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[Research]

Age determination and morphology of otolith in *Alburnus chalcoides* (Guldenstaedt, 1772) in the southern Caspian Sea

F. Amouei[1*], T. Valinassab[2], A. Haitov[2]

1-Tajik Agrarian University, Doshanbeh, Tajikistan
2- Iranian Fisheries Research Organization, Tehran, Iran
** Corresponding author's E-mail: amouein83@yahoo.com*

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ABSTRACT
The aim of this study was to provide necessary information on the age, growth and sex ratio of one of commercially important cyprinid species, *Alburnus chalcoides* in the southern Caspian Sea (North of Iran) from 2010 through 2011. 53 specimens of both sexes (males and females) were collected monthly. The samples were transported to the laboratory for further biological measurements and otolith extraction. The maximum and minimum age of *A.chalcoides* was 4^+(FL=26.0 cm) and 1^+ (FL= 18.1 cm), respectively. The mean fork length of *A. chalcoides* was 20.21±0.287 cm; and the sex ratio was 1.00: 2.12. The calculated length-weight relationships for all individuals were as $W=0.141TL^{2.199}$ (R^2= 0.648). Different regressions were prepared between age and morphological measurements of fish and otolith, of which the highest correlation was between age and body length as a linear regression of TL=1.959Age+16.32 (r= 0.66). There was no significant correlation between morphometric measurements of otolith and fish morphological parameters ($p > 0.05$), and an allometric growth was found with a slope of b=1.926 for the study area.

Keywords: Fish, Age, Otolith, *Alburnus chalcoides*, Caspian Sea

INTRODUCTION
Population characteristics and age composition of a fish stock are important parameters used in stock assessment models in fisheries management. The age profile of a stock gives an indication on how healthy the stock is (Metin and Ilkyaz, 2008).
Age determination in bony fishes can be carried out using the anatomical method by counting growth annuli appearing on hard structures such as otoliths.
Depositions of annual growth rings formed in this tissue are caused by seasonal changes in the environment (İlkyaz *et al.*, 2011). Each year of growth is composed of an opaque and a translucent zone (corresponding to summer and winter growth, respectively). Thus the age of an individual fish can be determined by reading the pattern of bands on otoliths. By determining the age of a large number of individuals, it is possible to build up a picture of the age structure of the whole population. Knowledge of the age structure provides an indication of how the stock is measuring up to exploitation (Metin and Ilkyaz, 2008).
Caspian Shemaya, *Alburnus chalcoides* (Guldenstaedt, 1772), is one of the commercial fish species in the Caspian Sea in which migrates toward the rivers and lagoons for spawning. This species belongs to Cypriniformes order, Cyprinoidei suborder and Cyprinidae family (Nelson, 2006), and the other common names of this species are Caspian bleak or Danube bleak (Naderi and Abdoli, 2004) with Persian name of "Shahkooli". Up to now, a total of 311 species of cyprinids have been identified all around the world (Banyankimbona *et al.*, 2011) and 40 species of them inhabit in the Caspian Sea (Naderi and Abdoli, 2004; Valinassab, 2013).
In the last three decades, the total catch of *A. chalcoides* were highly decreased and in the previous years, and at present is so rarely found in the Caspian Sea and is considered as an endangered species (IUCN List). The total catch of bleak fishes has been less than 20 tons per year in the

southern Caspian Sea (Ghaninedjad *et al*., 2005; Daryanabard *et al*., 2009). There are few studies have been carried out on this species consist of: Rahbar *et al*. (2001) estimated the absolute fecundity (Min-Max= 4448-8301) and relative fecundity (821.8 per g of body weight); Rahmani *et al*. (2001) studied some biological aspects of *A. chalcoides* consist of sex ratio, length and weight and maximum age of 5^{+}; Azari Takami and Rajabnejad (2002) did another study on fecundity and aging of this species in the most important river of the southern Caspian Sea, Sefidrood River and they reported the total fecundity with the range of 2929-18860. The morphological differentiation between two populations of Caspian Shemaya in Haraz and Shirud rivers were investigated by Bagherian and Rahmani (2007) and some differentiations were observed. Rahmani *et al*. (2007) did a study on morphological analysis of *A. chalcoides* in Haraz River and Shirud River and no population differentiation was determined. Rahmani *et al*. (2009) carried out a research project on genetic diversity of *A. Chalcoides* in three different rivers of Haraz, Shirud and Gazafroud and it was found that all specimens belong to the unique population.

In 1759, the first experience for age determination was carried out by using vertebra as a hard tissue (Henderstrum, 1959). Then Reibisch (1899) was the first researcher succeeded to do aging of *Pleuronectes platessa* by using otolith and from then otolith was introduced as very important and suitable structure for aging of bony fishes (Ricker, 1975). It should be noted that in many teleosts species such as croaker, grunt, grouper, Kutum among three otoliths of inner ear (sagitta, asteriscus & lapillus), sagitta is the biggest otolith and the best hard tissue for age determination (Campana and Thorrold, 2001; Campana, 2005) but for cyprinids, the biggest one is asteriscus. Azari Takami and Rajabinijad (2002) determined the maximum age of Caspian shemayaup to 8^{+}yr in Sefidrood River, western part of south Caspian Sea but Rahmani *et al*. (2009) reported maximum age of 5^{+} in Shirud River in which this result is more confidential.

There are some reports on age determination of cyprinids. Afzal and Shaista (2009) determined the age of three cyprinids' species. Chen *et al*. (2002) reported that using of lapillus otolith is so précised comparing to scale for aging of *Gymnocyris selincuensis*. The age of *Barbus sclateri* was determined by lapillus otolith (Escot and Granado-Lovencio, 2001). Amoie *et al*. (2013) determined the age of *B. brachycephalus caspius* and *B. capito* as maximum of 5^{+} and 4^{+} yr, respectively. Because of the commercial and ecological importance of Caspian shemaya, this research was carried out to attain the following objectives:

- Age determination of *A. chalcoides*
- To estimate the length-weight relationship
- To determine the relationship between measured morphological parameters both on otolith and fish body.

MATERIALS AND METHODS

Specimens were collected seasonally from artisanal fishermen at several landing sites such as Shirud River, Haraz River, Gazafroud and even fish markets. The study area restricted to the southern Caspian Sea, from longitude 49° 00´E to 54° 00´E (Fig. 1) during the period of 2011-2012. Fish samples were mostly caught by beach seine method and very few with gillnet (Daryanabard *et al*., 2009).

Collected Caspian shemaya were preserved in ice boxes and were transferred to the laboratory for further biological measurements and otolith extraction. A total of 53 specimens were collected. Noteworthy, the Caspian shemaya resources have been highly decreased in the last decade and it is considered as an endangered species; therefore it was not possible to collect more specimens.

The fork length (FL) and total length (TL) of fish were measured on biometry board (to the nearest 0.1 mm) and weighed to the nearest 0.01 g. Length-weight relationship was described using the potential function (Sparre *et al*., 1992; King, 1995) as:

$$BW = a \times FL^{b}$$

where:

BW is the body weight (g), FL: the fork length (mm).

a: intercept of regression; and b: regression coefficient.

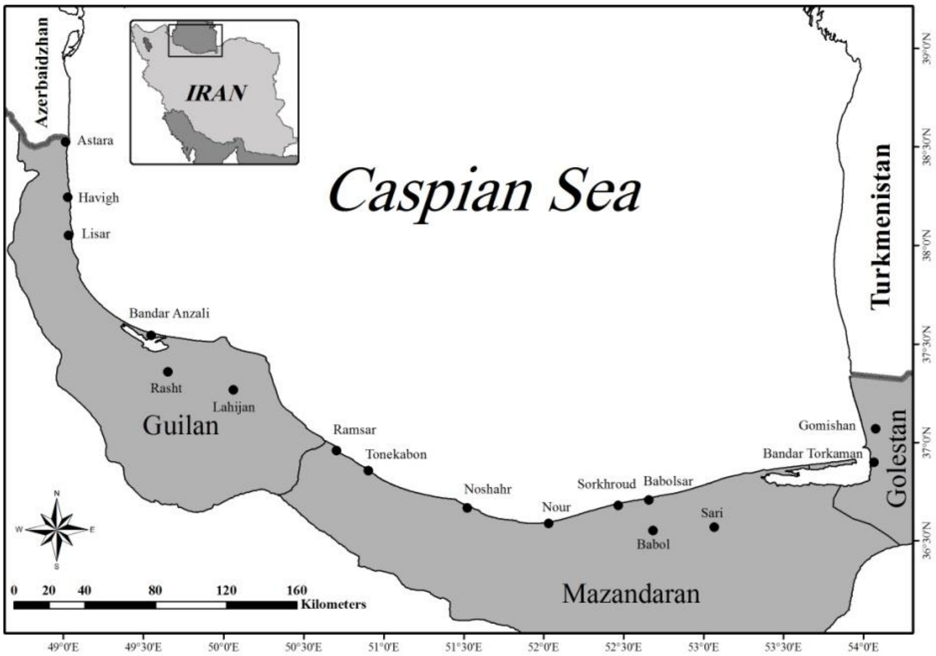

Fig 1. The map of study area in the southern Caspian Sea

An abdominal incision was made to determine sex of specimens. Asteriscus otoliths were taken from each specimen, cleaned and stored dry in glass vials for later age determination. Total asteriscus otolith weight (OW) was measured using an electronic balance with 0.0001 sensitivity. Otolith length (OL) was defined as the longest dimension between the posterior edges of otolith; and otolith width (OH) as the dimension from the dorsal to ventral edge. Also SEM Images (Scanning Electron Microscope) were prepared from all extracted sagitta otoliths (Figs. 2 and 3).

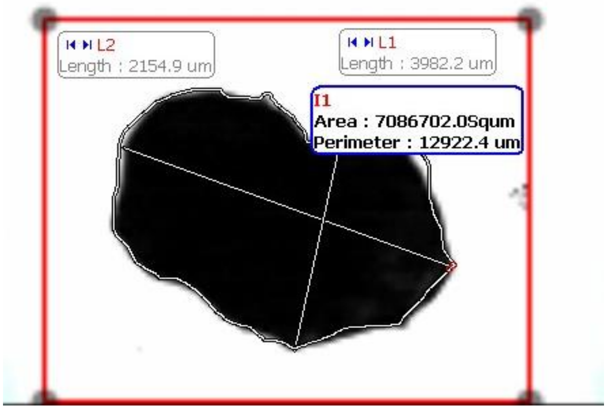

Fig. 2. Morphometric measurements of asteriscusotolith using MATIC programme

Then 30 asteriscus otoliths were selected in different fish sizes and were embedded in clear epoxy resin and were sectioned using a Buehler low-speed saw containing a diamond blade which cut a thin section (300 μm) through the nucleus. A grinding wheel fitted with silicon carbide paper with different grit sizes flushed with water was used to remove excess resin to provide a polished face for viewing. The sections were then mounted on a glass slide and were read under a Nikon compound microscope. The sectioned otoliths were read independently twice with no reference to the previous readings and without any knowledge of the fish specifications.

Morphometric and age relationships were built using exponential regression models which best fit the data distribution such as: BW vs. TL, OW vs. BW, OL vs. TL, Age vs. OL.

RESULTS

Summary results of all measurements of fish and otolith have been tabulated in Table 1. The results of biometric measurements of *A. chalcoides* specimens revealed that the minimum, maximum and mean (± SE) fork length were 18.1, 26.0 and 20.21 ± 0.287 cm, respectively and these values for asteriscus otoliths were 3004, 4200 and 3614.06 ± 86.18µm, respectively (Table 1). Fig. 3 shows the SEM image of *A. chalchoides* in the southern Caspian Sea.

Table 1. Some morphometric measurements of fish body and otolith of *A. chalcoides* in the southern Caspian Sea

Parameters	Min	Max	Mean	SE
Total weight (g)	79	280	128.22	7.086
Total Length (cm)	19.0	28.3	22.00	0.310
Fork Length (cm)	18.1	26.0	20.21	0.287
Otolith Weight (g)	4.1	7.2	4.86	0.152
Otolith Length (µm)	3004	4200	3614.06	86.179
Otolith Width (µm)	1930	2968	2468.80	76.026

The sex ratio of males to females *A. chalcoides* in the Caspian Sea was 1.00:2.12 with significant differences ($p>0.05$). Also, there were no significant differences in morphology of otoliths between males and females for examined species based on SEM images (Fig. 3).

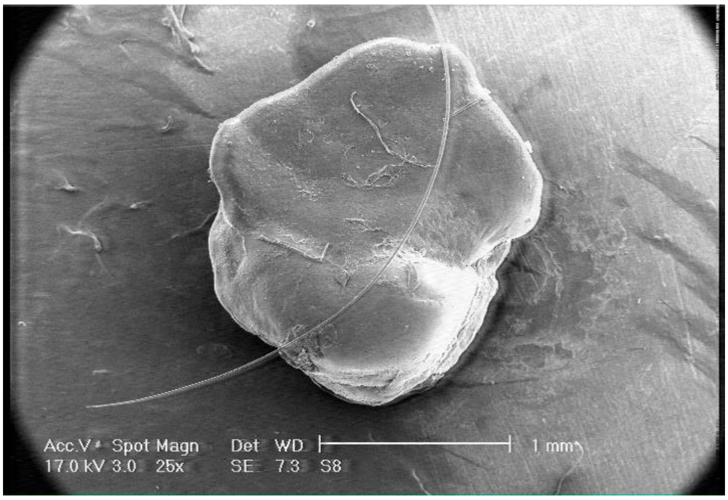

Fig 3. SEM image of asteriscus otolith of *A. chalcoides* from the Caspian Sea using Electron Microscope

The regression between total length (TL, cm) and otolith weight (OW, µm) for Caspian Shemaya was calculated as: OW= 0.0003TL- 0.0001 (R^2=0.3143) (Fig. 5), and the regression for body weight (BW) and total length (TL) was: BW= $0.1408TL^{2.199}$ (R^2=0.602) and it showed a negative allometric growth in this species (Fig.4).

The results revealed that the minimum and maximum age were 1^+ to 4^+ years for Caspian shemaya with the otolith length and weight 3004-4200 µm and 0.0041-0.0078 g respectively. The following linear relationship between age and total length with high correlation (r= 0.67) was defined as OW=0.0003Age + 0.0001 with weak correlation of r= 0.561 (Fig. 5).

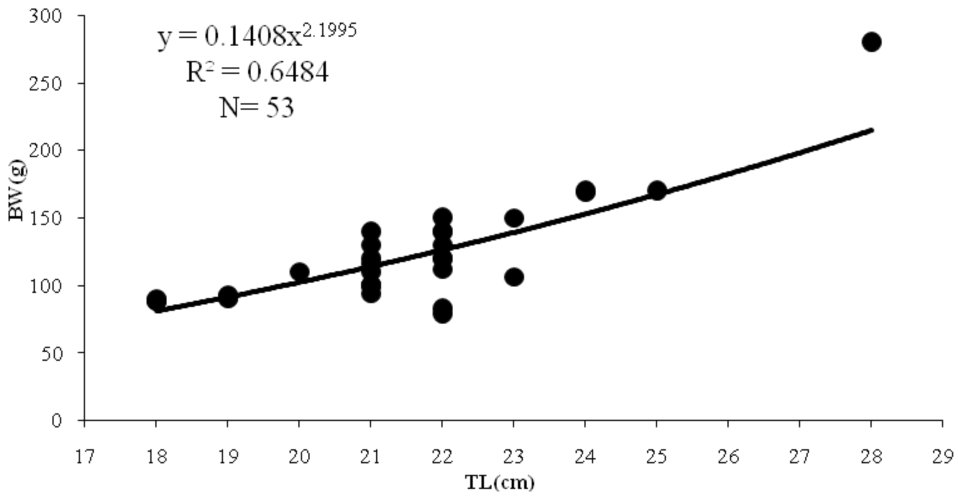

Fig 4. Length-Weight relationship for *A. chalcoides* in the southern Caspian Sea

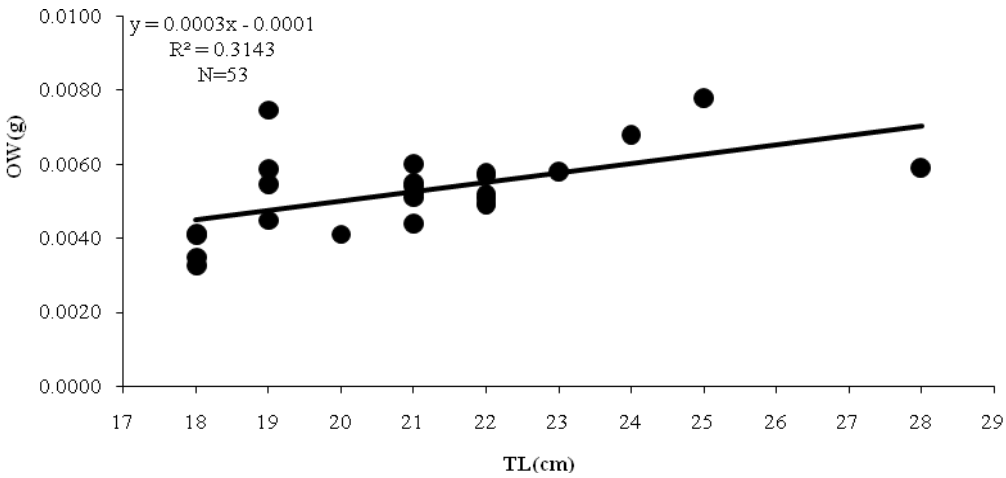

Fig 5. Total Length-Otolith Weight relationship for *A. chalcoides* in the southern Caspian Sea

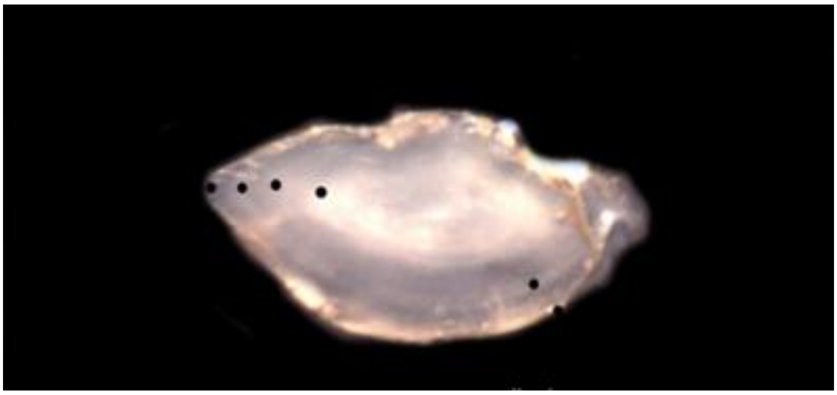

Fig 6. A section of asteriscus otolith of *A. chalcoides* from the Caspian Sea

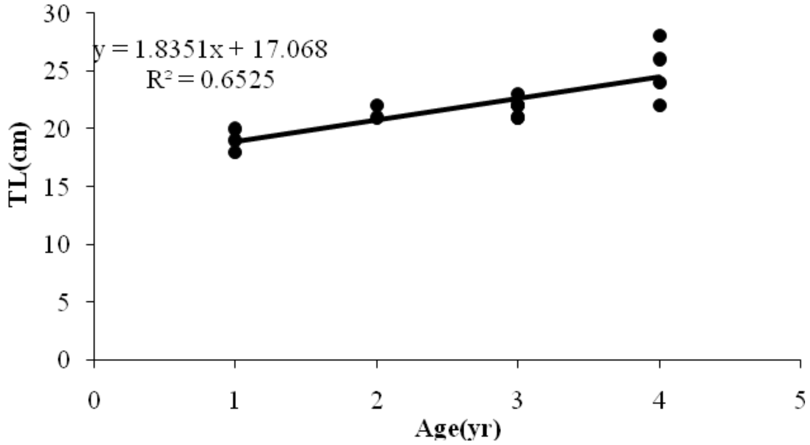

Fig 7. The linear relationship between age and otolith length for *A. chalcoides*
There was no significant relationship between TL and Age for *A. chalcoides* ($p > 0.05$), and also significant correlationwas not observed between age and otolith morphometric parameters ($r = 0.17$, $p > 0.05$).

DISCUSSION

Caspian Sea located in the north of Iran and ecologically is considered as temperate waters. There are different methods for aging of fishes in temperate areas, of those, the reading of increments on scales is the easiest, but the most precise method is using of otolith. About 110 fish species have been identified in the South Caspian Sea; of those, Caspian shemaya or Danube bleak is considered as an important one (Naderi and Abdoli, 2004), and also as an endangered species in this area. At present, very few individuals of shemaya are found in the Caspian Sea due to overexploitation, climate changes, ecosystem disturbance and increasing amounts of pollution (Daryanabard *et al.*, 2009).The aging of aquatics is an important parameter for further fisheries management and stock assessment (Campana, 2005) and measuring the morphological parameters is an applied key for food items identification in feeding behavior investigations (Valinassab *et al.*, 2012).

In this study, age of *A.chalcoides* from the Caspian Sea ranged from 1^+ to 4^+ using sectioning the sagitta otolith. Escot and Granado-Lorencio (2001) determined the age of *B. sclateri* using Lapillus otolith too. In the previous studies, the maximum age of Caspian Shemaya had been reported to be up to 8^+ years old from Sefidrood River (Azari Takami and Rajabnejad, 2002) and 5^+years old from Shirud River (Rahmani *et al.*, 2009); but at present old individuals were not found in the study area. Nikolsky (1980) suggested that, the situation in wide range of age distribution in a population is acceptable as an indication of enough level in the food of water system. The decrease of individuals in old age groups in the population will cause increase of individual in young age groups, decreasing the food competition. Some cyprinids has long-life span and as an instance, the age of *Ptychobarbus dipogon* (Cyprinidae family) ranged from 1^+ up to 44^+(Li and Chen, 2008). This situation may be caused by genetics structures of populations.

By increasing growth and age of fish, the weight of otolith increased significantly ($R = 0.997$) in a linear trend in *Trisopterus minutus* (Metin and Ilkyaz, 2008). In the present study, the same results werefound in *A. chalcoides* with high correlation ($r = 0.670$) between age and otolith length (OL)(Fig. 7). Lychacov and Rebeane (2000) revealed that lack of correlation between otolith morphology and fish length could be acceptable in the nature for aquatics.

The sex ratio of males to females of *A. chalcoides* in the Caspian Sea is 1.00:2.12 with significant differences. The same result was reported by Rahmani *et al.* (2009) who reported the ratio of M:F=1.02:2.36 in *A. chalcoides* from Shirud River. However, the ratio found in our study is not in agreement with the normalratio of 1.00:1.00 given for a number of species (Nikolsky, 1980). According to Nikolsky (1980), sex ratio varies considerably from species to species, but in the majority of species it is close to one. In the present study, no significant differences were found between males and females of examined species from the point view of otolith morphology.

The regression between different morphological parameters of otolith and fish body were measured consist of: TL vs. otolith area (OA, µm); body weight (BW) vs. OA; TL vs. otolith weight (OW); BW vs. OW; and many other measurements but the best fit was found between two parameters:TL vs. OW (R=0.835). This finding was not in accordance with the finding of Hunt (1992) who proposed an exponential relationship between otolith volume and fish length.

The SEM picture of otolith was illustrated in Fig. 3 which can be used for identifying stomach contents of predators of cyprinids. Finding the otoliths among stomach contents of predators, the shape and form of rostrum and antirostrum is highly emphasized to be taken into consideration and is a valuable key for prey identification. Amoie *et al*. (2013) reportedthe same findings for other cyprinid species consisting of *Barbus capito* and *B. brachycephalus* from the southern Caspian Sea. On the other hand, no separate populations have been found in different rivers entering into the Caspian Sea and also in its delta (Rahmani *et al*., 2007), therefore the shape and morphology of Caspian shemaya otolith can be used for all regions of the southern Caspian Sea for species identification and prey/predator food chain.

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تعیین سن و ریخت شناسی اتولیت *Alburnus chalcoides* (Guldenstaedt,1772) در جنوب دریای خزر

ف. عمویی[1]*، ت. ولی نسب[2]، ا. هایتو[2]

۱- دانشگاه آگراریان تاجیک، دوشنبه، تاجیکستان
۲- مؤسسه تحقیقات شیلات ایران، تهران، ایران

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چکیده

هدف از این تحقیق، بررسی سنی و رشد ماهی شاه کولی به عنوان یکی از گونه‌های مهم خانوده کپور ماهیان در حوضه جنوبی دریای خزر در طول سال‌های ۲۰۱۱-۲۰۱۰ می‌باشد. نمونه‌برداری ماهانه و در مجموع ۵۳ نمونه جمع‌آوری و برای انجام بررسی های زیستی و زیست‌سنجی و خروج اتولیت به آزمایشگاه منتقل شدند. حداقل و حداکثر سن این گونه ۱ و ۴ سال به ترتیب مربوط به ماهیان به طول چنگالی ۱۸/۱ و ۲۶/۰ سانتیمتر تعیین گردیدند. میانگین طول چنگالی برابر با ۰/۰۲۸۷ ± ۲۰/۲۱ سانتی متر و نسبت جنسی نر به ماده ۱/۰ به ۲/۱۲ اندازه‌گیری شد. همچنین رابطه طول- وزن برای شاه کولی محاسبه شد. رگرسیون‌های مختلف میان پارامترهای سن و اندازه‌گیری‌های ریخت‌سنجی ماهی و اتولیت تعیین شدند و بالاترین همبستگی میان سن و طول بدن به دست آمد. همبستگی معنی داری میان اندازه‌گیری‌های ریختی اتولیت و اندازه‌گیری‌های ماهی مشاهده نشد. نتایج نشان داد که ماهی شاه کولی دارای رشد آلومتریک است.

* مولف مسئول