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Histomorphological development study for tadpoles and different regions of skin to the frogs (*Bufotes variabilis*) in Babylon city of Iraq

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Abstract

Iraq's reproductive and developmental seasons for the frog vary depending on the region and ecological, geographical, and climatic factors, but they always take place from February to April. This study set out to observe the impact factor of water temperature on frog embryo development and tadpoles' development in the frog genus *Bufotes variabilis*. In central Iraq (semi-arid zones), temperatures are high, and precipitation is low; the temperature plays an important role in the timing of metamorphosis, especially in amphibian populations of Iraq. In the three months of February, March, and April, a hand net was used to gather a total of 100 eggs from the bank of the Babylon River and divided into three groups, tadpole's total snout to vent length (SVL) at stages 25, 35, and 46 were measured during this period. This group experiences varying stages when the water temperature varies from 10°C in February to 25°C in April. Histologically, frog skin is composed of an epidermal and dermal layer. The epidermal layer comprises a mucous-stratified squamous epithelium (keratinized or non-keratinized) with three strata of keratinocytes (basal, intermediate, and apical). The dermis is divided into two strata, a loose connective tissue stratum underneath the epidermis that contains melanin pigment cells, blood vessels, mucous and granular glands, and a dense irregular connective tissue stratum rich in crisscrossed collagen fibers. Histological specimens were taken to study the mucous and granular glands of the frog's skin during the same period using rotund methods; the skin showed changes in mucous and granular gland diameter in April is larger than that in February. We registered in dorsal pectoral skin the mucous gland I, spherical in shape and constituted by a single layer of relatively tall prismatic secretory cells with basal nuclei. While the Mucous gland II is constituted of low cuboidal secretory cells with middle or basal nuclei surrounding a somewhat demanding lumen. In conclusion, the temperature plays a big role in tadpole size and time of metamorphosis as well as skin changes.

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Introduction

Ten species, representing five families, of amphibian species have been identified in Iraq (1,2), and it has been discovered that in North Africa, the Middle East, some Mediterranean Islands, the Arabian Peninsula, and Europe, *Viridis* is widely dispersed (3,4). Ectotherm animal populations depend highly on ambient temperature because they lack an effective physiological thermoregulation mechanism (5-7). *Viridis* is considered the frog that can withstand arid circumstances the best compared to other amphibians. It can be found in various habitats, including grasslands, woodlands, deserts, gardens, or wetlands (8,9). It appears that *Viridis* might survive in a hostile desert environment. Temperature and length of the tadpole stage impact an amphibian's time to metamorphosis when exposed to various temperature settings in temporary ponds, particularly in semi-arid areas where rainfall and pond duration are uncertain (10,11). Tadpoles in these conditions must hasten metamorphosis when ponds dry out and postpone the transformation when ponds dry out later in the season. The influence of temperature substantially conditions the expression of this fitness trade-off. An ideal metamorphic phenotype in aquatic animals with complicated life cycles, such as amphibians (12,13). The length of the embryonic and tadpole phases in amphibians is influenced by temperature. The impacts of climatic conditions on the growth and development of *Bufotes variabilis*, however, have not previously been investigated. Semi-arid regions have *Viridis*. Tadpoles produced at higher temperatures should be smaller than those produced at lower temperatures because amphibians living at relatively high temperatures are likely to suffer from acute hypoxia or anoxia near their centers (14,15). The comparatively high metabolic rates of embryos at high temperatures force eggs to be tiny (16,17). Low temperatures, however, cause the embryonic phase to be prolonged. On the other hand, higher temperatures cause a faster growth rate toward a smaller final size due to the distinct differences between catabolism and anabolism (18,19).

We aimed in current study to show the effect of temperature on size, timing of metamorphosis, Survival, growth developing stop as well as the skin changes in in the dorsal pectoral region.

Materials and methods

Ethical approval

Ethical approval was examined and accepted from the medical research ethical committee of Al-Qasim Green University, Babylon, Iraq numbered UOQASIM /COM/MREC/23-24 (10).

Samples collection and the study design

The eggs were collected by net monthly from the bank of the Babylon River roughly 100 eggs/spawn (20-22), and divided into three groups, and placed in three small net cages in the river. The tadpoles were fed twice daily with rinsed and frozen leaves, such as lettuce, broccoli, or baby spinach. Every 12 hours during the experiment, the stage of development was examined and documented.

Tadpole measurement and developing examine

The tadpole size was measured from February 2021 to end of April 2021, and the temperature was measured by using a mercury thermometer. A digital camera (dic-HX9V, 3.6V) SONY is used and fixed on 30 cm above the sample to measuring the tadpoles' monthly snout-to-vent length (SVL). Corel draw 11 was used to estimate each tadpole's total (SVL) at stages 25, 35, and 46. Stage size was measured for each tadpole at each temperature condition (23-25). The developmental stage was deemed to have changed when 70% of the tadpoles in the same sample had reached a specific stage because individuals of the same spawn developed at different rates.

Histological preparation

Histological specimens were taken from the skin to study the mucous and granular glands of the frog at different ages, the histochemical study was begun with fixation that is carried out in 85 percent alcohol at -5°C. The tissue was embedded in paraffin and sectioned at 7µm. First samples were stained with hematoxylin and eosin, and second sample staining with periodic acid Schiff (PAS), for coloring the basement membrane and neutral mucopolysaccharide materials, while the third samples staining with Masson's trichrome stain for appearance of the connective tissue and fiber (26,27).

Statistical analysis

One way ANOVA test was applied to find the difference in mean values between the result samples, at $P \leq 0.05$ significant level. The data were processed using statistical package for society software (SPSS) / version 14 for Windows to analyze the data by computer (28).

Results

The result showed an approximately 1-cm-wide translucent jelly capsule encircles each tiny black egg. *Bufotes variabilis* frog eggs are normally small and black, yet you can discern golden specks with a magnifying glass. As they grow older, they start to develop faint bronze speckles. Additionally, many frog embryos grow inside inner chambers surrounded by shells of jelly, and occasionally, after hatching, larvae swim inside chambers surrounded by outer shells of foam. The study notes that oxygen distribution is probably best explained by a model in which oxygen diffuses through an outside shell and is consumed in an inner convection chamber. Larvae of *Bufotes variabilis* swim after hatching in a chamber within an outer shell of foam. After maturing, we registered the creature had a body that was mostly transparent with a few pigment cells here and there, a large mouth with fully formed mouthparts, and operculum-covered gills.

The result shows dorsally rounded tail fin tapers to a tip and may have a faint pattern. A portion of the intestinal coil is visible. Large papillae and an emarginated oral disc are present. In table 1 we demonstrate how length fluctuates depending on temperature (month of the hatch) in February, March, and April 16.10± 1.60, 18.71± 1.57, and 17.51± 1.57, respectively. The size at metamorphosis showed a significant difference of 19.151±10, 17.11±1.54, and 15.11±1.50 mm in February, March, and April, respectively, by shrinking and raising the temperature. On the other hand, we registered the time to metamorphosis as measured in days shows that February 105.40±7.80 takes the longest, followed by April 87.28±12.40 and March 82.48±10.47 (Table 1). The growth period continued in March for 90 days, while it lasted for 88 days for tadpole hatching in April, but it decreased to 55 days for tadpole hatching in February (Table 2). The development growth stops in different stages according to Gosner stages of tadpole development related to the month of the hatch; it shows a highly significant difference between the stage of growth stop in April, 42 stages related to 35 stages in March, and 23 stages in February groups related to different temperatures of this month's (Table 2). Furthermore, absorption of the tail began in stage 43 and was finished in stage 46. The metamorphosis was completed with the fully developed coloration at this stage.

Table 1: The effects of temperature on *Bufotes variabilis* tadpole in effects of a different month of growing

Source of variation	Mean ± Standard Diversion		
	February group	March group	April group
SVL (mm)	16.10±1.60	18.71±1.57**	17.51±1.57*
Size at metamorphosis (mm)	19.15±1.10**	17.11±1.54*	15.11±1.50
Time to metamorphosis (day)	105.40±7.80**	82.48±10.47	87.28±12.40*
Survival (%)	22.87±4.20	74.00±22.22**	64.00±22.20*

* Significant difference at P<0.05. **Highly significant difference at P<0.05.

Table 2: The effects of different months of growing on growth and stage developing stop of *Bufotes variabilis.*

Source of variation	Mean ± Standard Diversion		
	February group	March group	April group
Water temperature (ºC)	10±0.02 ºC	15±0.04 ºC	25±0.06 ºC
Growth stop (day)	55±1.10 days	90±1.30 day**	88±1.20 days*
Gosner stage stops (UNIT)	23±0.08 stage	35±0.09 stage*	42±1.00 stage**

* Significant difference at P<0.05. **Highly significant difference at P<0.05.

Mucous and granular glands

The skin's outside layer, its ectoderm-derived from the epidermis, and the inner mesoderm-derived from the dermis, comprise the basic skin structure. The multilayered epidermis comprises a thin stratum corneum crossed by ectodermal gland ducts buried in the dermis. These glands play an important role in skin function and are divided into two types based on the secretion they produce: mucous and granular. According to their morphological and histological properties, we generally identified four types of simple glands in the skin within the stratum spongiosum: serous glands, granular or poison glands, mucous glands I, and mucous glands II (Figures 1 and 2). The granular glands are oval and densely packed with juxtaposed spherical granules.

A duct connects the alveolus to the skin on the outside and is surrounded by a layer of myoepithelial cells (Figures 3 and 4). We registered the mucous glands I in the dorsal pectoral skin, which are spherical and constituted by a single layer of relatively tall prismatic secretory cells with basal nuclei. The monocytes around an obvious lumen diminish in height from the glandular bottom to the neck. The neck's basal epithelium appears connected to the gland duct's flattened cells. Mucous glands II are a different form of mucous glands than mucous glands I; these elliptic glands are found in the dermis of the dorsal regions. Low cuboidal secretory cells with middle or basal nuclei surround a demanding lumen. At the glandular neck, monocytes flatten and form an imbricated squamous epithelium. The result showed that the diameter of the mucous gland and granular gland in April was larger than in February (Figure 5).

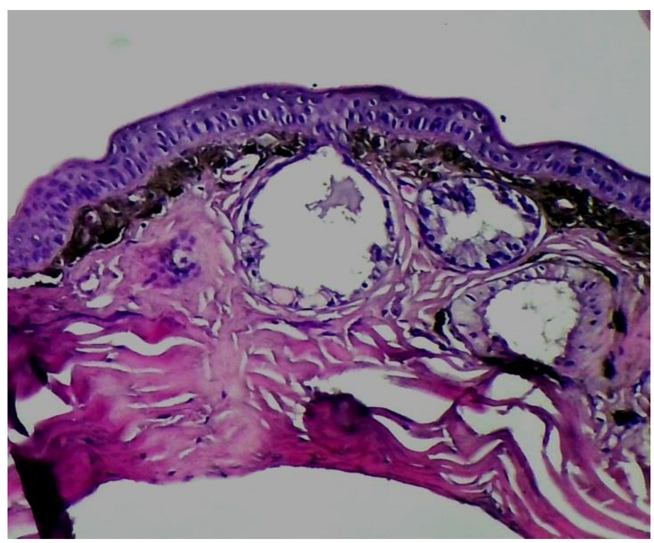

Figure 1: Histological section of mucus gland in the dorsal pectoral region of frog skin at 10ºC. 10x H&E.

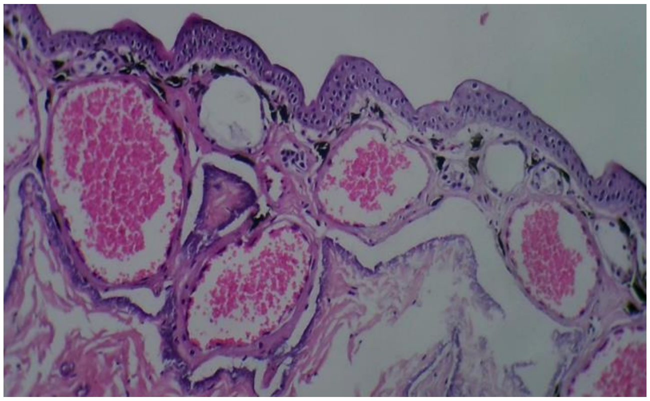

Figure 2: Histological section of mucus gland in the dorsal pectoral region of frog skin at 25ºC. 10x H&E.

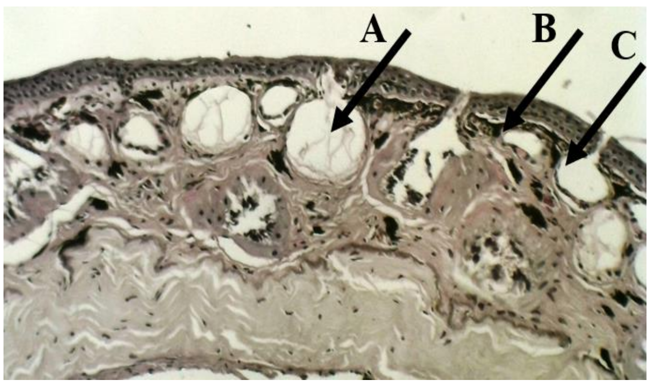

Figure 3: Histological section of frog skin glands in the dorsal pectoral region showing: A. Mucous gland (I); B. Melanophores C. Mucous gland (II); The intensity of the PSA reaction is weak in the lumen, 10ºC. (20X PAS stain).

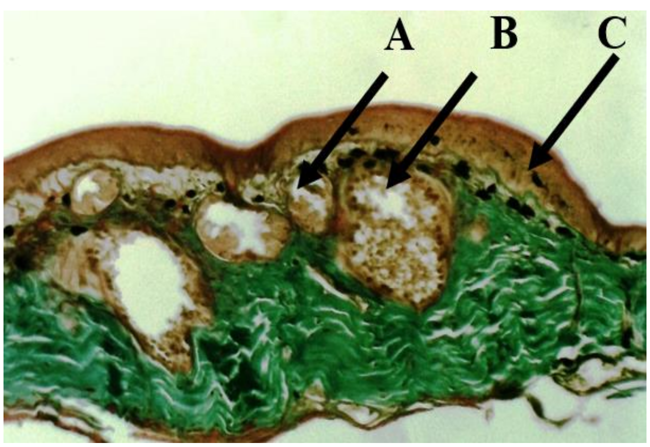

Figure 4: Histological section of frog skin glands in the dorsal pectoral region showing: A. Melanophores; B. Granular gland; C. Epidermis layer (10X Masson's trichrome stain).

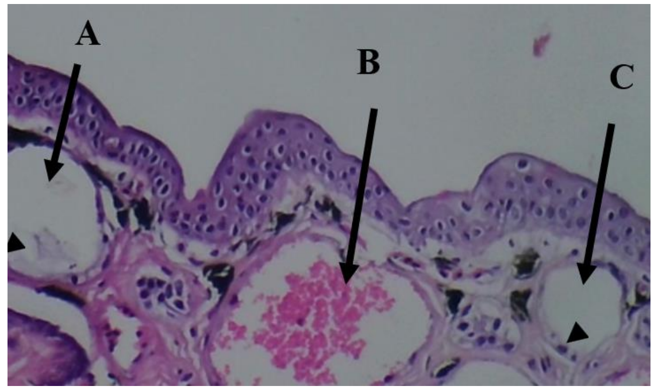

Figure 5: Histological section of frog skin glands in the dorsal pectoral region showing: A. Mucous gland (I); B. Melanophores C. Mucous gland (II); The mucous glands were lined mainly by simple squamous or cuboidal cells; the nuclei were flat and lay mainly in the apical portion of the cells (arrowheads) 25ºC. 40x H&E.

Discussion

The result shows the size, color, and larva growing inside the inner chambers with an outer shell, similar to earlier studies Álvarez *et al.* (29) and Arendt (30). The length and the size at metamorphosis depend on the hatch's month, February, March, and April, consistent with Álvarez *et al*. (29). Many frog species concur with that, including the wood frog, which grows to an adult size of 42 to 48 mm after emerging from the tadpole stage Arendt (30) explains that the thyroid hormone is the most important in frog metamorphosis; this hormone requires the presence of iodine in water for its production. If the water in which tadpoles are growing lacks iodine, the frog metamorphosis cannot be complete. While the time to metamorphosis as measured in February, March, and April, this result is consistent with Blouin *et al*. (31) and that because warm water speeds up the animals' metabolic need for oxygen to such an extent that it causes them to suffer from fatal respiratory distress, theory the environment that amphibians encounter during the larval development can affect not only the larvae's growth and development but also the characteristics of frog lets after the metamorphosis, in natural conditions the colder environment causes delayed metamorphosis of foothill populations. The negative effects of severe hypoxia on embryos are the reason why March has the highest survival rate, followed by April and then February; this is the same conclusion reached by Laugen *et al.* (32) and Newman (33) explains that an inverse relationship between the increase in temperature and the amount of oxygen in the water

However, rapid larval growth and development can increase the growth rate and the chance of survival even after metamorphosis. A lower water temperature significantly increases the tadpole period of *Bufotes variabilis*, slowing down the developmental rate so that the tadpole reared at 10 °C required approximately 20% more time to reach metamorphosis. This is similar to previous research by Mohammad *et al.* (34); if the environment of origin is colder in nature, their development can take longer than those of populations living in warmer climates. These phenomena have been frequently described as cases of counter-gradient variation. The growth period related to the month is similar to research that explains the ability to grow and develop faster for tadpoles living in moderate temperate climates than others living in warmer or cold conditions, giving the negative effect by slowing down the growth rate Garstecki *et al.* (35) explain it the frogs are ectothermic amphibians who are unable to regulate their temperatures internally like birds or mammals. Instead, they need to warm up using other things outside their bodies- this action is called thermoregulation.

According to their morphologies and histological, we generally identified four types of glands, and this corresponds to Gong *et al.* (36), where they studied bio-structural and functional assessment of the glands. The largest gland in the frog's granular glands was discovered lengthways superficially in the stratum spongiosum of the dermis of the dorsal pectoral skin area, with an immense number of chromophores. The granular glands shape and has a duct that connects the alveolus to the skin to the outside; this corresponds to Thomas *et al.* (37), where they studied bio-structural and functional assessment of the glands, where they found four types of glands in the skin of a frog.

The result for mucous gland I and mucous gland II related to the shape, the cells surrounding the lumen, and the place where found this result corresponds to Gong *et al.* (36), where they studied bio structural and functional assessment of the glands, where they found four types of glands in the skin of a frog, and this corresponds to Thomas *et al.* (37), Toledo *et al.* (38), Al-Khakani *et al.* (39) and Al-Niaeemi *et al.* (40) when they studied granulocytic glands and amphibian toxins, where they found that Serous (granular, venom, or venom), mucous membranes, lipids (wax), and mixed glands are the four basic types of skin glands (seromucosal).

Conclusion

The size and time at metamorphosis and survival rate for the frog *Bufotes variabilis* vary depending on the month of hatching in Iraq from February, March, and April.

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Conflict of interest

There is no conflict of interest

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دراسة شكلائية نسجية لتطور الشرغوف وجلد الضفادع في مدينة بابل، العراق

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فرع التشريح والأنسجة، كلية الطب البيطري، جامعة القاسم الخضراء، بابل، العراق

الخلاصة

تختلف مواسم التكاثر والنمو للضفادع في العراق حسب المنطقة والعوامل البيئية والجغرافية والمناخية، ولكنها تحدث دائما بين الأشهر شباط إلى نيسان. تهدف هذه الدراسة لمعرفة تأثير درجة حرارة الماء على تطور أجنة الضفادع من جنس الضفدع الأخضر المختلف. في مناطق وسط العراق (المناطق شبه القاحلة)، حيث تكون درجات الحرارة مرتفعة مع قلة في هطول الأمطار، وحيث تلعب درجة الحرارة دورا مهما في توقيتات التحول في مجموعات البرمائيات في العراق. وذلك في الأشهر الثلاثة من شباط وأذار ونيسان، حيث تم استخدام شبكة اليد لجمع ١٠٠ بيضة من ضفاف نهر بابل (الحلة) وقسمت الى ثلاث مجاميع وتم قياس يرقات الضفادع الصغيرة خلال هذه الفترة من الخطم الى فتحة المخرج خلال المراحل التطورية ٢٥ و ٣٥ و ٤٦ يوما. حيث لوحظ تواجد تغير في مراحل اليرقات وكذلك اختلاف في نمو اليرقات تبعا لتغير الحاصل في درجة حرارة الماء من ١٠ درجة مئوية في شباط الى ٢٥ درجة مئوية في نيسان. من الناحية النسيجية، يتكون جلد الضفدع من أدمة خارجية وأدمة داخلية. تتكون الأدمة الخارجية من ظهارة حرشفية مخاطية (متقرنة أو غير متقرنة) مع ثلاث طبقات من الخلايا المتقرنة (قاعدية، وسطية، وقممية). تنقسم الأدمة الداخلية إلى طبقتين، طبقة نسيج ضام رخوة تحت الأدمة تحتوي على خلايا صبغة الميلانين والأوعية الدموية والغدد المخاطية والحبيبية وطبقة نسيج ضام كثيفة غير منتظمة غنية بألياف الكولاجين المتقاطعة. كما أظهرت الدراسة النسيجية للضفادع في نفس هذه الفترة من السنة عن حدوث تغيرات في الجلد والغدد المخاطية والغدد الحبيبية مع تغير درجة حرارة الوسط المائي للمحيط حيث تم اخذ عينات نسيجية لدراسة الغدد المخاطية والحبيبية لجلد الضفدع في نفس الفترة باستخدام الطرق النسيجية الروتينية، حيث أظهرت النتائج زيادة في قطر الغدد المخاطية والحبيبية في شهر نيسان عن شهر شباط. وتم ملاحظة الغدد المخاطية الأولى في منطقة الجلد الصدرية الظهرية حيث كانت بشكل كروي وتتكون من طبقة واحدة من الخلايا الإفرازية المنشورية الطويلة نسبيا ذات النواة القاعدية. بينما تتكون الغدة المخاطي الثانية من خلايا إفرازية مكعبة مع نوى وسطية أو قاعدية تحاط بتجويف. نستنتج من هذه الدراسة بان الحرارة تلعب دورا كبيرا في تطور يرقات الضفادع وفي توقيت التحول لها وكذلك تلعب دورا كبيرا في التغيرات الحاصلة في الجلد.