

Fisheries Research 35 (1998) 171-181



# Reproductive strategy of *Aphanius fasciatus* Nardo, 1827 (Pisces: Cyprinodontidae) in the Mesolongi and Etolikon lagoons (W. Greece)

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Received 5 June 1997; accepted 21 December 1997

#### Abstract

The reproduction and fecundity of *Aphanius fasciatus* were studied in 2034 fish originating from three locations in the Mesolongi and Etolikon Lagoon system. The sampling stations differed in water temperature and salinity. The species exhibits early sexual maturation and is able to reproduce several months after birth. The reproductive effort (GSI) and histological studies of the ovaries indicate that reproduction is extended and lasts from April to July. Differences in the GSI values were observed between the sites. Fish originating from the sites with higher salinity has significantly lower GSI values and in addition, lay fewer eggs per unit of size (total length, body weight, age) than those from the other sampling sites. The maturation of oocytes stops when the environmental conditions are not satisfactory for the survival of eggs and larvae, and oocytes are subsequently absorbed. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Aphanius fasciatus; Fish fecundity; Reproduction strategy; Greek lagoons; Cyprinodontidae

#### 1. Introduction

Aphanius fasciatus (Nardo, 1827) is known in Greece by the common name 'zabarola'. It is distributed in the central and eastern coastal zones of the Mediterranean (Tortonese, 1975; Whitehead et al., 1986; Fischer et al., 1987). Populations of *A.* fasciatus live in geographically isolated waters and in environments of high salinity, which are unsuitable for other species. It is an abundant species in lagoons, salt marshes, brackish water habitats of relatively shallow depth, and also in fresh waters. It is included in the Annex II of the European Council Directive 92/43 'The Conservation of Natural Habitats, Wildlife and Flora'.

There are four representatives of the family Cyprinodontidae in the Mediterranean: *Aphanius fasciatus* (Nardo, 1827), *A. iberus* (Cuv. and Val., 1846), *A. dispar* (Ruppel, 1828) and *A. mento* (Heckel, 1843) (Kiener and Schachter, 1974; Tigano, 1982). Hybrids between *A. dispar–A. mento* (Goren and Rychwalski, 1974) and *A. fasciatus–A. dispar* (Villwock, 1982, 1985; Fouda, 1995) have also been reported. The phylogenetic relationships between

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*Aphanius* species have been studied by Scholl et al. (1978), Villwock et al. (1983) and Tigano and Parenti (1988). Fernandez-Delgado (1988) studied the age, growth and reproduction of *A. iberus*. Haas (1982) and Fouda (1995) provided information on the ecology, feeding behavior and the life-history of *A. dispar. A. fasciatus* has been studied with respect to its morphometric characteristics (Tigano, 1982, 1991; Tigano and Ferrito, 1983, 1985), their polymorphisms (Kiener and Schachter, 1974; Parenti and Tigano, 1993), population structure (Penaz and Zaki, 1985) and to some extent its reproduction (Boumaiza et al., 1979). Also, Leonardos et al. (1996) examined the biology, growth and mortality of *A. fasciatus* in relation to the environmental conditions and Leonardos and Sinis (1996, 1997) the age, growth, mortality and the early development. In the present study, information on the reproduction and fecundity of *A. fasciatus* is presented, based on individuals collected from three locations in the Mesolongi and Etolikon lagoons.

## 2. Study area

The lagoon system of Mesolongi, Etolikon and Klisova (38° 18' 36'' N-21° 32' 00"E) (Fig. 1) is



Fig. 1. Map of the Mesolongi and Etolikon Lagoons. Sampling stations are indicated by the spots.

among the largest in the Mediterranean, with a surface area of about 150 km<sup>2</sup>. The Etolikon lagoon is of tectonic origin and makes up the northern part of the system. The Mesolongi lagoon has been formed through the silting action of two adjacent rivers, the Acheloos and Evinos. The Mesolongi lagoon comprises the central and southern part of the lagoon system and connects to the south with the Patraikos Gulf. The Alvkes (saltworks) are located in the eastern part of the Mesolongi lagoon and occupy an area of about 12.4 km<sup>2</sup>. The sampling sites were considered to be practically separate and the movement of fish from one site to the other was virtually impossible. In the salt pans of the Alvkes station. only water from the Klisova lagoon is supplied using electric pumps. Fish were trapped many years ago in the Alvkes, when the saltworks were created. The sampling station in the Mesolongi lagoon is a salt marsh, from which fish can not pass to the Etolikon Lagoon.

## 3. Materials and methods

Three sampling stations were chosen in the Mesolongi and Etolikon lagoon system (Fig. 1). Monthly samples were collected from April 1989 to January 1991. A total of 2034 specimens were caught, ranging in total length from 21.13 mm to 70.63 mm.

Samples were collected in a fry fishing (drag) net with a mesh size of 2.5 mm, length of 15 m, a height of 1.5 m at the ends and 2 m in the center and which terminated in a bag, 3 m long and 1.5 m in diameter. The fish were rinsed in fresh water and immediately placed in neutralised formalin (4%) until examination. Water temperature and salinity were recorded at the time of each sampling.

Total fish length (TL) and body weight (NW) were measured, after removal of the intestines and gonads. Sex was determined from external characteristics since *A. fasciatus* exhibits external dimorphism. Age was determined from scales from the left side of the body, between the posterior end of the pectoral fin and the anterior end of the dorsal fin. A stereoscope with transmitted and reflected light was used for the observations (Jearld, 1983; Leonardos and Sinis, 1996).

To examine the monthly changes in the gonads, the gonadosomatic index (GSI) was calculated for male and female specimens separately, according to the following relationship:  $GSI = (GW/NW) \times 100$ . Where GW is the weight of the gonads and NW is the body weight (Grant and Spain, 1975; Wooton, 1990). To compare the gonad weight between the sampling stations, an analysis of covariance (ANCOVA) was performed, with length as the covariate and gonad weight as the dependent variable (both log transformed) (Zar, 1984).

Ovaries representative of each developmental stage were set in paraffin. Thin sections  $(3-4 \mu)$ were stained using haematoxylin-eosin (H and E) (Hinton, 1990). Ovary developmental stage was assessed microscopically. To investigate fecundity, size composition and the macroscopical study of oocvte maturity, the gonads of female specimens collected in April, May, June and July 1990 at the three sampling sites, were examined. Gonads were placed individually in dishes containing Gilson's solution (Bagenal and Braum, 1978) for about 6 months. The process of maturation was evaluated using a size frequency distribution of the oocvtes in relation to the sampling station and month. The gonads were passed through a series of four sieves, with mesh size from 1.50 mm, 1 mm, 0.5 mm and 0.2 mm under a slow flow of water. The number of oocytes retained on each sieve was counted using a stereoscope. The sieve mesh sizes were chosen after a preliminary macroscopical examination of the oocytes during the reproductive period and also from a histological study of the gonads, such that each sieve mesh would retain chiefly, only one category of oocytes.

The ovaries were examined before the beginning of the reproductive period and fecundity was measured in terms of the number of oocytes with a diameter greater than 0.2 mm, using 60 gonads from female fish collected in April 1990.

The relationship between the number of eggs and fish body size was expressed by the equation:  $F = aX^b$  which after logarithmic transformation has the form: log  $F = \log a + b \log X$ , where F is the number of eggs per specimen, X is the length, weight or age of the fish, a is 'a' constant and 'b' the slope (Bagenal and Braum, 1978; Elliott, 1995). The equations F-X were calculated for each sam-

pling site separately and the slopes were compared using an ANCOVA (Tukey-test) (Zar, 1984).

## 4. Results

#### 4.1. Physicochemical parameters

The Mesolongi Lagoon had an average depth of 0.8 m and the bottom was covered by vegetation. The water temperature ranged from 7 to 27.8°C and the salinity from 14 to 23.5 ppt, during the study period (Fig. 2). The Etolikon Lagoon is a meromictic

lagoon of tectonic origin, with an average depth of 12 m and maximum depth of 33 m. Sampling was carried out near the shore, where the depth was 1.5 m to 2 m, and the bottom was sandy. The water temperature ranged from 8 to 28.7°C and the salinity from 10 to 22 ppt. The sampling station Alykes was located in the first pond of the Mesolongi saltworks, from which water is supplied to the main evaporating ponds of the saltworks. The average depth of Alykes was less than 0.5 m, the bottom was covered with large amounts of mud and the vegetation was restricted. The temperature ranged from 6 to 39°C and the salinity from 19 to 80 ppt.



Fig. 2. Monthly variations in temperature and salinity at the sampling stations at the time of sampling.

## 4.2. Reproduction

Significant differences were observed in gonad weight of each sex between the three sampling stations (ANCOVA, F = 14.53, P < 0.001; F = 20.44, P < 0.001 for females and males, respectively). The monthly mean GSI values reach 13.61% and 3.19% for the Mesolongi Lagoon, 14.73% and 3.51% for the Etolikon Lagoon, and 5.68% and 2.17% for Alykes, for female and male specimens, respectively (Fig. 3). The GSI values increased during January

and February, peaking in the middle of spring. The mean GSI values (and the 95% c.i.) during the reproductive period, indicate that the females, and males to a smaller extent from, the Mesolongi and Etolikon Lagoons demonstrate a significantly higher reproductive effort than the fish from Alykes.

Part of the reproductive strategy of *A. fasciatus* is the extended duration of the reproductive period. This is evident from the duration of the high gonadosomatic index (GSI) values (Fig. 3), as well as from the histological sections of the ovaries in which



Fig. 3. Gonadosomatic index values for female and male A. fasciatus from the three sampling stations for the period July 1989–July 1990.



1 mm

Fig. 4. Histological sections of *A. fasciatus* ovaries from Alykes sampling station for the period November, 1989–July, 1990; (a) November, 1989; (b) January, 1990; (c) February, 1990; (d) April, 1990; (e) June, 1990; (f) July, 1990. (PO) perinucleolar oocytes. (RO) oocytes that were not discharged in the previous reproductive period and which are in an advanced stage of absorption. Early yolk vesicle oocytes (EV). Primary yolk granule oocytes (PG). Tertiary yolk granule oocytes (TG). Oocytes ready to be discharged and in which oil globules (OG) can be distinguished. Empty egg case after discharge of oocytes (EC), arrows point to sites of follicles rupture.

mature, hydrated oocytes are observed over a long time period (Fig. 4), showing that the reproductive period has a duration of about 6 months. Gonadosomatic index values were relatively low (Fig. 3) since *A. fasciatus* is a batch spawner.

Four categories of oocytes were identified in histological sections of ovaries (Fig. 4):

- 1. Small, round transparent oocytes with a central nucleus and with an average diameter 0.4 mm.
- 2. Developing oocytes containing yolk vesicles with

an average diameter of 0.9 mm (yolk vesicle stage).

- 3. Oocytes with large yolk vesicles, mainly central which were formed by the fusion of smaller vesicles with an average diameter of 1.3 mm (secondary yolk stage).
- 4. Round oocytes full of yolk with an average diameter of 1.7 mm (tertiary yolk granule).

The size frequency distribution of oocyte diameter during the spawning season shows differences between sampling stations. At Alykes the ratio of ready-to-be released oocytes was lower than from the other stations (Fig. 5). Observations of the ovaries show that oocytes are present at different developmental stages. A large reserve of resting, immature oocytes always exists, some of which mature and are liberated, while many do not mature (Figs. 4 and 5). At Alykes, a higher percentage of oocytes, than at the other two sampling sites (Fig. 5), do not mature and are eventually were absorbed after the reproductive period (Fig. 4).

#### 4.3. Fecundity

The relationship between absolute fecundity and fish size (total length, body weight or age) was



Fig. 5. Size composition of A. fasciatus oocytes from the three sampling stations for the period April-July 1990.

Table 1

Relationship between fecundity (F) and fish size (total length, body weight and age) with respect to sampling station

Sampling station	Equation	95% c.i. of b	Ν	$R^2$	Р
Relationship between fe	cundity (F) and total length (TL	L) (cm)			
Mesolongi lagoon	$F = 1.89 \text{ TL}^{2.86}$	2.02-3.70	20	0.83	< 0.001
Etolikon lagoon	$F = 0.42 \text{ TL}^{3.62} *$	2.42-4.82	20	0.68	< 0.001
Alykes	$F = 8.89 \text{ TL}^{1.52} *$	0.68-2.35	20	0.46	0.0015
Relationship between fe	cundity (F) and body weight (N	W) (g)			
Mesolongi lagoon	$F = 5.49 \text{ NW}^{0.84}$	0.60 - 1.07	20	0.83	< 0.001
Etolikon lagoon	$F = 0.05 \text{ NW}^{1.09} *$	0.71 - 1.46	20	0.65	< 0.001
Alykes	$F = 3.02 \text{ NW}^{0.49} *$	0.24-0.73	20	0.49	0.0005
Relationship between fe	cundity (F) and age (t) (years)				
Mesolongi lagoon	$F = 86.90 t^{0.83}$	0.49-1.15	20	0.59	< 0.001
Etolikon lagoon	$F = 71.71 t^{1.13}$	0.68 - 1.58	19	0.60	< 0.001
Alykes	$F = 55.65 t^{0.75}$	0.41 - 1.09	20	0.55	0.0002

\* Statistically significant difference.

examined for each sampling station and the slopes of the equations were compared using an ANCOVA. The slopes of the equation for absolute fecundity-total length for both Mesolongi and Etolikon Lagoons were higher, than that for Alykes (2.86 = 3.62 > 1.52)for Mesolongi, Etolikon and Alykes, respectively; F = 5.87, P = 0.004, Table 1). For absolute fecundity-body weight, an ANCOVA indicated that the slopes of the equation for the Mesolongi and Etolikon Lagoons were significantly higher, than that for Alykes (0.84 = 1.09 > 0.49 for Mesolongi, Etolikon and Alykes, respectively; F = 5.44, P = 0.006, Table 1). For the relationship between absolute fecundityage. ANCOVA indicated that there were no significant differences between the sampling sites, even though the slopes of the equations for Alykes were much lower than those for the other two sites (Table 1). At Alykes, the number of eggs that could be laid by a female is significantly smaller than that which could be laid by a female of the same size originating from the Mesolongi and Etolikon Lagoons. The maximum absolute fecundity for the Mesolongi Lagoon was 102.19 eggs/cm, for the Etolikon Lagoon it was 68.22 eggs/cm and for Alykes it was 47.62 eggs/cm.

Relative fecundity, which is calculated as the number of eggs per unit weight was also, studied (Bagenal and Braum, 1978; Snyder, 1983). Given the allometry of the fecundity-weight relationship (Table 1), the average relative fecundity was 136.20

 $\pm$  20.87 eggs  $\times$  g<sup>-1</sup> for the Mesolongi Lagoon, 108.40  $\pm$  13.10 eggs  $\times$  g<sup>-1</sup> and 94.69  $\pm$  54.10 eggs  $\times$  g<sup>-1</sup> for Alykes.

## 5. Discussion

A. fasciatus in the Mesolongi and Etolikon Lagoons first reproduces after completion of the first year of life, although a number of new-born individuals of both sexes are found to have well developed gonads. These are individuals, which are born at the beginning of the reproductive period and reached sexual maturity towards the end. One reproductive strategy of those species which live in unstable and unpredictable habitats is early sexual maturation. Nikolsky (1963) and Wooton (1990) have reported that cyprinodontids, which are encountered in temporary pools in tropical and subtropical areas, can reach sexual maturity in a few weeks. Reproduction in the age class 0 + has also been observed in populations of A. *iberus* from the marshlands of the River Guadalquivir, where individuals form gonads and reproduce 3-4 months after hatching (Fernandez-Delgado, 1988), and also in populations of A. boyeri from the Mediterranean coast of the Sinai peninsula, where the fish reach sexual maturity at an age of 2-3months (Henderson and Bamber, 1987).

Batch spawning is undoubtedly an advantage for *A. fasciatus*, which live in unstable and changing environments, such as lagoons. It increases individ-

ual fecundity and overcomes the constraints on fecundity imposed by the volume of the abdominal cavity. In addition, it also allows relatively large eggs to be laid which have a greater chance of survival (Wooton, 1990).

Since A. fasciatus is a batch spawner, it is difficult to calculate fecundity, because during the reproductive period oocvtes increase in size, mature, hydrate and are spawned. Many oocytes remain in the ovary and are eventually absorbed (atresia). To estimate fecundity, the total number of oocytes with a diameter greater than 0.2 mm was counted, using graded sieves, but probably some immature oocvtes were retained. This process was preferred since it was found that during the long reproductive period. large numbers of immature oocvtes, filled with volk, matured and were eventually spawned. The technique used runs the risk of overestimating fecundity. because some of the oocvtes are not discharged and are eventually absorbed at the end of the reproductive period. This means that the fecundity is perhaps lower than estimated, especially at Alykes where a higher percentage of oocytes remain immature (Fig. 5), compared to the other sampling sites, and which are eventually absorbed (Fig. 4).

The fecundity at Alykes, was significantly lower than that at the other two sampling stations (Table 1). It appears that the high temperatures and particularly salinities (Fig. 2) affect fecundity, resulting in a significantly lower fecundity at Alykes. Differences in fecundity in relation to different biotopes have also been observed for populations of *A. fasciatus* in Tunisia (Boumaiza et al., 1979).

The large increases in temperature and salinity (Fig. 2), perhaps in combination with other environmental factors during the reproductive period of *A. fasciatus*, inhibit the maturation of the oocytes (Fig. 5). Bye (1984) reports that daylength, temperature and food availability are probably the most important factors determining all the processes of oocyte maturation and spawning. In changing environments, however, it is possible that the later phases of gametogenesis may be initiated or synchronised by environmental factors such as monsoons, flooding, salinity changes or upwelling in the sea and in freshwater (Taber and Taber, 1983; Bye, 1984) or abrupt changes in temperature (Hotalling and Taber, 1986). The stimulus for initiating the new period of gonad

maturation appears to be related to a fall in water temperature, which occurs in December. The gradual decrease in temperature, which is at its lowest in December (Fig. 2), and its subsequent increase, stimulates the initiation of gonad maturation and the accumulation of yolk (Fig. 4) in the oocytes of *A. fasciatus*. The size composition of the oocytes (size frequency distribution) (Fig. 5) indicated that lower values of fecundity were probably a result of the adaptation to the unfavorable environmental conditions which occur in Alykes (Fig. 2) during the reproductive period.

The study of some biological and population characteristics of A. fasciatus showed that these characteristics demonstrate phenotypic plasticity in relation to environmental parameters (Leonardos et al., 1996). At extreme values of environmental parameters, this species responds with internal adjustments which relate to the duration of reproduction, the recurrence of reproduction, the high percentage of hatching (Leonardos and Sinis, 1997), early sexual maturation, the high survival rate of new-born individuals. the high mortality of old individuals and the high growth rate (Leonardos et al., 1996). The observed differences do not appear to result from genetic differences between the populations, but rather to population adaptations which minimize the effect of the environment. Slobodkin and Rapoport (1974) report that each organism which has a unique genotype shows maximal survival and fecundity at the optimal environmental conditions for the species. Deviations from those environmental conditions results in a reduction of survival and fecundity. Ware (1984) reports that the reproductive strategy of fish is an adaptive mechanism, and that finally combinations of the partial characteristics of the biological strategies are observed, which tend to increase the adaptability of the fish. One of the characteristics of the reproductive strategy of A. fasciatus is the interruption of reproductive processes and the absorption of oocytes (Figs. 4 and 5) under unfavorable environmental conditions (Fig. 2). The resources can be allocated for growth of the organism or for maintaining homeostasis, in this case osmotic regulation. Mann (1980) suggested also that natural selection generates differences in the ways in which species allocate their resources for growth, reproduction and survival. The persistence of a species is eventually

determined by the ability of individual fish to reproduce successfully in changing environments and to produce viable offspring.

A. fasciatus demonstrates plasticity in the parameters of the reproductive strategy according to the characteristics of its habitat. The phenotypic plasticity demonstrated by the populations in response to changes in environmental parameters minimizes the cost to the populations and appears to be a homeostatic mechanism. In this study, since the environmental parameters (temperature and chiefly salinity) have values which would be expected to decrease the survival of eggs and larvae, in *A. fasciatus* the maturation of oocytes stops and oocytes are subsequently absorbed. This probably saves resources for homeostatic mechanisms, such as, temperature and osmotic regulation.

## Acknowledgements

We thank Mrs. A. Kokkinidou, Mr. S. Kapetanakis, Mr. C. Kaloudis and Mr. K. Maltezos for their help with the sampling and collection of material.

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