal ölçüm değerleri*

Date	t (°C)	DO (mg/l)	pH	EC (µS)	Ca (mg/l)	Mg (mg/l)
Jul-07	25.9	7.65	9.03	279.0	36.16	9.85
Aug-07	26.8	8.25	8.95	301.2	37.46	10.47
Sep-07	22.1	7.90	8.80	330.2	31.33	9.21
Oct-07	18.2	7.60	8.75	387.1	24.00	8.41
Nov-07	8.3	8.72	7.14	229.1	23.08	8.11
Dec-07	5.7	11.80	8.68	269.5	36.43	9.77
Jan-08	8.2	8.50	8.34	320.1	21.06	8.91
Feb-08	9.1	10.20	7.86	344.6	24.43	8.02
Mar-08	9.6	12.85	9.03	382.3	38.27	7.88
Apr-08	13.9	9.53	6.11	311.6	23.85	6.91
May-08	15.9	9.02	5.50	265.8	34.31	7.44
Jun-08	22.4	10.69	6.85	234.5	36.50	8.11

Table 2. *Numbers of males and females sampled during the reproductive periods*
Tablo 2. *Üreme periyotlarındaki erkek ve dişi birey sayıları*

Date	Male			Females		
	Total	Mating Period	Non-mating Period	Total	Non-reproductive	Reproductive
Jul-07	22			17		
Aug-07	48			31		
Sep-07	9			12	10	
Oct-07	30	29		90	90	
Nov-07	15	14		10	9	
Dec-07	19	19		12	6	
Jan-08	36		31	10		1
Feb-08	17		16	8		8
Mar-08	6		5	18		17
Apr-08	35		33	16		12
May-08	11		6	14		13
Jun-08	7			53		

start of hatching *(Table 2, Fig. 2)*. A total of 51 of females carried pleopodal eggs. However, for statistical purposes the single female sampled in January 2008 was not used in the analysis due to missing replication.

Mean number of eggs in the ovaries were estimated as 275 in the non-reproductive period. The mean number of pleopodal eggs, on the other hand, was 240 in the reproductive period. The mean RSW was 0.42 g in the non-mating period and 0.56 g in the mating period *(Table 3)*.

During the mating period 62 individuals and during the non-mating 91 individuals of males were sampled. During the reproduction period 51 females were captured. Whereas during the non- reproduction period a total of 115 females were sampled.

In terms of length measurements, CL, AL, and AWi of female were significantly ($P<0.05$) greater than that of male's when analyzing the data of reproductive and non-reproductive periods were combined. In terms of weight, females had a significantly ($P<0.05$) heavier AW than males had. The degrees of importance of metric obtained from male and female crayfishes, the comparisons and differences between gender groups of these, have been determined statistically. In terms of, females had significantly larger CL, AL and AWi than the males had. In terms of weight measurements, only AW showed significant differences between sexes. No differences of CWi and CW were evident between the sexes *(Table 4)*.

Stress and correlation coefficients obtained from the analysis and the mean of dissimilarities measures of the appropriateness of the used model *(Table 5)*.

The relations in the males after non-mating are shown in *Fig. 3*. When the figure is examined, it is seen that the temperature is effective on RSW. And it is also determined that other morphological features are associated with the Ca level of water.

In the case of the explanation of the relationships

Table 3. *Descriptive statistics for males and females in each period (OEW, ovarian egg weight; OEN, ovarian egg number; RSW, reproductive system weight; PEW, pleopodal eggs weight; PEN, pleopodal egg number; PINEW, individual pleopodal egg weight; PEVOL, pleopodal egg volume)*

Tablo 3. *Her bir periyotta erkek ve dişi bireyler için tanımlayıcı istatistikler (OEW, ovaryum yumurtası ağırlığı; OEN, ovaryum yumurta sayısı; RSW, üreme sistemi ağılığı; PEW, pleopodal yumurta ağılığı; PEN, pleopodal yumurta sayısı; PINEW, tek pleopodal yumurta ağılığı; PEVOL, pleopodal yumurta hacmi)*

Sex	Period	N	Reproduction Variable	Mean ± SE	Min-Max
Male	Mating	62	RSW	0.42 ± 0.032	0.01-1.70
	Non-mating	91	RSW	0.56 ± 0.04	0.13-1.50
Female	Non-reproductive	115	OEW	0.93 ± 0.051	0.11-3.25
			OEN	275 ± 8.04	123-522
	Reproductive	50	PEW	3.08 ± 0.16	0.70-5.55
			PEN	240 ± 14.46	74-436
			PINEW	0.013 ± 0.0003	0.009-0.018
			PEVOL	2.31 ± 0.07	1.29-3.28

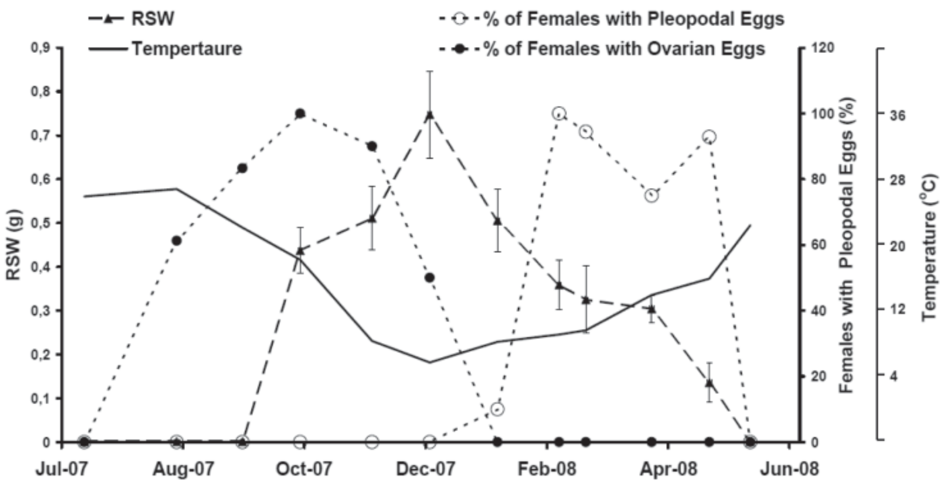

Fig 2. Water temperature (–), mean of the males' reproductive system weights (RSW; N = 153) (–▲–) with standard error bars, percentage of females (N = 115) that carry ovarian eggs (-●-), and percentage of females (N = 50) that carry pleopodal eggs (-O-) between July 2007 and June 2008

Şekil 2. Temmuz 2007 ile Haziran 2008 tarihleri arasındaki su sıcaklığı (–), ortalama erkek üreme sistemi ağırlığı (RSW; N = 153) (–▲–) ve standart hata çubukları, ovaryum yumurtaları taşıyan dişilerin (N = 115) yüzdeleri (-●-) ve pleopodal yumurta taşıyan dişilerin (N = 50) yüzdeleri (-O-)

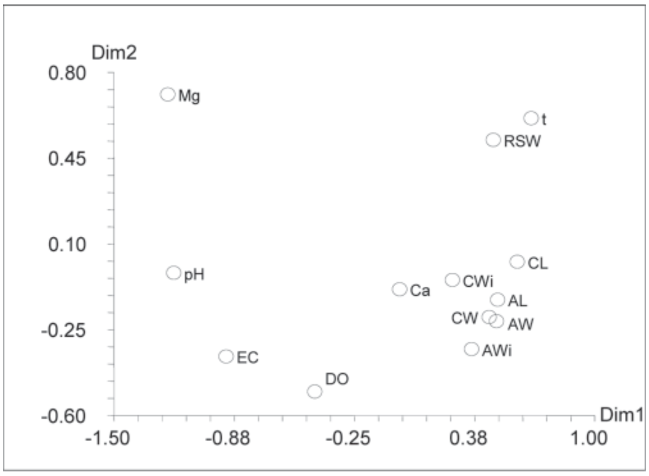

Fig 3. MDS map for males in the non-mating period

Şekil 3. Çiftleşme periyodu dışındaki erkek tatlısu istakozlarının MDS haritası

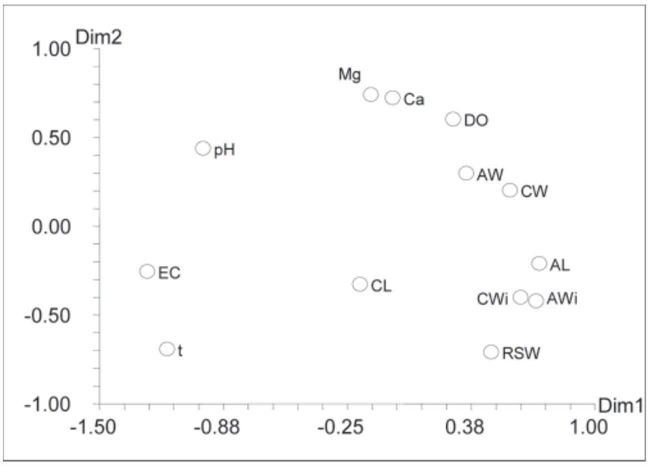

Fig 4. MDS map for males in the mating period

Şekil 4. Çiftleşme periyodundaki erkek tatlısu istakozlarının MDS haritası

between morphometric and reproduction features of male crayfishes in mating period and some physicochemical parameters of water by using the MDS technique, the 73.54% of total variation could be explained. In the determination of MDS technique is how much appropriate to the investigation of these relationships, these criteria were used; R^2 = 82.75, Stress Coefficient S = 0.199, Mean of Dissimilarities MS = 1.113 *(Table 6)*.

It was found that AW and CW were associated with DO, Mg, and Ca. Therefore, it is expected that the changes in these three water parameter affect AW and CW in the same direction. When the relationships between physico-

Table 4. *Descriptive statistics for morphometric measurements*
Tablo 4. *Morfometrik ölçümler için tanımlayıcı istatistikler*

Period	Morphometric Traits	Sex	N	Mean±SE	Min-Max	P Value
Non-reproductive (Females) Mating (Males)	CL	Female	115	51.27±0.638	35.00-80.63	P<0.001
		Male	62	46.57±0.859	32.60-68.85	
	AL	Female	115	54.91±0.684	35.50-81.62	P<0.001
		Male	62	45.50±0.841	31.30-66.20	
	CWi	Female	115	25.60±0.461	17.40-57.54	P=0.013
		Male	62	23.65±0.621	15.40-63.21	
	AWi	Female	115	29.59±0.587	15.56-47.37	P=0.001
		Male	62	22.43±0.486	13.60-31.30	
	CW	Female	115	21.68±0.914	6.50-81.12	P=0.473
		Male	62	20.56±1.250	6.50-64.13	
	AW	Female	115	8.07±0.317	2.60-26.80	P<0.001
		Male	62	5.38±0.280	1.80-12.60	
Reproductive (Females) Non-mating (Males)	CL	Female	50	52.61±0.741	42.31-68.63	P=0.634
		Male	91	51.96±1.136	36.05-72.76	
	AL	Female	50	56.86±0.936	44.25-77.15	P<0.001
		Male	91	50.64±1.085	35.50-70.04	
	CWi	Female	50	25.98±0.501	19.28-37.11	P=0.790
		Male	91	26.20±0.664	18.02-40.72	
	AWi	Female	50	29.59±0.587	20.50-42.39	P<0.001
		Male	91	22.43±0.486	15.28-30.57	
	CW	Female	50	24.04±1.218	10.41-55.85	P=0.051
		Male	91	28.63±1.980	8.32-82.48	
	AW	Female	50	8.76±0.372	4.42-18.78	P=0.004
		Male	91	7.07±0.425	1.80-17.03	
Combined	CL	Female	165	51.67±0.499	35.00-80.63	P<0.001
		Male	153	48.75±0.718	32.60-72.76	
	AL	Female	165	55.50±0.557	35.50-81.62	P<0.001
		Male	153	47.58±0.694	31.30-70.04	
	CWi	Female	165	25.71±0.354	17.40-57.54	P=0.080
		Male	153	24.68±0.467	15.40-63.21	
	AWi	Female	165	28.60±0.372	15.56-47.37	P<0.001
		Male	153	21.10±0.316	13.60-31.30	
	CW	Female	165	22.39±0.739	6.50-81.12	P=0.290
		Male	153	23.83±1.136	6.50-82.48	
	AW	Female	165	8.28±0.248	2.60-26.80	P<0.001
		Male	153	6.07±0.248	1.80-17.03	

Table 5. *Goodness of fit criteria*
Tablo 5. *Uyum iyiliği kriterleri*

Sex	Period	Stress	Mean of Dissimilarities	R^2
Female	Non-reproduction	0.106	1.221	90.98
	Reproduction	0.073	1.266	96.46
Male	Non-mating	0.059	1.113	98.11
	Mating	0.199	1.113	82.57

chemical parameters of water and morphological features of male crayfishes were examined, it was seen that DO and Ca was associated with AW *(Fig. 4)*. When figures 3 and 4 were evaluated together, period difference in pre-mating and post-mating periods affected these relationships significantly *(Table 6)*.

When *Fig. 5*, which is related with these relationships for females, was examined, it was found that EC was associated with OEN in general and T was associated with other morphological and reproduction features. When the relationships between some morphological features of female crayfishes and the physicochemical parameters of water (especially OEN) were examined, it was pointed out that it was associated with DO *(Fig. 5, Fig. 6)*.

DISCUSSION

The study has been carried out in a central irrigation pond of Yenice Town, Çanakkale, Turkey to identify the relationship between morphometric and reproduction characteristics of crayfish and various physicochemical

Table 6. *Accounted variation based on dimensions obtained from the MDS analysis for the males and females*
Tablo 6. *Erkekler için yapılan MDS analizinde boyutlardaki açıklanan varyanslar*

Sex	Period	Dimensions	Eigenvalue	Individual (%)	Cumulative (%)
Male	Non-mating	1	5.87	58.40	58.40
		2 (Used)	1.76	17.49	75.89
	Mating	1	6.10	47.78	47.78
		2 (Used)	3.29	25.75	73.54
Female	Non-reproduction	1	5.69	49.29	49.29
		2 (Used)	2.20	19.06	68.34
	Reproduction	1	8.00	50.95	50.95
		2 (Used)	3.54	22.55	73.50

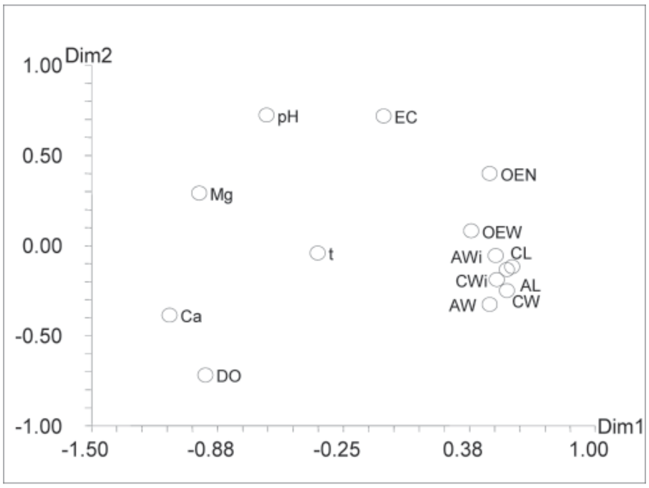

Fig 5. MDS map for females in the non- reproduction period
Şekil 5. Üreme periyodu dışındaki dişi tatlısu istakozlarının MDS haritası

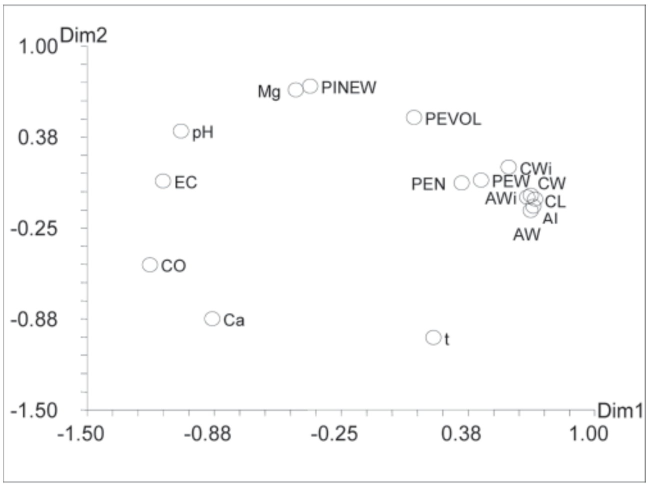

Fig 6. MDS map for females in the reproduction period
Şekil 6. Üreme periyodundaki dişi tatlısu istakozlarının MDS haritası

parameters of water. Obtained stress and correlation coefficients from the result of MDS analyses and the mean of dissimilarities have been given as the appropriateness of the model used [28-30]. Examination of above mentioned coefficients indicated that relationship between morphometric and reproduction characteristics of crayfish and the parameters of water quality could be explained at different rates (R^2).

One of the key features of arthropods is their protective shells that can adapt to almost all habitats [31,32]. Although the crayfish can adapt to all habitats, it is argued that some members of the species such as *Astacus pallipes* need habitat with higher water quality [33-35]. Moreover, it has been found that similar species are bioindicators of high water quality and can be used to monitor temporal and local changes. Therefore various studies have been carried out by using physical and chemical standards of water in order to define habitats where crayfish can dwell [34,36-38]. Aiken [20] stressed that growth rates of crayfish were affected by changes in various environmental conditions. The research conducted on this field puts forward that abiotic factors such as temperature, dissolved oxygen, conductivity, Ca^{+2} and Mg^{+2} can affect on the lives of coldwater crayfishes [39]. For instance, while crayfish plague is known to be the main reason for the quantitative reduction of European fresh-water crayfish reserves, it is also argued that one of the other main factors is the change of quality in their habitat [40-42].

As a result of MDS analyses, it can be seen that gender difference plays an important role in the relationship *(Fig. 2* and *Fig. 3)* among morphometric, reproductive and the physicochemical parameters of water. Therefore, it can be proposed that the evaluation of these relationships without considering gender difference, may lead to misleading conclusions.

When the relationships between morphological and reproduction characteristics in males and water quality parameters are considered together, it is seen that the period difference between pre-mating and post-mating periods is efficiently significant. Thus it can be said that period differences are necessary to be kept in mind while the relationship between above mentioned parameters are assessed among male species of crayfish. In MDS analyses, it is seen that the temperature in males is associated with RSW in the post-mating period. From this point, the weights of crayfish sperms, majority of which is lost during mating period, also increase with the rise in temperature. It was found that the temperature in females was associated mostly with PEN and PEW, and CL, CWi, AL, AWi, CW and AW from the morphometric parameters during spawning and incubation period. The feeding activities decrease especially in cold winter period and the incubation period, but accelerate with the increase in temperature. The increase in growth as both in length and weight is provided with the incremental feeding. In this direction, the relationships between temperature and CL, CWi, AL, AWi, CW and AW features can be explained. Some of the major effects of temperature on crayfish, either by keeping under control or regulating, are the shell change, growth, survival of juveniles and reproduction characteristics [43]. Typically, the shell change and intensive feeding are limited to the hot summer months [44]. While the temperature affects the intermoult period, it is also efficient on the shell change and thus the growth. Fluctuations in the optimum temperature values can lead to delays in changing the shell, and this may cause an increase in the mortality rate following the periods after the shell change [32].

According to MDS analyses, for male species of crayfish a significant relationship among AW, CW and DO values have been found both generally and during mating periods. In other words, it has been found that the changes in the level of dissolved oxygen in water affect weights of crayfish in the same way. When the female species of crayfish are evaluated generally, it has been found that reproduction and morphologic characteristics (especially OEN) are largely influenced by DO. One of the major problems that is faced in areas where cultivation of crayfish is conducted is low levels of dissolved oxygen concentration and dramatic fluctuations of the oxygen concentrations [45]. The oxygen concentration is the restrictive factor in the growth of crayfish. Generally, slowdown in growth and feeding occurs in crayfish, which are exposed to constant low oxygen concentration [46]. It has been found that *A. leptodactylus* species, which demonstrate a natural dispersion in Turkey, can tolerate a minimum of 3.97 mg/l and needs an oxygen concentration higher than 6 mg/l in optimal [13,47-50]. The high amount of dissolved oxygen in culture environment is usually associated with short intermoult periods and rapid growth. The large variation in the changes of dissolved oxygen in intraday, slows down the growth with the changes in temperature [21].

Male crayfish have been evaluated during mating, and post-mating period. According to MDS analyses, it has been found that Ca concentration of water is associated with t and DO as well as together with the weight measurements. In times of increased levels of Ca, increases in weight have also been found. Calcium concentration of aquatic environments is one of the most influential factors in the growth of crayfish [51,52]. Calcium accumulates in the outer shells of many crustaceans and the adequate amounts of calcium is required in each change of the shell as in crayfish [53]. These types of organisms have to change their shell for during growth. Thus growth is recorded after removing of the former shell. Crayfish grow by filling a larger shell gap both in length and in weight until another shell change time. During the time of calcium deficiency, the activity of shell change advances more slowly. For this reason, the soft shell period (intermoult) takes longer and in this period the crayfish are exposed to predators' attacks intensely since they are vulnerable. Cannibalism also increases during this period [54,55]. In times when calcium saturation level is 5 mg/l or under, the achievement in the process of creating a new shell is not be obtained, since shell calcification can not be completed by crayfish [52,53,56,57]. For *A. leptodactylus* type of crayfish, the optimal calcium levels are 50-100 mg/l and it is specified that the limit values within 5-130 mg/l [36]. When these given values are considered, Ca concentrations between 32.06-38.27 mg/l, which have been obtained from the pond, are among the limit values.

Furthermore, the results of MDS indicated an advanced relation between EC and OEN especially. It is reported that high electrolyte content of water affect the crayfish plague disease zoospores adversely, since it causes a loss in their ability to survive [58-60]. The changes in EC, which one generally associated with crayfish in terms of diseases, also influence OEN in the same way, which is one of the remarkable findings in the present study.

It is found that Mg concentration of water in male crayfish during reproduction period is associated with DO and Ca together with AW and CW. Cerenius and Söderhäll [58] found that the calcium and magnesium rates in water have antagonistic effect on zoospore production that is caused by *Aphanomyces astaci*, which is the factor of crayfish plague disease. The calcium concentration in water shows an incentive effect in production of zoospores at high rates. Magnesium prevents the formation of zoospore. It is reported that if it is in dominant concentrations, it keeps zoospore oogenesis under pressure [61,62]. In the female species of crayfish, a close relationship with PINEW and PEVOL has been found. Another remarkable finding of our analysis is that the Mg values of water which affect crayfish biology in terms of disease such as in the value of EC, closely influence the weight characteristics and especially pleopodal egg weights and volumes in the females

Identification of the relationship between morphological and reproduction characteristics of crayfish and physicochemical characteristics of water resources plays an important role in many aspects. Identification of reproduction characteristics of crayfish helps protecting the balance and management of crayfish reserves efficiently, which have grown naturally or artificially [63,64]. Especially the number of pleopodal eggs is one of the most important data used in the estimation of potential young individual members. They constitute the potential data in the selection of productive maternal females especially in culture environments and in making productive cultivation plannings in this manner. Understanding the ecological requirements of crayfish has a great importance in improving management plannings of crayfish reserves in natural or culture environments. Uncontrolled vaccination method without prior research to identify ecological requirements of freshwater crayfish has resulted in a failure especially in countries which try to increase their crayfish production by vaccination method. In the case of vaccination of crayfish to water sources in which they can not adapt fully, they continue to survive but constitute very weak populations [65]. In this sense in our research results, a close relationship was found between reproduction characteristics such as fecundity and sperm production, and water quality parameters such as temperature, Mg, EC, DO. Moreover a close relationship was also found between morphological characteristics such as AW, CW, AWi, CWi, AL, CL and water quality parameters DO, Mg, Ca and t. Identified relationships may play an important role for the selection of maternal females in culture environments for the management of productive reserves and in the selection of water sources which can be suitable for crayfish reserves that are generated by vaccination. Interpreting the biological features of organisms and the physicochemical characteristics of their habitats may help understanding the ecological requirements and suggest new potential approaches according to changing environmental conditions.

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