



## The Reproductive Biology of Forkbeard, *Phycis phycis* (Linnaeus, 1766) (Phycidae) in the Adriatic Sea (Croatia)

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### Abstract

The forkbeard, *Phycis phycis* (Linnaeus, 1766), is a species widely distributed throughout the Mediterranean and Adriatic Sea and although it is of great importance for fishing industry, little is known about its reproductive characteristics. This study provides first data on reproductive characteristics of this species in the Mediterranean, important for management and stock assessment. For this purpose a total of 550 individuals ranging from 19.9 to 45.8 cm in total length were collected monthly in a period of one year using trammel nets. Sex ratio of males to females was 0.62:1. The estimated length where 50% of analysed individuals were sexually mature was 30.98 cm for females and 32.98 cm males. The peak value of gonadosomatic index was recorded in November and continued throughout the December, indicating the highest spawning activity. This period is characterized by presence of oocytes with migrated nucleus and yolk coalesces in ovary and spermatozoa in testes.

**Keywords:** Gadiform fish, spawning period, histology, gametogenesis.

### Introduction

The forkbeard, *Phycis phycis* (Linnaeus, 1766), (Phycidae) is a gadoid fish distributed throughout the Mediterranean Sea and inhabiting Northeast Atlantic from Bay of Biscay to Morocco, south to Cape Verde, including the Macaronesian Archipelagos (Abecasis *et al.*, 2009). On the eastern Adriatic coast, forkbeard is predominantly widespread in middle and south part (Pallaoro and Jardas 2002), especially on the south side of outer islands (Jardas, 1996). The forkbeard as bentopelagic species inhabits all types of bottoms: muddy, sandy or rocky, it is more active during the night while it hides in holes during the day. It is present at depth from 50 to 270 m, but sometimes it can be found even deeper (Jardas, 1996).

While it has an ecological importance in the Mediterranean fishing industry (Farjallah *et al.*, 2006), it is also one of the most important commercial demersal species in the Azores (Abecasis *et al.*, 2009). However, in spite of its relatively broad geographic distribution and high economic value, the life history of the forkbeard is poorly known. Some aspects, such as age, growth and reproduction have been investigated in the Azorean archipelago (Silva, 1986; Abecasis *et al.*, 2009). Matić-Skoko *et al.* (2011) investigated age and growth of this species in

the Adriatic Sea. However, other life history features, such as reproduction, remain largely unknown for the Mediterranean. Early studies on eggs and larvae (Lo Bianco, 1909) indicate their occurrence between January and April, but other determinants of reproductive strategy are unknown.

The lack of basic biological information for forkbeard prevents ability to assess population sustainability and management of this commercially important species therefore the aim of this study is to describe reproductive parameters required for the stock assessments of this species not only in the Adriatic but also in the Mediterranean Sea.

### Materials and Methods

A total of 550 individuals were collected monthly from January to December 2008 in the south-eastern Adriatic Sea (Elafiti Islands). Fish were caught using trammel nets with 80 and 300 mm stretched mesh size (inner and outer panel). The specimens were frozen immediately after catch and transported to the laboratory for further analysis. For each fish, total length (TL) was measured using a simple calliper to the nearest 0.1 cm and weighed (Wt) to the nearest 0.1 g. Fishes were gutted, and gonads were removed and weighed (Wg) with three

decimal accuracy. Sex was determined by macroscopic observation of the gonads (Macer, 1974). Sex ratio was examined using  $\chi^2$  (Chi-square) test with a probability level of 0.05 to test differences in relation to the expected ratio 1:1. The gonadosomatic index (GSI) was estimated as:

$$\text{GSI} = W_g / W_t \times 100.$$

To estimate size at first sexual maturity, the data were fitted in equation:

$$P = 1/(1+e^{(a-bxL)});$$

where P is probability that individuals are sexually matured and L is their length. The length when 50% of analysed individuals were mature was calculated according to Sparre and Venema (1998):

$$L_{50\%}=a/b.$$

Gonad development stage were adopted from Murua and Motos (2006) and modified for this species. Classification of maturity was given to each individual, fish were classified as immature (I), undergoing maturation (II), ripening (III), ripe (IV), spawning (V) or spent (VI) (Table 1). After weighing, gonads were fixed in 4% formalin and processed histologically to enable the observation of the gonadal development processes. A small piece of tissue from the middle of gonad was tested. Fixation was followed by dehydration in increasingly concentrated ethanol (70%, 80%, 95% to 100%) and tissue

clearing. Finally, tissue was embedded in paraffin, sectioned on microtome (5  $\mu$ m) and stained by hematoxylin and eosine dyes. Classification of oocyte development was based on criteria used by Selman and Wallace (1989). Each ovary was staged according to the most advanced group of oocytes present in the sample: unyolked and perinucleolar, early yolked and previtellogenic, yolked and vitellogenic, migratory nucleus or hydrated. Measurement of oocyte diameters was performed using an image analysis system (AxioVision Release 4.8.2) and statistical differences in oocyte diameter between different developing stages of gonads were tested using Kruskal-Wallis test.

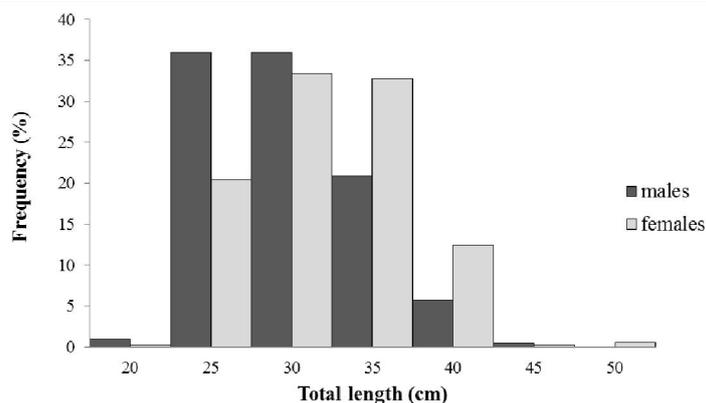
## Results

Total of 550 individuals were examined, out of which 211 were males (38%) and 339 were females (62%). Total length for males ranged from 19.8 to 42 cm (27.25 $\pm$ 4.73 cm (average $\pm$ sd) and females from 20 to 45.2 cm (29.52 $\pm$ 4.94 cm) (Figure 1). Weight of analysed males ranged from 72 to 898 g (228.55 $\pm$ 138.2 g) and females from 72 to 1060 g (301.16 $\pm$ 161.79 g). The overall sex ratio of males to females was 0.62:1 and it was statistically significantly different from 1:1 ratio ( $\chi^2 = 29.79$ ;  $P < 0.05$ ).

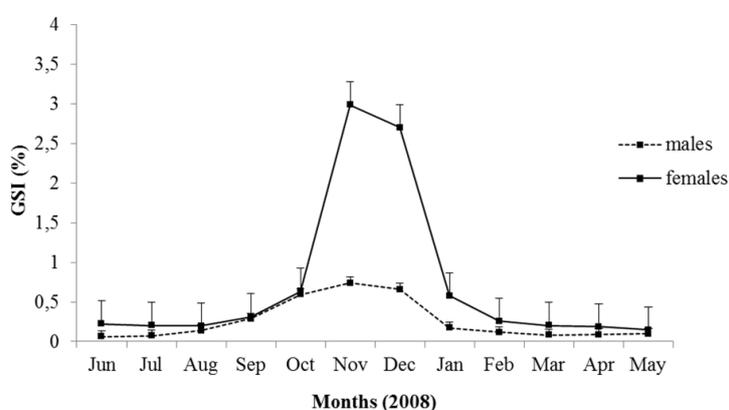
Monthly mean GSI values, except in October, November and December were lower than 1. The gonadosomatic index peaked in November and December indicating the highest spawning activity. After January, GSI values declined sharply indicating

**Table 1.** Description of gonad development stages for female and male forkbeard, *Phycis phycis* in the Adriatic Sea (adopted and modified from Murua and Moto, 2006)

	Stage	Description
Females		
I	Immature	Ovaries were small and transparent. Oocytes in perinucleolar stage were very small and nucleus was surrounded by dark stained cytoplasm
II	Maturation	Ovaries increase in weight, and covered with well-developed blood vessels. Oocytes beginning to undergo vitelogenesis with oil globules and cortical alveoli in cytoplasm. Nucleus starts to migrate.
III	Ripening	Oocytes are large and could be seen with naked eye in ovaries. Nucleoli are in the centre and more yolk starts to accumulate.
IV	Ripe	Ovaries fill most of the ventral cavity. Oocytes were undergoing hydration.
V	Spawning	Under slight pressure oocytes are released from the genital aperture. Most eggs are hydrated and ruptured follicles were present.
VI	Spent	Ovaries were shrunken. Oocytes had been discharged and scattered unspawned eggs could be seen.
Males		
I	Immature	Testes were small and elongated and undergoing spermatogenesis. Many spermatogonia within lobules.
II	Maturation	Testes increase in size but still with no visible vascularization. Number of spermatogonia increase between sperm ducts that are more visible than in immature stage.
III	Ripening	Testes large and less translucent. Ripe spermatozoa and all stages of spermatogenesis are present within spermatic duct.
IV	Ripe	Testes occupied half the ventral cavity. There is abundance of spermatozoa with little spermatogenetic activity. On slight pressure products are not released from genital aperture.
V	Spawning	Testes are milky white and sperm was easily released with slight pressure.
VI	Spent	Testes were shrunken in size. Sperm ducts empty with very few residual spermatozoa



**Figure 1.** Length-frequency distribution of male (n = 212) and female (n = 339) forkbeard, *Phycis phycis* in the Adriatic Sea.



**Figure 2.** Monthly gonadosomatic index values for males (n = 212) and females (n = 339) of forkbeard, *Phycis phycis* in the Adriatic Sea.

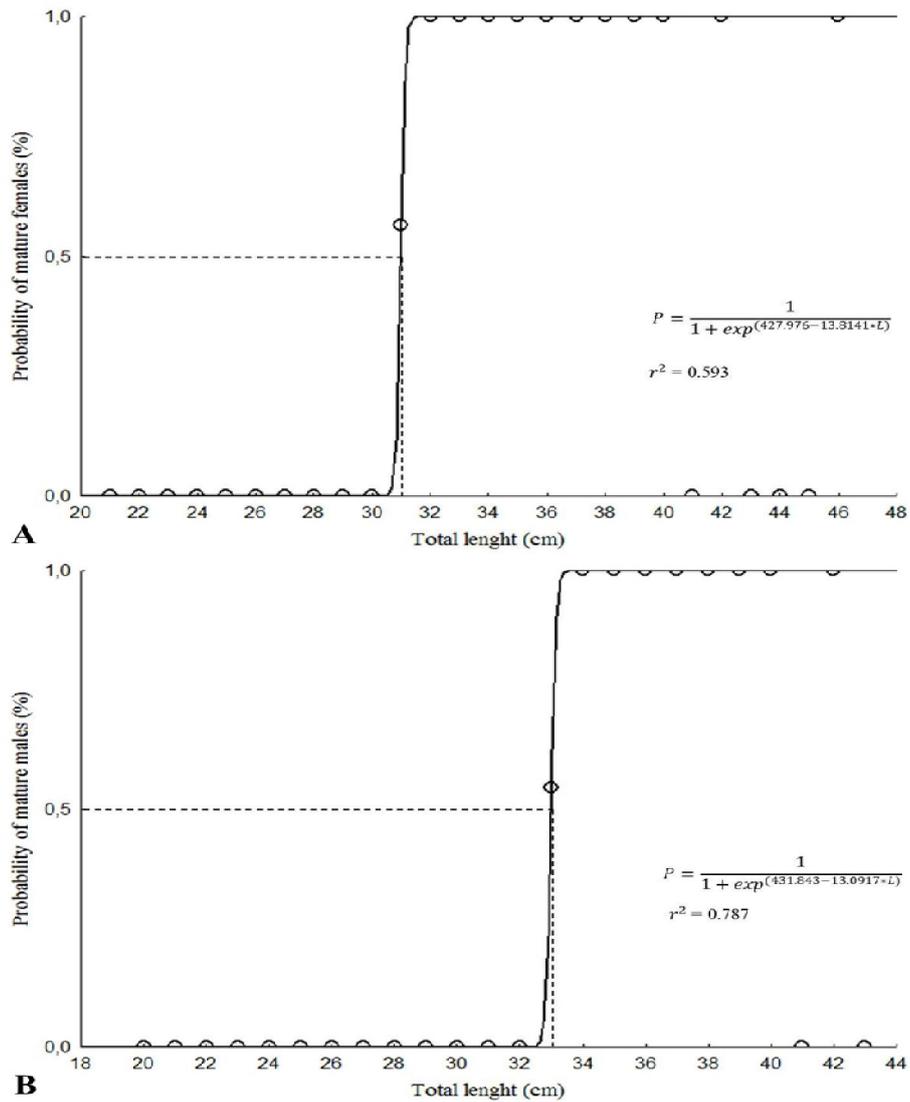
that the majority of reproductive products have already been released (Figure 2).

To determine first sexual maturity 139 females rearing in total length from 21.1 to 45.2 cm and 69 males rearing from 19.8 to 42 cm were sampled during the period of late gametogenesis and maximum gonad activity (from September to January). The estimated length where 50% of analysed individuals were sexually mature was 30.98 cm for females (Figure 3A) and 32.98 cm males (Figure 3B). Immature developing phase was characterized by presence of unyolked and perinucleolar oocytes in ovaries of forkbeard (Figure 4A). After July oocytes started to mature. In the ripening phase cortical alveoli and oil globules were present in cytoplasm and yolk plates were forming (Figure 4B). Ovaries in ripe phase contained oocytes with nucleolus that have started migration to periphery (Figure 4C). Spawning period from October to January was characterized by presence of oocytes with migrated nucleus and yolk coalesces were completed (Figure 4D). In testes of forkbeard during maturation different stages of spermatogenesis were present (Figure 5A). In the spawning season large number of spermatozoa filled the main sperm duct (Figure 5B) and when spawning is finished ripe testes were almost empty (Figure 5C).

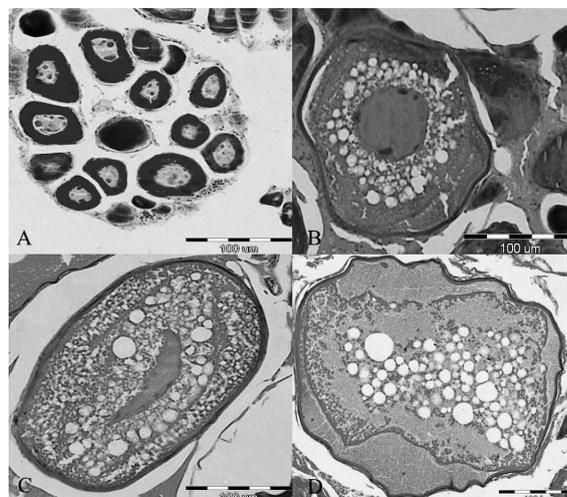
Statistically significant mean differences in oocyte diameter between developing stages were observed (Kruskal-Wallis,  $H=525.5014$ ,  $P<0.05$ ). In immature phase of ovarian development oocyte diameter ranged from 11 to 72  $\mu\text{m}$ . During the vitellogenesis, sizes of the oocytes increased from 26 to 200  $\mu\text{m}$  and in ripening phase they reached their maximum size of 430  $\mu\text{m}$ . Oocytes with diameter from 15 to 71  $\mu\text{m}$  were present in ovary after spawning (Figure 6).

## Discussion

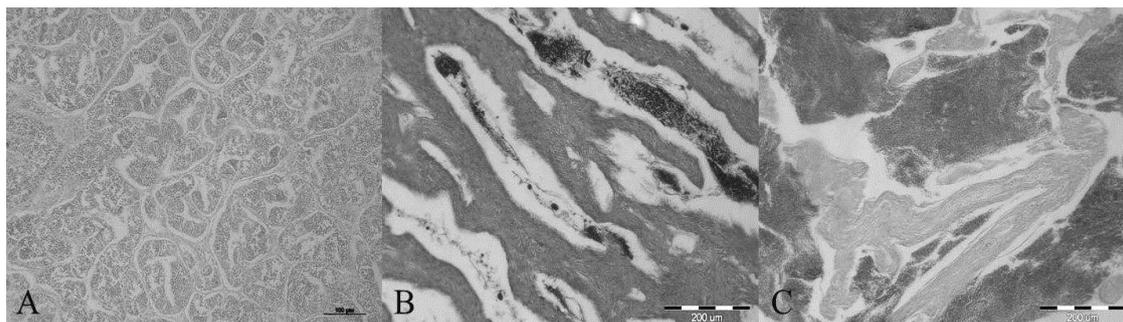
This study presents first data of reproductive characteristics of forkbeard in the Mediterranean and therefore results were compared with other gadiform species common for this area. In this study maximum total length of analysed specimens was 45.2 cm and maximum reported length for the Adriatic Sea was 64 cm (Jardas, 1996). Proportion of females of forkbeard in the Adriatic Sea was higher than males (1:0.62). It is different than sex ratio of *Trisopterus minutus* in the central Aegean Sea, where males were more numerous than females (1.29:1) (Metin *et al.*, 2008). Sex ratio varies, not only between different species but also between populations of the same species



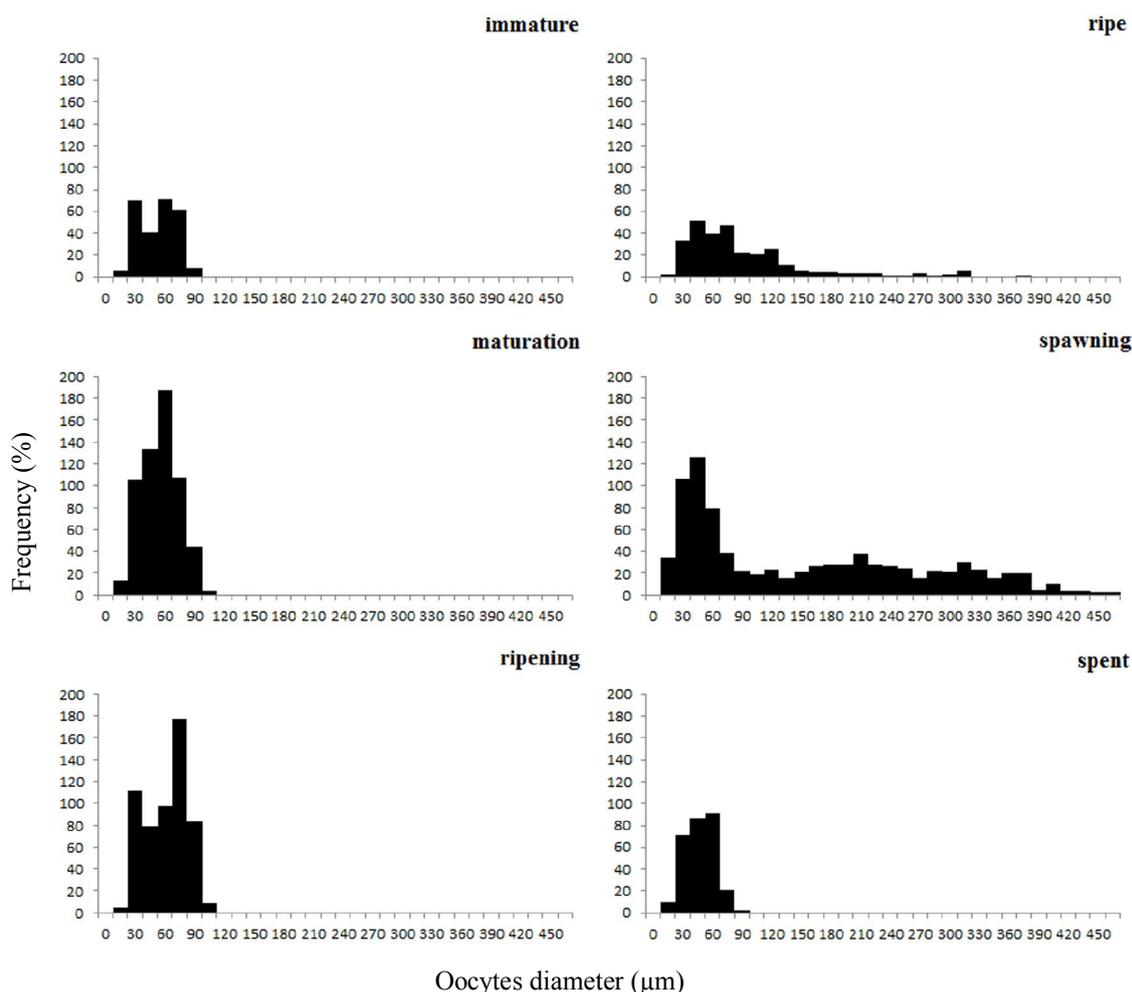
**Figure 3.** Total length of the first sexual maturity calculated for females (A) and males (B) of forkbeard, *Phycis phycis* in the Adriatic Sea.



**Figure 4.** Histological section of forkbeard, *Phycis phycis* ovaries showing oocytes in different reproductive development stage: (A) immature – unyolked and perinucleolar oocytes; (B) ripening – advanced yolked oocyte; (C) ripe – nucleus has started migration to periphery of oocyte; (D) spawning – nucleus has migrated to the periphery and yolk coalesces are completely (scale bar 100 µm).



**Figure 5.** Histological section of forkbeard, *Phycis phycis* testes showing different reproductive development stage: (A) spermatids and spermatocytes during maturation (scale bar 100  $\mu\text{m}$ ); (B) spawning sperm duct filled with spermatozoa (scale bar 200  $\mu\text{m}$ ); (C) ripe testes after the spawning season (scale bar 200  $\mu\text{m}$ ).



**Figure 6.** Oocyte diameter ( $n=1800$ ) during different developing stages in ovarian of forkbeard, *Phycis phycis* in the Adriatic Sea.

from year to year (Metin *et al.*, 2008).

The analysis of gonadosomatic index (GSI) can provide a quantitative assessment of the degree of gonad development and spawning season (Gutiérrez-Estrada *et al.*, 2000). GSI of forkbeard in the Adriatic Sea peaked in November and December, which indicates the highest spawning activity in this period. Spawning time is similar with *Phycis blennoides* in

Ionian Sea (Materrese *et al.*, 1998). Although they found mature males between August and March, presence of ripe females indicated that spawning of *Phycis blennoides* occurs during late autumn to early winter. Rotllant *et al.* (2002) investigated population of *Phycis blennoides* in the western Mediterranean Sea. Mature females in their study were found only in autumn, while mature males from summer to early

autumn. Values of GSI obtained in this study are similar with those obtained for *Trisopterus minutus* in eastern Adriatic Sea which started to increase in December, peaked in February and then slowly decreased to minimum in September (Šantić et al., 2010). Metin et al. (2008) observed that reproduction of *Trisopterus minutus* reached its maximum in January and continued until April, but in both sexes gonads started to mature in October same like in forkbeard. In *Trisopterus luscus* from Galician shelf, north-western Spain, GSI values rapidly increased between December and January with peak between January and March (Alonso-Fernández et al., 2008). Intensive spawning of *Merlangius merlangus* in the Black Sea occurs three times in a year; at the end of the summer, in mid-autumn and in early winter (Bilgin et al., 2012). From above mentioned data and data obtained from the study of Tsikliras et al. (2010) it can be concluded that reproduction period of different gadids species in Mediterranean is similar. Silva (1986) calculated length at first maturity at 41 cm for females and 36 cm for males in Azorean waters and in this study that length was lower for both sexes.

The oocyte development in most teleost fish follows a similar pattern and was described by Selman and Wallace (1989), West (1990) and Tyler and Sumpter (1996). Gonad analysis revealed that forkbeard in the Adriatic Sea has group-synchronous ovarian development. In ovaries with this development at least two populations of oocyte can be recognized at any time (Wallace and Selman, 1981; Murua and Saborido-Ray, 2003). In the ovaries of forkbeard oocytes gradually increase in size along with the ovarian development. From February to September mean value of oocyte diameter is not higher than 100 µm. These previtelogenic oocytes were characterized by large cytoplasm. After the primary growth phase and the beginning of vitellogenesis, characterized by formation of zone granulose and zone radiate and appearance of cortical alveoli in cytoplasm, the oocyte diameter starts to increase more rapidly. From October to January oocytes grow and reach diameter of 430 µm. During this period histological observation revealed that ovaries of forkbeard contain high presence of oocytes in migrates nucleus, yolk and hydrated stage and in testes high abundance of spermatozoa. There are no data about gonadal histology of other species of this family except that the telolecithal eggs of *Phycis blennoides* from Ionian Sea had a diameter from 200 to 590 µm (Matarrese et al., 1993).

In the conclusion, this study shows that forkbeard has group-synchronous ovarian development and spawning season from late October to early January. The obtained results from this study are important input data for management and stock assessment of this commercially important fish species.

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