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# Age and growth of *Aphia minuta* (Pisces, Gobiidae) from the central Adriatic Sea\*

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SUMMARY: *Aphia minuta* (Risso, 1810) is by far the most common pelagic gobiid of the Adriatic Sea and it is seasonally exploited by some local small-scale fishing fleets. Despite this, very few data on its biology in this area have been reported to date. Accordingly, age and growth of *A. minuta* were investigated by counting microincrements (daily rings) in the sagittal otoliths of 262 specimens caught in the central Adriatic between May 1996 and March 1997. Age of fish from 14 to 55 mm total length ranged between 42 and 275 days, confirming the short life span of this species. The von Bertalanffy growth parameters estimated by the age-length data pairs for the whole population were:  $L_{\infty} = 68.7$  mm; K = 1.55 year<sup>-1</sup>; t<sub>0</sub> = 0.077 year. The analysis of the monthly length-frequency distributions showed the simultaneous presence in late spring-early summer of two main cohorts which, together with the back-calculated hatch date distribution, indicated a more extended spawning period than previously reported in the literature. Because the fishery for *A. minuta* in the central Adriatic exploits the spawning stock it should be monitored and, if necessary, regulated.

Key words: Aphia minuta, age and growth, Adriatic

#### **INTRODUCTION**

The transparent goby *Aphia minuta* (Risso, 1810), best known along the Italian coasts by the common name of "rossetto", is a small fish belonging to a monotypic genus of Gobiidae, a large family of teleosts which comprises typically inshore marine as well as estuarine and freshwater species (Miller, 1986).

Aphia minuta, along with Crystallogobius linearis (von Düben, 1845) and Pseudaphya ferreri (De Buen and Fage, 1908), are the only gobies in the Mediterranean Sea which show pelagic habits, at least for most of their life (Iglesias *et al.*, 1997).

Such species are of particular zoological interest for the persistence in the adult of some postlarval features of habit and anatomy that are considered to be of adaptive significance for their planktotrophic life in midwater (Brunelli and Atella, 1914). These features include the scarcity of melanophores, the lateral compression of the body and positioning of the eyes, as well as the persistence of the swimbladder and possession of a short and straight alimentary canal (Miller, 1973). Furthermore, there are also evolutionary trends for reduction of the first dorsal fin (two rays in males and complete loss in female of C. linearis), conversion of ctenoid to cycloid scales (to entire loss in C. linearis) and enlarged caniniform teeth in the jaws of adult males (Miller, 1973).

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*A. minuta* is widely distributed throughout the north-eastern Atlantic region, from the western Baltic and Norway to Morocco, as well as in the Mediterranean and Black Seas (Tortonese, 1975; Miller, 1986). De Buen (1931) recognized two subspecies which he named *Aphia minuta atlantica* and *Aphia minuta mediterranea*, attaining different maximum adult sizes, total length of 5.8 and 4.5 cm, respectively.

Few studies have been carried out on the biology of *A. minuta* to date, and the data available from the literature deal mainly with reproduction, feeding habits and life history.

The spawning period of A. minuta is very different throughout its distribution, taking place from June to August in the Atlantic Ocean, from December to March in the western Mediterranean, in April in the northern Tyrrhenian, in May in the Adriatic and from May to July in the Black Sea (Mancini and Cavinato, 1969; Tortonese, 1975; Miller, 1986; Fischer et al., 1987; Froglia and Gramitto, 1989; Serena et al., 1990; Baino et al., 1996). The eggs of the transparent goby are demersal and similar to those of the genus Gobius (Tortonese, 1975). A. minuta feeds on zooplankton such as copepods, cirripede larvae and mysids (Miller, 1986). As with many other gobiids, the life span has been reported to be very short (about one year) and it dies after breeding (Miller, 1986). As the breeding season approaches, adult A. minuta migrate toward coastal waters, where they form large shoals close to the bottom.

As a consequence of this seasonal aggregation, small-scale fishing fleets of several Mediterranean localities exploit this resource, mainly in the winter months. Such a small-scale fishery is conducted in Spain off the Balearic Islands (Iglesias and Morales-Nin, 1992) and off Murcia (Martinez-Baño *et al.* 1990,1993), as well as in Italy in the Ligurian Sea (Relini *et al.*, 1996), in the northern Tyrrhenian Sea (Serena *et al.*, 1990; Baino *et al.*, 1996) and in the central and southern Adriatic Sea (Froglia and Gramitto, 1989; Ungaro *et al.*, 1994).

The study of age and growth of fish is considered of fundamental importance in fishery science. The knowledge of age and growth is used to determine the effect of fishing on the stocks, the efficacy of management policies, to understand life history events, and to maximize yield while still ensuring the future of the resource (Jones, 1992). Despite the importance of the fishery for *A. minuta* along the Italian coasts, little information on age and growth has been reported to date, although the age and growth of this species has been studied from fish collected off Majorca (Iglesias *et al.*, 1997).

In 1996 a research programme on the *A. minuta* fishery was funded by the Italian MRAAF (Ministero delle Risorse Agricole, Alimentari e Forestali). An ichthyological survey was carried out all year round in a coastal area of the central Adriatic, to provide information on several aspects of its biology (reproduction, age and growth, spatial distribution, etc.) useful for a correct management of the fishery. This paper deals with the study of age and growth and, indirectly, on reproduction, by counting the daily growth increments in otoliths as well as the analysis of size frequency distributions.

## MATERIALS AND METHODS

#### Sampling and laboratory activities

Sampling was carried out in the coastal area off the harbour of Ortona (central Adriatic Sea) from May 1996 to March 1997, including the 1997 fishing season (January to March). Nine monthly samples were collected from a local fishing vessel from 10 to 40 m depth on the sea bed, to follow the seasonal migrations and shoaling of *A. minuta*. The sampling gear was a small-meshed semipelagic trawl, with three concentric bags with a codend from 16 to 5 mm (stretched) mesh size.

For each sample, the total catch weight was recorded and from a weighed subsample the total length (TL) of A. minuta fresh specimens was measured to the mm below. The resultant size frequency distributions were then extrapolated to the whole sample. From a winter sample, size (TL, mm) and wet weight (mg) of the fresh specimens were recorded to determine the length-weight relationship. In order to study age and growth, three specimens per 1 mm size interval were selected each month, measured (TL) and sexed under a dissecting microscope. Sagittal otoliths were extracted and dried for some days in vials. Left otoliths were mounted on microscope slides with an epoxy resin (Petropoxy) and kept on a stove for polymerization. Each otolith was polished by hand on a wet polishing cloth using 0.05  $\mu$ m alumina powder, in order to make as much as possible of the whole otolith surface readable. Care was taken to locate the position of the nucleus, as well as the outermost increments. From these preparations two counts were made from the primordium to the margin and vice versa, and the mean value

considered. When the counts differed by more than ten increments they were discarded. Increment counts were performed using a light microscope at magnification 630x-1000x, with immersion oil. The microscope was equipped with a television camera connected to a video-analysis image program (OPTIMAS 6.0). The software was also used to measure the maximum diameter of each otolith specimen to an accuracy of 0.01 mm.

### **Data processing**

By means of the program FISHPARM of the statistical package FSAS (Saila *et al.*, 1988), which implements the Marquardt algorithm for non-linear least squares parameter estimation, the von Bertalanffy growth function (VBGF) was fitted to our age-length data pairs obtained by the increment counts of otoliths. The von Bertalanffy growth parameters ( $L_{\infty}$ , K and  $t_0$ ) were calculated for each sex and for the whole population. The growth curves obtained for males and females were compared using the multivariate Hotelling's T<sup>2</sup>-test, following the procedure of Cerrato (1990). Furthermore, from the ageing data and the date of capture, the monthly distribution of birthdates of our specimens was backcalculated.

In order to support the results derived from the otolith microincrement counts, further estimates of the growth parameters were also obtained by a length frequency distribution analysis of the monthly samples carried out by means of the programs BHATTAC and MODALPR of the LFSA package (Sparre *et al.*, 1987), for the whole sampling period.

Derived from the von Bertalanffy growth parameters, the growth performance index ( $\phi' = \log K + 2 \log L_{\infty}$ ) (Munro and Pauly, 1983; Pauly and Munro, 1984) was also calculated to compare the growth of the Adriatic and other populations of *A. minuta*.

The relationship between total fish length and maximum otolith diameter was investigated by means of linear regression analysis.

Finally, the length-weight relationship was calculated both for the whole population and for each sex and indeterminates independently. The exponential equation in the commonly used form:

$$W = a T_{I}^{b}$$

where W is the total wet weight (mg), TL the total length of fish (mm) and a and b are regression parameters, was fitted to the data. By linearization of the above equation, the log-transformed length-weight data were computed to determine the regression parameters. The allometric indexes (b) obtained for males, females and indeterminates were then compared, using a F-test for difference between two regression coefficients (Sokal and Rohlf, 1969).

#### RESULTS

#### **Otolith size and shape**

The maximum otolith diameter recorded in our samples shows a wide range, from 0.26 to 1.56 mm (range of fish length used was from 14 to 55 mm TL). The relationship between maximum otolith diameter (OD) and total fish length (TL) was linear and it is summarized in the following equation:

### $OD = -0.10482 + 0.02881 \text{ TL} \text{ } n = 496 \text{ } r^2 = 0.96$

The external morphology of the sagittal otoliths of *A. minuta* changed with the ontogeny, being circular in small specimens and slightly ovate in large specimens due to a progressive increase in the relative size of the rostrum. Moreover, the inner side of the otolith was concave and showed a heterosulcoid sulcus acusticus (Fig. 1).



FIG. 1. – External morphology of the otolith of an *A. minuta* specimen (36 mm TL), showing the sulcus acusticus. Maximum otolith diameter = 0.95 mm.



FIG. 2. – Internal microstructure of the otolith section of A. minuta showing the elongated primordium (arrow). Scale bar =  $10 \mu m$ .



FIG. 3. – Internal microstructure of the otolith section of A. minuta showing the microincrements narrowing close to the margin. Scale  $bar = 10 \mu m$ .

The internal microstructure under the light microscope showed the elongated primordium (Fig. 2) with a slight central constriction which is characteristic of gobiids (Brothers, 1984), and a series of microincrements consisting of alternate dark and light zones. Accessory primordia or clear transition zones were lacking in all the specimens. The thickness of the microincrements varied considerably along the otolith growth axis, from 1 to about  $4 \mu m$ . As a general trend, the increments were very narrow close to the primordium, their thickness increased towards the otolith margin, where they became narrow again (Fig. 3). This pattern was evident only in the large specimens; narrow increments on the marginal zone of otolith were not found in small and medium sized specimens. The change of thickness of increments generally occurred progressively, but in some cases the change was abrupt.

TABLE 1. - Age-length key of whole population. (TL total length)



### Age and growth

The validation of daily increments deposition in *A. minuta* has been carried out in aquaria by means of a marking procedure (Iglesias *et al.*, 1997) and we have assumed that the microincrements were also laid down daily in our samples. When calculating hatchdates the time elapsed between the hatching and the first increment deposition was ignored.

Of the 262 otoliths examined, about 21% (56) were discarded because they were unreadable. The size range of the fish almost covered that of the sample collected at sea. In total, 86 otoliths of males ranging from 21 to 55 mm, 80 otoliths of females ranging from 18 to 47 mm and 40 otoliths of indeterminates ranging from 14 to 22 mm were aged. The age range for both sexes was from 65 to 275 days (2-9 months), whereas it was from 42 to 82 days (1-2 months) for indeterminates. The age data are summarized in the age-length keys reported for males and females as well as for the population as a whole including indeterminates (Table 1, 2 and 3).

The von Bertalanffy growth curves fitted to the age-length data for each sex including indetermi-

TABLE 2. – Age-length	key of femal	es. (TL total length)
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TL (mm)	II	III	IV	A; V	ge classes months) VI	s VII	VIII	IX
$\begin{array}{c} 18\\ 19\\ 20\\ 21\\ 22\\ 23\\ 24\\ 25\\ 26\\ 27\\ 28\\ 29\\ 30\\ 31\\ 32\\ 33\\ 34\\ 35\\ 36\\ 37\\ 38\\ 39\\ 40\\ 41\\ 42\\ 43\\ 44\\ 45\\ 46\\ 47\\ \end{array}$	1 1 2 3 5 3 2 2 1 1	1 3 3 5 5 2 1 1	1 2 2 2 2 2 1 1	1 1 3 2 2 2 2 2 1 1	1 1 2 1 1	1	1	1

TABLE 3. - Age-length key of males. (TL total length)

TL (mm)	II	III	IV	Age o (mo V	classes nths) VI	VII	VIII	IX
$\begin{array}{c} 21\\ 22\\ 23\\ 24\\ 25\\ 26\\ 27\\ 28\\ 29\\ 30\\ 31\\ 32\\ 33\\ 34\\ 35\\ 36\\ 37\\ 38\\ 39\\ 40\\ 41\\ 42\\ 43\\ 44\\ 45\\ 46\\ 47\\ 48\\ 49\\ 50\\ 51\\ 52\\ 53\\ \end{array}$	1 4 3 3 3 1	$     \begin{array}{c}       1 \\       2 \\       1 \\       1 \\       2 \\       1 \\       2 \\       1     \end{array}   $	1 1 2 3 2 1 3 2 1 1 1	1 1 2 1 2 1 2 2 1	1 1 1 1 1 1 1	1 1 1 1 2 1 1	1	1

nates and for the whole population are displayed in Fig. 4. Generally, all curves seem to indicate a first phase of growth (about down to 100 days) which is faster than the latest (about from 100 to 300 days). The indeterminates included in the curves were only very small specimens (less than about 20 mm) and not adults. The inclusion of these indeterminates provided additional experimental data to the left side of both males and females growth curves, otherwise unavailable. The estimates of the von Bertalanffy growth parameters ( $L_{\infty}$ , K and  $t_0$ ), as well as their asymptotic standard errors are summarized in Table 4. They were quite different in the two sexes, the L and the related K values being respectively higher and lower in males than in females. Furthermore, the males generally attained a larger size than females. However, the comparison of the von Bertalanffy growth curves of the two sexes by the T<sup>2</sup> test did not show any statistically significant difference (P<0.01), in agreement with the considerable similarity between the growth performance indexes  $(\phi')$ calculated for each sex (Table 4).



FIG. 4. – Von Bertalanffy growth curves for whole population (above), females (+ indeterminates) (middle), and males (+ indeterminates) (below) of *A. minuta* from the Adriatic Sea.

TABLE 4. – Von Bertalanffy growth parameters, derived from agelength data pairs obtained by the increment counts of otoliths, and growth performance indexes; (ASE asymptotic standard error)

Parameter	Ma	les	Fema	ales	Whole pop	pulation
	Estimate	ASE	Estimate	e ASE	Estimate	e ASE
$\begin{matrix} L_{\infty} \\ K \\ T_{\rho} \\ \phi' \end{matrix}$	74.26 1.41 0.074 3.89	13.39 0.45 0.027	65.60 1.61 0.075 3.84	9.64 0.43 0.024	68.70 1.55 0.077 3.86	8.49 0.36 0.023

TABLE 5. – Von Bertalanffy growth parameters (L<sub> $\infty$ </sub> and K) and growth performance index ( $\phi'$ ) for A. minuta populations

Species	$L_{\infty}$	Κ	φ′	Source
Aphia minuta	68.70	1.55	3.86	This paper
<i>Aphia minuta</i> females	65.60	1.61	3.84	
<i>Aphia minuta</i> males	74.26	1.41	3.89	"
Aphia minuta	53.69	2.23	3.81	Iglesias et al. 1997
<i>Aphia minuta</i> females	49.58	2.78	3.84	e "
Aphia minuta males	61.01	1.68	3.79	"

In order to compare the growth of the Adriatic *A. minuta* with that of other populations, present and literature data of the von Bertalanffy growth parameters and  $\phi'$  values are reported in Table 5.

#### Length-frequency distributions

The monthly length-frequency distributions of *A. minuta* recorded for most of the year are shown in Fig. 5. Our catches included a wide size range (13-55 mm TL). The larger fish sampled were very close to the maximum size of this species reported in the Adriatic Sea (Mancini and Cavinato, 1969; Froglia and Gramitto, 1989). On the other hand, smaller fish (< 13 mm) were not sampled, although the fishing gear caught specimens of other species of about 10 mm. This was probably due to the marked pelagic habit as well as to the scattered distribution of the first stages of life of *A. minuta* in the study area.

The length-frequency distributions, clearly indicate the presence of two main cohorts from May to July, which refer to different years of birth. The first cohort, born in the previous year (i.e. 1995) and comprising large adult specimens, sharply decreased from May throughout July (1996) and then disappeared. The second cohort was born in the current year (i.e. 1996). It appeared in May and was the only cohort present in the area from September onwards.

The length frequency analysis conducted by means of the LFSA package allowed us to distin-



FIG. 5. – Monthly length-frequency distributions of *A. minuta* from the Adriatic Sea (n = total fish measured).

guish at least two "subcohorts" in the autumn-winter population. The von Bertalanffy growth parameters obtained by the modal progression analysis carried out on such subcohorts are reported below and closely resemble the growth parameters of the whole population derived from the age-length data (see Table 4):

$$L_{\infty} = 68.9 \text{ K} = 1.35 \text{ } \phi' = 3.81$$

The birthdates calculated for the two subcohorts identified were, respectively, the first half of May (spring cohort) and the first half of July (summer cohort). Similarly, the backcalculation of our ageing data providesd a wide hatch date distribution (six months, from April to September) (Fig. 6), showing two main peaks, in May and July/August.



FIG. 6. – Percentage distribution of birthdates of *A. minuta* derived from the backcalculation of the ageing data and the date of catch.

#### Length-weight relationship

The length-weight relationship was obtained from a sample of 245 specimens, comprising 137 females and 108 males. The length and weight ranges were, respectively, 24-48 mm and 66-569 mg for females, 25-52 mm and 53-831 mg for males. The values of parameters (a and b) of the allometric power equations, derived from linear regression of the log-transformed length-weight data, are the following:

Females:	$W = 0.00092 \text{ TL}^{3.46}$	$(r^2 = 0.97; n = 137)$
Males:	$W = 0.00100 \text{ TL}^{3.44}$	$(r^2 = 0.98; n = 108)$
Whole		
population:	$W = 0.00088 TL^{3.47}$	$(r^2 = 0.98; n = 245)$

Positive allometric growth (b>3) was found in each group. The F-test did not indicate any difference between the allometric coefficient (b) of males and females (P<0.05).

# DISCUSSION

Several characteristics of the internal microstructure of the otolith of A. minuta sampled in the Adriatic Sea, such as the absence of a settling mark and accessory primordia, closely resembled those described by Iglesias et al. (1997) for the Majorcan population. Furthermore, we also found very narrow increments (less than 1 µm) deposited on the otolith margin of adult fish. Such increment narrowing was most pronounced in the adults sampled during the spawning season (May to July), thus supporting the possible direct relation between narrow increments and sexual maturity of A. minuta suggested by Iglesias et al. (1997). Because of the close relationship between otolith size and fish length found in our samples, the decrease in thickness of the otolith microincrements occurring during the spawning season might reflect a simultaneous decrease of somatic growth.

The validation of daily periodicity of otolith increment formation carried out on the Majorcan population of A. minuta (Iglesias et al., 1997), allows us to determine the age of fish sampled in the Adriatic Sea by means of the otolith microincrements counts. As expected on the basis of the marked difference in maximum adult size, the von Bertalanffy growth parameters of the Adriatic population are rather different from those of Majorca. Indeed, the asymptotic length  $(L_{m})$  and the inversely related value of K are respectively higher and lower in the Adriatic than in the Majorcan population. However, it is noteworthy that the two populations show considerable similarity in the growth performance index  $\phi'$ , a feature generally considered a useful tool for comparing the growth curves of different populations of the same species and/or of different species belonging to the same family (Sparre et al., 1987).

The existence of several populations of *A. minuta* characterized by different maximum size could be

postulated both from our own and other data derived from the literature. The maximum size reported by De Buen (1931) for the two subspecies, Aphia minuta atlantica and Aphia minuta mediterranea, seems to be underestimated in the light of the recent data. Indeed, maximum lengths of 72 and 79 mm were found in Atlantic waters from the Ria de Arosa (NW Spain) (Iglesias, 1981) and from the Ria de Aveiro Lagoon (Portugal) (Arruda et al., 1993), respectively. On the other hand, the A. minuta populations of Spanish Mediterranean waters showed a maximum length of only 45 mm off Murcia (SE Spain) (Martinez-Baño et al., 1993) and 49 mm in the Balearic Islands (Iglesias et al., 1997). Our data and other data from the Adriatic Sea (Mancini and Cavinato, 1969; Froglia and Gramitto, 1989) indicate a maximum total length of 55-60 mm, which is between the above reported values.

The analyses of the length-frequency distributions recorded monthly throughout the year indicated the simultaneous presence in late spring-early summer of two main cohorts, the oldest of which quickly disappeared at the onset of autumn. Such a rapid disappearance is probably due to the collective death of the specimens of the previous year's cohort after the breeding season. Histological analyses of these specimens revealed a widespread degeneration of the intestinal epithelium that could partially explain their death (V. Caputo, pers. comm). A similar loss of function of the alimentary canal was pointed out also by Fage (1910) for the pelagic gobiid *Pseudaphya ferreri*.

The birthdates of *A. minuta* derived from the backcalculation of our ageing data, supported by histological data on the gametogenesis of males and females (Caputo *et al.*, 1997), suggest a wider spawning period than previously reported for the Adriatic population (Mancini and Cavinato, 1969; Froglia and Gramitto, 1989). The spawning season extends from April to September, with two main peaks in May and July-August, respectively. In addition, the simultaneous presence in the gonads of the same female of two distinct size-groups of oocytes suggests that *A. minuta* spawn more than once in its single breeding season (semelparous fractional spawner), at least in the Adriatic Sea (Caputo *et al.*, 1997).

The close link between the winter schooling and the reproductive activities of *A. minuta* reported in the western Mediterranean (Iglesias *et al.*, 1997) seems to be absent in the Adriatic population, which also gathers in schools during the winter season (from December to February) but breeds in spring and summer. Therefore, the schooling behaviour of A. minuta in the shallow waters of the Adriatic Sea during the cold season could be tentatively related to feeding rather than breeding.

As reported from the Spanish waters of Majorca (Iglesias et al., 1997), we observed that the schools of A. minuta tend progressively to scatter and to migrate into offshore waters from the end of February onwards, becoming unavailable to the fishery until the following winter. During the fishing season, from January to March, the A. minuta population exploited by the local fleet is composed of medium-large sized fishes (26- 50 mm TL), which would spawn in the following spring-summer season. Because the reproductive success in the springsummer period and the subsequent recruitment to the adult stock could be strongly affected by the number of specimens surviving the last fishing season, steps should be taken to monitor and, if necessary, to regulate the annual fishing effort.

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