Growth and Mortality Rates of *Cornu aspersum*: Organic Snail Culture System, Black Sea Region

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ABSTRACT

The study was aimed to examine a snail organic culture system and describe the cultivation properties of *Cornu aspersum.* The environmental parameters of the culture system and their effects on the growth and mortality rates of *C. aspersum* were determined between November 2014-October 2015. Snails were fed *Spinacia sp*. (spinach), *Urtica sp.* (nettles), Brassica *oleracea sp* (cabbage) and formulated diet. The feeding and growth rates increased with increasing temperature. Shell height growth rate was the highest in spring while the live weight growth rate was the highest in summer. Mortality rate of the baby snail was higher between November 2014 and May 2015 due to stress conditions such as handling and varying temperatures during their first stages of life. High mortality observed in adults could be associated with the spawning activity of the matured snails that caused physiological exhaustion. The result showed that the best culture cycle for *C. aspersum* was from spring to autumn in Black Sea region and in order to prevent post-reproductive mortality, snails reached to marketable size should be harvested.

Keyword: Land snail; Ecological culture; Extensive production

1. Introduction

Cornu aspersum (synonym: *Helix aspersa*), dominating the world market, are quite abundant in Turkey because of the topographical structure, favorable weather and environmental conditions of the country. Snail exporting is important, since the contribution to the maintenance of economic growth for Turkey (TUIK 2017). However, the snail production is only based on the gathering individuals from wild populations. On the other hand, overharvesting, human impacts and climate change have been affecting the natural snail population and it decreased in the last decade (FAO/Fishstatj 2018) in Turkey. Therefore, snail farming is necessary to prevent overexploitation and ensure sustainable production for the country.

Appropriate farming system for any given species provides optimal living conditions, supports growth and survival, minimizes risks and optimizes production. Humidity, temperature and feeding are vital factors to control growth and survival for heliciculture. Snails aestivate if the temperature is >30 °C and hibernate if the temperature is <5 °C (Cobbinah et al 2008). Growth of snails is

mainly determined by genetic factors, although it is influenced by many other parameters such as stocking density (Dupont-Nivet et al 2000), environmental conditions (Garcia et al 2006), the management and sexual maturation. Reproduction activity could affect the life history of snails. High reproductive activity drains somatic energy reserves (e.g. carbohydrate and lipid) and limits the energy available for biochemical systems. Thus, the cost of reproduction compromises immune function, decreases protection against stress and reduces adult survivorship (Harshman & Zera 2007). In addition, offspring's mortality is strongly affected by adverse environmental conditions. Adverse environmental conditions cause to increase maintenance costs under adverse environmental conditions and affects survival during early development stage of the gastropoda (Diederich & Pechenik 2013).

Generally, three snail culture systems are described; intensive (indoor), semi-intensive (indoor/outdoor-mixed) and extensive (outdoor) cultures (Cobbinah et al 2008). Extensive system involves the breeding site which has a protection from the wind, a sprinkler system to keep the substrate moist and troughs made from wood or building blocks covered with plastic netting to hinder predators, requiring minimal financial input (Bryant 1994). Extensive snail farming can be certified as ecological and organic if the soil's conditions and the management are appropriate to the principles of IFOAM (1998). The key principle relates to the integration of wildlife, habitats and farming is the principle of ecology (Toader-Williams & Golubkina 2009). Begg (2009) also detailed organic principles for snail farming.

C. aspersum, frequently used in snail farming since their high reproductive capacity, can adapt to every climatic and farming condition (Avagnina 2012). It is clear that the future of *C. aspersum* farming has an interesting lucrative potential in Turkey. In this study, we investigated the culture of one-month-old snails in an extensive system according to Begg's organic principles (2009) in Black sea region. It was aimed to test workability and profitability of the system for the Black sea

region, to describe the properties of *C. aspersum* farming and reveal practices of heliciculture.

2. Material and Methods

One-month-old snails with a mean shell height of 6.68±0.06 mm and a mean weight of 0.11±0.00 g were used in an extensive culture area located at the Scientific Research Center (SUBITAM) of Sinop University between November 2014-October 2015.

2.1. Study organism

Cornu aspersum (synonym: *Helix aspersa*), a hermaphroditic species, is a terrestrial pulmonate gastropod mollusc. The formation of a thickening and recurving lip at the edge of shell aperture indicates maturation (shell heigh>27 mm) (Daguzan 1982; Begg 2003). The reproductive behaviour involves that snails gets in touch with their tentacles, solidified dart comes out, the genital vebt pushed out and copulation happens (Avagnina, 2012).

2.2. Establishment of snail culture area

Extensive snail culture system was examined because of its suitability for the Black Sea regional climate. The culture system was maintained according to Begg's organic heliciculture principles (2009) which includes that a) crops are planted without the use of synthetic fertilizers, b) no chemicals are used in the snail fields, c) crops are planted densely to help prevent weed growth, d) finished crops are ploughed back into the ground as 'green manure' crops, e) physical controls are maintained for unwanted weeds and pests, f) ecological benefits of natural sunlight, organic soil.

110 square meters' area was fenced with a galvanized iron as the perimeter fence with a depth of 35-40 cm to the bottom and supported by iron posts. The sprinkler irrigation system, connected to water tanks filled with tap water, was established to provide water equitably to the production area. The culture area was divided into three pieces as parallels (B1, B2 and B3) with mesh fences of 9 m length and 1 m width and included 80 cm pathways between experimental fields (Figure 1). The downward

facing flaps and 4 pieces electric copper wires with a diameter of 0.80 mm (12 watts cm²) were integrated to the system to prevent snails from climbing up the sides of the fencing. 8 slight slope wooden boards (50 cm x 50 cm) were placed in each field for feeding and establishing shelter area for snails. The culture area was covered with greenhouse nylon until May 2015 due to prevent harsh winter conditions. The culture area was covered with a bird protection mesh after removing greenhouse nylon. The roof pitch of the system was oriented according to prevailing wind directions.

Soil in the experimental culture area was analyzed prior to the study. Sandy humus soil was added for improving water holding capacity and organic matter content of the culture area since the soil texture was mostly clayey. pH, organic matter and moisture contents of the soil then were determined.

After excavating soil, three preferred plant types were planted as a row of *Spinacia sp.* (spinach), *urtica sp.* (nettles) and *Brassica oleracea sp* (cabbage) to each experimental field in order to determine the most preferred plants. Plants development was monitored and unwanted plants were removed from the experimental areas.

Artificial diet was prepared according to the organic feed rules (Blair 2008). The formulation and biochemical composition of the diet were given in Table 1. Feed was supplied every other day on the wooden boards during sunset time (between 5 and 8 pm).

Table 1- Ingredient and biochemical composition of diet

Diet formulation		Biochemical composition of diet (based dry-weight, %)	
Soybean flour	16	Protein	33.33
Corn gluten	11	Lipid	3.85
Wheat flour	28	Carbohydrate	22.26
Canola oil	3	Ash	40.56
Dicalciu phosphate	5	Moisture	3.85
Pectin	1		
Limestone	33		
Sodium chloride	0.5		
Vitamin premix	1.5		
Mineral premix	1		

2.3. Environmental parameters

Temperature and humidity were measured every morning (9:00 am) and sunset time from inside and outside of the culture area by TFA 5013 (electronic equipment). Natural ventilation was supplied by opening door and window of the greenhouse when temperature increased inside the culture area. Supplemental irrigation was also supplied as needed before sunset time when moisture was deficient.

2.4. Growth and mortality rate

300 snails were placed in one square meter of unit (separated with wooden block) in each field (B1, B2 and B3). Then the unit was enlarged by one square meter for every month with increasing growth. One hundred snails was randomly sampled from each field (B1, B2, B3) for the biometric parameters in every week until June, then sampling were carried out every two weeks period. Live weight (total weight of snail) was measured by weighing live animals to the nearest 0.001 g and shell height was measured to the nearest 0.1 mm with a caliper (Figure 1).
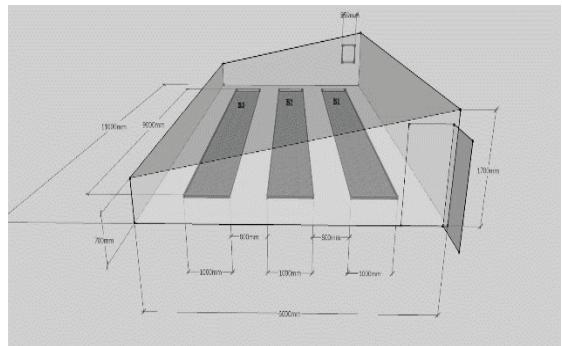

Figure 1- Schematic drawing of the organic snail culture system

Weekly shell height and live weight were calculated on a monthly base to allow for a clear pattern in the growth rates. Monthly shell height growth rate (SHGR %) and live weight growth rate (LWGR %) were calculated from the following formulate;

$$SHGR\ (\%) = [(L_2 - L_1) / L_1] \times 100 \qquad (1)$$

LWGR (%)= [(W_2-W_1) / W_1] x 100   (2)

Where; L_1 and L_2 are the mean shell height and W_1 and W_2 are the mean live weight in a month.

The mortality rate was not determined before May due to fragile shells. The first mortality rate was determined in May 2015 by subtracting snails in May from the starting number of snails. After May, empty snail shells were counted and removed to determine mortality rate and followed monthly.

Mortality rate (%)= 100 (N_t- N_0)   (3)

Where; N_t is the number of dead snails removed after t time and N_0 is the number of snail at the beginning of the experiment.

2.5. Statistical analyses

Data were analyzed for significant differences in means using ANOVA's, with significance levels set at $P<0.05$ and the normality of the variation of data was verified using the software program MINITAB 16 software. The variability of shell height and live weight were analyzed as the coefficient of variation (CV) in Microsoft Office Excel. A correlation matrix analysis was used to determine the relationships between the environmental and growth parameters.

3. Results and Discussion

3.1. Environmental factors

Monthly day and night temperature of the culture area (A), temperature of natural and culture environment (B), monthly day and night humidity of the culture area (C) and humidity of natural and cultured environment (D) were shown in Figure 2. Day and night temperature differences was the highest (7.72 ºC) in April ($P<0.05$). There was no difference in humidity between day and night inside the culture system when greenhouse nylon was covered ($P>0.05$) (Figure 3). However, there was significant difference in humidity between day and night after the greenhouse nylon was removed on May 2015 ($P<0.05$). pH, organic matter and

moisture values of the soil were 7.00, 30.67% and 24%, respectively.

It is reported that if soil structure is not suitable for snail culture, soil should be improved in order to ensure for healthy development of snails (Begg 2003). In the present study, organic matter in the soil increased by adding humus soil and water permeability increased by adding sandy soil. After soil improvement, the soil structure was suitable for snail and plant breeding. Lucas & Davis (1961) declared that if soil pH is around 7.2, it indicates that the soil is rich in calcium. Calcium rich soil is desirable property because it supports shell growth in snail culture.

The observation on developing plants showed that cabbages grew fast in the culture area. The spinach did not grow enough because of the shade of the enlarging cabbage leaves. The leaves of cabbage hardened quickly due to the high growth of the cabbage. However, this situation did not have an adverse effect on the snails feeding since cabbage mostly consumed by snails. Snails started to consume more formulated feed after cabbage leaves. In addition, enlarging cabbage leaves also obstructed to effectively use sprinkler irrigation system. On the other hand, the culture area covered greenhouse nylon was also regulated the circulation of humidity with preventing humidity lose when temperature was higher at the outside.

3.2. Growth rate

Matured snails were not within a certain size range because they were collected from nature, therefore no standard growth was achieved from the obtained offspring. Many studies revealed that cultured mature snails should be used for optimal and regular growth (Murphy 2001; Cobbinah et al 2008; Begg 2009). Monthly shell height growth was significantly different ($P<0.05$). SHGR was the highest (8.58%) in May while LWGR was the highest (4.11%) in June (Figure 3).

Temperature varied between 11-12 °C in the culture area until February and there was no significant growth rate in these time intervals. It was
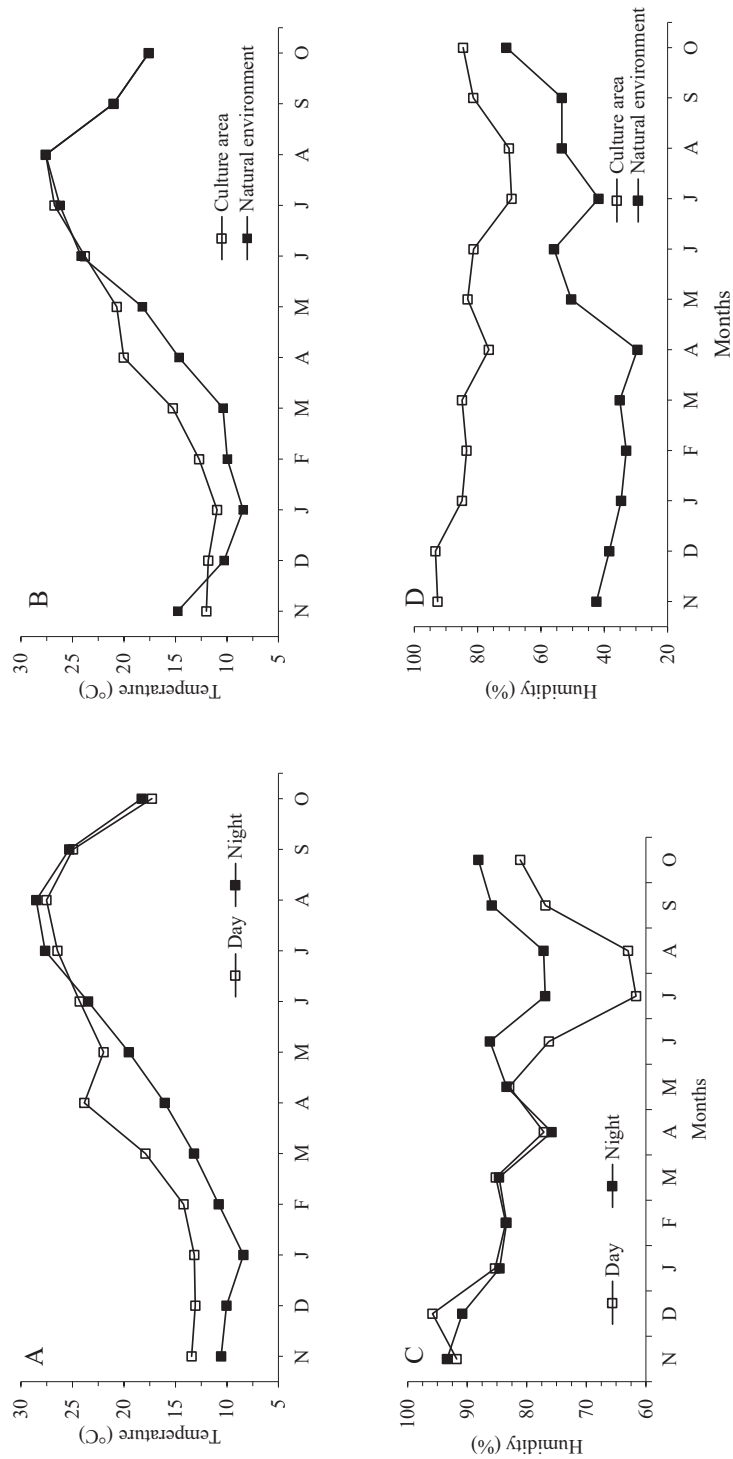
Figure 2- Monthly day and night temperature of the culture area (A), temperature of natural and culture environment (B), monthly day and night humidity of the culture area (C) and humidity of natural and cultured environment (D) during experiment
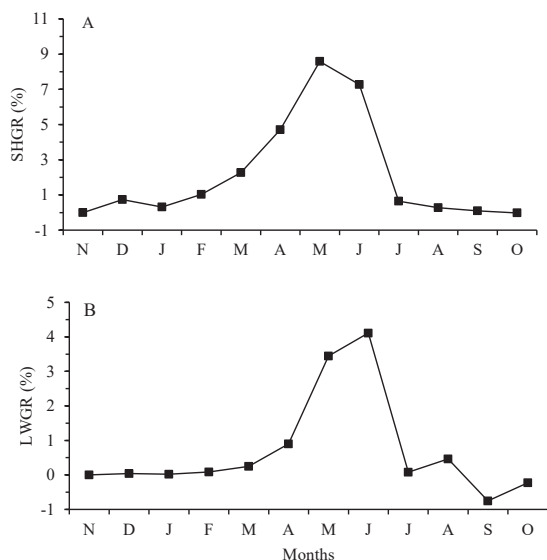
Figure 3- Monthly shell height growth rate (SHGR %) (A) and live weight growth rate (LWGR %) (B) of snails during experiment.

demonstrated that snails were not active below 12 °C and decreased or stopped feeding. After February, the growth rate increased with the increasing temperature above 12 °C. In April, the feeding rate of snails was increased with the increasing temperature about 19 °C. Furthermore, the highest growth was in May although the temperature difference was significant in April and May. It could be said that snails with the 13.91±0.28 mm shell height had better tolerance to environmental stress caused by the difference between night and day. Many studies showed that continuous growth was noticed in spring which demonstrated that most terrestrial snails show a faster growth rate during spring in nature (Staikou et al 1988; Hatziioannou et al 1989). Length growth of land snails' ceases with lip formation and reaches sexual maturation (Choat & Schiel 1980; Koene & Ter Maat 2004). In the present study, snails with 31.58±0.31 mm shell height growth rate started to decrease after May and reproductive behavior was observed. Daguzan (1982) reported that *H. aspersa* reached maturity with shell heights reaching over 27 mm and marketable size of this species is between 25 and 32 mm (Lazaridou-Dimitriadou et al 1998).

In the present study, 25% of snails reached shell heights of over 27 mm within seven months, while 90% of them reached to the same size in June within eight months. Approximately all of hatched snails reached marketable size in nine months (July 2015). Ligaszewski et al (2007) declared that *Helix pomatia* needed two year farming cycle from hatching to maturity in an unheated greenhouse farming system in Poland. In Greece, *C. aspersum* reached marketable size varied from 2.5-5 months indoor farming system (Lazaridou-Dimitriadou et al 1998). The life cycle of *C. aspersum* was highly affected by the climatic conditions of the region (Chevallier 1977) and farming system. In Australia, one third of the hatched snails reached marketable size before they were 12 months old at an extensive system. Lazaridou-Dimitriadou et al (1998) reported that snails reached marketable size in 4-5 months under intensive farming conditions, depending origin, instead of 18 months which is needed in nature.

3.3. Mortality rate

Our study showed that offsprings experienced high stress such as variation in weather conditions and handling stress in the first stages of their life-cycles between November and May (Figure 4).
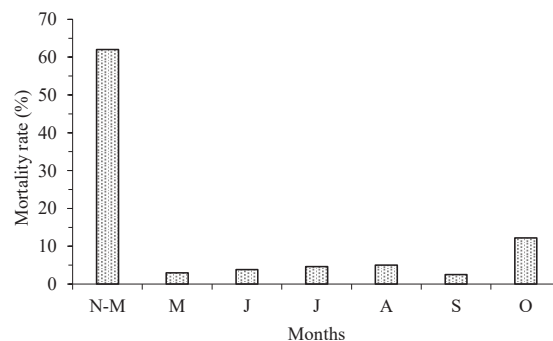

Figure 4- Mortality rate (%) between November 2014 and October 2015 (N-M: Mortality from November to May)

In November the juvenile snails (6.68 mm shell height) were taken from the hatchery (temperature range 18-20 °C; humidity range 84-87%) and

placed to the covered culture area where they were exposed to relatively cold weather and temperature difference. In addition, they were under handling stress caused by the weekly measurement procedure of this sensitive stages. Snails could be damaged during the measurement and were not able to repair their shells since they had low feeding or no feeding rate for generating enough energy for repairing their shells under inappropriate conditions like low temperature. Hence, high mortality was observed between November and May. Many studies revealed that animals has lower ability to tolerate stress during their first stages of life (Zippay & Hofmann 2010; Gheoca 2013; Diederich & Pechenik 2013).

Snails showed reproductive behaviors after reaching sexual maturity in May and continued throughout summer and autumn. This showed that snails were constantly mating and producing eggs throughout the summer when suitable environmental was provided. However, high mortality rates were recorded in August, September and October. Negative live weight growth rate was also observed after August due to death of mature snails (Figure 4). Thus, spawning activity of matured snails could affect immune system and caused to post-reproductive mortality. Many studies showed similar results that mortality rate was higher in animals showing higher reproductive efforts because of reducing body maintenance and immune capacity, and hence it might cause physiological exhaustion (Baur & Baur 2000; Barker 2001; Carvalho et al 2008). In addition, farming system types, snail species and rearing density also effect snails' mortality rate. Dupont-Nivent (2000) reported quite high mortality of C. *aspersum* as 21% in indoor system in France. Ogogo et al (2011) found 1.4% cumulative mortality of *Archactina* spp. in survey of snail farming (farming systems: concrete trench, wire quaze fence, wooden cage) in Nigeria.

4. Conclusions

Additional studies should be carried out to further investigate which plants should be grown together without competition with the others for snail farming at the Black sea region.

More controllable system should be established to minimize the difference between night and day temperatures in the winter months, and therefore, low mortality and increased growth rate could be obtained.

The largest and the same sized mature snails should be selected as much as possible for breeding stock from the initial collected wild snails to obtain uniform growth of offspring and for successful breeding.

The most suitable environmental conditions for snail culture were provided during the Spring-Summer-Autumn seasons in the Black Sea region. The production activities might be more efficient with the addition of shading and irrigation at these seasons to reduce the effect of hot and dry air in summer. However, irrigation should be carried out according to temperature value of the environment to prevent snails from thermal stress.

After snails reached the market size which is the same size at maturation, breeding stock should be separated and the rest should be immediately harvested. Otherwise, mature snails will continue to reproduce which causes mortality by physiological exhaustion.

Acknowledgements

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