Sex Control of the Japanese Eel by an Estrogen (DES-Na) in Feed

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The sex of the Japanese eel could be controlled by the oral administration of DES-Na (sodium diethylstilbestrol) which also promoted growth. Elvers were reared from glass eels for more than 500 days with a commercial feed which contained DES-Na (0, 0.5, 0.75, 1.0 ppm). All the eels were dissected at the end of the experiment to check the sex by both external appearance and histological examinations of gonads. The sex groups were divided into *male*, *male?*, *female*, *female?* and *unknown* by their external appearance, and into male, female, hermaphrodite, and non-sex by histological examination. The largest discrepancy (27.5%) in the two examinations was found in the *male*: the histological examination was found to be essential. The female ratio increased along with an increase in DES-Na. It was 7.7% in the control and 23.2–35.4% in DES-Na groups. The male ratio was 64.1% in the control and 32.0–40.4% in DES-Na groups. The hermaphrodite ratio was 1.1–4.6%, and the non-sexual ratio was 23.8–38.0%. Females were the largest of all sex groups. The growth promoting effect was found to vary with the growth stage.

There have been many papers reporting success in controlling the sex of fish by sex steroids: medaka *Oryzias latipes*,^{1,2)} goldfish *Carrasius auratus*,³⁾ black sea bream *Mylio macrocephalus*,⁴⁾ guppy *Poecilia reticulata*,^{5,6)} tilapia *Tilapia nilotica*,^{7,8)} rainbow trout *Oncorhynchus mykiss*,⁹⁾ coho salmon *Oncorhynchus kisutch*,¹⁰⁾ brook trout *Salvelinus fontinalis*,¹¹⁾ and masu salmon *Oncorhynchus masou*.¹²⁾ However, no paper except one job report^{*4} has reported the successful control of sex in eels, though broodstocks of Japanese eels have been urgently required for producing elvers because of the poor catch of catadromous eels as well as the very low female ratio of cultured eels (3.5%¹³⁾). This situation demands the development of techniques in controlling the sex of eels to obtain females.

This report deals with the effect of DES-Na (sodium diethylstilbestrol), an estrogen substance, on the sex differentiation of the Japanese eel, and also on the growth of each sex of the same age. It also describes the presence of a large number of non-sexual eels and of a small number of hermaphrodites, as well as a cross check between macro- and micro-morphological examinations of sex.

Materials and Methods

Elvers *Anguilla japonica* were reared from the glass eel stage for more than 500 days with a commercial feed which contained different doses of DES-Na (0, 0.5, 0.75, 1.0 ppm). They were the survivors from the previously described experiment,¹⁴⁾ and the rearing conditions were the same as before. However, all eels in each dose-group were reared together in a tank of 4.5 m² in the later period. The water temperature ranged between 14.5 and 25.5°C.

Total length (T. L.) was measured five times during the experiment as shown in Fig. 3, as well as body weight (B. W.). All the eels were dissected at the end of experiment to check the sex. The determination of sex was made by the external appearance of the gonad and also by histological examination. The sex groups were divided by the external appearance as follows: *male*, *male?*, *female*, *female?* and *unknown*. *Males* and *females* were clearly distinct, as described by Satoh *et al.*¹⁵⁾ However, *male?*, *female?* and *unknown* were difficult to distinguish. *Male?* and *female?* were guessed to be so, while *unknown* could not be guessed because of its thread-like gonad.

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Fig. 1-A. Testis: A large number of spermatogonia exist in dendriform arrangement, though a few oocytes may be seen in some cases like this one. T.L. 59.1 cm, B.W. 331 g. Scale Bar (S.B.) 300 μ . Sg: Spermatogonium, Oc: Oocyte.

Fig. 1-B. Ovary: Oogonia and Oocytes are found interspersed with each other. T.L. 57.4 cm, 338 g. S.B. 300 μ m. Og: Oogonium.

Fig. 1-C. Hermaphrodite: Spermatogonia and Oocytes exist in the same gonad. T.L. 53.4 cm, B.W. 212 g. S.B. 100 μ m.

Fig. 1-D. Non-sex: The gonad mainly consists of connective tissue and cells like Sertoli's cell, but has no germ cell. T.L. 54.9 cm, B.W. 206 g. S.B. 100 μ m.

Fig. 1-E. Magnification of Fig. 1-D. CT: Connective tissue, S: Cell like Sertoli's cell³²⁾. S. B. 10 μ m.

Pieces of gonad of all the eels were fixed with Zenker-Formol solution and embedded in paraffin, following the usual procedure. Gonadal sections cut at 7-8 μ m were stained with Mayer's hematoxylin eosin and Heidenhain's iron-hematoxylin and light green. The sex groups determined by histological examination were divided into the following 4; male, female, hermaphrodite and non-sex. Males and females were clearly distinct as described previously¹⁵⁻¹⁷⁾ (Figs. 1 A and B). The hermaphrodite had both female and male germ cells either in a single gonad or

in gonads of a single specimen, and it could not be guessed which sex it would become (Fig. 1C). The non-sexual eel had neither female nor male germ cells in the gonads and was not sexually differentiated (Figs. 1 D and E).

Results

The histologically determined sexes are shown in Table 1. The male ratio decreased with an increase in the dose of DES-Na. It was 64.1% in the control (group A) but 35.9% in the DES-

Table 1. Sex ratio determined by the histological examination of gonad

Group	Dose, Des-Na ppm	Male	Female per cent	Hermaphrodite	Non-sex	No. of eels examined
A	0	64.1	7.7	1.7	26.5	117
B	0.5	35.9	23.2	2.8	38.0	142
C	0.75	40.4	31.1	4.6	23.8	151
D	1.00	32.0	35.4	1.1	31.5	178
No. of eels		244	152	15	177	588

Table 2. Comparison between macro-morphological and histological examination

Group	Histologically determined sex	Male	Morphologically determined sex Male?	Female Number	Female?	Unknown	Total Number
A (Control)	Male	71	4	0	0	0	75
	Female	2	1	6	0	0	9
	Hermaphrodite	2	0	0	0	0	2
	Non-sex	17	6	0	3	5	31
	Total	92	11	6	3	5	117
B-D (Des-Na)	Male	150	11	0	1	7	169
	Female	16	7	98	10	12	143
	Hermaphrodite	11	1	1	0	0	13
	Non-sex	36	13	1	11	85	146
	Total	213	32	100	22	104	471

treated groups (mean of groups B-D). The female ratio increased with the increase in the dose. It was 30.4% in the DES-treated groups, though only 7.7% in the control. The hermaphrodite ratio was very low, but they appeared in every group irrespective of the dose. In this examination, every hermaphrodite had both germ cells in a single gonad. The ratio of non-sexual specimens, on the other hand, was very large and similar in every dose-group. The sum of male and female ratios was found to be similar in every dose-group by the contingency table (indifferent, 5%). Using the contingency table compiled from all the above data (groups A-D), the chi-square test revealed a significant difference in the sex ratio among groups (0.5%). The same treatment on groups (B-D) also showed a difference (1%). This means that the ratio of females increased with the dosage.

Table 2 shows a comparison between the macro- and micromorphologically determined sex. The hermaphrodite and non-sexual eels were apt to be mistaken for males. The macro-morphologically determined females were found to be almost correct. There was no difference between the control and the DES-treated groups. The males and females which were grouped in the *unknown* group had thread-like gonads which had a very

small number of germ cells.

The survival rate during the experiment is shown in Fig. 2. Death of eels due to disease was not observed in this experiment. In the early period of the experiment, mortality was low in every dose-group. Finally, however, it increased greatly with the decrease in dose. That in the control was found to be the highest of all by Yate's modification¹⁸⁾ of the chi-square test (contingency table, significant at 0.5%). The growth curves of each group are shown in Fig. 3. The total length of groups C and D was larger than that of groups A and B.

Table 3 shows the total length at the end of the experiment, and the ANOVA (analysis of variance) is shown in Table 4. The female was the longest of all (0.1%, t-test), and the mean total length of the other sexes was the same. Group C (0.75 ppm) was the longest of all (1%). Groups C and D were longer than groups A and B (0.5%). In group A, however, the female was shorter and the hermaphrodite was longer than those expected from the main effect. It made the interaction significant. Although large enough,¹⁵⁾ many eels were not sexually differentiated (cf. Range in non-sex group, Table 3). Therefore, they may not contribute to reproduction at all.

Table 5 shows the body weight at the end of

experiment, and the ANOVA is shown in Table 6. Group C (0.75 ppm) was the heaviest of all dose-groups (1%, t-test), and the female was the heaviest of the sexes (0.1%). In group A, how-

Fig. 2. Survival curve.

Solid, broken, chain, and dotted lines correspond to the doses of 0, 0.5, 0.75, and 1.0 ppm DES-Na, respectively.

ever, the female was lighter and the hermaphrodite was heavier than those expected from the main effects, as in the case of total length.

Table 7 shows the condition factor at the end of the experiment, and the ANOVA is shown in Table 8. The non-sex eels and group D (1.0 ppm) were the most slender of all as the mean (1%, t-test). The difference in condition factor be-

Fig. 3. Growth curve of total length.

Open and solid circles are those at the doses of 0 and 0.5 ppm DES-Na in the feed, and open and solid squares those at 0.75 and 1.0 ppm, respectively.

Table 3. Total length of eels in each group

Group	Dose, Des-Na ppm	Male	Female	Hermaphrodite	Non-sex	All sexes
Mean total length, cm						
A	0	51.57	50.38	54.15	49.63	51.00
B	0.5	51.31	56.01	51.90	49.47	51.72
C	0.75	43.30	62.00	53.64	52.63	55.86
D	1.00	52.78	57.45	51.65	53.09	54.51
Mean of each sex		52.23	58.12	52.98	51.29	53.49
Range of total length, cm						
A	0	27.4-66.3	33.3-76.9	51.4-56.9	28.6-70.9	27.4-76.9
B	0.5	26.0-63.0	32.6-67.6	46.9-59.8	20.2-63.7	20.2-67.6
C	0.75	38.2-62.1	40.0-73.3	46.2-56.8	31.7-65.2	31.7-73.3
D	1.00	35.3-61.9	44.5-66.9	49.9-53.4	26.4-68.3	26.4-68.3
Each sex		26.0-66.3	32.6-76.9	46.2-59.8	20.2-70.9	20.2-76.9

Table 4. ANOVA based on the total length

Sources of variation	df	SS	ms	F	Significant at
Total	587	43505.4	74.11	1.149	
Among all groups	15	6623.4	441.55	6.848	0.5%
Among sexes	3	3824.5	1275.16	19.776	0.5%
Among doses	3	1868.5	622.82	9.659	0.5%
Interaction	9	929.4	154.91	2.402	1.0%
Within each group	572	36882.1	64.48		

df: degrees of freedom, SS: sum of squares, ms: mean square, F: varians ratio.

Table 5. Body weight of eels in each group

Group	Dose, Des-Na ppm	Male	Female	Hermaphrodite	Non-sex	All sexes
Mean body weight, g						
A	0	247.2	261.2	283.0	243.6	247.9
B	0.5	223.8	300.3	229.8	198.8	232.2
C	0.75	240.8	390.4	236.7	240.6	287.2
D	1.00	228.3	287.5	209.0	237.7	252.0
Mean of each sex		236.3	320.6	237.3	227.5	255.4
Range of body weight, g						
A	0	17-467	36-781	252-314	24-610	17-781
B	0.5	16-428	35-527	181-351	8-394	8-527
C	0.75	60-381	62-747	171-282	30-450	30-747
D	1.00	34-381	103-539	206-212	15-538	15-539
Each sex		16-467	35-781	171-351	8-610	8-781

Table 6. ANOVA based on the body weight

Sources	df	SS	ms	F	Significant at
Total	587	7974745	13586	1.167	
Among all groups	15	1313346	87556	7.518	0.5%
Among sexes	3	824811	274937	23.608	0.5%
Among doses	3	222763	74254	6.376	0.5%
Interaction	9	265772	44295	3.804	0.5%
Within each group	572	6661399	11646		

Table 7. Condition factor of eels in each group

Group	Dose, Des-Na ppm	Male	Female	Hermaphrodite	Non-sex	All sexes
Mean condition factor, mg/ml						
A	0	1.589	1.363	1.780	1.545	1.563
B	0.5	1.539	1.574	1.607	1.410	1.500
C	0.75	1.558	1.573	1.531	1.425	1.530
D	1.00	1.503	1.471	1.525	1.423	1.467
Mean of each sex		1.551	1.519	1.584	1.441	1.510
Range of condition factor, mg/ml						
A	0	0.79-2.20	0.95-1.78	1.70-1.86	0.82-2.13	0.79-2.22
B	0.5	0.66-2.23	0.93-2.04	1.33-1.75	0.68-1.97	0.66-2.23
C	0.75	1.08-2.18	0.97-2.33	1.40-1.73	0.94-1.81	0.94-2.33
D	1.00	0.76-1.92	1.17-1.80	1.39-1.66	0.82-1.81	0.76-1.92
Each sex		0.66-2.23	0.93-2.33	1.33-1.86	0.68-2.13	0.66-2.33

Table 8. ANOVA based on the condition factor

Sources	df	SS	ms	F	Significant at
Total	587	37.937	0.0646	1.049	
Among all groups	15	2.708	0.1805	2.931	0.5%
Among sexes	3	1.340	0.4468	7.254	0.5%
Among doses	3	0.734	0.2448	3.975	1.0%
Interaction	9	0.633	0.1055	1.712	10.0%
Within each group	572	35.230	0.0616		

Table 9. Linear regression of the condition factor against the body length

Sex	Regression Equation*	No. of eels	Ratio of contribution	Significance of regression
Male	$CF = 0.016 * L + 0.696$	244	0.200	1%
Female	$CF = 0.016 * L + 0.587$	152	0.337	1%
Hermaphrodite	$CF = -0.01 * L + 2.470$	15	0.177	
Neutral	$CF = 0.017 * L + 0.525$	177	0.418	1%

* CF: condition factor (mg/ml), L: total length(cm).

tween males and females in the control (group A) was not so large (10%, ANOVA).

Table 9 shows the linear regression between the condition factor and the total length. The regression was significant in almost all sex groups. The condition factor in eels increases with total length as is commonly known, though the slope was very small.

Discussion

Although mortality in the early period was low, it increased greatly in the later period (Fig. 2). This increase is thought to be due to cannibalism, which was induced by the great difference in the size of eels. This can be reduced by segregating eels into different ponds according to size.

As shown in Fig. 3, the effect of DES on the growth of eels was similar to that already reported.¹⁴⁾ However, group C (0.75 ppm) was the largest of all. This was slightly different from the previous report,¹⁴⁾ probably because the most effective dose differed in size and/or age.

It is generally said that females grow larger than males.^{13,15,19-23)} However, those reports were based on eels of uncertain sex,^{13,20-23)} which it was difficult to ascertain without histological examination (Table 2). In this experiment, the eels used were raised from glass eels; they were certainly of the same age. The sex was determined histologically. Our data reliably confirmed the above mentioned phenomenon (Tables 3 and 5).

The sex ratio in European and American eels has been said to vary among different reaches of a river, but the descriptions have been conflicting.^{20,21,24,25)} Marukawa has also said that the sex ratio of the Japanese eel varies from river to river: the non-farmed *female* ratio ranges from 1.0 to 57.1%.²⁶⁾ Matsui¹³⁾ reports that the *female* ratio in natural Japanese eels collected from various places was 37.5% of all the eels whose sex could be determined. However, he also reports that he could not determine the sex

of 22.8% of natural eels and 26.5% of catadromous eels captured after October by the external appearance of gonads, and said that the *female* ratio in cultured eels ranged from 0.5 to 8.6% (mean=3.5%), excluding eels whose sex could not be determined. If those of *unknown* sex were included, the *female* ratio would have been reduced. In this experiment the female ratio in the control (group A) was 7.7%. This figure is thought to be slightly higher, probably because the mortalities during the experiment were mainly composed of slowly growing males and/or non-sexual eels which were thought to have been cannibalized.

The ratio of non-sexual eels was about one third, irrespective of the dose, and even large eels were not sexually differentiated (Tables 1, 3 and 5). Such large eels have no germ cell at all and are thought not to take part in reproduction throughout their life. Therefore, we named them non-sexual eels. This fact is very interesting, since it has never been noticed: only Matsui¹³⁾ described the presence of catadromous eels whose sex was unknown by macro-morphological examination, but he seemed unsure about whether they were completely non-sexual.

Although hermaphroditism has been recognized in juvenile European eels,^{20,27,28)} K. Bieniarz *et al.*²⁹⁾ report that European eels, like Japanese ones, do not pass through a stage of juvenile hermaphroditism. This has never been observed in any stage of Japanese eels. In this experiment, however, it was found even in large eels, though these eels were thought to acquire either sex at a later time. There can be two cases: each type of germ cell is separately located in a different gonad of a single specimen, or both types coexist in a single gonad. However, only the latter case was found in this examination.

As was described above, hermaphrodites and non-sexual eels were discovered. These sex groups can only be recognized by histological examination. For an exact determination of sex, therefore, it is absolutely necessary to examine the gonads his-

tologically. This necessity has been recognized for American and European eels as well.^{24, 25)}

This experiment reveals the possibility of sex control of eels by the oral administration of DES-Na. On the other hand, a favorable result was reported by the oral administration of Estradiol-17 β .^{*} The report was so short that we could not fully understand the result. Yamamoto^{1, 30)} succeeded in converting genetic males of Medaka *Oryzias latipes* to functional females by the oral administration of DES. The conversion percentage was 50% with 7.5 ppm and 100% with 25–250 ppm of DES. He did not describe the survival rate in his experiments, though many Japanese eels began to die about 20 days after feeding on the diet with 12.5 ppm DES-Na.¹⁴⁾ Using fingerlings of protandrous black sea bream, the feed with 2.0 and 2.5 ppm DES-Na was found to make 100% functional females, while a smaller dose also increased the female ratio.⁴⁾ Another estrogen, hexestrol, was reported to be more effective in making functional females in medaka *Oryzias latipes* than DES.³¹⁾

For the production of elvers, these facts are thought to encourage us to extend studies on estrogens and means of administering them, such as the effective dosage, the starting time, the duration, and so on.

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