## Induced Gonadal Maturation of Male European Eel (Anguilla anguilla L.) Inhabiting the Egyptian Lakes

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ABSTRACT. The European silver eel (Anguilla anguilla L.) obtained from the Egyptian Lake (Burollos) gave positive results when the males were induced by a human chorionic gonadotropin hormone (HCG). Males maintained in metallic tanks, supplied with sea water (salinity 37.5 ppt) and difused atmospheric air showed a response to hormone injections. Testes auained their nearly ripe condition after the 5th injection and full testicular ripeness and consequent spermiation were achieved after the 7th injection, in a period of 45 days. Several morphometric and biological changes occured with increasing the successive injections.

The European eel (Anguilla anguilla L.) undergoes an extensive catadromus migration from the continental fresh or brackish waters to the Atlantic Ocean for spawning (Bertin 1956 and Kokhnenko et al. 1977). The Egyptian Lakes are excellent feeding grounds for eel; more than 100 tons are landed annually and exported to Europe (Salnikov et al. 1980).

Many principal changes take place before the period of migration. The colour changes to brilliant white on belly and black on backs and the fish appear silvery at the time of migration. There are many changes in shape of the head which changes from broad to sharp and the eye diameter becomes larger (Bertin 1956, Kokhnenko 1969, and Amin 1974). In contrary to other catadromus fishes such as *Mugil spp* (spawn in the sea), which leave the Egyptian Lakes with nearly ripe gonads (Hussein 1974), the European eel starts its spawning migration with completely immature gonads (Amin 1974). The sexual maturation takes place in the sea water (Bertin 1956), far from any fishing operations. Therefore, many successful trials were carried out by several investigators to induce the spawning of male eel (Boucher *et al.* 1935, Fontaine 1936, Bruun *et al.* 1949, Olivereau 1961,

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Boetius and Boetius 1967, Yamamoto et al. 1972, and Kokhnenko and Bezdenegnek 1975). The successive injection with chorionic gonadotropin hormone (HCG) was found to be effective for inducing the gonadal maturation (Boetius and Boetius 1967, Kokhnenko and Bezdenegnek 1975).

This work represents a trial in the Egyptian Lakes for getting live spermatozoa from male silver eel by hormone injection as a first step in the process of artificial fertilization of this important commercial fish.

#### Material and methods

Fifty specimen of migrating silver eel (females and males) were obtained in the winter of 1979 from the commercial catch in lake Burollos (one of the Egyptian northern delta lakes). They were transported in tanks to the laboratory in Alexandria. Twenty specimen of migrating male silver eels were selected for the experiment according to their lengths and colours. Migrating silver eel were identified by their metallic gleaming colour on belly and blue on the back (Bertin 1956 and Amin 1974).

Ten male eels with total length from 42.9 to 43.5 cm (136 to 142 g) were used as the control fishes. The other ten migrating silver eel males of 40.8 to 42.0 cm total length (105 to 120g) were injected by human chorionic gonadotropin hormone (HCG) to induce their gonad maturation. The eels were first maintained in fresh-sea water aquaria for 25 days for acclimatization. Each pair of eels was then placed into metallic galvanised, tightly covered 100-liter tanks. All water (salinity 37.5 ppt) in the five tanks was replaced every four days. Compressed air was diffused into the water of each tank. Water temperature varied from 20 to 26°C. Eels were kept without food, and in complete darkness. Intramuscular injections were given weekly with 250 i.u (HCG). Measurements of eye diameter and total fish weight were recorded before each injection for all ten males. The stages of testicular maturation were defined according to the scale of Boetius and Boetius (1967), and Kokhnenko *et al.* (1977).

#### Results

#### I - State of testes

#### A. Controled male

The wall of the testes of the control silver eels (non injected) was found to be comparatively thin. Testes are divided by septa into lobules which are in turn subdivided into many cysts (Fig. 1). Spermatic tissue is grouped in cysts containing spherical spermatogonia, each with a conspicuous nucleus. Primary spermatocytes are few in number (Fig. 2). These features of the testes of the control silver eel correspond to a male in stage I of maturation, according to Amin (1974) which based on Boetius and Boetius (1967).

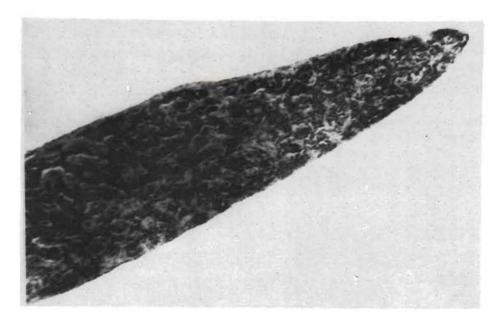


Fig. 1. Testis of male silver eel  $40 \times 15$ 

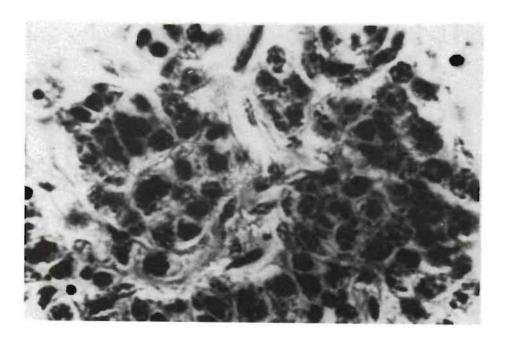


Fig. 2. Testis of control eel in stage I  $40 \times 15$ 

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#### B. Induced male

The testes of a male injected five times over 30 days with HCG had a creamy colour and almost filled the entire body cavity, and attained a nearly ripe condition of maturation.

Histological examination of testis showed lobules filled with spermatozoa, some grouped in a parachute-like structure with heads in one direction and tails in the opposite direction (Fig. 3). Lobules were covered with a very thin layer of connective tissue. In the anterior lobe and lumen of the sperm duct, a large number of spermatozoa were stored. These features of the testis of induced male correspond to stage IV of maturation.

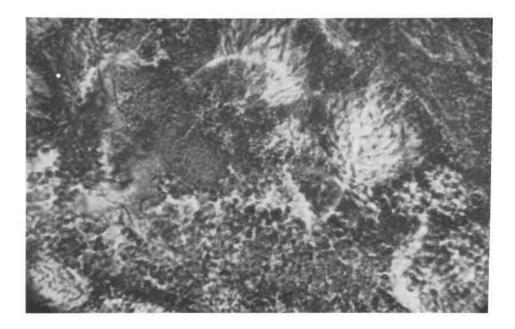


Fig. 3. Testis of induced male in stage IV  $100 \times 15$ 

Testes of male injected seven times with HCG attained the degree of ripeness in 45 days. The urogenital opening when stripped showed a milky fluid. Fully ripe testes in histological sections showed large numbers of free-swimming spermatozoa in the testicular fluid (Fig. 4). The average diameter of a sperm head varied from 2 to 3  $\mu$ . After discharge of spermatozoa and with continuous injection, testes became reduced in size, flaccid, and contained fewer spermatozoa inside and around the lumina of lobules.

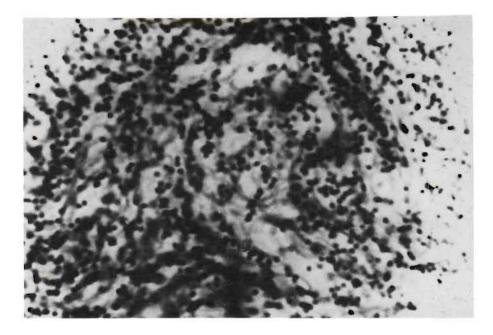


Fig. 4. Testis of induced male in stage V  $100 \times 15$ 

#### II - Gonadosomatic Index

The weight of the control testes of migrating silver eels (42.9 to 43.5 cm in length and 136 to 142 g in weight) ranged from 0.049 to 0.100 g. Gonadosomatic index (GSI), or gonad weight, as a percentage of gutted body weight amounted to 0.051 %. Total length of testes before urogenital opening was 2.5 cm.

During the period of induced maturation, the weight of testes is considerably increased. Testes of five times injected fishes (four males) reached 4.5 g in weight. The value of GSI increased to 5 and length of testes before urogenital opening increased to 6 cm.

On continuing the process to seven injections (with three other males) the testes attained the ripe condition. Weight of gonads reached 4.7 g with a corresponding GSI of 5.3 and total length before urogenital opening of 6.3 cm. Testes of males which received than seven injections (three males) revealed a drop in weight to 0.54 g with a corresponding GSI 0.63 %.

Plotted values of GSI against successive times of injection (Fig. 5) show that maximum gonad weight is attained after the fifth injection.

#### III - Change of external features

Silver eels ready to leave the lake for the Mediterranean Sea had eyes with an average diameter slightly greater than that of the non-migrating eels in the lake (Amin 1974).

During the process of injection (ten fishes) and the consequent gonadal development, the eye diameter of the male silver eels increased gradually (Fig. 5)

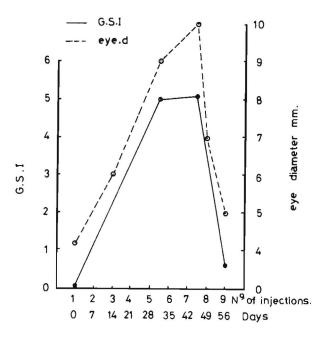


Fig. 5. Relation between the number of injections and values of GSI, eye diameter.

indicating that the continuous increase in eye diameter was a phenomenon related to the sexual maturation of gonads. At the begining of the experiment by the controlled males, the average eye diameter was 4.2 mm, and increased gradually with increasing number of injections to reach 10 mm at the ripe condition of gonad maturation. After discharging the sexual cells in the water the eye diameter came back to the normal diameter of 5 mm. Also, the body weight of hormone-treated males, injected 10 times, decreased about 2.6 g weekly (Fig. 6).

During the period of the experiment, an obvious change in the colour of the body took place. The colour became more bright on the belly and more dark on the back. The anus closed and urogenital opening began to open after the fourth injection.

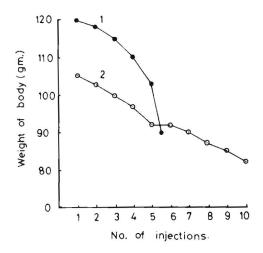


Fig. 6. Relation between the number of injections and body weight:

- 1. 5 times induced male
- 2. 10 times induced male

#### Discussion

The European eel does not develop its gonads in fresh or brackish water. Males start their spawning migration with testes in very early stages of maturation and the further development takes place in the spawning grounds of higher salinities (Amin 1974, Kokhnenko and Bezdenegnek 1975).

Injection with HCG could induce male silver eel of Stage I of maturation to spermiate; therefore, the injection of eel during its back migration to the sea in the winter is a suitable time for inducing testicular maturation. The very slow effect of hormones on the testicular maturation of eel was referred to the relative inactivity of the endocrine system by D'Ancona (1960).

In the experimental tanks no mortality occured among the induced eels with HCG at a weekly dose of 250 i.u., which may be considered as a standard dose. The water temperature in tanks ranged between 20 and 26°C and the adjusted water temperature was favourable for a successful induced testis maturation.

The acceleration of testicular development by injection of HCG was extended to 45 days and spermiation took place after the seventh injection. An extended course of injection was also carried by Kokhnenko and Bezdenegnek (1975) on the male silver eel of Baltic Sea. About the same extended period of induced maturation at similar conditions were also obtained by Boetius and Boetius (1967) and Yamamoto et al. (1972).

#### Conclusion

Males of European silver eel from Egyptian Lakes were successfully induced to gonadal maturation by a series of injections with HCG at 20-26°C.

Testes were found to undergo several changes up to the time of maturation. Spermatozoa with a head diameter of 2-3  $\mu$  were discharged in to the surrounding water in the tank.

Correlation exists between the increase in number of injections and gonad development, eye diameter, body weight, and testes weight until the maturation.

Maximum eye diameter of about 10 mm is achieved just before ripening of the testes, then it begins to decrease just after discharge of the sexual cells.

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# إنضاج ذكور ثعبان السمك الأوربي (أنجويللا أنجويللا) في البحيرات المصرية بالحقن الهرموني

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يشكل تعبان السمك الأوربي (أنجويللا أنجويللا) جزءً هاماً من إنتاج البحيرات المصرية ويدر عائداً مادياً لا بأس به من خلال تصديره حياً إلى أوربا. وهذا النوع من الأسماك لا يتوالد في البحيرات ـ التي يدخلها باحثاً عن الغذاء فيها ـ لكنه يقوم بهجرة طويلة حيث يعبر البحر المتوسط ليصل إلى المحيط الأطلنطي . . وهناك يتم التوالد .

ولقد أثبتت الدراسة التي أجريت على الذكور المهاجرة من ثعبان السمك أنها تعطي نتائج إيجابية عند حقنها بهورمون (كوريونيك جوناد وتروبين). . إذ أمكن الحصول على ذكور ناضجة جنسياً بعد حقنها ٧ مرات بالهرمون.

كما أوضحت الدراسة التغييرات المورفومترية والبيولوجية التي صاحبت تطور المناسل عند زيادة مرات الحقن، ومن هذه التغيرات: اتساع قطر العين وتحول لون الذكور إلى الفضي اللامع في منطقة البطن وأصبح الظهر أكثر دكانة.

ويمكن اعتبار هذه الدراسة بمثابة قاعدة علمية سليمة يمكن الأخذ بها عند تفريخ ثعبان السمك صناعياً.