Helgoländer wiss. Meeresunters. 27, 131–138 (1975)

The induction of maturation of female American eels through hormone injections*

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KURZFASSUNG: **Die Auslösung der Geschlechtsreife bei weiblichen amerikanischen Aalen durch Hormoninjektionen.** Nach intramuskulärer Injektion von zerriebenen Karpfenhypophysen in ♀♀ von *Anguilla rostrata* gelang es, eine Keimzellenreifung bei 6 Blankaalen herbeizuführen. Die abgelegten Eier ähneln denen europäischer und japanischer Aale *(A. anguilla* und *A. japonica)*, die experimentell zur Geschlechtsreife gebracht worden waren. Die Eier sind rundlich und haben einen Durchmesser von 1 mm. Da sie Öltropfen enthalten, scheinen sie pelagisch zu sein. Bei einem geschlechtsreifen ♀ lag die Eizahl zwischen 1 300 000 und 1 500 000. Bemerkenswert ist das Auftreten eines stechenden Geruchs, der aus der Haut und dem Schleim geschlechtsreifer oder heranreifender weiblicher Exemplare entweicht. Die mögliche Bedeutung dieses Geruchsstoffes für das Auffinden der Geschlechtspartner während der pelagischen Laichphase wird diskutiert.

INTRODUCTION

This study was conducted to induce complete maturation in female American eels (*Anguilla rostrata,* LE SUEUR) through the injection of hormone preparations. The American eel is one of three northern temperate species (the remaining two are the European eel, *A. anguilla* L. and the Japanese eel, *A. japonica* TEMMINCK & SCHLEGEL) that undergo extensive catadromous migrations to tropical, pelagic spawning areas. Because of the work of SCHMIDT (1922) we know that these eels spawn away from continents and at depths of from 50 to 500 m. Since no ripe adult eels have been seen or caught while in the spawning area, we know little of their physiology or behavior during the time of spawning. It is assumed that eel eggs are pelagic. The induction of complete maturation in the laboratory is one feasible method for studying the biology of mature eels.

Two earlier attempts to induce maturation in female American eels were not successful: HANSEN (1939) tried using frog pituitaries and chorionic gonadotropins, and BOETIUS et al. (1962) tried using human chorionic gonadotropin.

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* This paper will be submitted in partial fulfillment for the degree Doctor of Philosophy in Oceanography at the University of Rhode Island.

The first successful induction of maturation in the male *Anguilla* was reported by BOUCHER et al. (1935) and in the female by FONTAINE et al. (1964) for the European eel. They obtained one mature specimen which spontaneously emitted eggs in the aquarium after three months of being injected with acetone-dried, powdered carp pituitaries. OLIVEREAU & FONTAINE (1966) easily duplicated the achievement so the procedure of using carp pituitaries to induce maturation in eels was established.

Very recently, several Japanese investigators have been successful in inducing maturation in female Japanese eels. OCHIAI et al. (1972, 1974) and YAMAMOTO et al. (1974a, 1974b) obtained ripe females by injecting them with salmon pituitaries or a purified fish gonadotropin called Synahorin. YAMAMOTO & YAMAUCHI (1974) followed this up spectacularly with in vitro fertilization and hatching of larval Japanese eels.

MATERIALS AND METHODS

Large female eels were purchased from a local fisherman in the late summer of 1973 and 1974. The eels were caught in baited pots placed in Ninigret Pond, Charlestown, Rhode Island, USA. The specimens were selected according to their size and condition. In 1973 both yellow and silver eels were selected as I wanted to determine whether hypophysation (the injection of dissolved fish pituitaries) would force yellow eels to metamorphose into silver eels as well as induce the maturation of female silver eels. In 1974 only female silver eels and eels clearly in an advanced stage of the silvering process were used. These latter eels were characterized by the loss of yellow pigment in their ventral surface, metallic gleaming along the flanks and the appearance of a milky-blue color in the axil of the pectoral fin. The eels were held in live cars for 2–5 days before the experiments were begun; they were not fed for the duration of the experiment. Each eel was placed individually into an 80-liter-tank of fiberglass-reinforced plastic covered with hardware cloth to prevent escape. The tanks were contained in an isolated, windowless room which permitted the control of an artificial photoperiod and minimized disturbances. The eels were always held in sea water which was pumped into the research aquarium building from Narragansett Bay, Rhode Island. The sea water was flowing when the temperature was above 18° C but was shut off when the temperature fell below 15° C. BOETIUS & BOETIUS (1967) showed that the temperature range for experimental maturation of male silver eels was about 11 to 25° C but was optimal at 18 to 22° C corresponding to depths from about 300 m to the surface for the winter months when eels probably spawn. The temperature within the tanks was continuously monitored by a Bristol Telethermometer placed in one of them. In 1973 the temperature ranged from 12 to 20° C (mean 19.5° C) and in 1974 the temperature ranged from 14 to 24° C (mean 19.4° C). The tanks were always aerated and periodic salinity determinations showed very little variation from 32 ‰. The artificial photoperiod was set at 12 h of 10^3 lux at the water's surface (from 0600 to 1800 h) and 12 h of total darkness (any night observations were made with a red bulb). The lamps used were daylight-simulating fluorescents which augment the blue end of the visible spectrum.

The hormones used were fish gonadotropic hormone (GTH) as contained in

acetone-dried powdered carp pituitaries purchased from Stoller Fisheries, Spirit Lake, Iowa, USA, and luteinizing hormone (LH, specifically ovine NIH-LH-S18) donated by the National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland, USA. The powdered carp pituitaries were weighed, triturated and dissolved in 0.5 cc of physiological saline per injection per animal. The LH required no trituration and was easily soluble in saline solution. The doses used were modified from FONTAINE et al. (1964) for the pituitaries and from FONTAINE & GERARD (1963) for the purified LH. The pituitary dose was set at 10 mg dry weight per eel per injection whereas the LH dose was set at 0.8 mg per eel per injection.

The injection procedure was performed in the following manner. One eel was captured and placed into a polythene bucket containing an anesthetic solution (MS-222 in 1973 and Quinaldine in 1974) with 250 mg of chloramphenicol, a broad spectrum antibiotic. When fully anesthetized, usually after 3 to 9 min of immersion, the fish was put onto a V-shaped trough and injected through a 2.5 cm, 20 gauge needle intramuscularly (after BOETIUS et al., 1962) in alternate sides of the postvent dorsal muscle mass. Then the eel was photographed so that a serial record was maintained of any subtle external changes which occurred during treatment.

If an animal died naturally it was removed from its tank, photographed, weighed, and then dissected for the gonado-somatic index (GSI)* determination. Otherwise, the eels were individually removed from their tanks, anesthetised, weighed, photographed, dissected and the gonads photographed in situ.

RESULTS

The data obtained are presented in Table 1. In 1973 only one of the six eels injected with carp pituitaries showed any substantial increase in the GSI. That eel (experimental number 5) was one of two that had completely silvered prior to treatment. Gonadal enlargement became apparent in eel 5 after 7 injections (17 days) and continued until the eel was found dead on the 38th day. By that time she had been injected 16 times so a total dose of 160 mg of carp pituitaries was administered to her. Free eggs were found in the tank but it was unknown whether they were extruded before or after death. The perianal region was swollen and the vent was enlarged to an oval about 15 by 20 mm. Examination of the gonad showed eggs ranging in size from 0.59 to 1.25 mm but the mean diameter was 1.06 mm (Fig. 1). They were transparent and tended to be slightly ellipsoidal. Oil droplets were clearly visible. An aliquot sampling method gave estimates of 1.3 to 1.5×10^6 eggs. Morphologically the flanks became an intense copper color; the dorsal surface was dark but not black; the ventral surface which began as silvery-white became darkly mottled. The pectoral fins never became lanceolate and the eyes did not appear to enlarge. The caudal musculature was quite atrophied, a condition not sustained among the 5 remaining experimental eels and the 6 control eels starved for equal or longer times.

The remaining silver experimental eel (number 6) succumbed to vibriosis. No

* GSI = gonad weight × 100/whole body weight

gonadal enlargement was detectable. The 4 experimentals which began as yellow eels seemed to be in the process of silvering but gonadal enlargement was only slight. The 2 silver control eels which were maintained for over 100 days in 1973 did show some gonadal enlargement. Their GSI's of about 6 % are attributed to natural progression. The 4 yellow control eels showed no gonadal enlargement. They appeared to remain in the yellow form throughout the experiment.

In 1974 five of 6 experimental eels injected with dissolved carp pituitaries showed noticeable gonadal enlargement (Table 1). Eel 1 died of unknown causes on the 31st day of the experiment and, while completely silver prior to treatment, showed no gonadal enlargement. After receiving 6 injections in 12 days, eel 5's gonads became

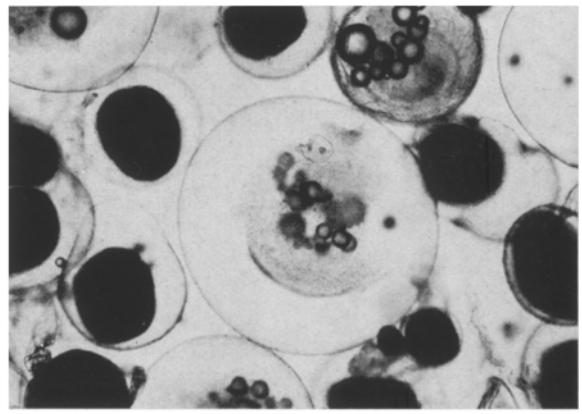

Figure 1: Whole eggs in sea water from eel 5, 1973. The large egg in the advanced stage measured 1.25 mm in greatest diameter. The smaller, less-advanced eggs (with dark yolks) measured 0.60 mm in greatest diameter. Oil droplets are visible in the yolk of the large egg

noticeably swollen. She progressed very similarly to her predecessor (eel 5 of 1973) but began extruding eggs on the 42nd day while still alive. Injections were discontinued at that time and her progress carefully monitored in order to see if there would be a climactic massive spawn or a steady rate of egg emission. Eight days after spontaneous egg emission began, eel 5 was discovered dead. Enough eggs remained in the gonad to give a GSI of 34.4. No egg count was deemed meaningful as so many were lost. The eggs were slightly smaller than those obtained in 1973 ranging in size from 0.50 to 0.99 mm (mean 0.78 mm). Morphologically, eel 5 of 1974 was identical to eel 5 of 1973. A phenomenon which was largely ignored in 1973 became grossly apparent, however, in 1974. Both matured eels had a unique, pungent odor which seemed to emanate from the skin and mucus. Although it was detectable from the living eels, after death the odor was gone.

The 4 remaining carp pituitary-injected experimentals showed noticeable gonadal enlargement by the 30th day of the experiment. None of them shed eggs, however, even though they received 3 injections more than eel 5 and were held at temperatures averaging 19.4° C for 103 days. The morphological characters approximated those of

Table 1

Induction of maturation in *Anguilla rostrata* females

Experimental group	Specimen number	Pre-injection condition	Length (cm)	Weight (g) Initial	Weight (g) Final	Gonad	Pituitary/ hormone per-injection	No. of injections	Duration eels held (days)	GSI
Controls 1973	1	yellow	51	418	435	4.9	Physiological	16	38	1.1
	2	silver	67	587	463	27.5	saline only	23	104	5.9
	3	yellow	59	457	391	4.8		14	33	1.2
21 IX 73 to	4	yellow	60	354	199	1.1		23	104	0.6
4 I 74	5	yellow	60	514	374	4.0		17	87	1.1
	6	silver	63	508	357	21.3		23	101	6.0
Experimentals 1973	1	yellow	74	678	569	8.7	10 mg carp	23	90	1.5
	2	yellow	59	440	364	6.8	pituitary	23	104	1.9
	3	yellow	62	430	255	7.2		23	104	2.8
21 IX to	4	yellow	64	474	410	13.5		18	90	3.3
4 I 74	5	silver	62	502	560	251.0		16	38	44.8
	6	silver	70	565	444	24.0		19	50	5.4
Experimentals 1974	1	silver	64	420	371	6.8	10 mg carp	13	31	1.8
	2	silver	65	500	454	162.2	pituitary	21	103	35.7
	3	silver	59	410	366	58.2		21	103	15.9
16 VIII 74 to	4	silver	55	300	215	60.1		21	103	27.9
27 XI 74	5	silver	65	615	600	206.1		18	52	34.4
	6	silver	59	400	330	82.2		21	103	24.9
13 IX 74 to	7	silver	53	382	320	13.6	0.8 mg LH	10	35	4.2
18 X 74	8	silver	65	677	543	16.5		10	35	3.0
	9	silver	59	565	467	17.3		10	35	3.7
	10	silver	67	773	695	18.3		10	35	2.6
	11	silver	62	534	496	17.2		10	35	3.5
	12	silver	76	891	809	23.1		10	35	2.9

fully mature eels (including mottled ventral skin) and all gave off the unique "odor of ripeness". Atrophy of the caudal musculature was apparent in all 5 eels with advanced GSI's.

The 6 silver female eels which were injected with purified ovine LH showed no noticeable external or internal changes even though they were initially larger than the other 6 eels injected with pituitaries and treatment began later in the migratory season. They each received a total dose of 8 mg (dry weight) of purified ovine LH which was determined by FONTAINE & GERARD (1963) to be equivalent in activity to 100 mg of powdered carp pituitaries. The 6 GSI's which resulted were lower than those obtained from the 2 silver controls in 1973.

DISCUSSION

Female American eels can be induced into full maturity through the injection of powdered carp pituitaries. Two eels showed externally noticeable signs of oogenesis and incipient maturation after $2^1/_2$ weeks of treatment. Both spontaneously emitted eggs in less than $1^1/_2$ months which appeared to be of a size (1 mm) and state (migratory nucleus, see OCHIAI et al., 1974 and YAMAMOTO et al., 1974), indicative of maturity and fertilisability. These times were considerably shorter than those required to induce maturation in the European (3 months) or Japanese eel (as much as 6 months) indicating that the American eel is more mature when it emigrates than either of the other species. Since different hormone preparations and techniques were used by each investigator this comparison of times required for maturation is tenuous. However, WENNER & MUSICK (1974) reported finding migratory female silver eels in the Chesapeake Bay with mean egg diameters greater than those of American eels from Newfoundland waters or European eels from Danish waters. At this writing the cause of death of both matured eels is unknown but is probably related to the treatment and consequent maturation. A complete histological report is planned for the future.

The number of eggs contained in eel 5 of 1973, 1.3 to 1.5×10^6, is in agreement with the results of WENNER & MUSICK (1974). For an eel the length and weight of eel 5, they found total fecundity to be between 1.3 and 2.0×10^6 although their specimens had a mean GSI of only 4.81 %.

It is not clear why 4 of the carp pituitary-injected experimental eels of 1974 never seemed to fully mature. All displayed noticeable gonadal enlargement and the morphological characters of the fully mature specimen but they appeared to advance only to a certain point and then no further. Declining sea-water temperatures may have been partly responsible. Furthermore, the injections were terminated about 40 days before the eels were sacrificied so some resorption is possible. Perhaps when the stimulating gonadotropins are withdrawn, the eels no longer use their skeletal musculature as an energy source for oogenesis but preserve it instead for the search for the spawning area or potential spawning partners.

The purified ovine LH was not efficacious in stimulating oogenesis in female American eels but the small dose and short experimental duration preclude statements on the usefulness and compatability of this hormone. Ovine LH was effective in

stimulating gonadal activity in frogs (FONTAINE & GERARD, 1963) but discordant results have been reported in fish (BURZAWA-GERARD & FONTAINE, 1972). Further studies are needed.

One of the most interesting aspects of this study was the unique "odor of ripeness" given off by the two fully matured eels and the 4 partially matured eels. The odor was the same for the 6 eels mentioned but uniquely different from any others handled. The fact that the odor seemed to emanate from the skin and mucus leads to the suggestion that it may be an attractant useful to the eels in their pelagic spawning area for orientation or maintenance of aggregations. Further studies are needed to demonstrate and quantify this hypothesis.

SUMMARY

1. Female American eels (*Anguilla rostrata* LE SUEUR) were induced to mature by injecting silver migratory forms with powdered carp pituitaries.
2. The eggs of mature American eels resemble closely those of the European and Japanese eels, i. e. they are small (1 mm), almost spherical and have oil droplets within; thus, they appear to be pelagic.
3. The number of eggs of one mature specimen was in the range 1.3 to 1.5 $\times$ 10^6.
4. Purified ovine LH was not an efficacious gonadal stimulant in female American eels.
5. A unique and pungent odor detectable from the skin and mucus of matured and partially matured eels is described for the first time.

Acknowledgements. The author was supported by an Office of Naval Research grant to Professor H. E. WINN. I thank the National Institute for Arthritis and Metabolic Diseases for donating the ovine LH and Profs. H. WINN and D. PRATT for reviewing the manuscript. Dr. H.-P. BULNHEIM kindly translated the abstract.

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