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Research Article

Production of *Aphanius mento* (Heckel, 1843) under controlled conditions

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Abstract. In this study, it was aimed to determine the adaptation capability to controlled conditions, reproductive behaviors, and production of *Aphanius mento* caught from Kirkgoz Springs, Antalya. Fish material for broodstock were caught using fine mesh tulle net and bag net with 2.5 mm mesh size. Twenty five female and 10 male breeding fish acclimated to experimental conditions in Aquarium Department Unit of Ministry of Food, Agriculture and Livestock Mediterranean Fisheries Research Production and Training Institute were spawned by providing egg collectors to the tank. *Aphanius mento* eggs were spherical, transparent and sticky. Examination with the microscope revealed that the eggs were full of pale yellow egg yolk with 7-8 lipid droplets and had fibrous sticking filaments on the surface. The average egg diameter was 1.59±0.50 mm (n=8). Hatching were observed at 11th day at 22.5±1.0ºC water temperature whereas at 8th day at 27.0±0.45ºC. Total length of newly hatched larvae was 5.1±0.07 mm, and swimming were competed at 4th day. Average size of one-week larvae was 5.6±0.2 mm. At this stage fins were fully developed, the larvae were able to swim freely and take exogenous food. Mean length of the fish was 2.7±0.2 cm on 7th month and a juvenile fish had an appearance of morphologically mature fish.

Key Words: *Cyprinodontidae*, *Aphanius mento*, reproductive characteristics, larval development.

Ozet. Bu calismada, Antalya Ili Kirkgoz Kaynagi'ndan yakalanan *Aphanius mento* turunun kontrollu ortam kosullarına uyumu, uretimi ve ureme davranislarinin belirlenmesi amaclanmistir. Damizlik olarak kullanilan erkek ve disi baliklar Kirkgoz Kaynagi'ndan ince gozlu tul ag ve 2,5 mm goz acikliginda torbali aglarla yakalanmistir. Gida Tarim ve Hayvancilik Bakanligi Akdeniz Su Urunleri Arastirma Uretim ve Egitim Enstitusu Akvaryum Birimi'nde hazirlanan ortam kosullarina uyumu gerceklestirilen 25 adet disi - 10 adet erkek damizlik balik, yumurta kolektorleri bulunan ureme tanklarina konularak, yumurtlamalari saglanmistir. *Aphanius mento* turunun yumurtalari kuresel sekilli, seffaf ve yapiskan ozellikte olup, mikroskop altinda incelemede 7-8 adet yag damlacigi icerdigi, solgun sari renkli yumurta sarisi ile dolu oldugu ve disinda ipliksi yapisma flamentleri icerdigi gorulmustur. 8 adet disi baliktan alinan yumurtalarda ortalama yumurta capi 1,589±0,50 mm olarak olculmustur. *Aphanius mento* yumurtalarinda, 22,5±1,0ºC su sicakliginda dollenmeden sonra 11. gunde, 27,0±0,45ºC' de ise 8. gunde larva cikisi gozlenmistir. Yumurtadan yeni cikmis larvanin total boyu 5,1±0,07 mm olarak olculmus, butun larvalar 4. gunde serbest yuzmeye gecmistir. 1 haftalik larvalarin boy ortalamasi 5,6±0,2 mm olup, yuzgecler tam olarak gelismistir, larvalar serbest olarak yuzmekte ve disaridan yem alabilmektedir. 7. ayda baliklar ortalama 2,7±0,2 cm'e ulasmis, yavru balik morfolojik olarak ergin balik gorunumu kazanmistir.

Anahtar Kelimeler: *Cyprinodontidae, Aphanius mento*, ureme ozellikleri, larval gelisim.

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Introduction

Fish play a significant role in examination of biological development from experimental and conceptual perspectives due to their enormous number of species among the animal kingdom and various living styles. Beside this, there is an increasing importance of knowing egg number of fish prior to spawning and survival rate after hatching because the early phase of development has a great impact on annual production volume (Sahandi 2011). For this reason, egg hatching and first feeding times, larval metabolism and relationship between chemical and physical factors that affect fish larvae populations can be regarded as approaches to better understand the fish population (Bagenal & Braum 1978).

As other Eurasian fish populations (Györe *et al* 2011; Lenhardt *et al* 2011), some *Aphanius* species populations belonging to *Cyprinodontidae* have greatly decreased due to anthropogenic drying processes of their habitats, pollutants, new parasites from South East Asia (Paladini *et al* 2011; Ebrahimzadeh Mousavi *et al* 2011) and limited competition ability with other introduced species such as *Gambusia* (Oltra & Todoli 2000). For instance, *Aphanius almiriensis*, *A. baeticus*, *A. burduricus*, *A. iberus*, *A. richardsoni*, *A. sirhani*, *A. splendens*, *A. transgrediens* have been reported as *Aphanius* species which are under the threat (Anonymous 2010). Investigation of reproductive characteristics of fish defines what should be done for the species future. Therefore, investigations of biological aspects of fish in artificial and natural ecosystems are of significance for more efficient protection of the environment (Blaustein & Byard 1993). During the acclimation, adaptation and domestication following transfer from the natural environment, certain sets of criteria should be considered. In this context, selection of local species has advantages such as becoming familiar with the artificial conditions faster resulting in less specific problems and more economic production. Moreover, fish caught from the wild for formation of broodstock can have difficulties with adaptation to the artificial environment and even die due to starvation and

stress resulting from limited feeding activity. Thus, some species may be started to get domesticated at early stages. Monitoring feeding activities of fish, determination of most appropriate culture form, good facility design, and controlling the reproduction are major strategies in aquaculture (Liao & Huang 2000). In 1955 the number of cultured exotic and aquarium species were about 410 and 40 respectively whereas today these figures are expressed in thousands (Fossa 2003). Almost every day a new species is discovered and transferred to culture environments to introduce to aquarium world. People are more interested in these new species rather than those which are easily accessible. Hence, potential species for aquarium sector should be investigated without damaging to the nature and introduced to sector by rearing in captivity. In this way, it would be possible to bringing value-added to an economically unexploited living organism.
Scientific studies in *Aphanius* genus have concentrated mainly on determination faunistic and biological aspects, and the degree of kinship among the species members (Wildekamp 1993; Frenkel & Goren 1997; Wildekamp *et al* 1999; Oltra & Todoli 2000; Hrbek & Meyer 2003; Hrbek & Wildekamp 2003; Bardakci *et al* 2004; Kucuk & Ikiz 2004; Sari *et al* 2007; Guclu & Kucuk 2008, 2011; Karsli & Aral 2010). Nevertheless, their production in the controlled conditions is extremely important in terms of protection of ecological balance as well as ensuring the species continuity. Therefore the present study was planned to acclimate *Aphanius mento* in view to investigate its potential to adapt to artificial production conditions, and to determine their behavior and production potential.

Materials and Methods

The study was carried out between March 2010 and March 2011 at the Aquarium Unit of Mediterranean Fisheries Research Production and Training Institute under the auspices of Ministry of Food, Agriculture and Livestock. The study material was the matured male and female individuals of Aphanius mento caught from Kirkgoz springs, Antalya (Figs 1 and 2).

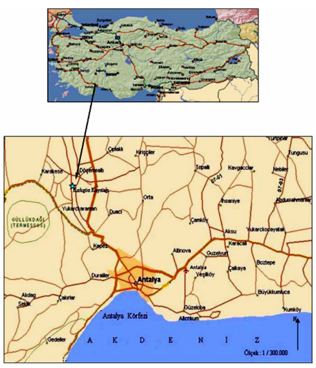

Figure 1. Kirkgoz springs, Antalya - Turkey (Guclu 2003).

For acclimation and adaptation of the species, firstly information regarding to natural living systems, water quality and environmental factors was gathered (Liao & Huang 2000). Accordingly, data of previous studies on characterization of population features (Guclu & Kucuk 2008, 2011) were accepted as a basis to the present investigation.

Figure 2. Male and female members of *Aphanius mento* used in the experiment

In adaptation of species to the artificial condition and determination of reproductive behaviors, 10 male with 4-6 cm length and 1.1-3.7g body weight, and 25 female with 4.5-7.6 cm length and 1.3-6.5 g body weight were used. The broodstock used were caught from Kirkgoz Springs, Antalya, using fine mesh tulle net and bag nets with 2.5 mm mesh size. The seized fish were then transferred using 10L plastic bags including 1/3 water and 2/3 pure oxygen (Berka 1986) to tank system in the Aquarium Unit. The system consisted of two fiber tanks one (1.20x1.20x0.50 m) for keeping the fish and the other (240 L) for filtration. Within the filtration tank, there were three floors shelves. Bioballs and coral parts and volcanic stones were placed into the first floor whereas zeolite and staple fiber into second and thirds floors respectively. A bath treatment using 10 g/L stock solution of methylene blue + formaldehyde at 12 mL/ton dose was applied against possible invasion of parasite and fungi (Noga 2000; Sahandi & Hajimoradloo 2011). To ease the acclimation of fish, pebbles and aquatic plants growing in the natural environment of fish were placed into the culture tank.
Tank water temperature was kept at the same level as the natural water at the beginning, and then gradually increased to 25°C using an electrical heater. PVC knee pipes were also provided to the tank to ensure shelter and reduce aggressive behavior of the males. Once the fish were adapted, all the materials were taken out from the tank and spawning time was tried to get controlled. Feeding was done thrice a day using live and dry feeds. Tank water was renewed weekly at a rate of 30%. Water temperature, dissolved oxygen and pH were monitored whereas nitrite and nitrate weekly.
A stable artificial photoperiod was applied over the study (14L: 10D) (Frenkel & Goren 1997). First feeding at the artificial conditions was done with *Daphnia sp*. and *Palaemon sp*.. During the acclimation period, commercial diets (Table 1) were also used together with the live food organisms. After acclimation, a combination of live and artificial dry feed was given thrice a day. Larvae after hatching were fed with rotifer for one week, and then artemia and rainbow trout starter feed. Raffia and stable fiber as egg collector were placed on the tank bottom for the acclimated broodstock to release their eggs easily (Ozen & Timur 1999). The existence of released eggs on the collectors were periodically checked. The collected eggs were treated with 2 mg L^{-1} methylene blue against fungi (Noga 2000). For observation of the development of fertilized eggs and measurements a binocular microscope was used while for measuring egg diameters and larval lengths on the pictures taken a software, Image J, was used.

Table 1. The nutrient content of commercial feeds used infeeding broodstock (%)*

Nutrients content	Feeds		
	Tetra Cichlid Feed	Sera Discus Feed	Trout Pellet Diet (2 mm)
Crude protein	31.6	46.2	50
Crude fat	2.75	6.2	15
Crude cellulose	-	3.7	3
Crude ash	5.04	11.2	13

(*) Declared values by the producers

Results and Discussion

Some physical and chemical characters of water samples taken from the spring where A. mento occur and experimental unit are given in Table 2.

Table 2. Some chemical parameters of water used in the experiment

Parameters	Kirkgoz springs	Experiment unit
Temperature (ºC)	14-17	18.5-28.5
pH	7.6	7.8
Dissolved oxygen (mg L^{-1})	5	8.4
Oxygen saturation (%)	50	89
Electrical conductivity (25 ºC µS cm^{-1})	809	744
Alkalinity (mg L^{-1} $CaCO_3$)	252	229

The minimum and maximum water temperatures in the experiment unit were recorded as 18.5ºC in winter and 28.5ºC in summer, pH 7.5 and 8.7, dissolved oxygen 3.8 and 6.8 mg L^{-1}, while nitrite and nitrate were never reached the threshold levels. Matured female and male individuals were collected from the places which are shallow, plentiful with aquatic plants and slow in water flow, using tulle nets. During the collection, body color particularly in males, got darkened but once arrival at the experiment unit, the darkening disappeared. During the acclimation period, fish appeared to hide between the plants and pebbles in the tank.

After the acclimation, feeding with daphnia and bait shrimp help the fish getting used to commercial dry food. Two weeks after acclimation matured male and female started to show reproductive behaviors and some eggs were seen on the plant leaves that had been previously placed into the tank. To take the spawning time under control, the materials onto which fish can lay the eggs were taken out. Only knee pipes were left as shelter for timid females especially in the case of fight between the males. But it was seen that the males tended to adopt these shelters. Staple fiber and raffia were put into the tank for the fish to lay their eggs on after color differentiation and chasing behaviors. In this way the male were forced to select these materials as nest. Dominant males became apparent with their darkening color and fight off the other males, and they started to attract the females with assault-like movements. From this moment on, regular egg control on the stable-raffia was started. Once removal of the staple-raffia with eggs from the tank and replaced with new ones, the female were seen to lay the eggs again. By this way, it was determined that the female does not lay the eggs at once but several times.

Based on spawning period in the nature, the microscopic examination of the eggs collected was done in May 2010. The examination revealed that the eggs are spherical, transparent and sticky, and full of egg yolks with 7-8 oil droplets, and have adhesion filaments on the surface. Average diameter of the eggs from eight females was 1.589±0.50 mm.

The raffias with eggs that were not used for measurement were kept in 10L incubation aquarium. The eggs that were examined under the microscope were hatched out in 11 days at 22.5±1.0ºC while those kept in 10L aquarium in 8 days at 27.0±0.45ºC (216.9 degree days). Newly hatched larvae tended to stay at the aquarium bottom; at three days after hatch (DAH) they started to swim freely and at 4 DAH all larvae completed to pass free swimming stage. Average body length at 7 DAH was 5.6±0.2 mm and the fins had not been completely formed at that time. During this period, the larvae could freely swim; take the rotifer and artemia easily. From this stage on, feeding was continued with live organisms together with the commercial dry foods and on seventh month average length reached 2.7±0.2 cm and morphologically resemble to adult individuals.

In the present study, centered on secondary freshwater fishes, reproductive features and larval development of *A. mento* under the artificial conditions were tried to be determined by observations and measurements. We think the reasons of color changes and tendency of hiding observed at the beginning of the acclimation period are stress factors. However, we also consider that a short-term-adaptation period observed in this study was probably due to very close water quality of the experiment unit to the spring water. Quality parameters of the water used in the experiment unit were always kept below the critical thresholds for fish health by setting up a filter system and regular renewal with fresh water.

In our study, spawning behaviors displayed by *A. mento* are similar to those observed in *Fundulus luciae* by Byrne (1978). In both species, the male adopts and protects his territory and then first approaches the female, displays assault-like behavior, tries to attract her to the collector to let it lay and the intercourse finally results in releasing the eggs. On the other hand, *Fundulus waccamensis* males approaches to incoming female to the nest by swimming up and down, show their body color and fin glows, lead the female to the nest and then spawning takes place (Shute *et al* 1983).

Guclu (2003) reported that natural spawning time of *A. mento* occurs between February and July but we observed it can takes place even in November under controlled conditions. *A. mento* resembles to Cyprinodontiformes species, which are annual fish species, in terms of spawning conditions and place. For example, *A. mento* experienced 25ºC water temperature in the experiment tank during spawning, while the spawning temperature for *Fundulus luciae* was reported as 20-25ºC (Byrne 1978). Likewise, staple - raffia egg collectors used in the present experiment resemble to the mop that was used by Byrne (1978). The eggs can be found in clusters including 2-4 eggs on the mop and raffias (Byrne 1978). Guclu (2003) determined the egg diameter of *A. mento* as 1034.783±50.262 µm. The minimum diameter was reported to be 36 µm whereas the maximum 1756 µm. Considering the spawning time, the egg diameters between this and our study are quite similar. The examination revealed that the eggs are spherical, transparent and sticky, and full of egg yolks with 7-8 oil droplets, and have adhesion filaments on the outer. Average diameter of the eggs from eight females was 1.589±0.50 mm. Where as in *Fundulus luciae*, average diameter of mature eggs are 1.96 mm (Byrne 1978). The eggs of *F. luciae* are slightly elliptic, sticky and have 21.8 oil droplets which are concentrated around the bottom (Byrne 1978). The egg diameter of *Cynolebias viarius* are 1.7 mm and they are transparent, covered with a spherical unicellular membrane and has a homogenous egg yolk except in the region of oil droplets (Arezo *et al* 2005). On the other hand, *Adinica xenica* eggs are yellow in color, their cell membranes have sticky filaments and the diameters can change between 1.70 and 1.90 depending on the water salinity. Size of oil droplets may change according as species genetic features as well as the age of the embryo (Cunningham & Balon 1985). Newly fertilized eggs of *F. waccamensis* are colorless, spherical and 2-3 mm in diameter and have countless oil droplets (Shute *et al* 1983). *F. heteroclitus* eggs are spherical in shape, 2.0 mm in diameter and clear yellow-amber in color. *F. majalis* eggs are spherical, 2.0-3.0 mm, translucent, yellow-amber and slightly sticky (Abraham 1985; Armstrong & Child 1965).

In the present investigation, hatching occurred in 11 days at 22.5±1.0ºC. This is on par with about 216.9ºC days. The differences between the results resulted from the difficulties experienced during the stabilization of water temperature. Iwamatsu (2004) reported that hatching occurs in 9 days at 26±0.1ºC in Japanese killifish (*Oryzias latipes*). Of Cyprinodontiformes species, in *A. xenica* hatching last for 9-10 days at 26-28ºC (Cunningham & Balon 1985). Whereas in *F. luciae*, eggs hatch 10-14 days at 20-25ºC after fertilization (Byrne 1978). The eggs of *F. heteroclitus* hatch out within 16 days at 20ºC (Armstrong & Child 1965). We can conclude from these reports that embryologic development and hatching duration can display variation depending on fish species and water temperature. Similarly, Markofsky & Matias (1977), who discuss the effects of water temperature on embryological development stated that a 5ºC increase in temperature could reduce the hatching down to 10 days (Arezo *et al* 2005).

In the current study, total length of newly hatching larvae of *A. mento* was measured as 5.1±0.07 mm. At this stage, it was seen that posterior, dorsal and anterior ventral sides of the head are colored and tail fin is not developed yet. In *F. heteroclitus*, a member of Cyprinidontiformes order, newly hatched larval length is 4.0-7.7 mm (mean 5.0 mm), whereas in *F. luciae* 7.0-11.0 mm (Abraham 1985). In *F. waccamensis*, coloration has been reported on the head, dorsal and abdomen regions. In addition, larval body length at hatching in this species is 8.1 mm while in *Fundulus diaphanus* 5.3-6.4 mm (Shute *et al* 1983). When *A. mento* is compared with other species, larval length at hatching in zebra fish (*Danio rerio)* is 3.1 mm, in medaka (*Oryzias latipes*) 3.8-4.2 mm, in red head goldfish (*Carassius auratus*) 2.54±0.01 mm, and inkalikoryukin goldfish 4.3-4.4 mm (Kimmel *et al* 1995; Iwamatsu 2004; Karsli *et al* 2007; Savas *et al* 2006).

In the present study, some information has been gathered based on obsevations and examinations of reproductive traits and larval developments *A. mento*, which has a significant place in terms of endemism, but not yet in danger of extinction, even so under the threat of environmental pollutions. The data obtained are of importance since they are the first in the field of the production in controlled conditions. Moreover, a production model and a database about the reproductive biology of this species, which would be used in aquarium sector because of their colorful and attractive appearance have been tried to be formed. Furthermore, with the production in captivity the chances of extinction of the species shall be reduced.

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