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THE EELS: FROM LIFE CYCLE TO REPRODUCTIVE ENDOCRINOLOGY

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Y.-A. Fontaine and S. Dufour (1991) The eels: from life cycle to reproductive endocrinology. *Bull. Inst. Zool., Academia Sinica, Monograph* **16**: 237-248. Eels are fish of great economical importance. Yet, they exhibit very complex life cycles which more and more endanger these species and, also, impede their culture.

In this review, some aspects of the life cycle of European eels are examined. No naturally mature eels have ever been observed (except maybe for a very few males) and it is assumed that eels spawn in deep seas, each species in a defined area. The gonadal development has to occur during the oceanic migration towards these areas because silver eels are still far from sexually mature when leaving continental waters.

Artificial sexual maturation was obtained by gonadotropin treatment and in a few cases fecundation was achieved, but the offspring did not survive more than a few weeks at the best. It may be that early development requires special environmental features such as a high hydrostatic pressure. Also, gametes obtained after artificially induced sexual maturation may be defective, due to an abnormal hormonal equilibrium during gametogenesis.

The endocrine mechanisms of the blockage of gonadal development in European silver eels have been investigated. This blockage is due to a lack of pituitary gonadotropin synthesis and release which results from both a lack of gonadoliberin secretion and a dopaminergic inhibition of its action.

Natural sexual maturation is dependent on environmental factors encountered during the oceanic migration. Our results suggest that a high hydrostatic pressure is important in triggering eel pituitary gonadotropin function.

The results of endocrine or environmental experiments are discussed. The interest of a comparative approach including various species of eels which exhibit differences in their life history is underlined.

Key words: Eel, Life cycle, Reproduction, Hormones, Environment.

For centuries, mankind has been interested in eels for the quality of its flesh and the mystery of its life cycle. Eels (*Anguilla* spp.) are distributed among two Atlantic species and some fifteen Indo-Pacific species. They are of great economical importance (with a production of more than 100,000 tons per year, Van de Vijdeven, 1990). Recent data indicate that, at least in Europe, eel production is decreasing dramatically (an approximate two-third decrease during the last 15 years); whatever the reasons are (overfishing, pollution, river dams and/or habitat reduction), eels may soon become an endangered species (Brusle, 1990). Eel culture, which should therefore be developed, is impeded by several biological characteristics of these fish. Here, we shall focus on problems related to the reproduction. The life cycle, which we shall deal with first, is such that natural reproduction is out of our control. No naturally mature eels have ever been observed (except may be for a very few males, see Tuzet and Fontaine, 1937). On another hand, artificial reproduction has not yet been successfully carried out and we shall analyse the complex of endocrinological and ecophysiological mechanisms which underlie these difficulties. Finally we shall consider research prospects which might improve our possibilities of action in the eel reproduction.

LIFE CYCLE AND NATURAL REPRODUCTION

The life cycles of various eel species appear to show a similar general pattern and the example of the European eel, *Anguilla anguilla*, will be considered here (Schmidt, 1922; Bertin, 1951; Sinha and Jones, 1975; Lecomte-Finiger, 1984; MacCleave and Kleckner, 1985). This life cycle includes larval oceanic stages (preleptocephals, then leptocephals). The smallest larvae found were caught in the Sargasso Sea indicating that the spawning ground was in this neighbourhood. With the help of oceanic corrents leptocephals migrate to Europe. Recent data indicate that the duration of this migration would be shorter (around 1 year) than previous estimations (2–3 years) (Lecomte-Finiger and Yahyaoui, 1989). When approaching the coast the larvae metamorphose into translucent small eels (glasseels) which enter fresh waters and acquire a yellow pigmentation. Sexual differentiation occurs (Colombo *et al.*, 1984), giving rise to an important dismorphism as the females will be come much larger than males. Yellow eels, which spread in a variety of continental water area, are still completely immature. After a long (5-20 years) sedentary period of growth, these fishes turn into "silver" eels. The details and the mechanisms of this metamorphosis are still poorly understood (Lecomte-Finiger, 1990). It includes changes in behaviour and the fish may be carried away by the current into the sea (Fontaine, 1975). Here lies a still mysterious part of the eel life cycle, very few data being available. No individual eels have ever been caught in the Sargasso Sea where they are supposed to reproduce.

How can we imagine the events leading to this reproduction? First, it should be underlined that silvering includes only very limited gonadal development. The fish leaving fresh water for their reproductive migration still have very poorly developed gonads (*e.g.* the ovaries only show just a beginning of vitellogenesis and the gonadosomatic ratio (GSI) is around 2 in females, Dufour *et al.*, 1988).

Second, if one keeps silver eels in aquaria, they do not undergo further sex maturation. Thus sexual maturation must depend on external factors encountered during their migration. In the absence of these factors, sexual development is blocked.

Very few eels have been caught during their pelagic, transoceanic, 6,000 km migration and only 2 have been described in some detail: one near Feroe Island (Ernst, 1975) and the other east of the Azores Islands (Bast and Klinkhardt, 1988). Both were caught some hundreds meters in depth and had a GSI higher than freshwater premigratory silver eels (2.9 and 9.8 respectively). Moreover, one eel was photographed by the "Alvin" submarine, in the neighbourhood of Bermuda Islands, 2,000 m in depth; it was apparently undergoing sexual maturation (Robins *et al.*, 1979).

These very rare direct data suggest a migration in deep sea, an idea which is corroborated by other indications.

—the silvering includes the appearance of characteristics which are found in abyssal fish (*e.g.* new retinal pigments and complexification of the swimbladder red body) (Kleckner and Kruger, 1981) and which it is tempting to consider as "anticipation adaptations" (Fontaine, 1983).

—tracking experiments (Tesch, 1987) showed that immersed silver eels quickly went down in the sea; further they revealed vertical migrations from 100 m in depth (during the night) to about 400-600 m in depth (during day time).

The reproduction itself is supposed to occur in Sargasso Sea, at a few hundreds meters depth (Tesch, 1979). In fact it could well occur at much greater depths as geographical considerations suggest that for all eel species, the spawning ground is probably associated with the existence of abyssal zones (Fontaine *et al.*, 1985b; Dufour, 1986). While in short-term laboratory tests, *Anguilla anguilla*, was found to convulse at 100 atm and to be paralysed at 150 atm (see Wardle *et al.*, 1987), recent experiments demonstrated that male and female silver eels, *Anguilla anguilla*, were able to survive a 3 month immersion at 1,650 m depth following a 1 month accommodation period at 800 m depth (Fontaine *et al.*, 1990 and unpublished data). This suggests that eels may acquire an increased tolerance to a high hydrostatic pressure during their oceanic migration.

In view of this astonishing life story, it is clear that eels are potential targets for many dangers, both during their stay in fresh water (specially from men) and during their marine migration and reproduction. As an example of a recent natural risk, we can mention the extension to Europe of the parasitosis by the nematode *Anguillicola crassus*. This worm inhabits the swimbladder of the eel. As this organ is supposed to play a role in deep sea accommodation, the parasitosis could be a new potential risk even though we have shown experimentally that infested eels can still support a high pressure exposition (Fontaine *et al.*, 1990).

REPRODUCTIVE ENDOCRINOLOGY

In fish, as in other vertebrates, gonadal development is stimulated by pituitary gonadotropin (GTH). A gonadotropin (GTH II) similar to the mammalian GTH's is present in all the fish species studied so far. A second GTH (GTH I) has been found in salmonids and cyprinids (Itoh *et al.*, 1988). Pituitary GTH II production is under the control of sex steroid and hypothalamic hormones (for review, see Fontaine *et al.*, in press).

Gonadotropin treatment of silver eels

Artificial reproduction was first attempted by injecting mammalian GTH's, specially human chorionic gonadotropin (hCG). Such experiments led to complete sexual maturation in males but not in females. After the pioneer work of Fontaine (1936), a number of papers have been concerned with this topic (*e.g.* Boetius and Boetius, 1967). A single injection of hCG is able to induce spermatogenesis and spermiation two to three months later (Khan *et al.*, 1987); this long lasting effect is at least partly due to the very long half life of hCG in the eels (Fontaine *et al.*, 1984).

After recognition of the species specificity of GTH, experiments were carried out with gonadotropic extracts of fish (carp) pituitary and, in those conditions, sexual maturation of female eels was also obtained and spawning was observed (Fontaine *et al.*, 1964). A similar result was obtained with the use of purified GTH II, indicating that this hormone is the main active factor (Burzawa-Gérard, 1974; Fontaine *et al.*, 1976). An artificial sexual maturation was also achieved in Japanese eels; in this case fecundation was carried out and larvae were obtained. However these larvae could not be kept alive more than about 2 weeks in aquaria (Yamamoto and Yamauchi, 1974; Yamauchi *et al.*, 1976).

This relative setback in obtaining the viable larvae may have several reasons. It maybe that special environmental factors, encountered in spawning areas, are necessary for precocious development. One may also suspect that eggs obtained by an artificial maturation show abnormalities because of the extreme pharmacological endocrine situation induced by the treatment.

Mechanisms of the silver stage prepubertal blockage

The mechanisms have been analyzed in female European silver eels. As the gonads are sensitive to fish GTH, both *in vivo* (see above) and *in vitro* (Fontaine-Bertrand *et al.*, 1978), the blockage of sexual development must be due to a lack of pituitary stimulation.

The development of a radioimmunoassay for eel GTH II (Dufour *et al.*, 1983a) has allowed us to study the pituitary gonadotropic function (for review, see Dufour *et al.*, 1988). Gonadotropin II is not detectable in the plasma and very low in the pituitary from the silver eels, indicating that both its synthesis and its release are poorly effective.

The pituitary gonadotropic function was activated by a dual treatment with a gonadoliberin (GnRH) agonist and an antidopaminergic drug, whereas each of these drugs was without effect when injected alone (Dufour *et al.*, 1991). This indicates a double blockage of pituitary gonadotropic function by a lack of GnRH and an inhibitory action of dopamine.

On the other hand, sexual steroids are the potent stimulators of GTH II synthesis. Administration of estradiol increased the pituitary GTH content up to 1,000 times (Dufour *et al.*, 1983b). A similar increase in pituitary GTH content was induced by endogenous steroids, the secretion of which was increased during experimental sexual maturation (Dufour *et al.*, 1989). The recent cloning of complementary DNAs for GTH II subunits (Querat *et al.*, 1990a, b) has allowed determination of the messenger RNAs (mRNA) levels: administration of estradiol increased the mRNA level for GTH II β subunit up to 150 times indicating a pretranslational effect of the steroid on GTH synthesis (Querat *et al.*, 1991 and unpublished data). However, estradiol did not stimulate the release of the hormone; again, in estradiol-treated eels, a dual treatment with GnRH analogous and antidopaminergic drugs induced GTH II secretion and GSI increase (Dufour *et al.*, 1988). As for GTH, sexual steroids appeared to stimulate the GnRH synthesis but not release (Dufour *et al.*, 1985, 1989). The positive control exerted by the sexual steroids on GTH and GnRH syntheses during sexual maturation in the eel would produce an amplification of the pubertal stimulation of the hypothalamo-pituitary-gonadal axis (Dufour *et al.*, 1989).

So far these endocrine manipulations have led to only a limited development of the ovary (GSI increasing from 2 to 3-4, compared to about 40 in the experimentally matured females). For further advances, several possibilities such as longer treatments or pulsatile administration of GnRH and antidopaminergic agents can be considered. Also, the possible role of GTH I, if present in eels has to be evaluated as well as of other pituitary factors, for instance growth hormone (Burzawa-Gérard and Delavallee-Fortier, 1989). The respective functions of the two GnRH forms (mammalian and chicken II) recently identified in this species have also to be investigated (King *et al.*, 1990).

ENVIRONMENTAL FACTORS AND SEXUAL MATURATION

Physiologically, the eel gonadotropic brain-pituitary axis should be triggered by external factors encountered during the oceanic migration towards the Sargasso Sea. As simple transfer to the sea water is ineffective, factors other than salinity must be involved. Several kinds of data, summarized above, led us to the hypothesis that the deep sea could be one of these and we undertook experiments to test this hypothesis. Female eels were placed in cages which were immersed from an oceanographical ship in the northern Mediterranean Sea. In a first experiment immersion was carried out at 450 m deep for 3 months. Compared to the controls, these animals showed a slight but significant ovarian development and especially, the pituitary GTH II level was multiplied by 27 (Fontaine *et al.*, 1985a, 1985b). Another experiment with immersion 850 m deep for 3 months gave similar results (Fontaine *et al.*, 1987).

Indeed, the deep sea conditions imply several parameters, two of which appear to prevail the obscurity and the hydrostatic pressure. As we have found that obscurity *per se* does not affect the pituitary gonadotropin levels, we suggest that the hydrostatic pressure is an important factor in triggering the eel gonadotropic brain-pituitary axis. If a high hydrostatic pressure is indeed necessary for sexual maturation of the eel, it would give meaning to the apparently extravagant oceanic migration, which is the only way to achieve the exposure to such conditions.

Deep sea immersion only led to limited ovarian development. Here also there may be several reasons for the relative unsuccessful results. Longer immersions could be necessary, as nobody actually knows the normal duration of sexual maturation. Also, a pulsatile application of high hydrostatic pressure might be more efficient than continuous exposure, in view of the vertical migrations observed by Tesch (1987). In this line, we carried out experiments in a hyperbaric caisson, in collaboration with Barthelemy's group in Brest (France). During one month, animals were compressed at 60 atm every day for 8 h (Fontaine *et al.*, 1990). Compared to the controls they showed a limited increase in pituitary GTH (4×) with no significant change to GSI (unpublished data). Finally parameters other than the hydrostatic pressure may well play a role and are likely to be different in our Mediterranean experimental site from those in the Atlantic routes of actual migration (*e.g.* water temperature, eel locomotion ...).

Indeed, the normal progress of sexual development is likely to need the interaction of several factors which may change from one step of this development to another.

From an evolutionary point of view we suggest (Dufour and Fontaine, 1985; Dufour, 1986; Fontaine, 1989) that the primary constraining adaptation has been the need for the deep sea spawning and that the various present characteristics (*e.g.* prepubertal endocrine blockage, preparation to migration) are secondary. If this is true, it would be of great interest to know which of the different physiological events which occur around spawning (final maturation and release of gametes, fertilization, embryonic development) require conditions found in abyssal zones, as well as the possible part played by external factors other than pressure. These points must be taken into consideration in experimentation on eel culture.

CONCLUSION

Gonadal development is blocked in the silver eels due to a lack of pituitary gonadotropin. Artificial reproduction has been obtained with animals treated by exogenous gonadotropins but larvae could not be kept alive more than a short time. The endocrine mechanisms involved in the blockage of pituitary GTH function in the European silver eels have been identified (lack of GnRH secretion, dopaminergic inhibition of GnRH action). However, pharmacological treatments intended to overcome the blockage have led so far to only a limited gonadal development. Further experiments could test longer treatments as well as the pulsatile administration of GnRH and antidopaminergic drugs; the effect of chicken GnRH should also be investigated, as only the mammalian form has been used so far. Normal sexual maturation is dependent on the environmental factors encountered during the oceanic migration towards the Sargasso Sea. We suggest that high hydrostatic pressure is one important triggering parameter, as deep sea immersion (but not obscurity) stimulated pituitary gonadotropin function. As only limited gonadal development was obtained we suggest that normal sexual maturation involves complex and changing interactions between factors.

Sexual blockage at the silver stage appears to exist in all eel species and its mechanisms are probably related to those elucidated in the case of the European eels; for instance GTH and GnRH levels are very similar in three Polynesian species to those in *Anguilla anguilla* (Le Belle *et al.*, 1988). However Pacific eels show much diversity in certain aspects of their life cycle (spawning area, length of the migration), which suggests diversity in biological adaptations (Tzeng, 1985). For instance, the sexual blockage at the silver stage might be less strict in some species than in the others; indeed silver animals appear to have undergone a much more important sexual development in two New Zealand species (*Anguilla australis* and *Anguilla dieffenbachii*) than in *Anguilla anguilla* (GSI 4.0-9.0 and 2.0 respectively) (Todd, 1981; Jellyman, 1987).

A thorough comparative study of the endocrine situation in several eel species and even in other Elopomorphs may be a promising approach to achieve a better understanding and control of sexual development.

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