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EMANUELE RODINÒ, MARTINO RIZZOTTI

Haemoglobin ontogenesis in the sea bass, Dicentrarchus labrax (L.)

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Biologia. — *Haemoglobin ontogenesis in the sea bass*, Dicentrarchus labrax (*L.*). Nota di EMANUELE RODINÒ e MARTINO RIZZOTTI, presentata (*) dal Corrisp. B. BATTAGLIA.

RIASSUNTO. — Il quadro elettroforetico della emoglobina della spigola o branzino, *Dicentrarchus labrax*, presenta inizialmente, nella post-larva, tre componenti anodici, mentre il quadro dell'adulto presenta cinque frazioni emoglobiniche catodiche. La transizione avviene gradualmente con comparsa di frazioni intermedie dopo il terzo mese dalla schiusa delle uova.

Il sangue dell'adulto presenta l'effetto Root, quindi può secernere ossigeno nella vescica natatoria. Anche il sangue della post-larva, in base alle caratteristiche elettroforetiche delle sue emoglobine, dovrebbe presentare la stessa proprietà e ciò potrebbe fornire una spiegazione per la malattia della bolla gassosa che si manifesta negli allevamenti intensivi di questa specie.

Among the relatively few described cases of ontogenetic variation of haemoglobin (1) in Teleosts, quite different patterns of variation were observed: in some cases the growth and the differentiation of the post-larva are correlated with the appearance of some new Hb component (Wilkins, 1968), whereas in other cases the disappearance of some component of the juvenile pattern occurs (Callegarini, 1968; Perez and Maclean, 1974; Rizzotti *et al.*, 1977). A complete substitution of the Hbs has been observed in the sea perch, *Embiotoca lateralis* (Ingermann and Terwilliger, 1981). A complex case was reported in the coho salmon: in this species the late reappearance of previously lost Hbi fractions was observed (Giles and Vanstone, 1976).

Moreover, in some cases the Hb ontogenetic variation is a protracted process occupying the greater part of the life cycle (Wilkins and Iles, 1966; Wilkins, 1970) whereas in other cases the variation is limited to an early phase.

As remarked by Sullivan (1977), in many species the post-larval juveniles inhabit environments different from those of the adults and it would be most interesting to attempt to correlate changes in haemoglobin(s) (1) patterns with changes in habitat. It is remarkable that some of the more typical cases of ontogenetic variation of Hb in fish were described in species which have a migratory

(*) Nella seduta del 26 gennaio 1985.

(1) Abbreviation: haemoglobin(s), Hb(s).

phase during their early life: the catadromous European eel, *Anguilla anguilla* (Rizzotti *et al.*, 1977), the anadromous Atlantic salmon, *Salmo salar* (Wilkins, 1970), and Pacific coho salmon, *Oncorhynchus kisutch* (Gilesand Vanstone, 1976).

Early changes in habitat, even if more limited, are also typical of many Teleost species living in estuarine environments. Some euryhaline species common in the North Adriatic lagoons, *e.g.*, the mullets, *Mugil* and *Liza* sp., and the sea bass, *D. labrax*, are actually characterized by a sort of catadromous migration: reproduction takes place at sea, then the young fry swim to coasts and often enter the brackish waters of coastal lagoons and river mouths where most of the growth takes place.

In this paper we report the description of the variation of the Hb pattern during the first year of growth from post-larva to juvenile in the sea bass, *D. labrax*, a typical euryhaline species with early migration to brackish waters. This species is of economic value; it is actively fished as 2-3 months old fry in coastal lagoons for restocking fishing ponds and is also widely utilized in local aquaculture plants for large scale artificial reproduction and rearing.

The presence of a multiple Hb pattern in the adult stage of *D. labrax* has already been reported (Perez and Mclean, 1976).

MATERIAL AND METHODS

Fry and adult animals 2 to 20 cm long were used. They were kindly supplied by the intensive fry rearing plant of the firm SIRAP in Pellestrina (Venice, Italy). As the different samples came from rearing basins and tanks meant for industrial production, their content was occasionally transferred and mixed. Therefore, it was not possible to follow the same stocks for the whole ontogenesis period. Environmental parameters, such as temperature and trophic conditions, might also have varied from tank to tank, affecting times and modes of growth and development. In consequence, age and length of the samples were not always found to be well correlated. Similar findings were reported by Calleca Barahona Fernandes (1978): large fluctuations in growth of sea bass farm-reared larvae were observed, depending on factors such as egg size and parents stock.

Blood was collected from the caudal vein in a glucose-citrate solution made isotonic with NaCl.

Erythrocytes were washed twice by centrifugation in buffered isotonic NaCl solution, and the pellet was haemolysed for 30 minutes in 0.005 M Tris-HCl buffer, pH 7.0, containing 5.10^{-4} M EDTA to prevent iron oxidation (Antonini and Brunori, 1971). The red cell pellet from a single fry appeared as a small dot at the bottom of the microtube; before haemolysis the inner walls of the microtube were carefully dried with absorbent paper.

The zone electrophoresis of the haemolysates was performed on strips of gelatinised cellulose acetate Cellogel (Chemetron, Italy) according to a method already described (Rizzotti *et al.*, 1977).

RESULTS

The Hb pattern of the sea bass shows an ontogenetic variation taking place within the first year after hatching (figs. 1 and 2). Multiple Hb components are present not only in the adult pattern (more cathodic), but also in the juvenile and post-larval ones (more anodic). No genetic polymorphism was observed in the over 100 individuals tested; the only observed variation was ontogenetic.

The age reported in fig. 1 is only approximate, as discussed before. All the individuals of *D. labrax* about 2 cm long (roughly 50-60 days from hatching, the smallest useful for analysis) showed the post-larval pattern with three anodic bands (fig. 1). Individuals more than 8-10 months old showed, in general, the adult pattern with five bands with higher isoelectric point (more cathodic) (fig. 2*a*) corresponding exactly to that reported by Perez and Maclean (1976). These individuals could be very different in length: the adult pattern was at times observed in specimens no more than 4-5 cm long, but in general it was typical of young fish 8-10 cm long. The ontogenetic variation in this species was not detected by the cited Authors, because they only examined specimens longer than 10 cm.

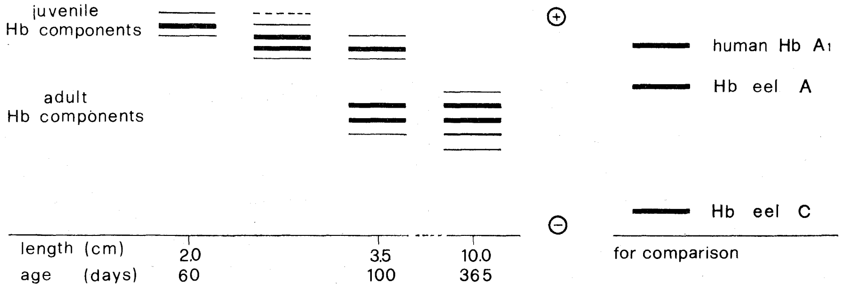

Fig. 1. – Variations of the elctrophoretic pattern of sea bass haemoglobins during the ontogenesis. For a comparison of the electrophoretic mobility, the main human and eel haemoglobin components are shown.

Generally in the third month after hatching, the larval Hb pattern starts changing with the appearance of bands of lower mobility; the most anodic larval components are the first to disappear. Very typical is the intermediate pattern which can be observed after the third month: the central adult bands are present together with a group of bands of electrophoretic mobility intermediate between the larval and the more cathodic fractions of the adult (fig. 2 *c*, *d*). Once established, the adult pattern is stable and seems not to undergo further variation.

DISCUSSION

The Hb ontogenetic variation in *D. labrax* has some peculiar characteristics in comparison with those described for other Teleosts species. The variation takes place during a large part of the first year of growth; moreover,

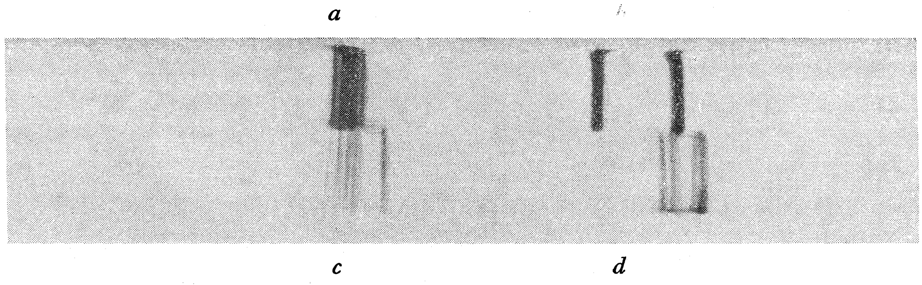

Fig. 2. – An electrophoretic strip showing parallel migration of haemoglobins from an adult sea bass (*a*), from an individual which presents both adult and juvenile haemoglobins (*c* and *d*) and from an adult eel (*b*).

at variance with the majority of the other reported cases, the components of the adult Hbs are totally different from those present in the post-larva.

It is also worth noting that the transition from the anodic larval Hbs to the intermediate juvenile pattern, seems to take place with good time and age correlation with the migration of the fry towards the coasts.

In many cases the different components of the multiple Hb pattern present in most Teleosts have been shown to possess very different physiological properties which are thought to be adaptive (Riggs, 1970, 1979; Bonaventura *et al.* 1975; Powers, 1980). In particular, one or more of the Hb fractions are not saturated with oxygen at low physiological pH, even at high partial pressure of the gas (the Root effect). The evolutionary origin of these fractions is assumed to be related to the choroid *rete* of the retina, rather than to the *rete mirabile* of the swimbladder (Farmer *et al.*, 1979; Ingermann and Terwilliger, 1982). Nevertheless, these fractions are thought to be responsible for the oxygen secretion into the swimbladder, where the pH can be low, mainly due to a countercurrent concentration of lactic acid (Steen, 1970; Fänge, 1973; Blaxtler and Tytler, 1978; Perutz and Brunori, 1982).

D. labrax has both a swimbladder and a choroid *rete*, and its haemolysate presents the Root effect (Corda *et al.*, in press). The separated Hb components were not analyzed. However, the observation made by Perez and Maclean (1976), that all the adult Hb components in this species have a polypeptide in common, may suggest a common function for the five components. If this is true, then *D. labrax* can be assigned to Class I, according to the subdivision of Teleosts on the basis of the functional properties of their Hb systems proposed by Bonaventura *et al.* (1975). Class I includes species which have single or multiple Hbs, but are similar in their sensitivity to pH and temperature. As yet, nothing is known about the functional properties of the larval Hbs of *D. labrax*. In Teleosts, however, anodal moving (low isoelectric point) Hbs generally tend to be pH-sensitive with Root effect (Brunori, 1975; Bonaventura *et al.*, 1975; Weber and De Wilde, 1976). Therefore, the anodal post-larval fractions in the sea bass might possess a Root effect as the adult fractions do.

This hypothesis suggests a possible explanation for the notorious gas bubble disease, responsible for a large part of the mortality of sea bass fry in the intensive rearing plants. In fact the bubble can develop in the swimbladder of the fry two to three months after hatching, after the pneumatic duct, connecting the swimbladder to the oesophagus during the first three weeks in the larva has degenerated (Giavenni e Doimi, 1984), but before the start of the Hb transition to the adult pattern. Some metabolic alteration, due to the artificial rearing conditions, may cause acidosis in the young fish. A decrease in the pH of the blood, even slight, might be sufficient to alter the balance of gas exchange in the swimbladder and therefore might act on the Hb components with Root effect, inducing a major oxygen secretion and decreasing its absorption.

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