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Effect of Induced Gonadal Development and Starvation on some Haematological Parameters of *Anguilla anguilla* L.

E.M. Amin

National Institute of Oceanography and Fisheries, Alexandria, Egypt

ABSTRACT. Blood pictures of the European silver eel *Anguilla anguilla* L. were described. Blood was obtained from control (immature) male and female silver eels, at ripe stage after developmental maturation of gonads by intramuscular injection with chorionic gonadotropin hormone (HCG) for male and carp pituitary (CP) mixed with (HCG) for female, and after long starvation for both sexes.

In normal blood (control), the haematological parameters such as haemoglobin, haematocrit, red blood cells were higher in male than in female.

Changes in blood picture were used as criteria of systemic response to experimental conditions. The effects of developing testes led to significant decrease in haemoglobin, haematocrit, whereas developing ovaries showed more significant decrease in haemoglobin, haemotocrit, red blood cell count. As a result of such decrease in haematological parameters in both male and female, the experimented fish suffered from certain degrees of anaemia.

Fish of extended starvation revealed sharp decrease in all haematological parameters.

An abnormal blood film is seen in nutritional anaemia. There is marked anisocytosis with predominantly hypochromic irregular microcytes and macrocytes.

Alterations of fish blood cell morphology have been correlated to changes in environmental conditions such as periods of nutritional deficiency (Smith 1968, Ikeda *et al.* 1973 and Sakamoto and Yone 1978) and periods of thermal acclimation

(DeWilde and Houston 1967). Morphological alterations are also associated with the effect of pollution (Saad *et al.* 1983), or after exposure to toxic chemicals (Gardner and Yevich 1970, McKim *et al.* 1970).

Few studies have previously been carried out on the blood of eels by Sano (1957), Amin (1972), Hussein *et al.* (1974), Larsson (1975) and Ochiai *et al.* (1975).

The combined stresses of spawning under artificial conditions (fasting fish) and long time starvation are reflected in several blood charactreristics of the European eels (*Anguilla anguilla*), therefore the nature of blood changes have received particualar attention in this investigation.

Material and Methods

The used material consisted of 100 fasting specimens of seaward migrating male and female silver eels. Of these 60 males measuring from 36 to 45 cm in total length with average 39 cm, weight from 90 to 130 g and average 110 g. Females counted 40 samples measuring 55 to 70 cm total length and average 60 cm, weight ranged from 400 to 900 g with 600 g average weight. Specimens collected alive from lake Edku during the spawning migration extending from November to February 1988-1989. Three types of sea water aquaria were used for holding specimens, and no food supply was given during the period of experiments:

a) Control metalic tank,
b) Metalic tank under certain circumstances for gonadal development by hormone induction according to Amin (1986).
c) Plastic covered tanks containing starved males and females (separate).

Blood was taken from the caudal area of all samples. Haemoglobin content was determined adopting the method of Sahli (1909). Haemoglobin value measured after centrifuging the obtained blood in Wintrob's tube for 15 minutes on a standard micro haematocrit centrifuge according to Wintrobe (1933). Erythrocytes numbers (RBC) were counted in millions/mm^3 according to Predchensky (1960). A drop of blood, collected with a heparinized capillary tube, was transferred to a glass slide to make the smear and stained with a commercial preparation of May-Greenwald (M-G giemsa).

The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were estimated by Wintrope's formulae (1933).

Results

The results of blood analysis of European eel *Anguilla anguilla* L. are summarized in Table (1). Males of migrating silver eel showed the successive stages of gonadal maturation when treated with human chorionic gonadotropin (HCG). Females treated with a combination of carp pituitary (CP) and (HCG) showed the same gonadal development (Amin 1986, 1991b).

Females were starved for 150 days and males for 330 days. As a result of such starvation, a sharp loss in fish weight took place. Female's weight dropped from

Table 1. Haematological changes on fish

	Sex	Control	Ripe fish	Starved fish
Average weight (g)	♂	110 ± 10* (30)**	80 ± 5 (20)	60 ± 5 (10)
	♀	700 ± 20 (30)	700 ± 20 (6)	250 ± 10 (4)
Haemoglogin concentration (g/100 ml)	♂	11.9 ± 0.51 (30)	9.9 ± 0.92 (20)	3.8 ± 0.41 (10)
	♀	10.1 ± 0.91 (30)	4.2 ± 0.19 (6)	2.0 ± 0.21 (4)
Haematocrit (%)	♂	34.1 ± 2.4 (30)	27.1 ± 1.91 (20)	13.5 ± 1.53 (10)
	♀	30.1 ± 2.9 (30)	14.6 ± 2.01 (6)	10.1 ± 1.67 (4)
RBC (millions/mm)^3	♂	1.61 ± 0.19 (30)	1.63 ± 0.29 (20)	0.88 ± 0.28 (10)
	♀	1.47 ± 0.15 (30)	0.90 ± 0.12 (6)	0.67 ± 0.25 (4)
M.C.V. μ^3	♂	213.1 ± 5.0	169.4 ± 1.9	153.4 ± 2.1
	♀	200.6 ± 3.5	162.2 ± 2.0	144.3 ± 1.9
M.C.H. π	♂	73.9 ± 1.3	60.7 ± 1.4	43.2 ± 0.9
	♀	68.7 ± 1.7	46.7 ± 0.9	29.9 ± 0.8
M.C.H.C. % (%)	♂	34.9 ± 0.5	36.5 ± 0.8	28.1 ± 0.3
	♀	33.6 ± 0.6	28.8 ± 0.7	19.8 ± 0.5

* = mean + standard deviation
** = number of fish
RBC = red blood cells
MCV = mean corpuscular volume
MCH = mean corpuscular haemoglobin
MCHC = mean corpuscular haemoglobin concentration

700 g to 250 g, males from 150 g to 60 g. The marked drop in fish weight was earlier shown to be a result of significant decrease in lipid and protein contents (Amin 1991a). Such reduction in lipid, protein and consequently body weight during the gonadal development and complete starvation was further accompanied by considerable decrease of blood contents.

As shwon in Table (1), haemoglobin value in male silver eel dccreased from the control normal value of 11.9 g/100-ml blood to 9.9 g/100-ml, when the testes attained ripe condition. A continuous drop took place amounting to 3.8 g/100-ml blood after starvation. In control female, the value of haemoglobin amounted to 10.1 g/100-ml blood, thence, sharply dropped to 4.2 g/100-ml blood on attaining its sexual ripe condition. Furthermore, a continuous decrease took place leading to a level of 2.0 g/100-ml after starvation.

Haematocrit value of control silver male was estimated as 34.1%. When the testes of the experimented male became ripe, the value dropped to 27.1%. After starvation, the haematocrit amounted to 13.5%. Concerning female, in control, the haematocrit was estimated as 30.1% decreased to 14.6% in the ripe condition and further to 10.1% in starved fish.

The total counts of red blood cells (RBC) in the male silver eel with ripe gonads did not differ from the control immature fish amounting to $1.63 \times 10^6/mm^3$ and $1.61 \times 10^6/mm^3$, respectively. Female during its ripe condition, revealed RBC counts significantly lower than those of the control fish giving $0.90 \times 10^6/mm^3$ and $1.47 \times 10^6/mm^3$ respectively. After starvation, the count values of red blood cells showed greater decrease in both silver male and female.

The full mature formed erythrocytes of controlled silver eels were elliptical discoid elements with centrally located nucleus as visualized in fixed preparations. The fixed erythrocytes and nuclei measured an average 13.5×5.6 μ and 4.6×2.0 μ, volume of nucleus amounted to 16.0% of erythrocyte volume. The cytoplasm stained pale pink, and the nucleus stained magenta with darker staining chromatin clumps (Fig. 1). At ripe stage of male and female silver eels, and in starved fish, the red cell appears with a central pallor with a thin peripheral pink ring, also the film appears especially in starved fish young red blood cells (Fig. 2), hypochromic irregular microcytes (Fig. 3) and macrocytes (Fig. 4).

The crenated red cell which appears in the film (Fig. 5) has a uniformly serrated edge. The cell may be elliptocytic, appears as a dark-stained cell with a surface covered by numerous needle-like projections. The crenation is produced when the film dries slowly and is due to the development of increasing fluid hypertonicity immediately surrounding red cells (Harper 1974).

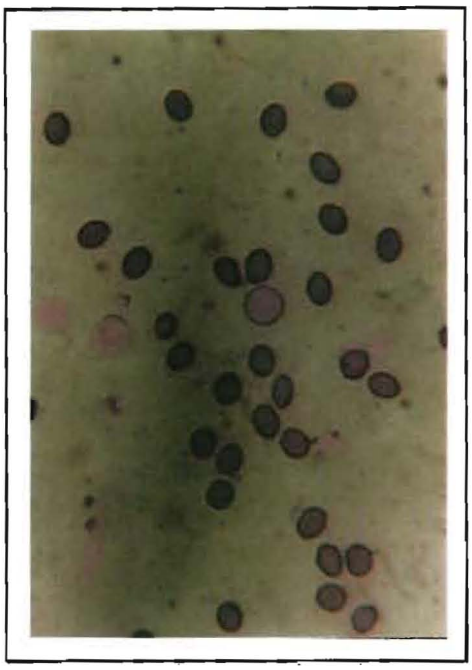

Fig. 1. Blood smear of normal blood cells (control), Giemsa stain. 264X

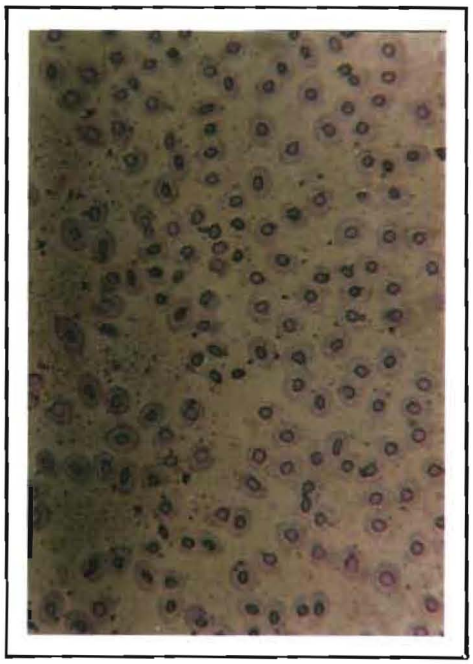

Fig. 2. Blood smear of anaemic male at ripe stage, showing young red blood cells (arrow). 264X

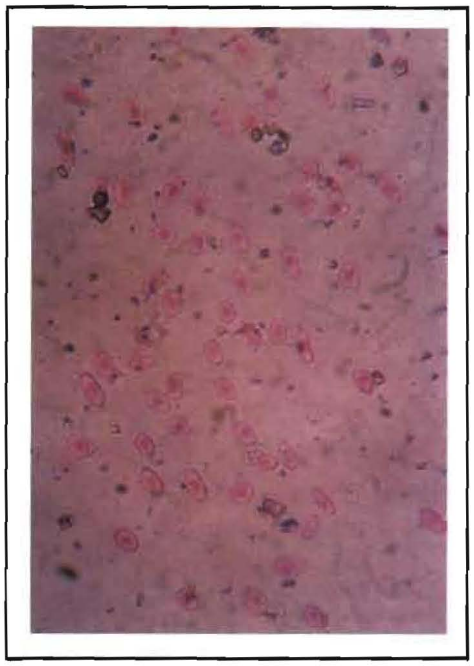

Fig. 3. Blood smear of anaemic male starved fish, showing hypochromic irregular microcytes. 264X

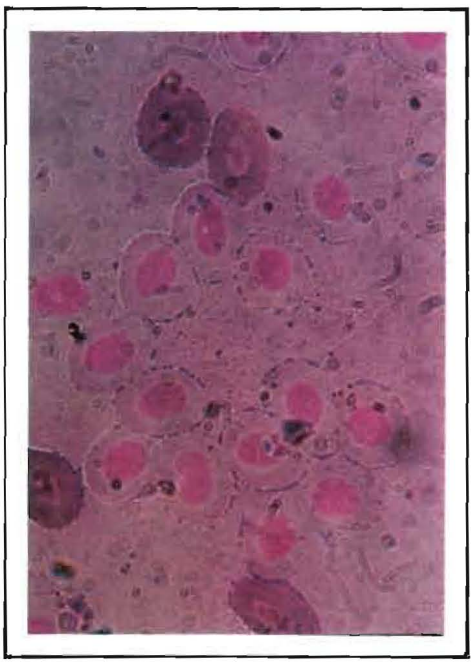

Fig. 4. Blood smear of anaemic female starved fish, showing hypchromic macrocytes. 660X

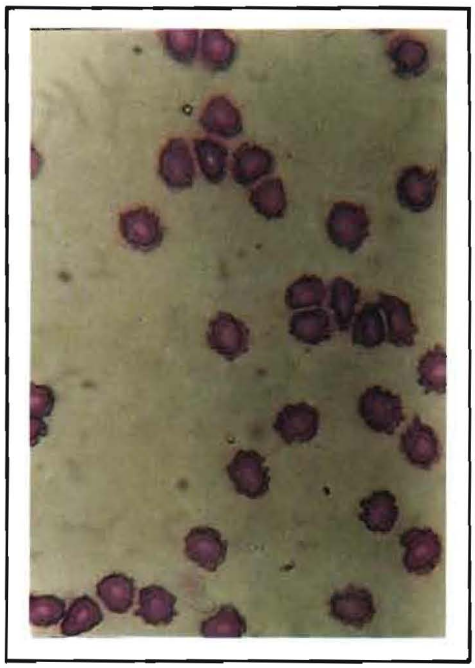

Fig. 5. Blood smear of normal blood cells showing crenated red cells. 264X

The mean corpuscular volume (MCV) and the mean corpuscular haemoglobin (MCH), in treated male and female were significantly lower than the control fish. On the other hand the mean corpuscular haemoglobin concentration (MCHC) estimated for ripe male was slightly higher than for the control. This percentage was more lower in ripe female. MCV, MCH and MCHC became lower on starvation.

Discussion

Judging from our results, and also from general observations made on the blood content of the same species and other fishes, particularly salmon, carp and trout, it seems that apparently healthy fishes may have a lower haematological value than the normal standards or they may suffer from the effects of some anaemia. The anaemia, in turn due to some dietary deficiency for normal blood production.

Experimented silver eels (control and treated fish to the ripe condition) revealed that the haemoglobin, haematocrit and red blood counts were significantly higher in male than those of female, (control fish were supposed to be under the

influence of endogenous gonadal hormones and related to endocrine activity which stimulates the fish to start migration with still immature gonads; on the other hand the treated samples up to the ripeness were actually under the influence of both endogenous and exogenous hormones (CP and HCG). The significant decrease of haematological levels in control and induced ripe females may be attributed to the effect of the expected secreation of male androgene hormone which increased metabolic rate in male than female (Guyton 1986), and to the fact that these are yolk constituents and were probably being utilised by the developing ova. Furthermore, it may be partly explained by the longer time of artificial ovarian maturation (70 days) than testes (45 days), taking into consideration the importance of the lipid, protein and vitamin contents, especially pantothenic acid in the reproductive growth of the gonads. Braekkan (1955) revealed that some differences exist in the pantothenic acid content of some species. He related them to the degree of ovarian ripening. The value being minimum at the time of spawning. Kokhnenko *et al.* (1977), Amin (1991a) reported that the degree of loss in body lipid and energy needed for ovarian maturation is greater than the energy destined to testicular maturation.

From the results obtained by Ochiai *et al.* (1975) during artificial maturation of Japanese eel and our present results the ripe male and female silver eels have lower haemoglobin, heaematocrit and red blood counts than control silver eels. Gonadal maturation resulted in a highly significant decrease in haematological values. This period is associated with profound changes in blood chemistry, and injection by excessive estrogens hormone may cause a positive electrolyte and water balance (Chung-Wai Ho and Vanstone 1961). Therefore the observed decreases of haematocrit value at sexually ripe silver eels may be partly due to the increase of reabsorption rate of sodium which causes hemodilution, and partly to etarvation as the fish were not fed during the extended period of the experiment. Reduction in haematocrit value, associated with gonadal development of silver eels was essentially similar to the result of Chung-Wai Ho and Vanstone (1961) who revealed the effect of estradiol monobenzoate on certain serum constituents in adult sockeye salmon during their spawning migration. Sano (1960) related such reduction in haematocrit value to the gonadal development of trout. Similar results were also obtained by Kaplan and Crouse (1956) when they determined the haematocrit of frog (Rana pipiens) during the spawning season especially in females having laid their eggs. No obvious reduction in the count of red blood cells was noted as a result of difficult separation between young and mature red cells when counted for male attaining its ripe stage.

Reduction in haematological parameters at ripe stage was shown to cause anaemia, the same result was noted by Kawatsu (1961) from vitamin deficiency in

rainbow trout, and by Ikeda *et al.* (1973) from iron deficiency in the diet of yellow-tail (Seriola quinqueradiata). Similar results were virtually obtained in mammalian pregnancy (Guyton 1986). Furthermore in the film appear young red blood cells, hypochromic microcytes with a few number of macrocytes which may be hypochromic, the pictures are usually seen in case of iron and vitamin deficiency anaemia. Sakamoto and Yone (1978) denoted that carp fed the diet without iron supplementation manifest a hypochromic microcytic anaemia. They found that haemoglobin content, haematocrit value of blood, the mean corpuscular constants and the mean corpuscular diameter of minor length were lower, and the percentage of immature erythrocytes was higher than those of the group fed the iron-supplemented diet.

The analysis of the quantitative parameters of red cells at the end of starvation period showed that long time starved fish were characterised by significantly lower parameters, even than fish under control or at the ripe stage. The blood of such anaemic fish is characterised by the presence of hypochromic irregular microcytic, macrocytic and are young red blood cells. This characteristic phenomenon is frequently noticed where nutritional deficiencies.

The hypochromia with irregular shape of a red cell microcytes is due to defective haemoglobinization. The degree resultant pallor depends mainly on the extent to which the haemoglobin concentration within the cell is reduced. Such reduction is virtually related to the inadequate quantities of transferring in the blood. Failure to transport iron to the erythroblasts in this manner causes severe hypochromic anaemia, besides significant decrease of red cells and cells containing little amount of hemoglobin. Deficiency of vitamin B and folic acid was suggested by Harper (1974) as result of the presence of macrocyte red cells in the blood film. The lack of vitamin inhibits the rat of red blood cell production and causes failure of nuclear mauration. Also the calculated MCV is not very small indicating the presence on macrocytes in blood film. Such reduction in haematological parameters after starvation is similar to that observed in Amia calva in which the red blood cell count dropped from 1.640.000 per cubic mm to 400.000 after starvaion of 20 months (Smallwood 1916). Similar results were observed by Murachi's (1959) in Cyprinus carpio when red cell volume (haematocrit) decreased from 50% to 33%, and a corresponding reduction in haemoglobin from 11 to 7 g per 100 ml, after starvation for 7 weeks.

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تأثير الانضاج الصناعي للمناسل والتجويع على بعض الصفات الهيماتولوجية لأسماك ثعبان السمك

اكرام محمد أمين

المعهد القومي لعلوم البحار والمصايد ـ الأسكندرية ـ مصر

تشكل مصايد أسماك الثعبان الأوروبي (أنجويللا أنجويللا) جزءاً هاماً من إنتاج البحيرات المصرية، كما أنها تدر عائداً مادياً لا بأس به من خلال تصدير الأسماك حية إلى أوروبا. هذا النوع من الأسماك لا يتوالد في البحيرات بل يدخلها وهو في الطور اليرقي ليتغذى وينمو، وعند بلوغه العمر المناسب للتوالد يقوم بهجرة طويلة وشاقة قاطعاً مسافة قدرها حوالي ٤٠٠٠ كم ليصل إلى المحيط الأطلنطي ثم بحر السرجاسو حيث يتم التوالد ويموت الآباء ويخرج إلى الحياة جيل جديد ليواصل مسيرة الآباء وهكذا تتوالى الحياة.

وعندما يهاجر ثعبان السمك الأوروبي من البحيرات المصرية إلى البحر ثم إلى المحيط من أجل التوالد تكون الأسماك قد أستعدت فسيولوجياً لهذه الهجرة من حيث :

١ ـ تخزين كميات كبيرة من الدهن في معظم أعضاء الجسم تستخدمها كطاقة لازمة للهجرة والنضوج الجنسي والتوالد خاصة أن هذه الأسماك تبدأ هجرتها وهي في حالة إنقطاع تام عن الطعام.
٢ ـ الاستعداد الهرموني للنضوج الجنسي الذي يتم أثناء الهجرة حيث أن هذه الاسماك تبدأ هجرتها ومناسلها في حالة عدم نضوج.

ولقد اتجهت الدراسة لتشمل الآتي :

١ ـ إجراء التجارب على الأسماك المهاجرة فقط وهي ثعبان السمك الفضي الأوروبي.
٢ ـ إنضاج مناسل الذكور والأناث معملياً.
٣ ـ تتبع تأثير الصيام والنضوج الجنسي لمناسل الجنسين على خلايا الدم.

جُمعت الأسماك المهاجرة الفضية من بحيرة أدكو (شمال مصر) وهي في طريقها للتوالد. أجريت التجارب في ثلاثة أحواض، أسماك الحوض الأول (كونترول) للمقارنة وأسماك الحوض الثاني لإنضاج المناسل والوصول بالخلايا الجنسية إلى درجة النضوج التام، أما أسماك الحوض الثالث للاستمرار في عملية التجويع.

ويمكن تلخيص نتائج هذا البحث في النقاط الآتية :

١ ـ عند فحص عينات دم الأسماك (كونترول) والأسماك التي وصلت مناسلها إلى حالة النضوج التام، اتضح أن قياس الهيموجلوبين والهيماتكريت وعدد كريات الدم الحمراء للذكور أكبر من قياسها لدى الأناث، ولقد فُسِّر هذا الاختلاف بأن عملية تكوين البيض في المبيض تحتاج للكثير من الدهون والبروتينات والفيتامينات والطاقة أكثر من احتياج مناسل الذكور لهذه الكميات، الأمر الذي انعكس على الشكل العام للدم.
٢ ـ القياسات العامة للدم عند الاسماك (الذكور والاناث) في حالة النضوج التام أقل من القياسات المأخوذة للأسماك (الكونترول) مما أدى إلى ظهور بعض من خلايا الدم الحمراء بشكل غير طبيعي من حيث الحجم واللون.
٣ ـ الأسماك التي تم تجويعها لمدة ١٥٠ يوماً للأناث و ٣٣٠ يوماً للذكور انخفض وزنها لدرجة ملحوظة من ٧٠٠ جراماً إلى ٢٥٠ جراماً للأناث أما بالنسبة للذكور فانخفض الوزن من ١٥٠ جراماً إلى ٦٠ جراماً. انخفاض وزن

الأسماك نتيجة التجويع أدى إلى الانخفاض الشديد في جميع القياسات الكمية للدم بالمقارنة بالقياسات التي تم نضجها جنسياً (والاسماك كونترول)، الأمر الذي أثر على عدد وحجم وشكل خلايا الدم الحمراء.

٤ - بصفة عامة عانت الاسماك بعد النضوج الجنسي وأيضاً بعد فترة التجويع ولكن بصورة أكثر وضوحاً بما تسبب في حدوث الأنيميا وأتضح هذا جلياً في شكل وحجم خلايا الدم الحمراء ولقد أرجعنا الأنيميا نتيجة فقد بعض عناصر التغذية خاصة الحديد والفيتامينات.