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Experimental Embryology in Xenopus Laevis

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Abstract: Xenopus laevis is a new experimental subject for Turkish scientists. Our main goal was to set up FETAX (Frog Embryos Teratogenesis Assay: Xenopus) in our laboratory. In this study, after discussing the wide-ranging uses of Xenopus laevis, we present the breeding, feeding, housing and population density conditions of Xenopus laevis in the laboratory.

To induce mating, males and females were given, respectively, 500 and 1000 I.U.'s of human chorionic gonadotropin via injection into the dorsal lymph sac. Amplexus normally ensued within 2 to 6 hours and deposition of eggs took place within 9 to 12 hours of injection. The embryos were then examined under microscope to determine if the eggs had been normally fertilized.

In order to determine how temperature, feeding, aeration, and population density affect the developmental ratio of Xenopus embryos, we studied two groups: a control and an experimental group.

It was concluded that optimum growth and development of larvae occur when they are kept at about 24-26 °C temperature, fed once a day, constantly aerated and given enough space for the growth of each individual.

Key Words: Xenopus, feeding, temperature, aeration, population density.

Xenopus Laevis'de Deneysel Embriyoloji

Özet: Xenopus laevis Türk bilim adamları için yeni bir hayvan modelidir. Ana amacımız, laboratuvarımızda FETAX testini (Frog Embryos Teratogenesis Assay: Xenopus) kurmaktır.

Bu çalışmada, Xenopus'un geniş kullanım alanları tanıtıldıktan sonra laboratuvar koşullarında üreme, besleme, havalandırma ve populasyon yoğunluğu parametreleri sunuldu.

Çiftleşmeyi indüklemek için dişiye 1000 I.U, erkeğe 500 I.U. human chorionic Gonadotropin (hcG) dorsal lenf bezlerine enjekte edildi. Ampleksus 2-6 saat içinde başladı ve yumurta bırakma enjeksiyondan sonra 9-12 saat kadar sürdü. Yumurtaların, normal ve fertil olup olmadıkları mikroskopla saptandı.

Sıcaklığın Xenopus embriyolarının gelişimini nasıl etkilediğini bulmak için kontrol grubu olarak 20-22 °C ve deney grubu olarak 24-26 °C sıcaklıklarda iki grup alındı. Beslenmenin etkisini araştırmak için kontrol grubu haftada iki kez, deney grubu her gün beslendi.

Sonuç olarak, larvaların 24-26 °C sıcaklıkta hergün beslenerek, kaplarından sürekli hava geçirilerek ve her birey için yeterli genişlikteki kaplarda en iyi şekilde geliştiği bulundu.

Anahtar Sözcükler : Xenopus, sıcaklık, besleme, havalandırma, populasyon yoğunluğu.

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Introduction

Over the past twenty years, amphibian species have become popular experimental animals. They are vertebrates, and can be easily and inexpensively maintained in laboratory conditions (1). The Xenopus toad has become one of the most widely used amphibian species in biomedical research (2).

There is no taxonomic rule to classify the Xenopus as a frog or toad. Being totally aquatic, it may be referred as a frog, since toads are primarily terrestrial. as a frog, however, Xenopus would be considered primitive, because it has no ears, no evolved eyelids and no prey-catching tongue. Approximately 20 different species have been identified. The genus originates from the southern region of Africa (Figure 1) (1, 3).

Xenopus embryos offer numerous advantages for screening assay. The frog embryo is a classical developmental system that undergoes many of the same developmental processes as mammalian embryos. Mating is inducible with human chorionic gonadotropin at any time of year in commercially available frogs, and development is external, therefore permitting non-destructive analysis of malformation at different stages of development (4). They are easily fed proprietary pellets diets that are readily available for mature animals (2). Xenopus laevis is fairly resistant to disease (1).

One of its first applications in biological experiments came in the 1950's with its use in human pregnancy tests. Early experimental uses of Xenopus included sex reversal by hormones and the embryological work of Balinsky and Niewkoop (1). In 1950, John Gurdon used Xenopus in early nuclear transplantation work. The Xenopus oocyte became very popular for identification of receptor components, following the 1971 finding of Gurdon and his colleagues that injected mRNA is efficiently translated (1).

In addition, embryos of Xenopus laevis have been used to determine the toxic and teratogenic effects of chemicals (5), aromatic amine (6), selenium (7), complex effluent (8), reconstituted water medium (9), heavy (10) and light metals (11).

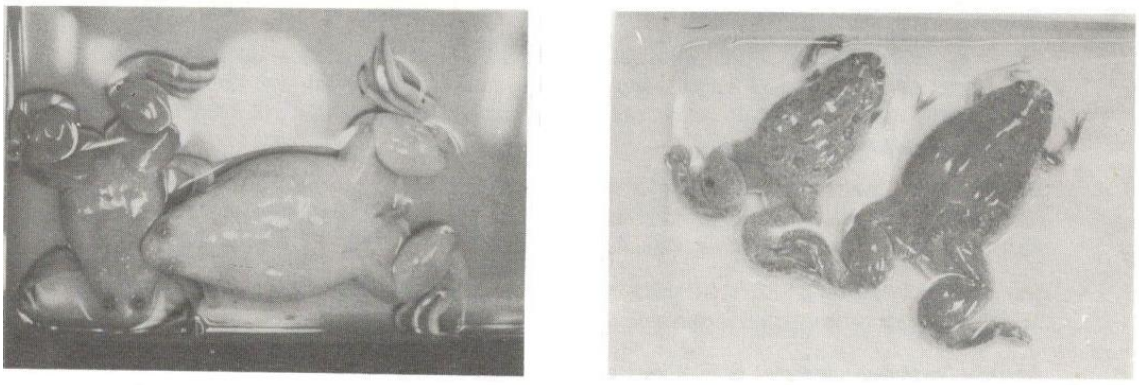

Figure 1. a) A pair albino Xenopus leavis b) A pair will Xenopus leavis.

We screened MED-LINE in order to find percent ratio of the use of different animals in laboratories and we observed that the use of Xenopus laevis had increased by 225% between the years 1983-1994 (Figure 2).

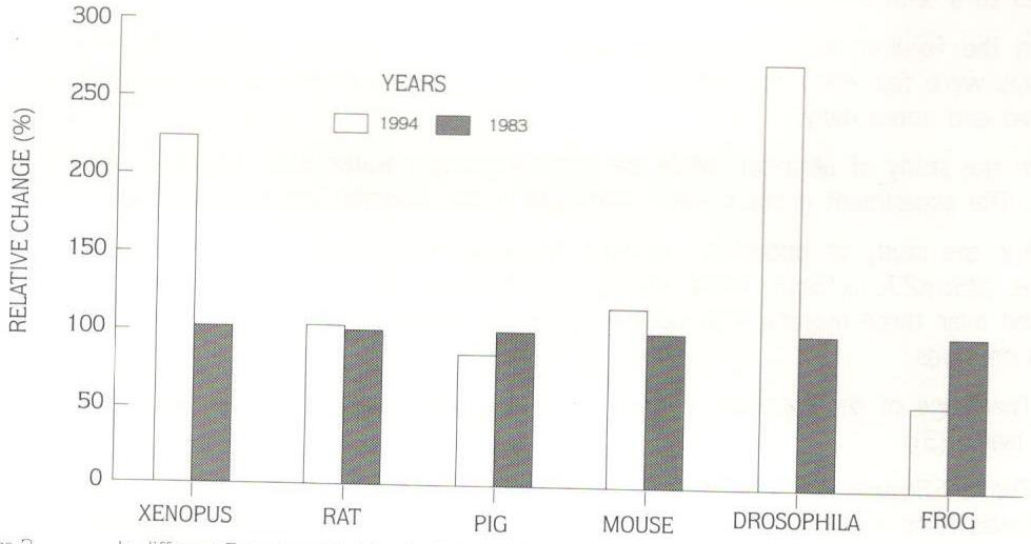

Figure 2. In different Experimental Animals Species. Percentage of Usage Between 1983-1994 Years.

Materials and Methods

The adult Xenopus were housed in clear glass aquariums (95cmx60cmx44cm) and care was taken to supply water absolutely free of copper and chlorine ions. Chlorine was removed by allowing the water to remain in contact with the air for several days (3). The aquarium was cleaned at regular intervals. The room temperatures for adults and larvae were 23±1 °C and 24±2 °C, respectively. The lighting schedule was eight hours of constant light and sixteen hours of dark.

Feeding of the animals was scheduled as follows: Xenopus were fed alternately on chicken liver and trout pellets twice a week. Larvae were fed on spinach extracts daily, starting from the fifth day. Larvae and tadpoles were also fed on newly sprouted wheat tiller and boiled lettuce once a month. Details of the feeding procedure may be found in reference number 10.

To induce mating, females were injected with 1000 and males with 500 I.U. of human chorionic gonadotropin (hcG) in the dorsal lymph sac (2). The animals were placed into a large aquarium covered with netting, sheltered from lights, and provided with twigs for oviposition. Amplexus normally ensued within 2 to 6 hours and deposition of eggs took place within 9 to 12 hours of injection (11). Following breeding, the embryos were collected in petri dishes, and the normally fertilized eggs were selected under dissection microscope. Abnormally cleaving embryos and necrotic eggs were removed from the collection of embryos (3, 9).

They were tested in terms of parameters of feeding, temperature and aeration. In the study of temperature, embryos were divided into two groups. The first group was control (n=267), and was exposed to a temperature of 20-22 °C, and the second group was exposed to a temperature of 24-26 °C (n=482).

In the feeding tests, the control groups were fed twice a week, and the experiment groups were fed every day (n=270). The stages of development of the embryos were observed and noted daily.

In the study of aeration, while the control group's water was maintained without aeration. The experiment group's water was kept under constant aeration (n=450).

For the study of population density, embryos were divided into two groups in plastic boxes (35cm27cmx15cm) filled with 5 cm of water; developmental stages were then observed over three months. The control group consisted of 38 and the experiment group of 273 embryos.

The stage of development of embryos was checked against the Normal Tables of Xenopus laevis (3).

The Chi-Square test was used for evaluation of temperature and aeration, and t-student test was used for evaluation of feeding and density of population. Statistical analysis of data averages was presented as mean±SE.

Results

The effects of temperatures on the vitality percentage of tadpoles were significant ($p<0.05$). The vitality percentage of tadpoles was found to be continuously higher in tadpoles maintained at 24-26 °C than in the control group (Figure 3).

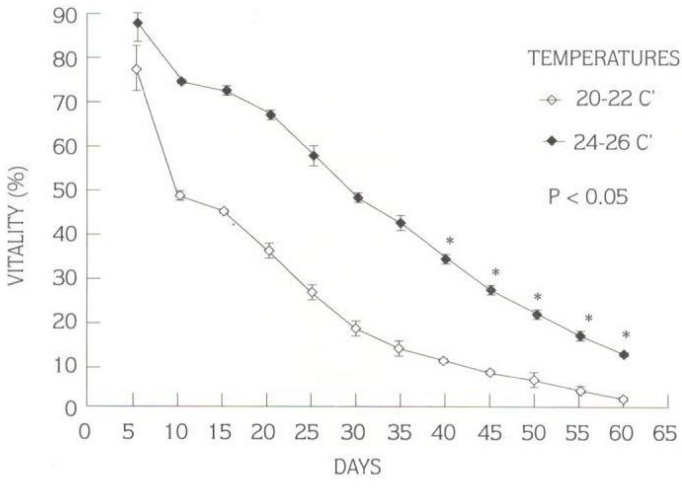

Figure 3. The Effects of Different Temperature on Vitality Ratio (%) of Xenopus leavis Embryos.

Differences in vitality between control and experiment groups were significant, particularly at 30-35 days (Figure 3). The vitality percentage at 30-35 days was 43±1.2 in the experiment group and 14±1.2 in the control group. Vitality at 55-60 days was 15±0.4 in the experiment group and 5±0.3 in the control group, showing the decreasing vitality ratio differences between two groups with the observation days. Also, the vitality percentage of the control and the experiment group ranged from 78% to 5% and from 98% to 15%, respectively, decreasing towards the end of the observation periods (Figure 3).

The effects of aeration on the vitality percentages of tadpoles were significant. Differences in vitality between the control and experiment groups were significant, particularly at 25-30 days during the 60-day observation period.

The vitality percentage at 25-30 days was 52±3.2 in the aerated group and 19.3±1.6 unaerated group, whereas at 55-60 days it was 14.6±1.1 in the aerated and 4.3±0.3 in the unaerated group, showing the decrease in the vitality raito between two groups towards the end of the observation period. Furthermore, the vitality percentages of groups with and without aeration ranged from 93% to 14.6% and from 84% to 4.3%, respectively, decreasing with the days of observation (Figure 4).

In the groups fed twice a week and daily during the 120-day observation period, there were significant differences in terms of development stages, with averages of 49.4±4.7 and 41.5±4.2 in the daily and twice-a-week groups, respectively ($p<0.05$). Development stages in both groups increased sharply up to 30 days, and then only slight increases were observed (Figure 5).

In the population density study, during the 90-day observation periods, there were significant differences in terms of development stages, with averages of 46.9±0.21 and 48.77±0.59 in the control and experiment groups, respectively ($p<0.001$). Development stages in the experiment group increased sharply from 10 to 20 days. Between 30 and 40

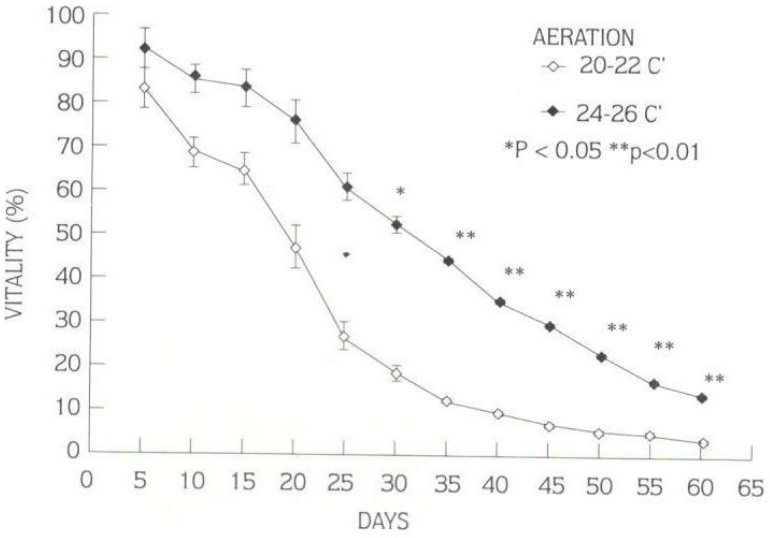

Figure 4. The effects of the Aeration through Aquarium Water on Vitality Ratio (%) of Xenopus levis Embryos.

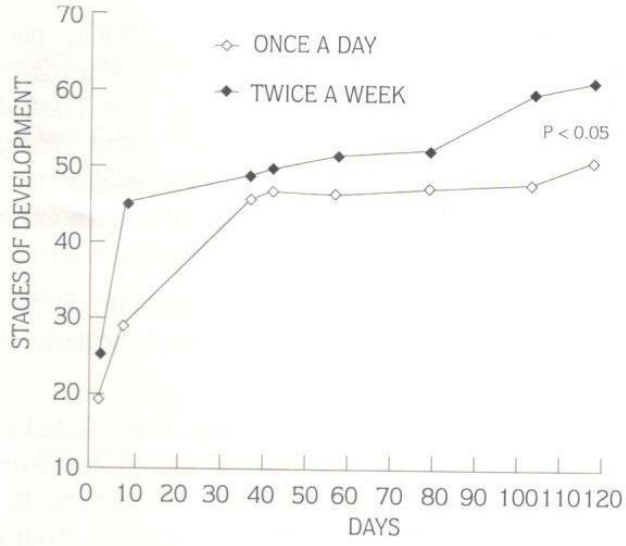

Figure 5. The Effects of Feeding Time on th Stages of Development of Xenopu Embryos.

days in both groups, stages were seen to be at a relatively static level. Then, at 40 to 65 days in both groups levels were silghtly increased. At 65 to 85 days in the experimen group, stages were almost constant, while in the control group, they decreased sharply (Figure 6).

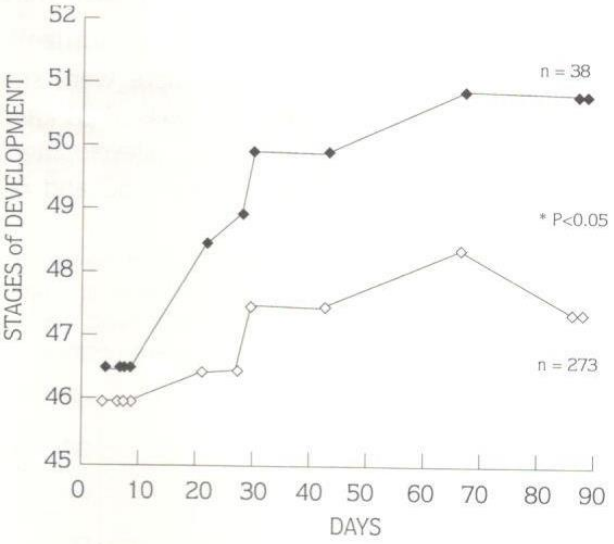

Figure 6. The Effects of Population Density on the Development Stages of Xenopus Embryos.

Discussion

The development of Xenopus laevis is adversely affected by prolonged exposure to temperatures below 14 °C and above 26 °C, and the quality of eggs and oocytes decreased in adult survivals. Strees can be followed by atresia (death and resorption) of the oocytes (12).

In this study, we aimed to find the most suitable environment for the development of the larvae.

At the end of the study we found that larvae kept at a temperature of 24-26 °C had a higher vitality ratio than did larvae kept at 20-22 °C temperature.

According to some authors, the water must be aerated until the larvae begin to breathe with their lungs (indicated by the appearance of air bubbles at the surface on the fifth day) (3, 12). This seems to accelerate tadpole growth and perhaps reduce the probably of occasional deaths caused by fouling of the water. At the fifth day we were unable to find any differences between the control and experiment groups. In this experiment, the differences began at 25-30 days.

Feeding starts during the fifth day (3, 12). Excellent results have been obtained with nettle powder (Herba urtica) or alpha leaf powder as food (Niewkoop, Faber, 1975 (3), (Özgünen T, 1994 (13), although in our experiment tadpoles grow faster on yeast-supplemented food. Others have obtained positive results with dried yeast (3, 12), liver powder or spinach infusion (12). There are many suggestions for feeding times. Authors variously suggested that feeding must be done twice a week, once a week and every day. Our data showed that the larvae must be feed every day.

Several studies stress the importance of keeping the population density low to optimize the rate of development (12). We found that development was better in the control group, which had 38 embryos. These results were in accordance with the findings of other studies (12, 13).

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