Biological and physiological characteristics of glass eels (*Anguilla anguilla*) in the Bay of Biscay.

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SUMMARY : The purpose of this work was to characterize the quality of glass-eel recruitment during season of migration and transition from the marine to the continental environment. Glass eels were sampled, in the south of the Bay of Biscay from November 1999 to March 2000. The individual degree of slimming was measured from the ratio DNA/dry weight and the water content. The larval history was studied by interpretation of the microstructures registered on the otoliths. During the sampling season, glass eels changed in pigmentation and length and weight decreased. Their energy and water budget remained constant but large individual variations inside each groups were observed. A slimming between glass eels captured at sea and in estuary was observed in December only whereas they were not pigmented. Samples of glass eel otoliths collected on different areas and periods presented microstructural differences. The otoliths of glass eels caught at sea in February presented a smaller radius and a transition mark in estuary. Monthly measurements of growth zones of otoliths showed a similar larval history during the transoceanic migration. These results indicate a mixing of flows of the glass eels between the marine and continental environment at the end of sampling season.

KEY-WORDS : glass eels, otolith, DNA/dry weight, estuarine migration, pigmentation

INTRODUCTION

Morphometric characteristics of glass eels when they enter estuaries have been studied in many works^{1,2,3}. Length and weight of glass eels decrease while pigmentation develops, during the principal season of migration. Such characteristics are insufficient to determine their physiological state when they colonize the continental waters². The purpose of this work was to characterize the physiological state and the larval history of glass eels groups during the main season of migration and the transition between the sea and the estuary. The energy reserves were quantified from the DNA/dry weight⁴ ratio. This index classically used on marine fish larvae, was for the first time measured on glass eels. It presents the interest to be used individually. The larval history was revealed through the microstructures registered on the otolith⁵. Parameters (trophic availability, courantology, temperature) of oceanic migration condition state of slimming of the glass eels entering the estuary. They were then at the end of the metamorphosis and in fasting⁶ phase and will be confronted with an environmental change inducing energy losses. The quality of the recruit was a significant parameter for their aptitude to colonize continental area.

MATERIALS AND METHODS

Glass eels were sampled from November 1999 to March 2000 along the coast of the Gulf of Biscay south-west of France, in the Adour estuary and in the marine coastal zone (tab.1).

Table 1- Sampling scheme during main migratory season. (n= fish number ; Flow in $m^3.s^{-1}$ C= tide coefficient ;)

Date	Place	n	Flow	C	SeaT°C	EstuaryT°C
11/1999	Estuary	25	145	80	15	11.3
12/1999	Sea	25	140	76	13	7.7
12/1999	Estuary	25	142	78	13	7.4
01/2000	Estuary	25	206	78	12	7.9
02/2000	Sea	25	120	48	12	7.0
02/2000	Estuary	25	157	87	12	9.4
03/2000	Estuary	25	219	97	12	10.6

Biology

Length (in mm), weight (in g) and pigmentation stage⁷ were recorded for each glass eel. The DNA/dry weight (in $\mu g.mg^{-1}$)⁸ condition index was obtained from crushed tissue following a 24h freeze drying period. The fluorimetric method suggested by le Pecq and Paoletti⁹ as modified by Karsten and Wollenberger¹⁰ was used to determine DNA quantities by larva. Proportioning of the nucleic acids were carried out according to the method of Karsten and Wollenberger¹¹. After extraction, *Sagittae* were embedded in resin then grounded with an abrasive paper. The grounded surface was polished and etched with EDTA (5%). Then otoliths were examined on a Scanning Electron

Microscope (SEM) (Hitachi S520) after gold coating (20nm). The magnifications used to measure the various phases of growth from the nucleus to the external edge of the otolith was of 600 times. The structure of the otoliths were determined according to the description of

Lecomte-Finiger and Yahyaoui¹²⁾.

The otoliths were classified in three categories. Type 1 don't show transition mark, type 2 present this mark on the edge of otolith. Type 3 presents a growing zone after the transition mark.

Measurements (μm) of the zones of growth were : R = total radius of the otolith, R_m = distance from the nucleus to the zone of leptocephalus-glass eel metamorphosis, R_t = distance between the nucleus and transition zone in the estuary, 3^{rd} = transition zone (representing the crossing of the salinity barrier, in the estuary), $R_p = R_t - R_m$ (across of the continental shelf).

Statistical processing

Length and weight variations were examined to confirm previous work showing a seasonal reduction⁴⁾. The proportion of the pigmentation stages was also taken into account. DNA/dry weight data have a strong variability which leads us to use non parametric tests to fill the conditions for application and to neglect the extreme values inducing a strong variability. For comparisons of individuals caught at sea and in estuary, the *Wilcoxon*¹³⁾ test of ranks was used. The comparison of the individuals sampled in estuary over the 5 months of migration was carried out, by the *Kruskal-Wallis*¹⁴⁾ test for the index of condition and by the test of the median rank with two levels of classification¹⁵⁾ for measurements of otolith structures. The independence condition of the samples was checked and the assumption of equality between the two samples was tested with the risk $\alpha = 0.05$. As far as possible, to eliminate intra-group variability from measurements of otoliths microstructures related to the metabolism of each glass eel and to highlight variability related to the studied phenomenon, an estimator ratio was used. The R_m distance was replaced by R_m/R and the distance R_p by R_p/R .

RESULTS

Biometry

Length and weight of glass eels decrease gradually during the season. They lose over the 5 studied months on average 0.6mm and 0.12g. Size and weight were equivalent between the two geographical sites (2).

The pigmentation stages present the same evolution during season and between the two geographical sites.

The V_B stage decreases at the end of the season to the benefit of V_{IA} stages. At sea, the stages V_B also dominate but with a stronger percentage of V_A than in the estuary especially in December (table 2).

Table 2 - Morphometric characteristic of glass eels sampled - Mean (std deviation) - Pigmentation stage (%).

Date	Place	Length	Weight	V_A	V_B	V_{IA}
11/1999	Estuary	7.44 (0.38)	0.41 (0.07)	6	92	2
12/1999	Sea	7.55 (0.36)	0.38 (0.06)	24	76	0
12/1999	Estuary	7.50 (0.39)	0.41 (0.07)	22	74	4
01/2000	Estuary	7.31 (0.35)	0.37 (0.06)	16	78	6
02/2000	Sea	7.32 (0.47)	0.33 (0.06)	14	76	10
02/2000	Estuary	6.83 (0.4)	0.3 (0.06)	4	58	38
03/2000	Estuary	6.79 (0.38)	0.28 (0.05)	10	68	22

Index of slimming

Figure 1- DNA/Dry weight box-plot for glass eels pigmentation stage 1999/2000 during season (A) and sampled at sea and in estuary (B). (NS = no significant ; * significant)

extreme values of DNA/dry weight were highlighted in many samples (figure 1). Median differs month by month with an asymmetry of the interquartile variations.

The tests applied show that during the season no significant difference was observed for the stage VB (Kruskal-Wallis=4.64-P=0.32). The comparison between sea and estuary samples, for a given month, showed a significant difference in December (Wilcoxon=2.96 - P=0.003) and none in February (Wilcoxon=0.74 - P=0.808).

Otolith Structures

The otoliths classification into 3 types shows significant differences between month during season and sampling area (figure 2). Type 1 otoliths were dominant at sea and at the beginning of season whereas type 3 otoliths dominate in the estuary and at the end of season.

Figure 2- Type of otolith according to season and sampling place.

The different measures taken on the otoliths show a relative stability during the migrating season and between the two sampled areas (table 3).

Table 3- Measures of the total radius of otoliths (R) the nucleus to the metamorphosis (Rm) the crossing of the continental shelf (Rp) for glass eel stage and their standard deviation () during sampling season and sites.

	Rm	Rp	R
November	113 (8.5)	76.6 (9.3)	191.7 (12.2)
December	112.9 (10)	77 (14.5)	193.7 (15)
December sea	110.1 (9.9)	72.1 (12.3)	183.4 (13)
January	116.9 (10.8)	69.6 (9.3)	190.7 (17.7)
February	121 (10.5)	68.1 (13.7)	198.1 (17.4)
February sea	111.8 (10.7)	70.6 (11.5)	185.7 (9.2)
March	116.45 (12)	72.9 (14.1)	199.8 (19.3)

The inter-monthly variations (median rank test) were not significant (table 4).

Table 4- Median rank test according to season and to the various types of otoliths.

	Type 1	Type 2	Type 3	χ^2	Decision (H_0)
R	190.3	195.6	202.8	12.43	accept
Rm/R	0.603	0.607	0.592	12.78	accept
Rp/R	0.396	0.371	0.348	13.11	accept
$\chi^2 = 15.5 - \alpha = 0.05$					

The glass eels sampled in the estuary presented otoliths significantly larger than those from the sea (table 3 and 5). The zones of growth of the otolith until the glass-eel phase were the same ; on the other hand the thickness of growth of the otolith during the cross of the continental shelf differs among the two samples in February (table 5).

Table 5- Comparison between samples at sea and estuary for December and February (*Student t* test)

	December			February		
	Sea-Estuary			Sea-Estuary		
	T	P	decision (H_0)	T	P	decision (H_0)
R	-2.52	0.015	reject	-2.94	0.005	reject
Rm/R	1.08	0.285	accept	-0.77	0.45	accept
Rp/R	-0.28	0.78	accept	2.29	0.03	reject

DISCUSSION

Seasonal fluctuations

Our results show that successive samples exhibited variation in length and weight and corroborates previous data ^{6,16)} Recent studies ⁴⁾ show that the glass eels were not really thin at the end of the season. The water content of glass eels ⁴⁾ show the same pattern. The variations would result from groups of individuals exhibiting different characteristics. The homogeneity of the growth of the microstructures on the otoliths seem to indicate similar conditions during the oceanic migration. Thus, the glass eels migrating on the European coasts could come from distinct geographical areas or spawning areas spread out in the time which would give larvae different mean length and weight ¹⁷⁾. To confirm this assumption additional studies focusing on microchemistry must be undertaken.

Transition sea-estuary

Biochemical and otolithometric results concerning the transition between marine coastal zones and estuaries were complementary. The two analyses confirm a mixing of incoming glass-eel waves at the end of the season. Thus, these results show that the glass eels have a certain ability to return to sea after a phase of adaptation in the estuarine freshwaters. This capability has been confirmed by research on glass-eel osmotic capacities ^{18,19)}. The strong thermal difference between marine and estuarine water in February (cf tab.1) could explain the return to sea because no water discharge was observed during the period of study. Moreover, variability of biometry and pigmentation at the end of the season corroborated these results. Nevertheless, several new information are to be confirmed, mainly on the rates of growth of the otoliths and on the formation

of the double mark supposed to be the transition in estuary⁶⁾. Otolith were growing whereas the glass eels were in phase of fast. This could explain why the otoliths of glass eels sampled at sea were smaller than those in the estuary. At the beginning of the migrating season, when groups were not yet mixed, the difference was also significant with respect to the level of classification of the types of otolith and also for the index of slimming.

CONCLUSION

This study in the Adour estuary allows to confirm previous hypothesis on the seasonal biometric variations, on the range of the larval period and on the supposed only way used by larvae during the transatlantic migration. Moreover, this work underlines the lack of knowledge on the origin of glass eels arriving on the European coasts.

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