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Wytrzymałość płazów na topienie — Tolerance to submersion in water in amphibians

by

Juliusz CZOPEK

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The survival capability of amphibians submerged in water and deprived of pulmonary respiration has been the subject of comparatively little research. Serfaty and Guental (1943) have found that *Rana esculenta* L. is able to survive two to three weeks in water, whose temperature is 14 to 15° C. Under similar conditions *Xenopus laevis* (Daud.) survives 28 days (de Graaf, 1958), and *Triturus vulgaris* L. and *T. cristatus* Laur. only from 8 hours (specimens without breeding dress) to 100 hours (specimens in full breeding dress) (Bannikov, 1948).

Morphological researches on the vascularization of the respiratory surfaces in amphibians have shown that the length of respiratory capillaries per gram of body mass in the above species is approximately the same and ranges from 15 to 18 m (Czopek, Pugaczewska, Sopoćko, 1954; Czopek, 1955 a, b; 1959; Broda, 1956). According to the same researches, in *R. esculenta* and *X. laevis* 1/3 of the respiratory capillaries is located in the skin, and 2/3 in the lungs, whereas in the newts 3/4 to 4/5 of all respiratory capillaries are found in the skin, and only 1/4 to 1/5 in the lungs. Moreover, the epidermis in newts is nearly by one half thinner than in most other species, which certainly makes it easier for the oxygen to penetrate into the subepidermal capillaries of the skin. It may therefore be induced that newts deprived of pulmonary respiration and submerged in well-aerated water should have a better possibility of supplying the organism with oxygen than specimens of *R. esculenta* and *X. laevis*.

Bannikov's results regarding endurance to submersion in newts are difficult to explain in the light of morphological studies. Moreover, his work contains certain rather serious shortcomings, e. g. the author gives no data on the oxygen content in the water before and after the experiments or on the number of specimens used in each experiment. For these reasons it was considered worthwhile to repeat Bannikov's experiments, extending them to other species, which had not been studied before, and using uniform methods of investigations.

Material and method

The following amphibian species were used in the experiments: *Triturus vulgaris* (L.), *Bombina bombina* (L.), *Bufo bufo* (L.), *Hyla arborea* (L.), *Rana temporaria* L. and *R. esculenta* L.

The experiment with newts were carried out in aquaria measuring 20 cm × 20 cm × 30 cm, specimens of the remaining species were placed in aquaria measuring 30 cm × 50 cm × 30 cm. A tightly fitting frame with orlon netting stretched on it had been placed inside each aquarium.

20 specimens of one species, 10 males and 10 females, were put under the netting of each aquarium. Only in the experiment with very large-sized individuals of *R. esculenta* 10 specimens were used, as no more specimens of that size could be obtained, and in the experiments with *T. vulgaris* and *R. temporaria* the males and the females were kept in separate aquaria.

The net had a closing aperture for removing dead specimens. The diameter of the meshes did not exceed 3—4 mm. In all the experiments the column of water was 25 cm high, and the net was 5 cm under the water surface. The water in the aquaria was saturated with oxygen by passing through it a stream of air bubbles 1—2 mm in diameter at a rate averaging 500 ml per minute. Before and after the experiments the oxygen content of the water was measured by the Winkler method.

To keep an adequate standard of purity of the water half of the contents of the aquaria was emptied every 48 hours and replaced with an equal amount of oxygen-saturated water from an emergency reservoir. While doing that, care was taken that the net with the animals underneath should be always covered with water. The temperature of the water during the experiments ranged from 15 to 16° C, except for a few hot days in July, when it reached 21° C.

The animals were captured in the environs of Toruń and immediately used for the experiments. The experiments for a given species were started during its breeding season. For *T. vulgaris* and *R. esculenta* they were repeated in autumn once again.

The present experiments have shown that the species studied survive in submersion a number of days; an exception to this is *H. arborea*, which under identical conditions survives only up to 25 hours. To find out whether the rapid death of the specimens of *H. arborea* was due to submersion and obstruction of pulmonary respiration or to submersion alone and the subsequent strong hydration of the organism, the following supplementary experiment was carried out. 10 specimens of *H. arborea* were put under the covering net, which was raised about 2 mm above the water surface. This position of the net made pulmonary respiration possible, while virtually the whole body of the animals was submerged. There was no need of similar control experiments for the other species, as they all survived a number of days in complete submersion; if they were given the possibility of respiring through the lungs, their surviving conditions would certainly not deteriorate.

No food was provided to the animals during the experiments. In an additional experiments with *T. vulgaris*, however, the animals were fed plentiful plankton.

An animal was considered dead when it showed no response to a pair of sharp tweezers being pressed against its web, the skin in its anal region and its cornea.

All the specimens which died in the course of the experiments were dissected. Those which survived 30 days in submersion and the control specimens kept for 30 days in terraria were killed by destroying their brain with a probe. Bits of the skin, the muscles, the liver, and the kidneys from all the specimens were fixed, dehydrated and embedded in paraffin for future use in histological studies, the results of which will be presented in a separate paper.

Results of the experiments

T. vulgaris. The experiment with specimens in full breeding dress was started on June 9. After being put into the aquarium the animals were very lively and tried to get out through the net. After several hours they calmed down and sat on the bottom, coming up to the net every 5 to 15 minutes. This performance became gradually less and less frequent until, 4 or 5 days from the beginning of the experiment up to its close, it was repeated on the average every 60 minutes by the females and every 40 minutes by the males. During the first four days of the experiment the females laid 95 eggs on the floor of the aquarium. Later on no egg-laying was observed.

Movements of the mouth floor occurred throughout the experiment, but they were far less frequent and slower than in animals staying on land. The rate of mouth floor movements averaged one per every 5 to 10 seconds, and the movements were accompanied fairly frequently by a slight opening of the mouth. On the ninth day of the experiment the breeding dress began to disappear in some of the specimens; after 24 days it disappeared completely in all the specimens. After living 30 days in submersion all the experimental specimens were transferred to a terrarium.

A similar experiment was carried out with non-breeding newts staying on land. The experiment was started on September 9. After being put under the net the animals behaved in a similar way as those already described. All the time of the experiment the animals were fed plankton. All the specimens used in this experiment survived 30 days in submersion (Fig. 1). After that time it was decided to continue the experiment. In mid February, i. e. after five months of submersion, and, consequently, of elimination of respiration with atmospheric air, both the males and females developed a breeding dress. The caudal and dorsal fin, however, were not so well developed as in a typical breeding dress. Starting from the end of March this incomplete breeding dress began gradually to disappear.

It should be noted that, due to intense heating of the room where the experiments were being conducted, the temperature of the water during the winter months ranged from 18 to 22° C. A break in current supply during the night from 9 to 10 April and the consequent stoppage in the work of the ventilator caused the death

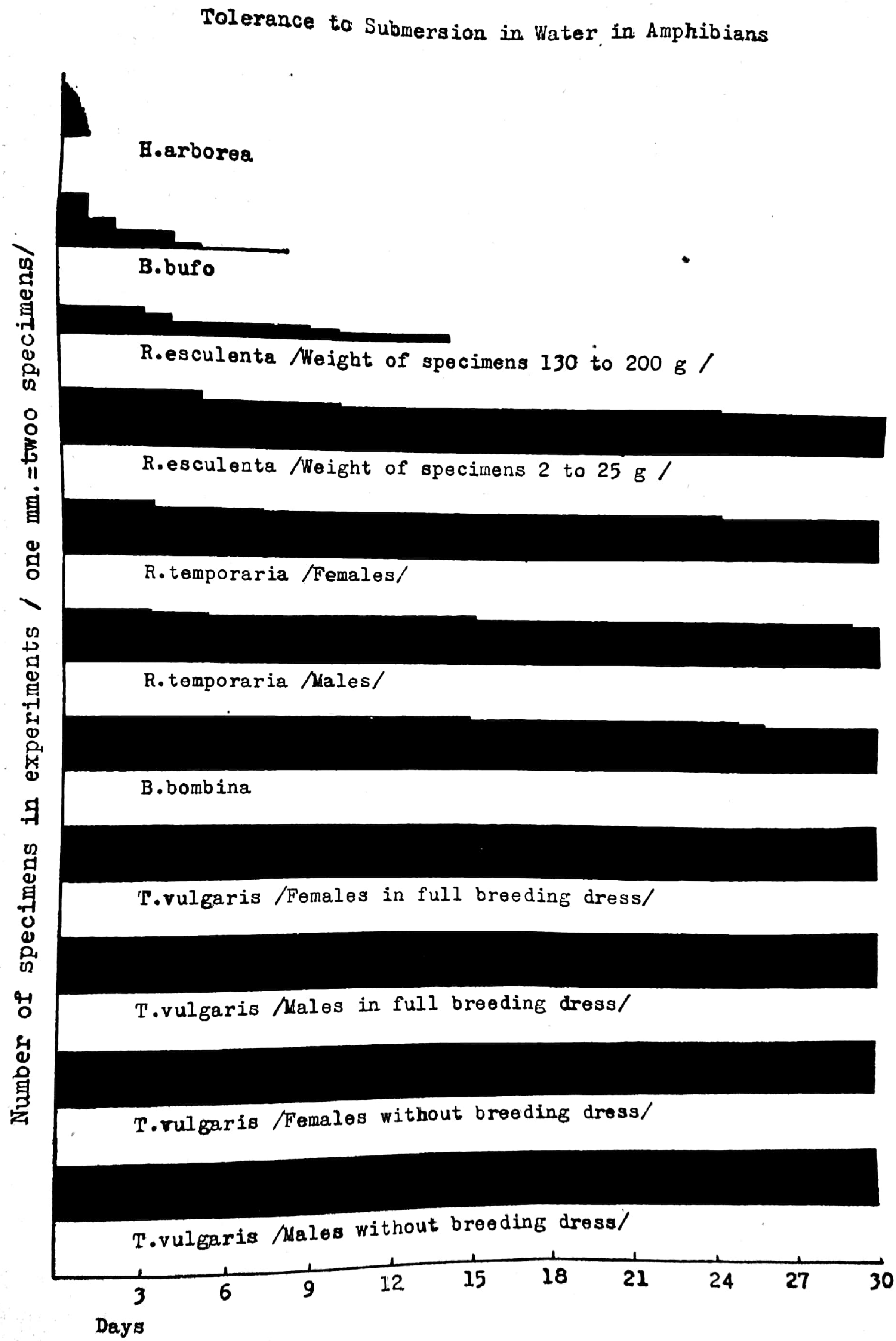
Tolerance to Submersion in Water in Amphibians
H.arborea
B.bufo
R.esculenta /Weight of specimens 130 to 200 g /
R.esculenta /Weight of specimens 2 to 25 g /
R.temporaria /Females/
R.temporaria /Males/
B.bombina
T.vulgaris /Females in full breeding dress/
T.vulgaris /Males in full breeding dress/
T.vulgaris /Females without breeding dress/
T.vulgaris /Males without breeding dress/
Number of specimens in experiments / one mm.=twoo specimens/
3
6
9
12
15
18
21
24
27
30
Days

Fig.1.

of all specimens due to insufficient saturation with oxygen of the water. The animals lived 7 months in submersion.

Salientia. After being put into the aquarium all the *Salientia* behaved in a similar way. For the first 2—10 minutes the animals sat quietly on the bottom of the aquarium, then tried to get out through the net for an average of 20 to 90 minutes. After that the animals calmed down and in most cases attached themselves by their forelimbs to the net and remained there with their trunks loosely outstretched and their hind limbs nearly straightened. After a period of 30 minutes to 2 hours the animals repeated their attempts to get out through the net, then resumed the former „hanging" position.

Movements of the mouth floor and releasing of air bubbles from the lungs were observed in all specimens. The mouth floor movements were performed with closed anterior nares. The movements ceased altogether after 3 hours (in *H. arborea*), or after 20 to 50 hours (in the remaining species), with the mouth floor adhering to the palate.

All the specimens showed many open vessels in the skin during the experiment; this was particularly apparent on the ventral side of the body (posterior portion of belly and anterior portion of thigh), where the vascular network is best developed and pigment cells are scarce.

As revealed by dissection, all the specimens that died in the course of the experiment (except *H. arborea*) had neither air nor water in the lungs. The ventricle of the heart was always strongly constricted, while the atria were dilated to the maximum. In *H. arborea* the heart was still showing faint contractions.

B. bombina. The experiment was started on June 16. The specimens ranged from 7 to 9 g in weight. Two males died on the 15th and 25th day of submersion, and one female on the 24th day. The remaining 17 specimens survived 30 days in submersion, after which the experiment was interrupted. The weight of both the surviving and the dead specimens was 5 to 8% lower after the experiment than before it.

B. bufo. The experiment was started on May 8. The specimens ranged in weight from 35 to 48 g. The first 3 specimens died already after 12 hours, the next 4 after 18 hours, and 10 more died between 22 and 35 hours after the beginning of the experiment. The last three specimens died in succession on the fifth, sixth and eighth day of the experiment. The dead specimens weighed an average of 15 to 20% more than while living.

The dissection of the specimens revealed large amounts of fluid in the subdermal lymphatic sacs and in the coelom. The urinary bladder was filled up.

H. arborea. The experiment was started on May 18. The weight of the specimens ranged from 7 to 9·5 g. The animals survived in submersion only from 4 to 26 hours. In all specimens a gradual swelling of the body was observed. 13 specimens died between the 4th and the 13th hour of the experiment. The remaining 7 survived 19 to 26 hours. All the dead specimens were strongly swollen and their weight averaged 24 to 29% higher than while living.

Dissection revealed large amounts of water in the lungs and plenty of fluid in the subdermal lymphatic sacs and in the coelom. The urinary bladder was tightly filled up and in 9 specimens it was thrust out of the body.

On May 22 an additional experiment was started, in which the net which 10 specimens of *H. arborea* underneath was just above the water surface. Under these conditions pulmonary respiration was possible while virtually the whole body of the animals was submerged in water. The experiment was interrupted after 7 days. Only one specimen died (on the 5th day of the experiment). In the course of the experiment only slight swelling of the body was noted in the experimental animals.

R. temporaria. The experiment was started on April 25. The specimens ranged from 28 to 42 g in weight. After 30 hours of submersion the females (in spite of the males being absent) laid some spawn. Spawn laying continued until May 1. 8 specimens died in the course of the experiment: 4 males and 4 females. The subsequent dissection showed that 2 of the males and 2 of the females had fractures of thigh bones and haemorrhagic exudates under the skin or in the coelom. These injuries had been probably inflicted while capturing the animals. The injured specimens died already after 3 days of submersion. The other 4 specimens survived much longer: the females died on the 9th and on the 24th day, the males on the 15th and on the 28th day of the experiment. 32 specimens were taken out alive after 30 days of submersion. The weight of the specimens was lower after than before the experiment: in the females the difference ranged from 20 to 28%, in the males from 4 to 8%.

R. esculenta. 10 very large-sized specimens were used in this experiment. Their weight ranged from 130 to 200 g, i. e. two or three times as much as in the most frequently found grown specimens of this species. The experiments were started on June 14. The two largest specimens died after 3 days, three further ones after 4 days, from the beginning of the experiment. The remaining specimens died in succession: on the 9th day (1 specimen), on the 10th day (1 specimen), and on the 14th day (3 specimens) of the experiment. The weight of the dead specimens averaged to 8—10% more than at the beginning of the experiment.

A similar experiment was carried out with 20 young specimens of *R. esculenta*. 17 of them ranged 2 to 4 g in weight, the remaining three weighed about 25 g each. The experiment was started on October 2. After 20 hours of submersion the small specimens were noted to swell gradually. Notwithstanding the swelling, from time to time the animals moved about alertly under the net. 50 hours from the beginning of the experiment the small specimens began gradually to return to normal appearance, whereas the medium-sized specimens began to swell and finally died in the 4th day of submersion. Their weight was 21 to 23% higher than at the beginning of the experiment. One of the small specimens died after 9 days of submersion, another one after 24 days. The remaining 15 small specimens were let out after surviving 30 days in submersion (Fig. 1). The weight of the small specimens, both the surviving and the dead ones, was 5 to 8% lower than before the experiment.

The oxygen content of the water before the experiments was 4·2 to 4·6 ml per litre. In the course of the experiments this value underwent a reduction of only about 10%.

Discussion

Poczopko (1956, 1957, 1958 a, b, 1959 a, b) has demonstrated that in individuals of *R. esculenta* subjected to treatment altering the conditions of cutaneous and pulmonary respiration occur reflexes of cutaneous vessels which may be regarded as vasomotor regulation of cutaneous respiration. This investigator has found that submersion of frogs in water produces an increase in the number of open capillaries in the skin, this increasing the surface of diffusion. Elimination of pulmonary respiration in the edible frog produces a decrease in the heart rate. According to Poczopko, increased circulation in the skin is possible due to decreased circulation in the internal organs. A similar increase in the number of open capillaries in the skin has been found by this author in frogs with pulmonary respiration artificially eliminated. Poczopko (1959 b) also studied gaseous exchange in frogs breathing with lungs and skin and in the same animals devoid of pulmonary respiration. The elimination of pulmonary respiration produced an immediate decrease in oxygen intake.

Laskowski (1929) has found that an edible frog kept for some time under water takes in more oxygen when taken out into the air. The oxygen intake reaches its maximum 40 to 90 minutes after emergence, then gradually decreases, which shows that the animal pays off the oxygen debt incurred while staying under water. Poczopko claims that in *R. esculenta* under conditions of limited oxygen supply occur both reduction of metabolism and incurring of oxygen debt. The oxygen debt is incurred immediately after submersion, and if this is protracted metabolism is reduced until balance is achieved between the new reduced demand for oxygen and its supply through the skin. Leivestad (1960) found that metabolism in *B. bufo* after 2 hours' submersion was only 20% of that found in the toads when breathing through the lungs. After emergence the oxygen uptake soon returned to the pre-submersion or slightly lower level, which meant that no paying off of the oxygen debt was taking place.

In the experiments under discussion all the species studied after being put into the aquarium under the net making pulmonary respiration impossible showed an intense blood flow through the skin, particularly apparent on the ventral side of the body. This confirms Poczopko's (1959 a) observation that submersion in water produces in the frog an increase in the number of open capillaries in the skin. Newts proved exceptionally tolerant to submersion. The specimens of *T. vulgaris* survived 7 months under water, and only a break in the current supply and the subsequent stoppage in the work of the ventilator caused the death of all the animals. An evidence of well-being of the animals during the experiment is the fact that in the middle of February, i. e. after 5 months of submersion, both in the males and females appeared a breeding dress, though less developed than normal. There is good reason to believe that newts can live in submersion without any harm for much longer periods. This is possible due to the fact that the majority of respiratory capillaries in the newts lie in the skin (Czopek et alii, 1954, Broda, 1956, Czopek, 1959), whereas the lungs in fact play only the role of a hydrostatic organ

(Spurway, Haldane, 1954). Moreover, the epithelium is much thinner in newts than in the remaining species, and therefore oxygen can more easily penetrate into the cutaneous capillaries (Table I).

TABLE I

Species	Thickness of epidermis in μ	Length of capillaries of all respiratory surfaces per 1 g of body weight in meters	Length of capillaries of all respiratory surfaces expressed in per cent of total			Remarks
			Skin	Lungs	Palate	
T. vulgaris	20·0	16·814	79·86	19·11	1·03	Males in full breeding dress
T. vulgaris	24·0	16·305	75·82	22·99	1·19	Females in full breeding dress
T. vulgaris	25·5	15·487	74·03	24·50	1·47	Specimens without breeding dress
T. alpestris	25·4	15·090	75·61	22·79	1·60	Specimens without breeding dress
T. cristatus	25·0	15·570	73·70	23·30	3·00 *	Specimens without breeding dress
B. bombina	22·8	14·620	53·70	45·20	1·10	
X. laevis	43·6	15·170	33·90	65·90	0·20	
B. bufo	51·9	19·680	27·60	71·50	0·90	
H. arborea	32·6	45·990	24·20	74·70	1·10	
R. temporaria	42·5	18·350	36·60	62·50	0·90	
R. esculenta	35·0	44·830	35·27	64·73	—	Newly metamorphosed frog
R. esculenta	39·1	17·870	34·30	64·80	0·90	Specimen weighing 53 g
R. esculenta	62·30	11·000	36·17	62·13	1·70 *	Specimen weighing 250 g

* The capillaries were counted both in the palate and in the floor of the mouth.

Bannikov (1948) obtained divergent results: in his experiments on tolerance to submersion in newts specimens of *T. cristatus* and *T. vulgaris* survived in submersion only from 8 hours (normal specimens) to 100 hours (specimens in full breeding dress). Bannikov, however, gives no data on the oxygen content of the water before and after the experiments. Neither does he give the number of specimens used in each experiment. The fact that in our experiments the newts died a few hours after the ventilator had stopped working indicates that the newts in Bannikov's experiments died most probably in consequence of insufficient oxygen content of the water.

Among the *Salientia* investigated the specimens of *B. bombina, R. temporaria,* and the small, 2 to 4 g specimens of *R. esculenta* survived 30 days in submersion. Mortality in the course of the experiment in any species studied did not exceed 10% of the total number of the specimens used in the experiment. This rate would no doubt be even lower but for the fact that some of the specimens which died during the experiments had many injuries caused probably by handling the animals while catching them. There is reason to believe that these species are capable of surviving under water much longer periods. Very prolonged submersion, however, would lead to death of starvation, as these animals are unable to feed under water.

From the *Salientia* mentioned in the present paper the best chance of surviving in submersion has *B. bombina*. In this species more than a half of all respiratory capillaries is located in the skin, which, moreover, is covered with an epithelium nearly by one half thinner than in the other species (Table I). In the genus *Rana* the skin capillaries constitute roughly 1/3 of the total amount of respiratory capillaries (Czopek 1955 a). The very large specimens of *R. esculenta,* weighing 130 to 200 g, survived in submersion only 3 to 9 days, the first to die being the largest ones. Only the three smallest specimens survived somewhat longer, namely 14 days. The much lower tolerance to submersion shown by the large-sized specimens of *R. esculenta* may be explained as follows: these specimens have only 3·970 m of skin capillaries per gram of body mass, i. e. over four times less than the specimens weighing 2 to 4 g (Strawiński, 1956, Andrzejewski et alii, 1962). So small an amount of capillaries may be not enough to ensure under submersion conditions adequate oxygen supply to the body, and particularly to the brain and kidneys, which eventually is fatal to the organism. Though, as demonstrated by Kreps (1960), amphibians have the highest capacity for anaerobic respiration with regard to nervous tissue among all vertebrates including even fish, anaerobic respiration certainly cannot continue for a very long time. The period of time survived in submersion by the toads was even shorter than that survived by the large-sized specimens of *R. esculenta*. Most of them died after 12 to 35 hours of submersion, only three specimens survived 5 to 8 days. The specimens of *H. arborea* survived in submersion only from 4 to 26 hours.

As demonstrated by morphological studies (Czopek, 1955 a), *B. bufo*, and even more *H. arborea* have more respiratory capillaries per gram of body mass than any of the species discussed before, which indicates higher metabolism in these animals. Another proof of high metabolism in *H. arborea* is the fact that its heart to body weight ratio is two or three times that of other amphibian species (Hesse, 1921). The skin capillaries in *B. bufo* and *H. arborea* constitute only 1/4 of the total amount of respiratory capillaries, which under submersion conditions may be not enough for adequate supplying the organism with oxygen. In *B. bufo*, and even more in *H. arborea* a gradual swelling of the body was observed during submersion until the death of the animals. In all the animals dissection revealed large amounts of fluid (15 to 29% of body weight) in the coelom and in the subdermal lymphatic sacs, and the urinary bladder was found to be filled up. This may be accounted for by insufficiency of the kidneys caused by inadequate oxygen supply.

The fact that specimens of *H. arborea* tolerate prolonged submersion if they have free access to atmospheric air proves well enough that the death of the experimental animals was caused by inadequate oxygen supply to the organism, the only source of oxygen being the gas dissolved in the water. Another premise to the same conclusion is the fact that small specimens of *R. esculenta* showed much greater tolerance to submersion than very large ones. As mentioned before, small specimens of *R. esculenta* have more than four times as much skin capillaries per gram of body mass than large ones. The precise mechanism of damage to the organism caused by oxygen deficiency is still unexplained.

Three alternatives are possible:

a) The lack of oxygen caused changes in the nervous system, this in turn produced disturbances in reflexes, flooding of the lungs with water and death.

b) The lack of oxygen reduced the efficiency of the kidneys in osmoregulation, the other changes are secondary phenomena.

c) The lack of oxygen produced parallel changes in the brain, kidneys, and perhaps in other organs as well. All these changes summed up caused death.

To decide which of these alternatives took place in our experiments further investigations would be necessary.

Particularly exposed to penetration of water into the organism were the small, 2 to 4-g specimens of *R. esculenta*, in which the relation of surface area of the body to its mass was especially favourable (Table I). During the first 5 days of submersion the animals were gradually swelling with excess of penetrating water. After that period they began to decrease in volume. This showed that the organism adapted itself to changed conditions, and the kidneys were efficiently eliminating excess water. In the remaining species only slight swelling was noted in the first few days of submersion, and this receded within a short time.

The tolerance to submersion in amphibians depends to a great extent on the temperature of the water. Serfaty and Guental (1943) demonstrated that specimens of *R. esculenta* submerged at 14 to 15° C survive without pulmonary respiration for 2 to 3 weeks. With rising temperature of the water the survival time of the animals becomes greatly reduced: at 19 to 20° C they survive 10 to 15 days, at 26 to 27° C only from 36 to 48 hours.

Summary

Investigations have been carried out on the tolerance to submersion in water of the following amphibians: *T. vulgaris*, *B. bombina*, *B. bufo*, *R. temporaria*, and *R. esculenta*.

T. vulgaris endures many months of submersion in well-aerated water without using the lungs. *B. bombina*, *R. temporaria*, and young specimens of *R. esculenta*

weighing 2 to 4 g survive under similar conditions at least 30 days. Much less tolerance to submersion show large-sized specimens of *R. esculenta* weighing 130 to 200 g and toads. The large specimens of *R. esculenta* die already after 3 to 9 days of submersion, whereas most of the toads die already after 12 to 35 hours. Still less tolerant to submersion is *H. arborea* which survives only 4 to 26 hours.

References

Andrzejewski H., Czopek J., Siankowa L., Szarski H. 1962. Unaczynienie powierzchni oddechowych u płazów osiągających znaczne rozmiary ciała (*Rana esculenta* L., *R. sphenocephala* (Cope) i *R. grylio* Stejneger). Studia Soc. Sci. Torunensis, Sec. E., 1—15.

Bannikov A. G. 1948. O sezonnom izmenenii dychatel'noj funkcii kozi u tritonov. Doklady A. N. S. S. S. R., 59, 1121—1124.

Broda G. 1956. Unaczynienie powierzchni oddechowych traszki górskiej (*Triturus alpestris* Laur.). Studia Soc. Sci. Torunensis, Sec. E, 3, 85—93.

Czopek J. 1955 a. The vascularization of the respiratory surfaces of some *Salientia*. Zool. Pol., 6, 101—134.

Czopek J. 1955 b. Vascularization of respiratory surfaces in *Leiopelma hochstetteri* Fitzinger and *Xenopus laevis* (Daudin). Acta Anat., 25, 346—360.

Czopek J. 1959. Skin and lung capillaries in European Common Newts. Copeia, 2, 91—96.

Czopek J., Pugaczewska H., Sopoćko I. 1954. Unaczynienie powierzchni oddechowych u traszki grzebieniastej (*Triturus cristatus* Laur.). Folia Morph., 5, 93—104.

Graaf de A. R. 1957. A note on the oxygen requirements of *Xenopus laevis*. J. Experim. Biol., 34, 173—176.

Hesse R. 1921. Das Herzgewicht der Wirbeltiere. Zool. Jahrb. Physiol., 38, 243—364.

Kreps E. M. 1958. Brain metabolism in the evolution of Vertebrates. XVth International Congress of Zoology, London, 16—23 July 1958, Proceedings, 510—512.

Laskowski M. 1929. O pobieraniu tlenu przez skórę u żab. Acta Biol. Exper., 4, 1—32.

Leivestad H. 1960. The effect of prolonged submersion on the metabolism and the heart rate in the toad (*B. bufo*). Univ. Bergen Arbok Nat. Rekke, 5, 1—15.

Poczopko P. 1956. Cutaneous vasomotor response in the edible frog (*Rana esculenta* L.) to changes in carbon dioxide and oxygen concentration. Bull. Acad. Polon. Sci. Cl. II, 4, 397—403.

Poczopko P. 1957. Further investigations on the cutaneous vasomotor reflexes in the edible frog in connexion with the problem of regulation of the cutaneous respiration in frogs. Zool. Pol., 8, 161—175.

Poczopko P. 1958 a. Wpływ atmosfery wzbogaconej w CO_2 i O_2 na naczynia włosowate skóry żaby wodnej (*Rana esculenta* L.). Studia Soc. Sci. Torunensis, Sec. E, 4, 1—28.

Poczopko P. 1958 b. On the seasonal variability of cutaneous vasomotor reflexes in the edible frog (*Rana esculenta* L.). Zool. Pol., 9, 115—129.

Poczopko P. 1959 a. Changes in blood circulation in *Rana esculenta* L. while diving. Zool. Pol., 10, 29—43.

Poczopko P. 1959 b. Respiratory exchange in *Rana esculenta* L. in different respiratory media. Zool. Pol., 10, 45—55.

Serfaty A., Guental J. 1943. La résistance de la grenouille à l'asphyxie lors d'une immersion prolongée. C. R. de la Soc. de Biol., 137, 154—156.

Spurway H., Haldane I. B. S. 1954. The comparative ethology of vertebrate breathing. Breathing in newts, with general survey. Behaviour, 6, 8—19.

Strawiński S. 1956. Vascularization of respiratory surfaces in ontogeny of edible frog, *Rana esculenta* L. Zool. Pol., 7, 327—365.