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STUDIES ON THE MICROSTRUCTURE OF OTOLITHS AND THE ESTIMATION OF THE DURATION OF LARVAL STAGE IN GLASS EEL (*Anguilla anguilla*) IN MAROCCAN COASTAL WATERS

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A B S T R A C T. Dimensional and microstructural analyses on the otoliths of glass eels (*Anguilla anguilla*) of two populations, one from the Atlantic coast (Sebou Estuary) and the other from the Mediterranean coast (Mouloya Estuary), were made using optical and scanning electron microscopy. A comparative study revealed that Atlantic and Mediterranean glass eel showed approximately one month difference in their migration periods. This difference was marked on the growth area of the otoliths, justifying the different migratory routes undertaken by the two populations, starting from the Sargasso Sea up to the continental waters.

Key words: OTOLITH MICROSTRUCTURE, LARVAL STAGE, GLASS EEL, MAROCCAN COASTAL WATERS

INTRODUCTION

Estimation of age is an important criterion for the understanding of growth and its pattern in all living organisms. In teleosts, growth patterns are reflected in the form of cyclic and periodic marks in certain bony formations (scales, otoliths, vertebrae, operculae, fin rays). In eel, a fish with a life cycle characterised by an oceanic and a continental ecophase, estimation of growth is fairly difficult. The principal handicap is an accurate determination of the age in course of the marine larval life.

Since Schmidt's publication (1922), different periods for the marine larval life of European eels *Anguilla anguilla* have been used. Variation of the results depends mainly on the methods employed to assess age from the otoliths (Moriarty and Steinmetz 1979, Vollestad 1985). In general, duration of the transoceanic migration is more than one year (Schmidt 1922, Boetius and Harding 1985, Van Utrecht and Holleboom 1985). These data, however, have not been confirmed by the recent oceanographic (Boetius and Harding 1985) and biological data based on the analyses of daily growth rings in the otolith (Lecomte-Finiger and Yahyaoui 1989, Yahyaoui 1991).

It has been accepted that European eel populating Atlantic and Mediterranean coasts of North Africa and Europe reproduce in the same spawning area, in the North West Atlantic: the Sargasso Sea (Boetius 1980, Comparini and Rodino 1980, Yahyaoui 1983, Williams and Koehn 1984, Avise 1986). However, the migration routes, starting from the Sargasso Sea up to the European and North African coasts, remain unknown. According to Tesch (1986), the size of leptocephalus larvae caught in the southern part of North Atlantic is 4 to 13 mm less than of those caught more northward and hence much younger.

As a contribution to the knowledge on the biological cycle of European eel in the south part of its geographical distribution and in the Gibraltar Straits, a comparative study was undertaken on the otolith dimension and microstructure of Atlantic and Mediterranean glass eel from Moroccan coastal waters.

MATERIAL AND METHODS

Glass eels were captured in December-January during their anadromic migration periods, from the Atlantic coast (Sebouu Estuary) and the Mediterranean coast (Mouloya Estuary).

The sagittae, after extraction, were incubated in a synthetic resin with fast polymerisation in cold. Their convex surfaces were polished and decalcified with HCl (1%) or EDTA (5%), and observed directly, or after staining with blue Toluidine (1%), in optical and scanning electron microscopy (Hitachi S 520 of the University of Perpignan, France) after metallising in gold (thickness of gold deposit: 200 A°).

RESULTS

1. DIMENSIONAL ANALYSES OF THE OTOLITHS

During their recruitment period, the glass eels have otoliths with a mean diameter of 0.3 mm (Table 1), which increases during the pigmentation. However, during any given stage of pigmentation, the otoliths of Mediterranean glass eels are larger (in mean diameter) than those of Atlantic ones. The glass eels from Sebou and those from Mouloya showed significant difference in the mean diameter of the otoliths.

TABLE 1

Mean diameter of otoliths (± 95% confidence limits)

Stages	Sebou (Atlantic)		Moulouya (Mediterranean)	
	N	Mean diameter (μm)	N	Mean diameter (μm)
VA	17	290.6 ± 13.6	-	-
VB	40	310.0 ± 8.9	9	328.6 ± 11.8
VIA0	5	317.0 ± 17.3	10	336.7 ± 11.4
VIA1	6	331.7 ± 23.5	22	340.8 ± 9.6
VIA2	8	340.9 ± 18.8	5	355.7 ± 18.3
Total	76	311.1 ± 7.2	46	339.1 ± 6.4

TABLE 2

Thickness and number of growth rings in marine environment. (The number of rings on the growth zone of leptocephalus is counted starting from the ring appearing during the first feeding activity)

Zone	Site	Moulouya	Sebou
	Number N	14	25
Growth zone of leptocephalus	Mean thickness (μm) ± 95% confidence limits	100.80 ± 5.55	91.49 ± 7.47
	Mean number of rings	259	232
Zone of metamorphosis	Mean thickness (μm) ± 95% confidence limits	60.93 ± 4.83	59.22 ± 5.24
	Mean number of rings	34	33

2. MICROSTRUCTURAL ANALYSES OF OTOLITHS

The observation of the otoliths using an optical and scanning electron microscopy helps to determine different growth zones in relation to their general aspects. Growth zone in marine environment is different than that in continental waters by a concentric limit: the zone of transition showing a deep and well marked double ring.

Even in a marine environment, the otolith is characterised by 2 well defined zones:

- Central growth zone of leptocephalus, large and with fairly visible concentric rings

- Peripheric metamorphosis zone of leptocephalus in glass eels, smaller and opaque.

Spatial comparison is possible basing on microstructural analyses of these two growth zones in glass eel from the Atlantic and Mediterranean coasts (Tab. 2).

In Mediterranean glass eel, both during the leptocephalus and metamorphosis phases, the growth zones are thickner than in the Atlantic glass eels. The mean number of daily rings in each zone also differs between the eels from the two sites. This number has been estimated taking into account thickness of each zone and the mean thickness of the corresponding daily rings.

DISCUSSION

The works of Pannella (1971) on *Gadidae* revealed for the first time the presence of daily rings on the fish otoliths.

Recent works on the otoliths of glass eel (*Anguilla japonica* and *A. anguilla*) have shown the presence of fine rings which are considered to be daily rings. However, description of the central part of the otoliths remains incomplete and the explanation of fine rings is difficult due to specific biological cycle of eel.

According to our observations, the otolith shows one central part, opaque, with a mean diameter of 9.61±1.39 μm and a nucleus having a mean diameter of 4.1±0.60 μm. A more transparent zone is followed by this first zone, limited on the centrifugal side by a well marked ring situated at 10.25 μm in diameter).

Tabeta et al. (1987) have noticed that 6-days old preleptocephalus larvae of *Anguilla japonica* have smaller otoliths, with a diameter of 13 to 17 μm, compared to those of older individuals, in which the diameter of the first ring is about 20 μm. Consequently the otolith diameter is much smaller at hatching. This first ring might be formed during the embryonic development; its centrifugal limit probably corresponds to the time of hatching. The same was also observed by Lecomte-Finiger and Yahyaoui (1989), Tzeng (1989), Yahyaoui (1991). In other fishes, formation of the otoliths takes place before hatching (Everson 1980).

The second deep mark corresponds to the initiation of the trophic activities of the larvae (Tabeta et al. 1987, Lecomte-Finiger and Yahyaoui 1989, Tzeng 1989). In fact, it has been demonstrated in other fishes that formation of daily rings on the otoliths starts after the reabsorption of the vitelline sac at first feeding (Radtke and Scherer 1982, Tsuiji and Aoyama 1984). Lee and Lee (1989) have noted the same phenomenon in eel at first feeding in continental environment after the metamorphosis.

Starting from the first ring at feeding, fine rings succeed towards the centrifugal direction. According to Tsukamoto (1989) and Tabeta et al. (1987), the microstructural marks of approximately 1 μm thickness are considered as daily marks.

However, one can observe variations in the thickness of daily rings during the growth phase of larvae in the marine environment. In all the glass eels captured at the two sites, the rings at the growth zone of leptocephalus had a mean thickness of 0.35 μm ± 0.07 μm. In the metamorphosis phase, the thickness is more important (1.8 ± 0.3 μm). Such variations in the thickness of growth rings reflect variations in daily growth rate. Moreover, during the metamorphosis, daily growth is faster. This is in accordance with the observations of Mochioka et al. (1989) on *Conger myriaster*. Variations in the growth rate are related to larvae migration in different environments and to the metamorphosis (Neilson et al. 1985, Victor 1986, Tzeng 1989).

A difference in growth between Atlantic and Mediterranean glass eel during their marine life was observed with respect to the diameter of the otoliths. This difference may be due to corporal differences between tha glass eels at the two sites and, consequently, to different routes undertaken by each population during marine migration.

Considering the mean thickness of daily ring at the zone of metamorphosis (1.8 μm), and at the growth zone of leptocephalus (0.35 μm), the metamorphosis seems to have taken place at the same dimensional limit for the glass eel from the two sites (the difference is 1.71 μm). On the contrary, growth of leptocephalus revealed a difference of 9.39 μm between the two sites. Consequently, growth of leptocephalus larvae revealed a time difference of 27 daily rings (more or less equal to 1 month) starting from the first feeding activity. Therefore, the difference of the mean diameter of the otoliths, observed between the glass eel of Sebou Estuary and of Moulouya Estuary, is due to a difference in growth of leptocephalus in oceanic environment. This is probably due to longer route taken by the Mediterranean individuals.

On the other hand, considering the same thickness of daily rings observed for individuals at the two sites, it is possible that the growth rates may be the same for all larvae that occur there. Therefore, the difference may be related to the growth period before metamorphosis which is a month longer for the Mediterranean population. This may justify the delay in the recruitment of glass eel at the Mediterranean coast compared to that at the Atlantic coast (Yahyaoui 1983, 1988 and 1991).

Moreover, according to our estimation, the metamorphosis phase which occurs near the continental platform, takes place more or less at the same time intervals (1 month) for all the individuals at the two sites. The same was observed for *Anguilla rostrata* (Kleckner and Mc Cleave 1985), *A. japonica* and *A. marmorata* (Tabeta et al 1987), but it was much less for *Anguilla anguilla* (Lecomte-Finiger and Yahyaoui 1989).

The results of our studies suggest that it is possible to estimate marine larval life of eel. With a period of approximately 15 days between hatching and formation of the first ring at feeding (Yamauchi et al. 1976, Sato 1979, Tabeta et al. 1987), the transoceanic migration period (including metamorphosis) will be less than one year (308 days for the Mediterranean population and 280 days for the Atlantic one). Moreover, populations recruited during December - May on the Atlantic coast of Morocco probably originate from larvae hatched during a prolonged spawning period of March to July.

In conclusion, the study supports the argument on the existance of only one spawning area in the North West part of the Atlantic (the Sargasso Sea) for the population of *Anguilla anguilla* which occur on the Mediterranean and Atlantic coasts of North Africa and Europa. At the same time, it explains the time difference in the recruitment and also the corporal differences between the glass eel at the two sites.

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STRESZCZENIE

BADANIA MIKROSTRUKTURY OTOLITÓW I OCENA CZASU TRWANIA STADIUM LARWALNEGO WĘGORZYKA SZKLISTEGO (*Anguilla anguilla* L.) W MAROKAŃSKICH WODACH PRZYBRZEŻNYCH

Mikrostrukturalną analizę otolitów węgorzyka szklistego pochodzącego z dwóch populacji, jednej z wybrzerza atlantyckiego (ujście rzeki Sebou), drugiej z wybrzeża Morza Śródziemnego (ujście rzeki Mouloya), przeprowadzono przy użyciu mikroskopu optycznego oraz elektronowego skaningowego. Porównawcze badania wykazały, że między węgorzykiem atlantyckim a śródziemnomorskim istnieje około jednomiesięczna różnica w okresie trwania wędrówki. Różnica ta uwidoczniła się na obszarze wzrostowym otolitu odzwierciedlając różne trasy wędrówki obu analizowanych populacji po opuszczeniu Morza Sargassowego.