

ON INDUCED MATURATION IN THE INDIAN SHORT-FINNED EEL
ANGUILLA BICOLOR BICOLOR McCLIBLAND

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ABSTRACT

After acclimatizing the eels *Anguilla bicolor bicolor* in sea water, various hormones viz., Gonadotrophin F. S. H., Gonadotrophin L. H. and Chorionic Gonadotropin (Profasid) and Chorionic Pituitary extracts were administered to different dosages through intramuscular injections. By repeated hormone injections maturation was induced in both the sexes. Finally one male eel which was given 2000 IU of Gonadotrophin L.H. through four injections, reached spawning condition 144 days after the first injection. After partial spawning, the eel died on the same day. The gonadosomatic index was 6.47 and eye index increased by 14.38%. Each spermatozoid has a comma shaped body and a long tail. One female eel on which was administered 240 mg of chorionic pituitary extract and 19,000 IU of Chorionic gonadotropin (Profasid) hormone through 16 injections, attained 'ripe' condition 98 days after the first injection and yielded 185.6 g of eggs on stripping. The gonadosomatic index was 33.5. The total weight and eye index of the eel increased by 2.9% and 24.1%, respectively. The number of stripped eggs was estimated to be 0.35 million. The mature eggs were spherical and measured 1.00 mm (+ 0.08 mm) in diameter. Attempts made for artificial fertilization were not successful. Fully developed ovary of *A. bicolor bicolor* is described¹.

INTRODUCTION

It is well known that the anguillid eels undertake a long spawning migration and breed at great depths of the ocean (Schmidt 1923) but their breeding biology has not yet been fully elucidated. In order to understand this aspect, attempts had been made in the past to induce eels to maturity by hormonal treatment (Branco et al 1949, Teach 1977). Out of the 17 species of *Anguilla*, artificial maturation has been achieved only in three species, namely, the European eel, *Anguilla anguilla* L. (Fontaine et al 1964, Boetius et al 1962, Boetius and Boetius 1967, 1980, Meske 1973, Sinfua and Jones 1975) the American eel, *A. rostrata* LeSueur (Boetius et al 1962, Edel 1975) and the Japanese eel, *A. japonica* Temminck and Schlegel (Nose 1971, Qafai et al 1972, Yamamoto et al 1974 Yamamoto and Yamauchi 1974, Yamauchi et al 1976). Among the two species of *Anguilla* (*A. bicolor bicolor* and *A. nebulosa nebulosa*) distributed in India, some work on the culture (Nair 1973, Dorairaj et al 1980 a, b) and other related aspects (Nair and Dorairaj 1975, Dorairaj 1980, Dorairaj and

Soundarairajan 1980) has been done on the short-finned eel, *A. bicolor bicolor* at the Regional Centre of Central Marine Fisheries Research Institute, Mandapam Camp. Induced breeding experiments on this species were initiated in 1980, with 20 male and 15 female adult eels of 6 years age, cultured in the laboratory from eelers, which were collected at SriVaikundam. And out on the River Tambraparni during November 1973-January 1974, Various hormones, viz., Gonaadotrophon F. S. H., Gonaadotrophon L. H. and Chorionic Gonadotropin (Profasi) and carp pituitary extracts in different dosages were used in the experiments. By repeated hormone injections, maturation was induced in both sexes (Pl. IA). One male eel reached 'Spawning' condition on 27-2-1981 and a female eel attained 'strip-ripe' condition on 18-3-1981. Artificial fertilisation was attempted without success. The results in induced maturation in *A. bicolor bicolor* are described here.

INDUCED MATURATION IN FEMALE EEL

The male eel (Pl. IA) was transferred from fresh water into sea water on 31-5-1980 and was maintained in aquarium tank with continuous flow of sea water for 130 days for acclimatisation and further experimentation. From 1-6-1980 the eel ceased feeding. To accelerate the maturation process, four intramuscular injections of hormone Gonaadotrophon L.H. (Mfd. by Paiaas & Byrne Ltd., England), at a dosage of 500 IU per injection, were given to the eel. The first injection was given on 7-10-1980 after recording the measurements of the eel which were: length 425 mm; weight 140.8 g; eye dia. horizontal 10.5 mm, vertical 9.8 mm; and eye index 102.9 sq.mm. On 10-10-1980 the second injection was given. The third injection was given after 65 days, i.e., on 15-12-1980 and the fourth on 22-12-1980. By the third injection the male eel had developed secondary sexual characters like enlargement of eyes, darkening of the pectoral fins and silver colouration on the lower half of the body. The eel was active and healthy throughout the period of the experiment.

On 23-2-1981, the eel became restless, very active and its vent was slightly protruding, bloodshot in colour. The same condition persisted for the next three days. Finally, on 27-2-1981, i.e. 144 days after the first hormone injection, the male eel reached 'spawning' condition. Oozing of milt was first observed by 11-30 a.m. At the time of 'spawning' the water temperature was 28.0°C and the room temperature was 28.4°C. A gentle pressure on the abdominal region yielded plenty of milt. At 4.30 p.m. weight of the eel was 78.4 g. The eel continued to be restless, after settling down at the bottom of the tank and respiring rapidly. During the period the eel released the milt at frequent intervals by vigorously twisting the body. By about 8.00 p.m. the eel made three quick release of milt, became exhausted and motionless. It convulsed twice and died by 8.30 p.m.

Immediately after death, measurements were taken: length 423 mm; weight 77.3 g; eye dia. horizontal 10.7 mm, vertical 11.0 mm; and eye index

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117.7 sq.mm. When, the measurements of the eel at death- were compared with those taken at the first injection, it was found that the total length and weight of the eel decreased by 0.47% and 45.1% respectively and eye index increased by 14.38%. The difference of 1.1 g in weight of the eel between 4.30 p.m. and 8.30 p.m. may be due to release of milt. The testes of the partially spawned eel occupied the entire body cavity, covering the digestive tube (PI. IB). In the left testis, there were 24 lobes; the length of the lobes ranged from 2 to 31 mm and the width from 2 to 9 mm. In the right testis there were 17 lobes; the length of the lobes ranged from 5 to 18 mm and the width from 3 to 310 mm. The total weight of the testes was 5.0 g which works out to 6.47% of the body weight.

Clusters of sperms were clearly seen when a small bit of preserved milt was examined under the microscope (PI. IC). Each spermatozoid has a comma shaped body and a long tail (PI. ID). Approximate measurements of a spermatozoid are as follows: head length 4 m.d.; width 1 m.d. and maximum tail length 32 m.d. (1 m.d. = 0.00114 mm).

During March-April, 1981, 6 male eels attained mature condition through hormone injections. 3 of them were given the same quantity of Gonadotrophin L. H. Maturity was reached in 165-171 days after the first hormone injection. The remaining 3 eels were given 1000 IU of Chorionic gonadotropin (Profasi) hormone through two injections on 11th and 15th of December, 1980. They reached mature condition in 97 days after the first injection. In all the six male eels, thick white milt oozed out when slight pressure was applied to the belly. Motility of the sperms was seen when the milt was observed in fresh condition after diluting it with sea water. On five occasions milt was stripped from these eels for artificial fertilisation. All the eels are still alive and healthy.

INDUCED MATURATION IN FEMALE EEL

The female eel (PL IA) was transferred from fresh water into sea water on 8-8-1980 and maintained for 123 days for acclimatisation. From 18-9-1980 onwards the eel was not fed. A total of 16 intramuscular injections were given. The hormones used were carp pituitary (CP) extracts and Chorionic Gonadotropin (HCG) (Profasi) made by Istitoto Farmacologico Sero, Italy) and they were given at a dosage of 15 mg and 1000 IU (respectively per injection, twice a week. The injections were administered from 11-12-1980 and continued till the 9th injection on 9-1-1981. The 10th injection was given after a lapse of 45 days. The 11th, 12th and 13th injections were given at a higher dosage of 15 mg CP and 2000 IU HCG (Profasi). Later on, the injections were given at a regular dosage of 15 mg CP and 1000 IU HCG (Profasi). The last injection was given on the night of 17-3-1981. During the 16 intramuscular injections a total of 240 mg of carp pituitary extracts and 19,000 IU of HCG (Profasi) had been administered to induce sexual maturity. Throughout the period of experiment the eel was active and healthy.

On the morning of 18-3-1981 by about 10-30 a.m., the eel was found in an inactive condition. On close examination slight oozing of eggs was noticed. Immediately stripping was done (PI. IIA). The eel had reached "strip-ripe" condition at the end of the 98th day after the first hormone injection. Complete stripping yielded 185.6 g of eggs (PI. ITB). About 50 g of eggs were taken in a finger-bowl into which milt from three male eels which had shown advanced secondary sexual characters was stripped (PI. III A). After mixing the eggs and milt in 30 ml of sea water with a feather at 1.00 p.m., the material was transferred into two glass troughs, each containing 5 litres of seasoned sea water provided with aeration. The glass troughs were kept at 20° C room temperature. Till 1.00 p.m. of 19-3-1981, i.e. 24 hours after the mixing, the eggs were not fertilised and the experiment was terminated.

The measurements of the female eel at first hormone treatment and at stripping are given below.

	<i>At first injection</i>	<i>At stripping</i>
Length	650 mm	643 mm
Weight	637.2 g	655.5 g
Eye diameter		
a) Horizontal	8.3 mm	9.0 mm
b) Vertical	8.3 mm	9.5 mm
Eye index (Horizontal x Vertical)	68.9 sq.mm	85.5 sq.mm

From the above measurements it was found that the length of the eel had decreased by 1.1% whereas the weight and eye index had increased by 2.9% and 24.1% respectively.

After stripping, the eel was cut open and the residual ovarian portions were dissected out which weighed 34.0 g. The total weight of the ovary was estimated to be 219.6 g (185.6 g stripped eggs + 34.0 g residual ovarian tissue). The gonadosomatic index (GSI) was found to be 33.5.

The number of stripped eggs was estimated to be 0.35 million. The mature eggs were ispherical, relatively uniform in size and measured 1.00 mm in diameter (s.d. 0.08 mm) (PI. III B). With regard to transparency and number of oil droplets in the eggs, great variations were noticed (PI. III B).

Subsequent to the stripping of the female eel on 18-3-1981, two more females reached mature condition on 31-3-1981. They were given 19 intramuscular injections of HOG (Profaisi) hormone and carp pituitary extracts at a dosage of 500 IU and 15 mg respectively per injection, twice a week. First injection was given on 11-12-1980. The ninth was given after a lapse of 49 days from the eighth. One female eel was found to release some eggs in the aquarium tank on the forenoon of 31-3-1981. A slight pressure on the belly resulted in oozing of eggs. Many of the eggs were shrunken while a few were

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spherical, transparent, with a few large-sized oil droplets. The eggs measured 1.00 mm in diameter. The fertilization attempts were again not successful both at 31.6°C and 20°C.

The developed ovary of the hormone-treated eel and an ovary of an untreated eel are shown in Plate IV. In the former, the entire body cavity was occupied by the ovary. The stomach and intestine have been reduced in size and were fully covered by the ovarian folds (Pl. IV C). The two lobes of the ovary looked like tight ruffles, light orange in colour in fresh condition. The left lobe was longer (184 mm) than the right lobe (170 mm), which extended anteriorly beyond the left lobe by 28 mm and posteriorly beyond anal opening by 25 mm. The left lobe extended posteriorly beyond the right lobe by 41 mm and beyond anal opening by 67 mm. The maximum width of the ovarian folds in both the lobes was 41 mm. The surface of one side of the ovarian lobe facing the intestine was smooth while the other side facing the body wall was uneven with protuberance in which the eggs were imbedded.

REMARKS

In the European male eel, spawning was reported to take place in about 60 days after first injection. When the eels were maintained at temperature range of 21.6°C-26.3°C and many eels died on the day of spawning (Boetius and Boetius 1967). In the present experiment on *A. bicolor bicolor* spontaneous spawning occurred in male after 144 days from first injection when the eel was maintained at a temperature range of 26.0°C-28.5°C and the eel died on the day of spawning. The total weight of the testes of the European eel at spawning was less than 5% of the body weight (Boetius and Boetius 1967, Marks 1973), whereas in Indian eel it was 6.47%. With regard to female European eel, Boetius and Boetius (1980) obtained 'strip-ripe' condition in 40-80 days by giving 15 mg carp pituitary and 500 IU HCG (Human Chorionic Gonadotropin, Physex, Leo mark) per injection twice a week and all eels having GSI over 40 proved to be 'strip-ripe.' But in *A. bicolor bicolor* the female reached 'strip-ripe' condition in 98 days after first injection (with a lapse of 45 days between the 9th and 10th injections). The GSI value for this species was 33.5 which is lower when compared to *A. anguilla*. The egg diameter of *A. bicolor bicolor* (1.00 ± 0.08 mm) is in close agreement with that of *A. anguilla* (0.9-1.4 mm), *A. japonica* (1.0-1.3 mm) and *A. rostrata* (1.1 mm) (Tesch 1977). Variations were found in respect of transparency, yolk granules and oil droplets in the eggs of *A. bicolor bicolor* as also found in the eggs of the other three eel species (Tesch 1977).

The estimated fecundity of the European eel ranged from 0.73 to 2.63 million eggs and that of the American eel from 0.5 to 2.6 million eggs (Tesch 1977). In terms of body weight the estimated fecundity for the above two species works out to 1.6 and 3.0 million eggs per kg respectively. In *A. bicolor bicolor* the estimated fecundity was 0.35 million eggs which is equivalent to

0.5 million eggs per kg. This estimate suggests that the fecundity in *A. bicolor bicolor* is about three and six times lower when compared to that of *A. anguilla* and *A. rostrata* respectively. Further investigations are in progress which may throw more light on this aspect.

Yaoimamoto and Yamauchi (1974) were the first to successfully carry out artificial fertilisation, hatching and rearing of larvae for six days of *A. japonica*. Subsequently Yamauchi et al (1976) succeeded in rearing the larvae up to 14 days. Boetkr and Boetius (1980) have also succeeded in artificial fertilisation in *A. anguilla* but the eggs did not develop beyond gastrula stage. In the present experiment with *A. bicolor bicolor*, the attempts at artificial fertilisation were not successful. The experiments are being continued.

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