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The Evaluation of the Reproductive Performance of Land Snail, *Cornu aspersum* (Müller, 1774) without Photoperiod in the Non-breeding Season

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ABSTRACT: The purpose of this study was to investige the reproductive success of land snails (*Cornu aspersum*) without photoperiod during the non-breeding season in the Black Sea Region between December 2019 and January 2020. Snails, which were placed in cold storage for a hundred days, were awakened in December. The reproductive performances were assessed under semi-controlled laboratory conditions using the natural daylight cycle and the ideal temperature and moisture. The experiment was designed as a single group with 3 replications. The experiment was designed 3 replications, with 10 snails in each replicate in total of 30 snails. The reproductive performance of the snails was observed daily and the first mating took place on the 9th day of the study and the first egg-laying was detected in the second week of the study. The fecundity of the snails was 43%. Spawning activity was continued through to 3 weeks. The mean number of eggs, hatching rate and the number of offsprings were 151.13±22.02, 90.33±2.39% and 138.13±21.09, respectively. The mean egg and offspring live weights were 0.034±0.001 g and 0.03±0.007 g, respectively. The findings showed that the reproductive performance was low. In conclusion, reproductive performance is unsuitable for commercial snail production without the photoperiod treatment during the non-breeding season.

Keywords- *Land snail, reproductive performance, breeding*

Cornu aspersum (Müller, 1774) Türü Salyangozlarda Üreme Mevsimi Dışında Fotoperiyot Uygulamadan Üreme Performansının Değerlendirilmesi

ÖZET: Bu çalışma, Karadeniz Bölgesinde *Cornu aspersum* türü kara salyangozlarının doğal üreme dönemi olmayan dönemde, Aralık 2019-Ocak 2020 tarihleri arasında fotoperiyot uygulanmadan üreme başarısını gözlemlemek amacıyla yapılmıştır. Yüz gün süreyle soğuk hava deposunda bekletilen anaç salyangozlar, aralık ayında uyandırılmıştır. Üreme performansları, doğal gün ışığı döngüsü, ideal nem ve sıcaklık kullanılarak yarı kontrollü laboratuvar koşullarında değerlendirilmiştir. Deneme tek grup ve üç tekerrürlü olacak şekilde tasarlanmıştır. Deneme başladıktan sonra salyangozların üreme performansları günlük olarak gözlemlenmiştir. İlk çiftleşme çalışmanın dokuzuncu gününde gerçekleşmiş ve çalışmanın ikinci haftasında ilk yumurtlama tespit edilmiştir. Çalışmada salyangozların üreme performansı % 43 olarak bulunmuş olup yumurtlama aktivitesi üç haftaya kadar azalarak devam etmiştir. Ortalama yumurta sayısı, yavru çıkış oranı ve yavru sayısı sırasıyla 151.13±22.02, %90.33±2.39 ve 138.13±21.09 olarak bulunmuştur. Ortalama yumurta ve yavru ağırlıkları sırasıyla 0.034±0.001 g ve 0.03±0.007 g olarak tespit edilmiştir. Çalışmadan elde edilen bulgulara göre salyangozların üreme performanslarının düşük olduğu belirlenmiştir. Sonuç olarak, doğal üreme dönemi dışındaki bir dönemde fotoperiyot uygulanmadan elde edilen üreme performansının ticari salyangoz üretimi için uygun olmadığı sonucuna varılmıştır.

Anahtar Kelimeler- *Kara salyangozu, üreme performansı, yetiştiricilik*

1. Introduction

Heliciculture is accepted as a profitable and sustainable agricultural activity with its economic, social and environmental aspects, low capital cost and high-profit margin (Hatziioannou et al. 2014). For this reason, snail farming is increasing in many countries, and also in Turkey. It is necessary to understand the physiology, reproductive behavior, and nutritional requirements of animals for successful snail breeding. The success of farming is closely related to the efficiency of reproductive performance of land snails (Charrier, 1980; Bailey, 1981; Enée et al. 1982; Aupinel and Daguzan, 1989; Gomot et al. 1990). All these studies showed the importance of photoperiod on reproductive regulation. Gomot et al. (1989) demonstrated a temperature-photoperiod relationship in snails and a photoperiod-dependent control of ovulation and egg-laying. The authors also evaluated the effect on the day length and temperature related reproduction. Photoperiods (18L:6D) and temperature of 20°C are the most favourable condition for egg-laying on long-day; photoperiods (18L:6D) and temperature of 15°C inhibits egg-laying, while a temperature of 20°C partially egg-lays (8L:16D) in short-day.

Today, it is possible to get offspring three or four times a year in intensive heliciculture. Reproduction can be achieved by regulating the photoperiod, favourable day length and optimal temperature in a non-breeding season. Another side, the current agricultural studies mainly focus on developing strategies for improving production methods with less labour. In this study, we aim to investigate the possibility of obtaining offspring from *C. aspersum* without photoperiod application with optimum temperature and optimum moisture in the non-breeding season.

2. Materials and Methods

Study Organism

Cornu aspersum (synonym: *Helix aspersa*) is abundant in Turkey, particularly in the Black Sea region. The climate condition of region is a major ecological component of phenological processes in *C. aspersum* populations. Optimal temperature, humidity and photoperiod were 20±1°C, 80±5 % and 16:8 light dark cycle for the reproduction of *C. aspersum* (Clutton-Brock, 1988). Shell diameter of adult snails is between 30 and 32 mm and maturation are characterized by a reflected lip at the shell aperture (Ansart and Vernon, 2004). In Black Sea region, *C. aspersum* has two main breeding seasons during the year, one in February to May and second in September to October (Çelik et al. 2018*). C. aspersum* is hermaphroditic and iteroparous, with a lifespan ranging from 4 to over 8 years depending on its geographic location and living conditions. Adult snails' mate 1 to 7 times every reproductive season under favorable conditions, with a mean clutch size of around 100 eggs (Madec et al. 1998).

Experimental Design

The experiment was conducted in the laboratory of the Faculty of Fisheries, Sinop University between December 19, 2019 and February 04, 2020. *C. aspersum* species with length of 33.25±0.49 mm and live weight of 10.53±0.08g were collected from their natural habitats on September 20, 2019. The effect of snail size on reproductive performance has been ignored

since snails are almost all the same size. They were placed in cold storage (+4ºC) on the same day and allowed to sleep for 100 days. The snails were removed from the cold storage on December 12 and have been allowed to adapt to room weather conditions (22ºC ±1) for a week. During this period, the snails were moistened and fed with lettuce, carrot, cucumber and apple. The experiment was designed as one group with 3 replications, and 30 snails were used with 10 snails in each replicate. After ten days of adaptation, the study was started in semi-controlled laboratory conditions under natural photoperiod, avoiding direct sunlight on December 21, 2019 and the temperature of the laboratory was kept constant by air-conditioning. The translucent plastic boxes (20.5x20.5x7.5 cm) were used as experimental boxes which were designed; with 2 cm wet natural pre-sterilized soil in the bottom, soil-filled containers with 7 cm depth, a water-filled petri dish, a plastic tray for the feeding area. The experimental boxes were cleaned daily to avoid the negative effects of excreta, mucus and leftover food. The relative humidity of plastic boxes was maintained at 80%- 85% by water spraying twice a day in the active and rest phases of snails.

Diet preparation and feeding

The snails were fed *ad libitum* with artificial feed in the form of flour, which was prepared according to organic diet rules (Blair 2008), and lettuce, green apple, carrot, cucumber and tomato were given alternately with feed every day. The main content of artificial diet was corn flour (60%), wheat flour (20%), limestone (15%), dicalcium phosphate (4.8) and vit-min. mixture (0.2%) and its main constituents were crude protein (16.52%), crude lipid (1.29%) and calcium (19.80%). Prepared feeds were stored at +4°C in a glass container until used in the study.

Measurements and Analyses

All snails were individually weighed at the beginning and end of the egg-laying, using a digital scale (with ±0.0001 precision). The experiment was started with snails 10.53±0.08g.

The soil container was checked for the presence of eggs and laid eggs were carefully removed from the containers. The number of spawning snails was determined. Eggs number (EgN) were determined. Ten eggs from each clutch were randomly taken and measured weight (EgW). All eggs were then incubated. The newly hatched offsprings was counted (OfN). Ten offspring were randomly selected and measured. ImageJ software was used for image analysis to obtain the egg and offspring sizes.

Hatching rate (HR) and spawning rate of groups (SpR) were determined according to the following formulas;

HR = (Hatched offsprings / Total eggs) × 100
SpR (%) = [(The number of total snails) / (The number of spawned snails)] × 100

Statistical Analyses

Data were tested for normal distribution. Then, an analysis of the Kruskal-Wallis test was used to determine statistical differences between weeks, with a significance level of $p<0.05$.

3. Results and Discussion

Generally, the natural breeding season of snails in the Black Sea region of Turkey is from March to May in the spring and from September to November-December in the autumn period (Duman and Çelik, 2019). Optimal temperature, humidity and photoperiod (20±1°C, 80±5% and 16:8 light dark cycle) encourage breeding activity of *C. aspersum* (Clutton-Brock, 1988). In this study, the environmental parameters of laboratory were kept stable; the average temperature was 23°C and, the average humidity was 85% in resting time (daytime). While food consumption was good at the beginning of the study, it decreased toward the last weeks. The reproductive performances of snails collected during the breeding season were evaluated by awakening them in January, which is nearly 15 hours a night and 9 hours a day. The first mating in snails began about nine days after awakening from hibernation. Spawning occurred 14 days after mating and, spawning activity continued for three weeks (Table 1). It was determined that 13 snails lay eggs only once. At the end of the study, the fecundity is 43% and the snails lay eggs once lay an egg. The obtained fecundity was low compared to the related studies. Çelik et al. (2018) reported that the hatching rate of *C. aspersum* was 90%. Snails reared in long-day photoperiods had more matings and layings, and longer reproduction durations, according to Benbellil-Tafoughalt et al. (2011). In contrast, snails hibernated in short-days, egg-laying stopped early and lasted only 6 weeks at 20°C. Bailey (1981) found a strong link between photoperiod and reproductive activity in *Cornu aspersum aspersa*. *Cornu aspersum aspersa* snails, obtained from natural habitats in France, stopped spawning after only four weeks when exposed to short daylight periods (6-12 hours of light), while those exposed to long daytime periods (18:6 light-dark cycle) continued to lay eggs for up to 13 weeks. (Enée et al. 1982). Stephens & Stephens (1966) declared that *Cornu aspersum aspersa* receiving 9h of light per day never laid eggs.to. The main factors controlling fecundity are both temperature (Gomot et al. 1986; Gomot and Deray, 1990) and photoperiod (Gomot and Griffond, 1993).

In the present study, the live weight after spawning was 9.31±0.24 g. As a result, the average live weight difference before) and after spawning was 1.22±0.32 g. Mean values of egg number (EgN), egg weight (EgW), egg length (EgL), hatching rate (HR), offspring number (OfN), offspring live weight (OfL), offspring length (OfL) is given in Table 1. In the statistical analysis, only HR and OFN data differed between the third week and the other weeks ($p<0.05$). In this study, the number of eggs per individual, egg and offspring sizes is similar to the data obtained by Çelik et al. (2018) during the breeding season. Çelik et al (2018) obtained 0.04 g egg weight from 32.22±0.06 mm matured snails in their study. It has been demonstrated by many studies the critical factor affecting egg size varies depending on the size of the snails. The size of the egg and juvenile was highly heritable characters and regulated by adult characteristics (Çelik et al. 2108). The heritability of body size, was a function of the parent's condition (Bernardo, 1996).

Table 1. Mean values of egg number (EgN), weight (EgW), egg length (EgL), hatching rate (HR), offspring number (OfN), offspring live weight (OfL), offspring length (OfL)

Çizelge 1. *Yumurta sayısının (EgN), yumurta ağırlığının (EgW), yumurta boyunun (EgL), yumurta açılma oranının (HR), yavru sayısının (OfN), yavru canlı ağırlığının (OfL), yavru boyunun (OfL) ortalama değerleri*

	1. week	2. week	3. week	Mean
EgN	105.00±12.71[a]	111.50±7.69[a]	99.00±6.36[a]	*151.13±22.02*
OfN	91.75±14.43[a]	99.00±8.44[a]	69.75±11.55[b]	*138.13±21.09*
HR (%)	86.40±4.33[a]	87.63±1.53[a]	72.10±1.53[b]	*90.33± 2.39*
EgW	0.035±0.002[a]	0.034±0.002[a]	0.032±0.004[a]	*0.034±0.001*
OfW	0.028±0.002[a]	0.028±0.002[a]	0.025±0.004[a]	*0.03±0.007*
EgL	3.97±0.10[a]	3.96±0.14[a]	3.97±0.17[a]	*3.96±0.07*
OfL	4.46±0.14[a]	4.36±0.018[a]	4.41±0.09[a]	*4.42±0.06*

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

In this study, snails awakened in January showed feeding behaviour decrease through the following weeks. The snails gradually started to shut down and tended to decrease feed intake and spawning activity towards the third week. Egg hatching rate and number of offspring decreased throughout the weeks that followed. It was determined that the hatching rate and the number of offspring were significantly lower in the third week ($p<0.05$). This situation was attributed to the low energy reserve status because of the snails were fed less in the following weeks of the study. In this regard, many previous studies supported our results. Hatchability is positively correlated with energy reserves of matured snail. (Roff, 1993; Madec et al. 1998; Gołąb and Lipińska, 2009; Angeloni et al. 2002).

In conclusion, the reproduction activity is strongly regulated by the photoperiod. *C. aspersum* snails have poor reproductive performance without the photoperiod during the non-breeding period.

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