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Effect of Salinity on the Growth and Survival of the Freshwater Mussel, *Unio crassus*, in an Environmentally Disturbed River

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Abstract.- The aim of this study was to examine the effects of salinity on the survival and growth of a freshwater mussel *Unio crassus*, from a Mediterranean stream (Sarıçay Stream) in south-west Anatolia, Turkey, which has highly variable environmental conditions and a salinity gradient. A total of 250 similarly-sized *U. crassus* were collected from a stream in the species' native range and transferred in ice to Sarıçay Stream. The length, width, height and weight of each individual were measured before and after the sampling period, a period of 286 days. Significant increases were detected in the height, width and weight of mussels at all sampling points during the study, with the exception of weight in the sampling station third with salinity of 3‰. Increase in mussel weight was highest at the second station (19.29%) with a moderate salinity (1.5‰), while that with a growth loss was observed at the third station (-1.56%) with the highest salinity concentration (3‰). Survival rates over the duration of the study were 60%, 56%, and 66% at stations 1-3, respectively. All mussels were dead at the fourth (salinity 4.5‰) and fifth stations (salinity 6‰) at the end of the experiment.

Keywords: Freshwater mussel, weight gain, salinity, temperature, nutrient enrichment, survival rate.

INTRODUCTION

Freshwater mussels are key components of freshwater ecosystems, and have worldwide ecological and economic significance. Filter feeding behavior by mussels removes phytoplankton and suspended particles from the water column (Ahmed, 1971; Kasprzak, 1986; Kryger and Riisgaerd, 1988; McMahon, 1991; Welker and Walz, 1998; Strayer *et al.*, 1999). When mussel biomass is large, filter feeding has a profound impact on ecological processes (Welker and Walz, 1998). Mussels also have an impact on the calcium budget of freshwater systems, and serve as food items for other organisms (McMahon, 1991).

Despite their importance, many aspects of their ecology are still unknown. In Turkey, the existed information is restricted to their growth (Ercan *et al.*, 2013a, b), ontogeny (Özdemir, 1991; Şereflişan, 2003; Çek and Şereflişan, 2006; Ercan *et al.*, 2013c), species description (Başçınar *et al.*, 2003; Bilgin, 1980; Çetinkaya, 1996; Demirsoy, 1999; Geldiay and Bilgin, 1969; Öktener, 2004; Özemsi 1999; Ercan *et al.*, 2013c), distribution (Başçınar *et al.*, 2003; Bilgin, 1980, 1987; Çetinkaya, 1996; Geldiay and Bilgin, 1969; Öktener, 2004; Özemsi, 1999; Ercan *et al.*, 2013c, d), abundance (Ercan *et al.*, 2013e) and effects of pollution (Celiloğlu-Begenirbaş, 2002).

Freshwater mussels are sensitive to a variety of pollutants, including the impacts of salinity. Elevated salinity in freshwaters can result from land-use change, pollution events, drought conditions and climate change, with consequences for the survival and viability of populations of freshwater mussels (Kaushal *et al.*, 2005; Sherif and Singh, 1999). Despite its potential significance, the impact of elevated salinity has only recently been recognised as having a significant impact on freshwaters and attempts to understand its effects have been limited to specific taxa; insects (Hassell *et al.*, 2006), macrophytes (James and Hart, 1993) and snails (Kefford and Nugegoda, 2005). Studies on salinity tolerance of freshwater mussels are limited and include experimental studies focusing on the zebra mussel *Dreissena polymorpha*, which is considered as one of the most invasive mussel species that causes serious economic losses. Another recent experimental study on the effects of salinity on mussels was on the multiple life stages of a common freshwater mussel, *Elliptio complanata*, which demonstrated that low levels of salinity could have a dramatic effect on the reproduction, physiology, and survival of this species (Blakeslee

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et al., 2013). An impact of salinity on the reproduction of freshwater unionids was also shown through a decrease in glochidia viability in the freshwater mussel *Lampsilis fasciola* (Gillis, 2011).

Unio crassus Philipsson, 1788 is a native mussel species in Turkish waters. It was classified as having an 'endangered' status in 1990, which was later changed in 1994 to 'threatened' by IUCN (the International Union for Conservation of Nature). Although there have been several studies on this species in Turkey (İlhan *et al.*, 2004; Gürelli and Özbek, 2012; Ercan *et al.*, 2013c,e), its distribution and population status are poorly known. In addition, there have been no studies conducted on its salinity tolerance or the effects of salinity on its growth in natural conditions, despite its ecological importance and endangered status.

The aim of the present study was therefore to investigate the survival and growth of *U. crassus* from a Mediterranean-type stream in south-west Anatolia, Turkey, in a highly variable environment and showing a distinct salinity gradient. Our specific goals were to find out the salinity tolerance and effects of salinity on growth of *U. crassus* among different stream zones with variable salinity concentrations.

MATERIALS AND METHODS

Study site

Sarıçay Stream is situated in the south-western region of Anatolia, part of Muğla Province and drains into Güllük Bay (Fig. 1). The stream has a large reservoir, named the Geyik Reservoir, on its course and experiences a typical Mediterranean climate, with hot, dry summers and mild, wet winters. There are a number of human disturbances on the stream, mainly river channel regulations, destruction of the riparian zone, water abstraction for agriculture and solid waste inputs from fish feed and yogurt industries. The influx zone of the stream has brackish water springs, which makes the surrounding area suitable for earth pond marine aquaculture facilities. Salt concentrations in the water increase steadily towards the sea, reaching 6‰ in the estuary zone. Five sites were selected on the stream that had similar physical environments, but differed in their salinity regime of 0, 1.5, 3.0, 4.5, 6.0‰ for stations 1-5, respectively. Three of these sites (0, 1.5, 3‰) (*i.e.* first three stations) were located on the stream, whereas the other two sites (4.5, 6.0‰) (*i.e.* the fourth and fifth stations) were located on channels connected to the stream (Fig. 1, Table I).

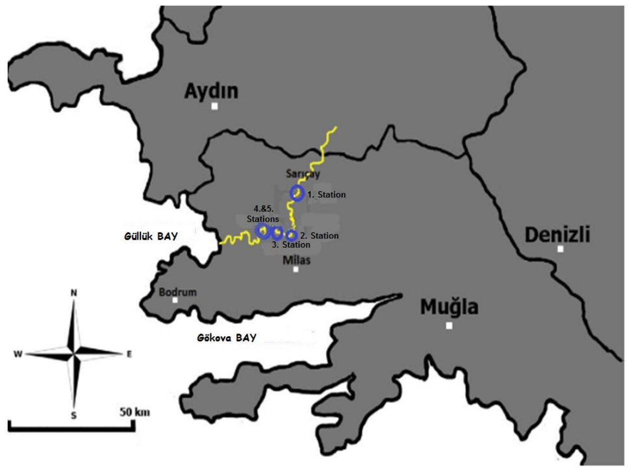

Fig. 1. Map of the study site (Sarıçay Stream) and the sampling stations.

The study was conducted between February and November 2011. A total of 250 similarly-size *U. crassus* specimens was collected by hand within its native range in the Tersakan Stream (Dalaman, Muğla) and transferred in ice to the Sarıçay Stream. Before collection, all individuals were checked for eggs availability and individuals that were not carrying eggs were selected for further analyses. All individuals were measured in three dimensions (width, height, and length) using digital calipers to the nearest 0.1 mm and their dry weight measured on an electronic balance to the nearest 0.01 g. To avoid biased calculation in dry weight, the mussels were first taken out of water and they were measured after flushing the water inside of the mussels by opening their shells. All measured mussels were placed into buckets with 5 mm mesh sizes and 50 L volume. Fifty individuals were placed in each bucket. No modifications were carried out on the mussels. All groups were placed at each station with a 1m s^{-1} flow rate, which was the same flow condition with the site of collection in the Tersakan Stream. Experimental mussels were checked monthly for a total of 286 days. Mussel size and weight were measured before and after the sampling period. The number of dead mussels was counted on each monthly visit. However, died mussels were not included in growth calculations, as all mussels were individually tagged.

The instantaneous growth rate (K) for length, width, height and weight, was calculated by modifying the following equation for each measurement (Malouf and Bricelj, 1989): $K = (\ln S_2 – \ln S_1)/t_2\text{-}t_1)$, where S_1, S_2 are respective size measurements (length, L; width, W; height, H; weight, We) at the beginning and end of the study, respectively. The duration of the study (in months) is expressed by t, (t_2-t_1). Relative growth rate (RGR %) was calculated: (W_2-W_1/W_1) × 100 where W_2 and W_1 is the final and initial weight, respectively.

Survival rate (%) was estimated according to the formula; $S = (N_t/N_0) \times 100$, where N_t is the number of live mussels removed from the buckets after t and N_0 is the number of mussels at the beginning of the study.

Water samples for nutrient analyses were collected monthly at each of the study sites throughout the sampling period and analyzed by Standard Methods for the Examination of Water and Wastewater (APHA *et al.*, 1985). Water samples for Chlorophyll-*a* content was filtered and extracted through ethanol. Following centrifugation, absorbance was measured before and after acidification in a spectrophotometer and then estimated (Ryther and Yentsch, 1957). Temperature, dissolved oxygen, conductivity, TDS, salinity and pH were measured in the field using a multi-parameter probe (YSI 556 MPS).

Differences in mean size of the mussels (length, weight and height) between the beginning and end of the experiment were tested with a t-test while size variations in growth rate among the study sites were determined using a one-way ANOVA, using population means for each variable. When significant differences among the sites were found, a Tukey HSD test was used to determine which sites were different. Chi-squared (x^2) tests were used for survival rate comparisons. $P < 0.05$ was accepted as the level of significance for all analyses.

RESULTS

Survival rates in three study sites were 60%, 56% and 66%, respectively by the end of the study (Table I). There were no statistical differences among the study sites ($\chi^2 = 0.196$, $P>0.05$), although mussels in station 3 had the highest survival rate. All mussels were dead in the fourth and fifth stations at the end of the first week of the experiment.

Length measurements of *U. crassus* at the beginning and at the end of the experiment showed no significant differences in all stations (t-tests, $P >$ 0.05). However, there were significant increases detected in other size measurements (height, width and weight) (t-tests, $P < 0.05$) for all study sites, with the exception of weight (t-test, $P = 0.571$) at the third station with a 3‰ salinity concentration (Table I, Fig. 2). Growth rates measured as length (ANOVA, $F = 0.111$, $P = 0.895$), width (ANOVA, $F = 0.248$, $P = 0.781$), height (ANOVA, $F = 0.893$, $P = 0.413$) did not differ significantly among study sites, but weight showed a significant difference, with the mean weight of mussels at the third station lower than the other two stations (ANOVA, Tukey HSD, $F = 31.331$, $P < 0.001$). Increase in weight was highest at the second station (19.29%) with moderate salinity concentration (1.5‰), while there was a weight loss at station 3 (-1.56%) with a higher salinity concentration (3‰) (Table II).

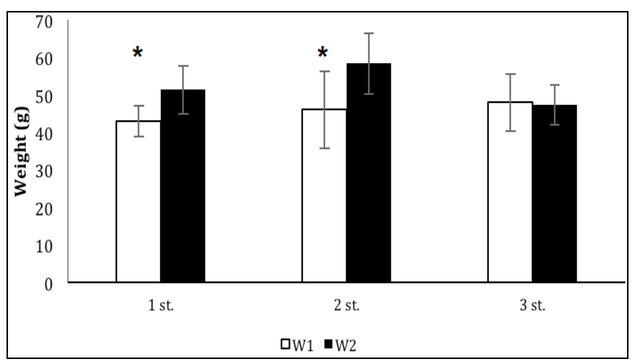

Fig. 2. Weight (g) of *Unio crassus* at study stations 1-3 at different salinity concentrations between the beginning (open-W1) and end of the experiment (dark-W2). Significant differences at $P = 0.05$ are denoted with an asterisk. Vertical bars on each column indicate standard deviations.

Table I.- **Differences in the number of individuals, survival rates, size measurements (weight, length, width and height) and relative growth rate as percentage of *Unio crassus* at the beginning and the end of the study from five sampling stations in the Sarıçay Stream.**

	Stations				
	1	**2**	**3**	**4**	**5**
Initial no of individuals	50	50	50	50	50
Final no of individuals	31	28	34	0	0
Survival rate (%)	62	56	68	0	0
Initial weight	42.92±4.06	46.03±10.21	47.97±7.67	47.05±8.17	49.03±9.23
Final weight	51.29±6.46	58.32±8.18	47.37±5.32	-	-
Initial length	64.39±2.09	66.07±3.97	64.27±2.57	66.82±2.68	68.31±4.12
Final length	64.63±2.39	66.70±3.80	64.74±2.74	-	-
Initial width	42.41±1.69	42.54±2.43	41.48±1.60	43.52±1.82	44.21±1.96
Final width	44.46±1.71	45.12±2.04	44.18±1.65	-	-
Initial height	25.15±1.20	25.5±2.18	23.76±1.32	25.38±1.44	26.17±1.23
Final height	26.67±1.27	28.05±2.58	26.13±1.23	-	-
RGR (%)	17.38	19.29	-1.56	-	-

Table II.- **The instantaneous growth rate (*K*) of *Unio crassus* for length (L), width (W), height (H) and weight (We) at study stations 1-3 on the Sarıçay Stream.**

K	K (L)	K(W)	K (H)	K (We)
1st station	0.0006	0.005	0.005	0.010
2nd station	0.0004	0.005	0.007	0.020
3rd station	0.0007	0.004	0.007	0.001

Mean environmental parameters measured in Sarıçay Stream during the study period are presented in Table III. The first two stations had similar values for physico-chemical measurements, showing higher temperature but lower conductivity, TDS, *orto*-phosphate and chlorophyll-*a* values than those at other stations (Table III). Higher TDS and conductivity values in the last two stations (fourth and fifth) are probably due to the high correlation of these variables with salinity. However, both these stations, along with third station, had notably higher chlorophyll-*a* concentrations (Table III). Other chemical components, which are indicative of trophic status of the water, were similar among all sampling stations (Table III).

DISCUSSION

The results of the present study represent the first findings of salinity tolerances and growth responses of the freshwater mussel, *U. crassus* and indicated that moderate salinity concentrations (1.5‰) supported the greatest weight increase but least survival rate among different salinity levels examined, although these rates were not significantly different among the sampling sites with different salinity concentrations, with the exception of 4.5‰ and 6‰ where all mussels died. This finding suggests that the salt concentrations are increasingly ideal for growth up to 1.5‰ for *U. crassus*, but higher salt concentrations than 3‰ were lethal for this freshwater mussel species. This would be expected as elevated salinity can trigger the growth of aquatic organisms within certain limits, a well-known phenomenon for several fish and mussel species (Blakeslee *et al.*, 2013; Gonzalez, 2011).

Unfortunately, there were no available data on the effect of salinity on the survivorship and the growth of *U. crassus* from other field studies that can be used to compare with our data. Other available studies have usually been conducted on common marine mussel species, such as *Meretrix meretrix*, *Katelysia opima*, *Mytilus viridis* and *Mytilus edulis* and freshwater species *D. polymorpha*, *E. complanata*, and *L. fasciola* (Ranade and Kulkarni, 1973; Fong *et al.*, 1995; Gillis, 2011; Blakeslee *et al.*, 2013). These studies have clearly shown profound effects of salinity on growth, breeding and distribution of the mussel

Table III.- Mean environmental variables at the sampling stations on the Sarıçay Stream.

	Stations				
	1	2	3	4	5
Number	10	10	10	1	1
Temperature (°C)	21±5.91	20.6±5.40	16.9±1.33	18	17.5
Conductivity (µS/m)	356.5±100.53	384.5±98.07	431±178.80	672	1071
TDS (mg/L)	0.24±0.05	0.27±0.06	128.09±63.92	3.88	6.62
Salinity (‰)	0.1±0.04	1.5±0.05	3±0.08	4.5	6
DO (mg/L)	7.38±2.51	9.14±3.13	9.89±2.42	8.3	8.9
pH	7.79±0.95	7.22±0.93	7.09±0.66	6.78	7.06
Nitrite-N (mg/L)	0	0	0	0	0
Nitrate –N (mg/L)	0.70±0.01	1.32±0.02	0.75±0.01	1.95	1.78
Ammonium- N (mg/L)	0.11±0.02	0.01±0.001	0.11±0.01	0.29	0.18
o-phosphate (mg/L)	1.05±0.03	0	1.99±0.04	2.08	1.53
Suspended solids (mg/L)	0.13±0.01	0.13±0.01	0.14±0.01	0.32	0.26
Cl-*a* (mg/m^3)	10.12±0.21	4.18±0.07	21.35±4.98	25.16	20.56

species. Similarly, the larvae of mussel species seem to be strongly influenced by salinity (Tham *et al.*, 1972). Blakeslee *et al.* (2013) studied the effects of salinity on several life stages of the freshwater mussel *E. complanata* in experimental conditions, demonstrating that salt concentrations as low as 4‰ resulted in mortality after only 48 hours of exposure, concentrations as low as 3‰ disrupted reproductive success, and concentrations as low as 2‰ significantly depressed metabolic rate. These results are in accordance with our findings, which showed the survivorship at 3‰ was the limit for the survival of *U. crassus* in nature. However, it should be noted that there could be some differences between experimental and natural conditions, where there are many more confounding environmental variables.

The growth of the mussels, measured as mean weight or size gain, may dependent on several factors. Indeed, growth of *U. crassus* has been demonstrated as variable depending on the environment in which they live (Akyurt and Erdoğan, 1993; Ercan *et al.*, 2013e). Therefore, in addition to salinity, it is expected that growth may vary along a gradient of ambient natural factors, such as nutrient availability and water temperature. Temperature, for instance, is known to have a major impact on mussel growth because it affects metabolism and the timing of reproduction (McMahon, 1991). In the present study, the temperature was higher (21±5.91) in the first two stations, with the second station slightly colder (20.6±5.40) than first station while the third station had notably lower (16.9±1.33) than that in other stations. This difference would explain the reduced growth of *U. crassus* in station 3, which had a 3‰ salinity concentrations. However, higher weight gain at the second station could not be attributed to temperature since the first two stations had similar mean temperatures throughout the study period.

In parallel with temperature, nutrient availability had a similar role for growth variation of *U. crassus* among study sites, which were highly variable. Despite significantly higher chlorophyll-*a* values at station 3, weight increase was limited. This would suggest that the growth of *U. crassus* was limited by salinity rather than other environmental conditions, such as temperature and food availability (Ranade and Kulkarni, 1973; Gillis, 2011; Blakeslee *et al.*, 2013; Ercan *et al.*, 2013e). Increased growth of the species at a certain salinity concentrations (1.5‰) is indicative of importance of this variable (Ercan *et al.*, 2013b). The study site (Sarıçay Stream) receives numerous inputs from agricultural, industrial and municipal sources, which might lead variable growth and survival patterns for mussels in different sections of the stream. However, the effects of the analyzed variables for *U. crassus* were strongly dependent on salinity variation in the stream. This effect can be explained by the fact that *U. crassus* is tolerant of relatively low water quality conditions and could be associated with nutrient-enriched waters (Ercan *et al.*, 2013e). Alternatively, this might be due to additional variables, which were not revealed as key causative factors. The measurements taken in the present study were obtained from water only, not sediments, yet it is known that mussels receive energy from both resources (Salazar and Salazar, 2001).

The low survival rate of *U. crassus* has been linked to nitrate concentrations between 2 and 10 mg L^{-1} (Köhler, 2006). This was not the case in Sarıçay Stream since nitrate concentrations measured at all of stations never exceed 2 mg L^{-1}. Healthy *U. crassus* populations exposed to nitrate levels varying from 0.4 mg L^{-1} to 0.6 mg L^{-1} have been reported from a similar stream in northwestern Turkey (Ercan *et al.*, 2013e). Salinity, on the other hand, is certainly a limiting factor for the survival of *U. crassus*, as all mussels were dead at salinity levels of 4.5‰ and 6‰. Salinity is known as the most limiting factor for the widely-distributed mussel species *D. polymorpha* in European brackish waters (Strayer and Smith, 1993), although reproduction of this species was reported to be possible in brackish water below 7‰ when mussels are acclimated to brackish water (Fong *et al.*, 1995). This may suggest that mechanisms may have evolved for surviving and reproducing when mussels are exposed to brackish waters. This seems to be case for *U. crassus*, which adapt to varying salinity concentrations, within certain limits, in Sarıçay Stream. However, while not examined in the present study, a negative effect of increased salinity on glochidia production has been observed (Blakeslee *et al.*, 2013). Other similar studies have also reported that 24 h of salt exposure from 0.1‰ to 2.0‰ decreased glochidia viability in several mussel species, but the lethal concentrations are variable among mussel species (Bringolf *et al.*, 2007; Cope *et al.*, 2008; Gillis, 2011). These studies clearly indicated that salinity had a dramatic effect on the reproduction of freshwater mussels by reducing successful host infestation of glochidia (Gillis, 2011; Blakeslee *et al.*, 2013).

From the results of this study, which constitute the first evaluation of the effect of salinity on *U. crassus* under natural conditions it can be concluded that *U. crassus* could successfully be reared in a salinity range 0-3‰, ideally at 1.5‰. It should be noted that our results represent a long-term evaluation and should be further re-studied with short-term experiments for assessing survivorship and growth. Furthermore, since the effects of salinity could differ depending on season, seasonal effects should also be experimentally examined. If salinity stressors have important consequences for the reproductive success of freshwater mussels, salinity is also likely to impact their population dynamics and persistence. This may become an increasingly important issue given increased worldwide salinization of freshwater ecosystems.

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