Effect of shell height on the reproductive success and survival of *Cornu aspersum* (O. F. Müller, 1774)

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Abstract. The study was carried on *Cornu aspersum* (synonym: *Helix aspersa*) in the Black Sea region, Turkey. The snails were placed in two separate treatments called G1 (28.00-33.99 mm) and G2 (34.00-39.99 mm) at the artificial hatchery. Mean shell height and live weight were 32.22±0.06 mm and 8.96±0.07 g in G1; 35.40±0.06 mm and 11.76±0.09 g in G2, respectively. The spawning rate in G1 was lower than G2 ($p \leq 0.05$). The survival rate was 78.18% in G1 and 81.14 % in G2 after spawning; 86.63% in G1 and 94.12% in G2 after hibernation, respectively. The mean egg weight was 0.04 g in G1 and 0.05 g in G2 while the shell height and live weight of juvenile were 5.56±0.05 mm and 0.06g in G1; 5.81±0.07 mm and 0.07 g in G2, respectively. The results showed that larger eggs were laid by bigger size adults and after hatching bigger size juveniles were obtained due to larger egg diameter. In addition, the results of experiment confirmed the hypothesis suggested about survival that newly matured individuals could have insufficient energy reserves while larger ones can not withstand the excess energy for reproduction. Thus, the body size of *C. aspersum* is correlated with reproductive performance and survival rate.

Key Words: *Cornu aspersum*, land snail, reproduction, survivorship.

Introduction. *C. aspersum* (synonym: *Helix aspersa*) is a land snail species in the family Helicidae which is hermaphroditic and iteroparous, living from 4 to more than 8 years according to its geographical location or conditions of life (Dekle et al 2014). Heliciculture is regarded as lucrative and sustainable agricultural activity with economic, social and environmental aspects and has high profit margin with a low capital cost (Hatziioannou et al 2014). Nowadays, the main producers of snails were turn to farming facility not only for a country's balance of trade, but the security and health of its population as well (Adinya 2010). Therefore, snail farming in many countries is gradually raising up. Knowledge on physiology, growth, reproduction behavior and nutrition requirement of animal is important for successful snail farming. However, the profit is related closely to reproductive efficiency. It is significant to properly improve the reproductive performance of animal during farming period. Thus, the correlations between egg production, clutch dimension and snail size have been studied previously (Madec et al 1998; Dillen et al 2010).

Turkey has the geographical and climatic potential for land snail (Cook 1997). The production is an important source of income and export item while only based on gathering individuals from wild populations. In recent years, the statistical data of TUIK (2017) reported that terrestrial snail production has a decline from 2.227 tons in 2009 to 1.547 tons in 2014 which is related to over harvesting, heavily-used agricultural chemicals and global climate change. When this situation is taken into account, farming facilities should be developed and supplied as soon as possible in Turkey. Thus some methodological approach is needed for the development of this sector (Yıldırım et al 2014). There are no known studies available on farming as models for entrepreneurs interested in snail breeding and no data are available on the reproduction of *C. aspersum* at the Black sea region. The present study aimed to investigate the effects of snail size on survival, fertility and egg production.

Material and Method

Experimental design. The study was carried on *C. aspersum* (Gastropoda: Pulmonata: Helicidae) in Sinop, Black Sea region, Turkey between September 2014 and January 2015. A galvanized steel container (240 x 600 x 250 cm) was designed as snail hatchery containing six hutch shelf (75 x 200 cm) which was made in wooden for the experiment. The moisture was supplied by fogging machine (75-90% humidity) and temperature (17-20°C) was arranged by air conditioning system in the hatchery. Daily temperature and humidity were measured during the morning (8.00-9.00 am) and night periods (08.00-09.00 pm) with a direct-reading instrument (TFA Dostmann elektronisches Thermo-Hygrometer 30.5013). Artificial hatchery remained on natural period (12h light: 12 h dark) for Sinop. Some plastic flowerpots (10 × 11 × 10 cm) were filled with moist soil with depth of 10 cm and also placed in the containers. All snails were fed ad libitum with the same composite snail food (Murphy 2001).

The snails (989 individuals) with a mean height of 33.79±0.06 mm with a range of as 28.32 and 39.95 mm and a mean live weight of 9.31±0.07 g with a range of as 4.05 and 18.04 g were collected from wild populations between May and August 2014. They subsequently artificial aestivated until 10 September in polythene boxes in the faculty building until starting the experiment. The snails were housed in the artificial hatchery at the 10th of September when optimal reproduction condition was supplied (75-90% humidity and 17-20°C temperature) and snails became active (Daguzan 1982) in two days. Afterwards, the healthy snails (without shell anomaly, actively feeding and moving) were selected for the experiment from the samples at the 15th of September. The shell height of each individual was measured to the nearest 0.1 mm with a caliper before separating the snail into two treatments. The experimental design was completely randomized with triplicate for each treatment. Each group in triplicate consisted of 100 ind m^{-2} (each shelf of 150 individuals). Snails were placed in two separate treatments; G1 (28.00-33.99 mm) and G2 (34.00-39.99 mm). Mean shell height was 32.22±0.06 mm in G1 and 35.40±0.06 mm in G2; mean live weight was 8.96±0.07 g in G1 and 11.76±0.09 g in G2, respectively. Initial shell height of each treatment were significantly different ($p \leq 0.05$).

Reproduction performance. Reproduction performance of groups is determined by evaluating fecundity and hatchability. Fecundity is defined as the number of eggs produced by an individual in each treatment; the number of eggs per egg-mass per snail were recorded every 24 hr. White transparent plastic boxes 18 x 13 x 4 cm with depth of 2 cm moist soil were used to keep eggs for incubation. The incubation period was carried out under artificial hatchery condition. Egg hatching was monitored every 24 h to determine hatchability until all eggs were hatched. Baby snails were removed as soon as possible after birth and immediately started feeding to avoid egg and juvenile cannibalism. Egg weight in each treatment were determined during egg-laying period. One of plastic boxes was selected randomly and eggs were measured for egg volume. Hatchability rate was found from following formulates:

Hatchability (%) = (total number of snail hatched out)/(total number of eggs)

After hatching period, one week old juveniles were counted and transferred to another plastic box with density of 2 ind cm^{-2} for per group (García et al 2006). Shell height and live weights of juveniles for each group were measured on randomly selected of 100 snails at weekly intervals and also counted every week to determine weekly survival rate from 1st week to 5th week.

After laying period, adult snails were counted and artificial hibernated at 4°C and darkness from 14 November to 26 January (74 days) at the hatchery. After hibernation time, the condition of hatchery regulated optimal temperature and humidity value, then snails became active and after that counted again for survival rate according to groups. The following formula was used for determining the survival rate:

$$\text{Survival rate (\%)} = 100-[100\ (Nt/N_0)]$$

where: Nt is the number of death snails removed after t time and N_0 is the number of live snails at the beginning.

Analyses. Data were tested for normal distribution and one-way ANOVAs was applied to compare groups, with significance level of $p < 0.05$. Statistical analyses were carried out by software program MINITAB 17 (Minitab Inc., State College, PA, USA).

Results and Discussion. The environmental condition was relatively stable in the hatchery. The mean temperature was found 19.29±0.34°C and ranged from 18.00±0.71 to 20.57±0.49°C and mean humidity was 82.85±0.83% and ranged from 78.91±0.68 to 85.93±0.82%. Daily temperature and humidity values were not differed significantly ($p > 0.05$).

Shell height frequency of snails before reproduction, after spawning and after hibernation were shown in Figure 1.

Height-weight relationships of G1 was found as W = 0.0014xL2.5227 (r = 0.5084) while the relationship of G2 was determined as W = 0.006L2.7606 (r = 0.6329) before spawning time. The mean shell height and live weight of snails were determined before and after spawning and after hibernation and shown in Table 1.

Table 1

The shell height (SH) and live weight (LW) of snails before and after spawning and after hibernation

	G1			*G2*		
	SH (mm)	*LW (g)*	*Number*	*SH (mm)*	*LW (g)*	*Number*
Before spawning	$32.22±0.06^a$	$8.96±0.07^a$	440	$35.4±0.06^a$	$11.76±0.09^a$	440
After spawning	$31.87±0.06^a$	$9.18±0.06^b$	364	$35.2±0.06^a$	$15.41±0.07^a$	357
After hibernation	$32.14±0.07^b$	$8.36±0.06^c$	319	$35.29±0.06^a$	$10.29±0.07^b$	336

Different superscripts are in the same row significantly different ($p < 0.05$).

After acclimatization of two weeks, snails started laying eggs in 22th of September and lasted 41 days. Spawning rate of snails were lower in G1 (38.40%) than G2 (47.05%) ($p \leq 0.05$). Partial spawning was also observed. Fecundity in G1 was higher (79.90±4.08) than G2 (75.15±4.43) but no statistical differences were determined ($p \geq 0.05$). The survival rate was found as 78.18% in G1 and 81.14% in G2 after spawning; 86.63% in G1 and 94.12% in G2 after hibernation, respectively. The significant difference was determined between two groups in survival rates after spawning and hibernation ($p < 0.05$).

Hatching time of eggs was recorded between 12 and 16 days. The mean egg weight was 0.04 g in G1 and 0.05 g in G2 while coefficient of variation (CV) was 10.41 in G1 and 20.51 in G2. There was significant difference between the groups weight ($p < 0.01$). Juveniles, hatched within the same time, were selected and measured shell height and live weight for five weeks (Table 2).

Table 2

The shell heigh (SH) and live weight (LW) of juvenile snails in five weeks

	G1		*G2*	
	SH (mm)	*LW (g)*	*SH (mm)*	*LW (g)*
First week	$4.66±0.04^a$	0.03^a	$4.90±0.04^a$	0.04^a
Second week	$4.83±0.04^b$	0.04^b	$5.12±0.04^b$	0.04^a
Third week	$4.95±0.04^c$	0.04^c	$5.16±0.05^c$	0.05^b
4th week	$5.15±0.04^c$	0.05^d	$5.62±0.05^c$	0.07^b
5th week	$5.56±0.05^d$	0.06^e	$5.81±0.07^c$	0.07^c

Different superscripts are in the same row significantly different ($p < 0.05$).

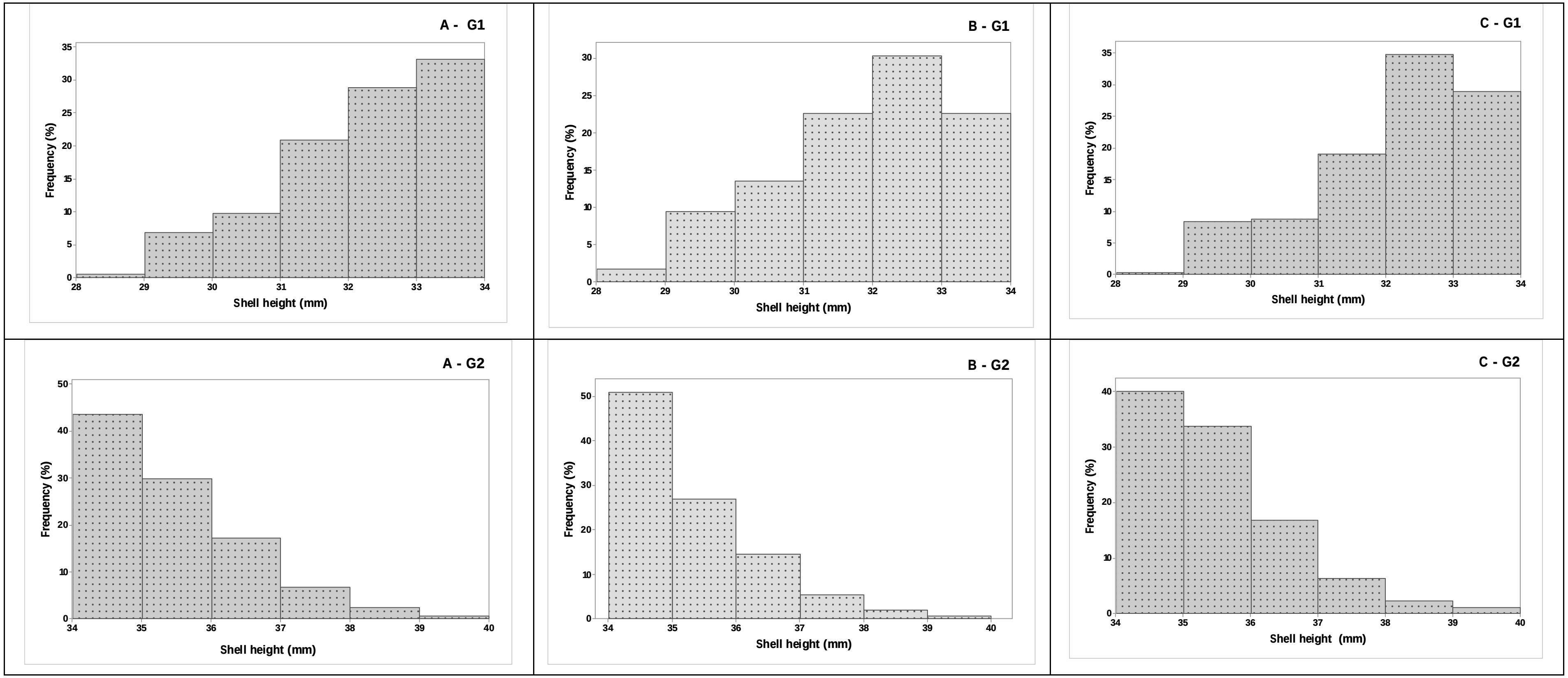

Figure 1. Shell height of snails on before spawning (A), after spawning (B) and after hibernation (C) in G1 and G2.

CV of shell height was 10.67 in G1 and 8.10 in G2 at the first week while it was 11.39 in G1 and 10.41 was in G2 at the fifth week. The significant differences were determined with regard to shell height and live weight, between the G1 and G2 ($p < 0.01$). The shell height–weight relationships of juveniles was showed $r = 0.8588$ in G1 and $r = 0.9366$ in G2. After five weeks, survival rates of juveniles were 88.24±2.65% in G1 and 89.24±2.04% in G2 (Figure 2).

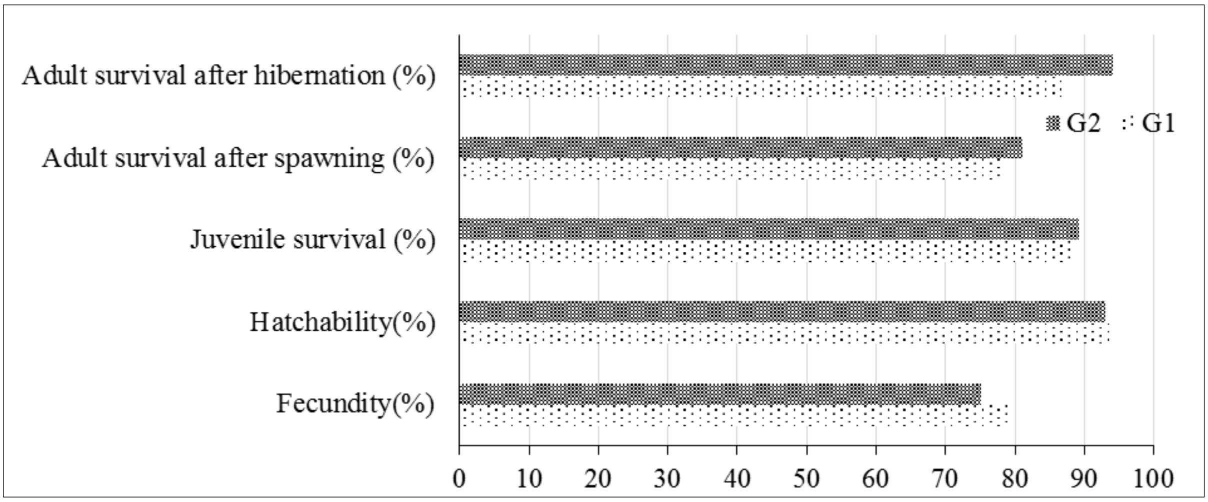

Figure 2. The comparative graphic representing the differences of the fecundity (%), hatchability (%), survival rate before spawning (%), survival rate after spawning and hibernation (%) and also survival rates of juvenile snails (%) in each group.

Environmental conditions affects reproductive function of land snail. Optimal temperature, humidity and photoperiod (20±1°C, 80±5% and 16:8 light dark cycle) encourage breeding activity of *C. aspersum* (Jeppesen & Nygard 1976; Daguzan 1982; Clutton-Brock 1988). In the present study, no problems were counted in spawning because snails were awaked on an annual life cycle autumn reproduction and temperature and humidity conditions were arranged optimal and sufficient food supplied to allow ad libitum feeding and also photoperiod synchronized with sunrise and sunset in September at the artificial hatchery for encouraging spawning. The maintenance of optimal interior temperature and humidity levels was a key component to encourage spawning of snails in the hatchery. There was not statistical difference between the two different size groups in fecundity. This was conflicting data compared to the many research. Reproductive output is an increasing function of body size (Jeppesen & Nygard 1976). Larger snails from the same population tended to have greater reproductive output (Carter & Ashdown 1984). The reproduction of land snails has positive relationship between snail size and fecundity (Cowie 1984; Baur & Baur 2000). But Foster & Stiven (1994) reported that reproductive output is independent of size in the land snail *Mesodon normalis* because of that experimental snails were collected from different populations. This report supported our results that the matured snails in our study were collected from the wild and represented different populations.

Larger individuals produced heavier eggs (Dillen et al 2010). We also identified the egg size of G2 was bigger than G1 which showed that high resource of G2 for reproduction therefore invested a greater proportion for egg production. These results demonstrated that body size variation affected egg production. Snail size illustrates total energy budget which can be invested in reproduction thus large individuals have more resources to invest in reproduction. Many authors reported similar results that larger snails have more energy reserves for reproduction than smaller ones (Roff 1993; Madec et al 1998; Angeloni et al 2002). The egg size is one sign of reproductive investment, along with nutrient content, yolk quantity etc., which affects larval fitness. Larval survival was related to egg size (Ito 1997; Barker 2001; Gołąb & Lipińska 2009). The data of present study provides a better understanding of parental effects on juvenile size. The results showed that larger eggs were laid by bigger snails (G2) and after hatching bigger juveniles were obtained from G2 due to larger egg diameter. Based on the present study, we suggested that egg weight and juvenile size were highly heritable characters and regulated by adult characteristics. Bernardo (1996) reported that heritability of body size, was a function of the parent's condition and reflects habitat quality. The body size of juveniles in early growth stages was correlated with the parent size, however there was no such correlation when offspring aged 2 and 3 months (Gołąb & Lipińska 2009). Our experiment demonstrated similar results with Gołąb & Lipińska (2009) that some snails grew faster and depressed the growth and as a result, percent coefficient of variation in height and weight of juveniles in both groups was gradually increased especially in larger size group. This situation might lead to developed social hierarchy among individuals and cause to increase size variation. Increased competition can lead to increased size variation (Peacor et al 2007).

Land snails display reproductive strategy that serves to decrease the mortality in different conditions. Various authors reported similar results. Higher fecundity brings along the risk of mortality, therefore adults might postpone spawning season for survival (Nicolai et al 2010). Reproduction before hibernation represents an additional cost that decreases the survival rate during hibernation (Lazaridou-Dimitriadou & Kattoulas 1991). Larger snails have a certain thermal inertia, which makes them cool more slowly and will have an impact on their survival (Ansart & Vernon 2004). Smaller animals cool more quickly and are less tolerant to exposure to low temperatures (Loomis 1991). Different size in land snails reflected different water content of body which reflects their differential ability to enter into hibernation and stay at a low metabolic rate (Charrier 1980). Murphy & Johnson (1980) declared that an increase in the body weight caused a significant increase in freezing tolerance (Murphy & Johnson 1980). All these studies on the effects of body size on survival supported our results. After spawning period, the height-frequency histogram of G1 clearly indicated that the distribution in 33-34 mm decreased nearly 11% while according to the G2, the bar in 35-38 mm was decreased nearly 7% which showed that the most of mortality rate in two groups might be occurred in the 33-34 mm in G1 and 35-38 mm in G2 corresponded to reproduction. At the same way, after hibernation the survival in G2 was higher than G1. When the results are evaluated in terms of death after spawning, matured snail mortality strongly coincided with the reproductive and energy needed for gonadal development might be resulted physiological exhaustion and thus, death is an organism's response. The reason of death of newly matured individuals in G1 could be due to insufficient energy reserves. Baur & Raboud (1988) findings support our hypothesis that reproductive investment was negatively correlated with shell size and body weight in all populations which means that one egg represents a higher investment for a smaller snail than for a larger one. Thus the extent of reproductive investment affects survivorship. High production could contribute to the reduced life span recorded in younger adults (Madec et al 2000). The present study also suggested that the larger size might not withstand the cost of reproduction because of consuming excess or insufficient energy for reproduction which threatens the survival of snails in G2. Reproduction might drain energy from somatic stores, resulting in a decreased capacity to mount an effective immune response or to produce enough protein, or other molecules, to protect against damage from stress and toxicity (Baur & Raboud 1988). Resources that are diverted into reproduction cannot also be used for somatic repair and maintenance, and reproduction will hence shorten life-span (Harshman & Zera 2007). Snails reduce their egg output to save energy for hibernation, and increased survival. Thus, the body size of *C. aspersum* correlated with reproductive performance and survival rate.

Conclusions. The results of this study supported hypothesis that snail size influence reproductive performance and related mortality. Bigger size adults laid larger eggs and after hatching bigger juveniles.

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