Albumin concentration in plasma of two related species of *Ceratophrys* (Anura Leptodactylidae) from two different environments

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Summary. Albumin concentration in plasma of two related species of *Ceratophrys, C. cranwelli* and *C. ornata* was analyzed and shown to be higher in the former species, which is the adaptive form found in dry areas.
Key words. Ceratophrys; plasma albumin; species differences; osmotic regulation; hydric balance.

Amphibians represent a transition between aquatic vertebrates and those whose development is totally terrestrial. Among the different problems they have to cope with in terrestrial life, one of the most important is dessication. Because of water shortage important adaptations occur, especially in those species inhabiting arid regions. These adaptations, which tend to reduce the loss of water, are shown through epidermic changes such as skin sculpturing[1], seasonal variation in skin sensitivity to neurohypophysial hormones[2], or cocoon formation[3]; at the renal level, through reduction of urine formation during the dehydration stress[4] and uricotelism[5]. Behavior patterns in amphibia such as skin lipid wiping[6] or burrowing in the dry season[7], also constitute major adaptations to terrestrial life. Furthermore, the possibility of synthesizing major quantities of seroalbumin, which in amphibians increases in the later embryonal stage[8], could be one of the osmotic regulation factors that made possible the colonization of the dry environments.

This work analyzes the albumin concentration in plasma of two sibling species[9] of the genus *Ceratophrys* from different climatic areas of Argentina. One, *C. cranwelli,* lives in a subtropical dry region, with a relatively low humidity and an intense solar radiation. Rain falls mostly in summer[10]. The other, *C. ornata,* is found in humid subtropical regions with a moderately warm climate and rain throughout the year. A predominant characteristic of this region is the relatively high humidity[11].

Materials and methods. C. ornata were captured in Maschwitz and Benavídez, in the outskirts of Buenos Aires, while *C. cranwelli* were collected in Gobernador Crespo (Santa Fé Province). 12 animals, males and females, were used for this study (6 of each species). The frogs were anesthesized with ether (Rhodia Argentina SA) and the blood extracted by heart puncture was collected in test tubes with 0.1 ml of heparin (Abbott Laboratories Argentina S.R.L.).

For the electrophoretic analysis on cellulose acetate[12] of the plasma proteins, barbital buffer (Merck) 8.24 g/l, pH 8.6 was used. The volume of plasma applied was about 3–4 µl/16 mm. Voltage was adjusted to 200 V for 60 min. The proteins were stained with Ponceau S 0.5% solution.

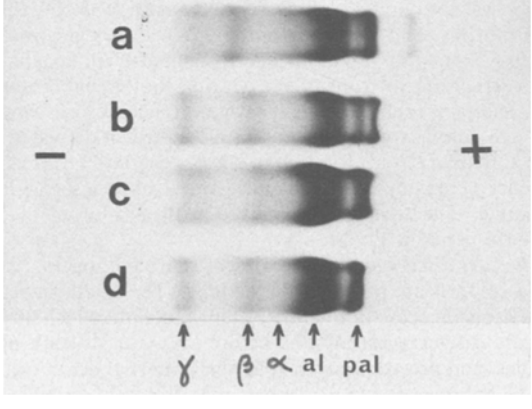
The protein fractions were measured quantitatively by elution with 80% acetic acid. Absorbance was read in a Crudo Caamaño 3, 14 B Spectral colorimeter at 530 nm.

Results. The figure shows four plasma samples; one from a specimen of *C. ornata* (fig., a) and three from specimens of *C. cranwelli* (fig., b, c, d). The albumin fraction is clearly evident in each sample, preceded by one or two bands of prealbumin. The fractions corresponding to alpha, beta and gammaglobulins appear towards the cathode. The table shows the absolute concentrations of albumin and total globulins and the albumin to globulins (A/G) ratio of each specimen. Differences between the two species were tested for albumin and total globulins concentrations and for the A/G ratio. As the distributions of the variables we are dealing with are unknown, and because few animals were examined, the U Man-Whitney test was applied. Significant differences were observed for albumin concentration and for the A/G ratio with $0.1 < p < 0.025$ for both. On the contrary, when the absolute values of total globulins were compared no significant difference was found.

Discussion. The osmotic regulation factors are very important in the complex hydric balance of amphibians[13]. Albumin is recognized as the protein with the highest capacity to attract and retain water[14]. Besides, by virtue of its small molecular weight and its highly charged state at physiological pH's, it seems to be the most suitable protein to ensure the osmotic balance. The capacity for synthesizing more or less albumin, or any other protein, is genetically determined. The different concentrations of albumin found in the plasma of these two species is due to the fact that they differ in their number of chromosomes: *C. cranwelli* is diploid (2n = 26) and *C. ornata* is octoploid (2n = 104). These major karyotype alterations as well as tetraploidy and translocations have also contributed to speciation events in other amphibians, as indicated by Mahony[15] in the case of the Australian leptodactylid *Neobatrachus.* The A/G ratio analyzed by electrophoresis in different species of South American amphibians seems to be higher in those whose ecology shows an evident independence of aquatic environments, especially in some adaptive species of the arid regions[16]. In accordance with the above-mentioned data, our results show a higher A/G ration in *C. cranwelli* than in *C. ornata* because of the higher concentration of albumin in the former species, which among other features, allows it to inhabit desert areas where water is only present in the rainy season.

Absolute concentrations of albumin and total globulins and the A/G ratio of each specimen. [a]absolute concentrations in g/100 ml.

	Ceratophrys ornata						*Ceratophrys cranwelli*					
Animal	1	2	3	4	5	6	1	2	3	4	5	6
Albumin[a]	3.11	3.28	2.78	3.58	2.57	2.44	3.82	3.24	3.80	3.30	4.33	3.49
Total globulins[a]	2.98	2.35	2.80	3.40	2.81	2.59	3.43	3.10	2.36	2.80	2.30	2.23
A/G	1.04	1.39	0.99	1.05	0.91	0.94	1.11	1.04	1.61	1.18	1.88	1.56



Electrophoresis on cellulose acetate of four plasma samples. a: *C. ornata:* b, c, d: *C. cranwelli;* pal: prealbumins, al: albumin, α: alphaglobulins, β: betaglobulins, γ: gammaglobulins.

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Senescence and dying signals in a reef building coral

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Summary. Decreases in two physiological processes (reproduction and growth) have been shown to precede the advent of colony death in the branching coral *Stylophora pistillata*. These diminutions were sometimes detectable even 6 months and more before any first visible sign of tissue mortality was observed.
Key words. Coral; dying signals; growth rate; reproduction; senescence; *Stylophora pistillata.*

There is little disagreement with the principle that an individual organism eventually accumulates physiological decrements that increase its likelihood of dying[2]. However, there appears to be some controversy regarding the presence of aging and dying processes in the phylum Coelenterata. For example, Strehler[3] indicates that among the coelenterates 'there are probably nonaging species, animals which may not undergo a regular senescence and animals which quite regularly and systematically die as individuals but their contents being returned to the colony'. He[3] summarizes that coelenterates of the class Anthozoa may fit into a category of organisms which fail to show aging as a whole because of a continual replacement regimen. Moreover, regarding reef building corals, it was long ago stated and has never been disproved that a coral 'is a living thing that knows no time of youthful vigour, no waxing to a period of adult life, no waning to senility – it knows no age – it practically knows no natural death'[4]. Therefore, when the death of a coral was recorded in field experiments, while other neighbor colonies still survived, it was suggested to be a result of competitive interactions and natural enemies[5], or was related to both physical and biological disturbances[6] rather than to internal aging and dying processes. In this report we describe decrements of two important physiological processes (reproduction and growth rate) in the branching coral *Stylophora pistillata,* preceding the advent of colony death. These diminutions could sometimes be detected even 6 months and more before any visible sign of damage or partial tissue mortality was observed. We conclude that such physiological decline may be the first harbinger of forthcoming natural mortality of the whole colony.

Materials and methods. Two sets of experiments were conducted on the reproduction and growth rate in the reef building coral *Stylophora pistillata.* The reproduction of *S. pistillata* was studied extensively in Eilat, Gulf of Eilat, Red Sea during 1974–1982[7–9]. During the course of this study we also followed the reproductive status of 20 mature colonies first sampled in Dec. 1976, and thereafter sampled 2–3-times each year during three reproductive seasons. These colonies were carefully chosen for their size (large colonies, more than 20 cm in diameter) and for their state of health (without dead branches or tissue damage). This study was ended 39 months later with the death of the last colony. Gonads were regularly examined every month using histological sections and planula-larvae were collected in situ with plankton nets or in the laboratory by putting mature colonies in aerated aquaria[7,8]. *S. pistillata* has a long period of reproduction, lasting approximately 8 months (from December to July). During the reproductive period synchronization in breeding exists in different branches within a single colony. However, no such synchronization has been found among colonies within the population. *S. pistillata* is a hermaphroditic species. Ovaries and testes are situated in the same polyp and attached to the septae by stalks. In the first year of reproduction the vast majority of the population contains male gonads only. With increase in size there is a tendency towards an increase in the percentage of hermaphroditic colonies[7,8].

Rates of growth of *S. pistillata* populations were studied during 1978–1981 in shallow (3–5 m) and deep water (27–30 m) colonies by using a radioisotopic tracer, ^{45}Ca[9,10]. In one set of experiments we traced the calcification rates of three different populations, sampled regularly every three months, during a period of 21 months, between Feb. 1978 and Nov. 1979: deep water colonies (n = 41), shallow purple (n = 30), and shallow yellow (n = 30). In deep water colonies it is impossible to distinguish between the color morphs, and these colonies exhibit a dark-brown color resulting from the zooxanthellae's pigments. All incubations were carried out in front of the Marine Biology laboratory at Eilat. Samples, each containing several branches, were carefully cut from colonies using pliers and incubated underwater with $^{45}CaCl_2$ (0.05 μ Ci/ml) for 24 h in closed transparent tanks. Tip branches were sampled and the surface area of each segment was calculated. The tissue was removed with a solution of 30% hydrogen peroxide[9,10].

Results and discussion. The modes of reproduction in 20 examined colonies are presented in the table. The results indicate a remarkable decrease in the rate of reproduction, which appears in many cases several months before the natural death of the colonies, and precedes any visible sign of partial death or damage. In colonies 6 and 14 (table) this dramatic decrease was recorded even 10–12 months before the first visible degeneration of tissue appeared. Of 6 colonies which were found dead following a sampling period of high reproductive rate (h+ or h++,