Aquatic Respiration: An Unusual Strategy in the Hellbender Cryptobranchus alleganiensis alleganiensis (Daudin)

Abstract. *Separate and simultaneous determinations of aerial and aquatic gas exchange in the giant salamander,* Cryptobranchus alleganiensis alleganiensis *(Daudin) were made at 5°, 15°, and 25°C. The aquatic respiration of this animal accounts for over 90 percent of the total volume of oxygen consumed and 97 percent of the total volume of carbon dioxide released at all temperatures. The lungs of these individuals are large transparent sacs which are poor respiratory organs; the lungs probably function more as hydrostatic structures than as gas exchangers. This animal is the largest aquatic vertebrate that lacks gills and yet utilizes almost exclusively an aquatic mode of respiration. Specialized cutaneous modifications, a unique body form, and a peculiar behavioral mechanism are of considerable adaptative significance, and confer to the skin the effectiveness of a veritable "gill."*

The physiology of amphibian respiration has been extensively investigated in recent years (*1–3*). However, the sparse information on the respiration of the large aquatic salamander, the hellbender (*Cryptobranchus alleganiensis alleganiensis*), is based largely on indirect evidence from anatomical investigations which were often contradictory. For example, some researchers ascribed considerable importance to aquatic buccopharyngeal respiration in *Cryptobranchus* (*4–6*), while others discounted it (*7, 8*). Still others have described the lungs as elaborate and well developed (*8–10*), yet most investigators acknowledge that these organs are simple transparent sacs which probably function poorly in respiration. Most authors attributed prominence to cutaneous respiration, but others contended that the lungs can also be important in gas exchange (*5, 6, 8, 10*). Another investigator (*11*) suggested that the internal gills (structures that are nonexistent in the adult) were the principal organs of respiration.

Because of the aforementioned inconsistencies, we examined the relative role of pulmonary and cutaneous surfaces in the external respiration of *Cryptobranchus* at 5°, 15°, and 25°C and attempted to determine whether there were significant changes in gas exchange partitioning at the different temperatures. The respirometric techniques have been described previously (*12, 13*). A special Plexiglas respirometer with separate aquatic and superadjacent aerial compartments was used; manometric, titrametric, and polarographic techniques were utilized to monitor oxygen consumption ($\dot{V}_{\mathrm{O}_2}$) and carbon dioxide release ($\dot{V}_{\mathrm{CO}_2}$). All mean gas exchange values are in microliters per gram per hour (standard temperature and pressure dry) in Table 1.

The external respiration of *Cryptobranchus* was predominantly aquatic at all temperatures. The aquatic $\dot{V}_{\mathrm{O}_2}$ represented 93.89 percent of the total $\dot{V}_{\mathrm{O}_2}$ at 5°C, 89.42 percent at 15°C, and 92.24 at 25°C. The aquatic $\dot{V}_{\mathrm{CO}_2}$ prevailed and accounted for over 97 percent of the total $\dot{V}_{\mathrm{CO}_2}$ at all temperatures. Our findings, therefore, establish *Cryptobranchus* as principally a water breather and substantiate a number of previous inferences based on indirect evidence (*4, 5,*

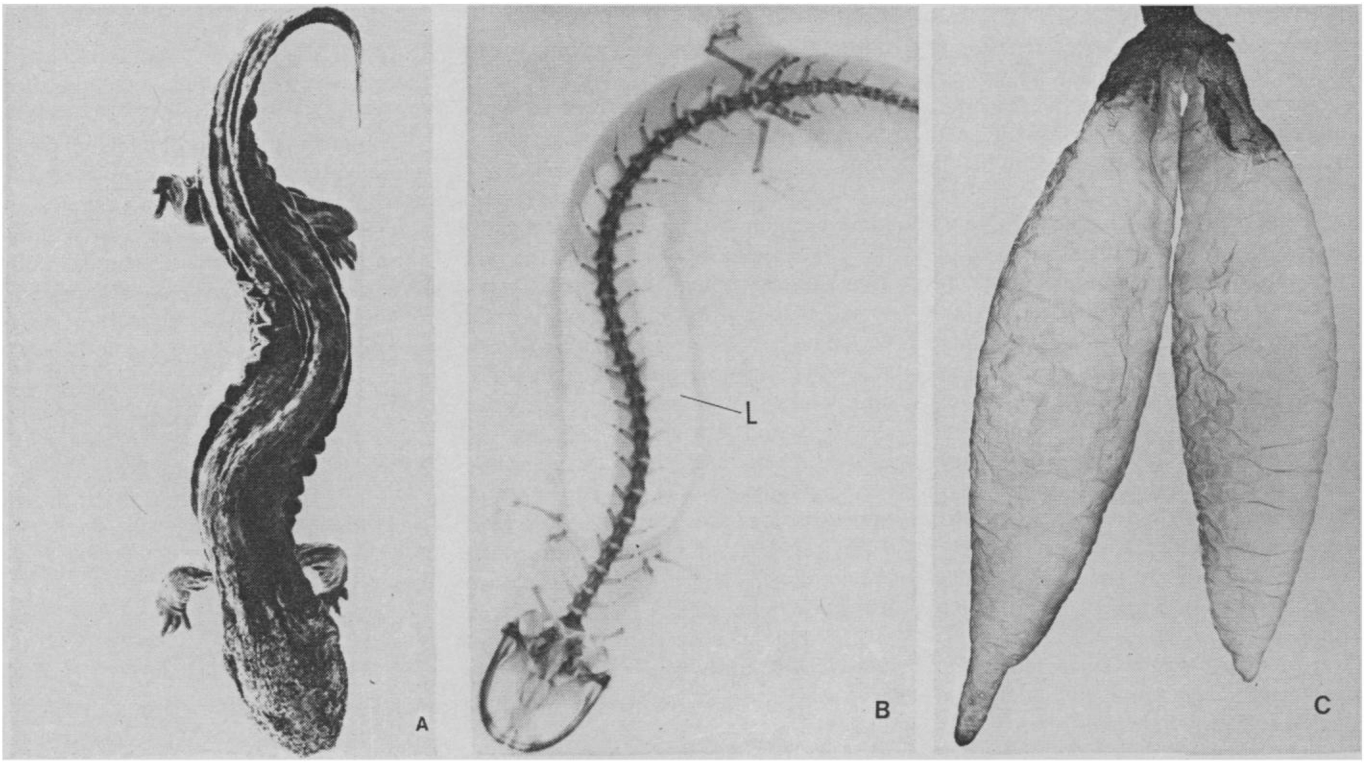

Fig. 1. (A) Dorsal view of the large aquatic salamander *Cryptobranchus*. Note the extremely dorsoventrally flattened body form and the numerous folds of skin along the margins of the body, both of which contribute to the efficient cutaneous respiration of this animal. [Photograph courtesy of I. Kneeland, J. Bent, and P. Samuelson] (B) Radiograph of a live *Cryptobranchus* showing the large volume the lungs (*L*) occupy in the body cavity. [Radiograph by R. E. Frates and J. T. Hanks] (C) Inflated air-dried lungs of a 400-g *Cryptobranchus*. The lungs appear to be no more than large, transparent, and poorly vascularized air sacs. [Photograph by P. Samuelson]

Table 1. Aerial and aquatic oxygen consumption (V_{O_2}) and carbon dioxide release (V_{CO_2}) in the hellbender, *Cryptobranchus alleganiensis*. The abbreviations are: S.E., standard error of the mean; *N*, number of specimens.

Temperature (°C)	Mean weight (g)	Total hours	*N*	V_{O_2} (μl g^{-1} hour^{-1})		Aerial O_2 (%)	V_{CO_2} (μl g^{-1} hour^{-1})		Aquatic CO_2 (%)
				Aerial (mean ± S.E.)	Aquatic (mean ± S.E.)		Aerial (mean ± S.E.)	Aquatic (mean ± S.E.)	
5	514	120	24	0.53 ± 0.10	8.15 ± 0.65	6.11	0.19 ± 0.07	6.40 ± 0.58	97.12
15	511	115	23	2.29 ± .27	18.57 ± .93	10.98	.26 ± .08	15.84 ± .99	98.39
25	423	115	23	2.40 ± .17	28.52 ± .97	7.76	.54 ± .10	24.54 ± 1.15	97.85

7, 9). Although the lungs of this animal are not suited as efficient respiratory organs, they do occupy a large volume of the body cavity (Fig. 1B). When extirpated and air-dried, the lungs appear as large, transparent, and poorly vascularized sacs (Fig. 1C). These organs, therefore, probably function more as hydrostatic organs than as gas exchangers.

How can such a large animal (adults attain lengths of 25 to 60 cm and can weigh over 1 kg) whose gas exchange is primarily aquatic survive without gills? Several investigators have concluded that the buccopharyngeal oscillations of amphibians have little to do with gas exchange and are chiefly associated with olfaction. Czopek (*14*), for example, noted that little gas exchange can take place in the buccopharynx since only 1 to 3 percent of the total respiratory capillaries of amphibians are located in that region. Other authors (*2, 15*) contended that the continued renewal of buccal air compensates for the paucity of respiratory capillaries, and in certain lungless salamanders the buccal cavity accounts for 15 to 24 percent of the total $\dot{V}_{O_2}$ (*1*).

The ventilatory mechanism of *Cryptobranchus*, however, differs substantially from that of most anurans and urodeles. This salamander does not exhibit separate buccal oscillations interrupted by intermittent lung ventilations; we observed only infrequent pulmonary ventilations and no consistent aquatic buccal ventilations (*16*). These findings show that the aquatic respiration in *Cryptobranchus* represents cutaneous gas exchange only and does not support the previous speculations (*4–6*) that buccopharyngeal respiration is of significance.

A few basic strategies are utilized in the external respiration of aquatic amphibians. Those organisms, such as *Necturus*, with poorly developed lungs have elaborate and efficient gills that can account for as much as 60 percent of their total gas exchange (*12*). On the other hand, efficient air-breathing aquatic salamanders have an elaborate lung structure, but gills are either poorly evolved or are completely absent as in *Amphiuma* and *Siren* (*13, 17*). However, *Cryptobranchus* has neither elaborate lungs nor gills.

Previous investigations have shown that the unmodified integument of amphibians is important in gas exchange, particularly in $\dot{V}_{CO_2}$ (*1, 2, 12, 13*). *Cryptobranchus* has additional cutaneous modifications that confer to the skin exceptional capabilities for gas exchange. Unlike those of most amphibians, the cutaneous capillaries of *Cryptobranchus* penetrate through the epidermis to the surface cell layer, greatly reducing the O_2 and CO_2 diffusion barriers between the water and the cutaneous blood supply (*7, 18*). In addition, the animal has an exaggerated dorsoventrally flattened body with reticulated folds and flaps of skin (Fig. 1A). These folds, which hang conspicuously from the margins of the body and legs, not only increase the integumentary respiratory surface area, but are also richly supplied with an extensive capillary network (*7*); thus, the skin functions as a veritable "gill."

Cryptobranchus also exhibits a behavioral peculiarity that further enhances the efficiency of the cutaneous surface as a gas exchanger. We frequently observed that resting *Cryptobranchus* will periodically rock or sway from side to side. This rocking maneuver continually breaks the boundary layer between the water and skin and ensures that the body surface and, in particular, the hanging folds of skin are kept in continuous contact with oxygenated water. These rocking motions were also noted previously (*7–11*), but the significance was never alluded to.

Twenty-two specimens of *Cryptobranchus* (mean weight, 355 g) were kept submerged in water saturated with air for 2 weeks at 5°, 15°, and 25°C. All the animals survived the 2-week period, even at the higher temperatures, which illustrates that the lungs are not essential for adequate respiratory gas exchange. These results were in sharp contrast to those obtained with the Congo eel, *Amphiuma means* (11 animals; mean weight, 174 g), which perished when submerged longer than 2 days at 25°C (*13*). The latter aquatic species, therefore, is an obligate air breather at higher metabolic rates. Other studies of the cardiovascular system of *Cryptobranchus* also support the finding that the lungs function very little in gas exchange. Efficient air-breathing amphibians always possess a complete atrial septum and well-developed spiral valve; the latter structure, situated in the truncus arteriosus, serves to divert the oxygenated blood to the systemic circuit while the venous blood is directed to the pulmonary circuit (*7, 19*). *Cryptobranchus* and other water-breathing amphibians typically have hearts that have greatly perforated atrial septa and lack a spiral valve. As a consequence, blood coming into the heart from the pulmonary circuit becomes thoroughly mixed with the venous blood before it leaves the heart and reduces the efficiency of pulmonary respiration (*7, 19*).

The large aquatic salamander, *Cryptobranchus*, therefore, employs an unusual strategy in carrying out its external respiration: it utilizes special cutaneous modifications, a unique body form, and a peculiar behavioral pattern, which collectively confer to the integument an exceptional respiratory capability.

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Polymorphism in Plastosciara perniciosa

Abstract. *Two widely divergent morphotypes of both adult males and adult females were found in laboratory colonies of* Plastosciara perniciosa *in Hawaii. Extraordinary modification of all features except the genitalia, with associated behavioral differences, enables the micropterous morphotype to take maximum advantage of a stable larval habitat. Capabilities for migration from adverse habitats, dispersal, and maintenance of gene flow are retained in the macropterous morphotype.*

An unusual degree of behavioral and morphological polymorphism was discovered in laboratory colonies of *Plastosciara perniciosa* Edwards, a small fly (Diptera, Sciaridae) common around potted plants and in greenhouses. Four basic morphotypes are involved: (i) macropterous males; (ii) macropterous females; (iii) micropterous males; and (iv) micropterous females (*1*). Some intermediates were noted but these were rare. Colonies were reared for 4 years and one, started from a single gravid macropterous female, was maintained for 28 generations. The mean developmental time of eggs, larvae, and pupae at 20° ± 2°C was 27.3 days (*2*). The larval, pupal, and adult behavior of the micropterous morphotypes differed significantly from that of the macropterous morphotypes.

The male and female macropterous morphotypes (Fig. 1) resemble most winged Sciaridae, except that they have reduced two-segmented maxillary palpi and are small in size. Sexual dimorphism includes differences in genitalia and minor differences in wing and antennal lengths (*3*).

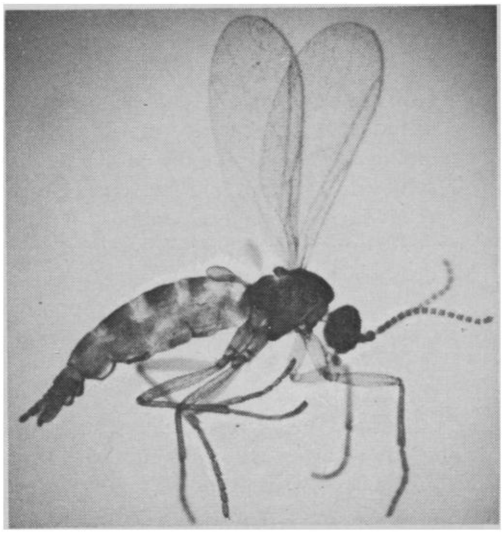
The micropterous morphotype of each sex (Fig. 2) differs so markedly from the corresponding macropterous morphotype that, except for the fortunate circumstance that they were reared from a single macropterous female, they would not be considered conspecific or even congeneric (*3*). In fact, according to most existing keys to families the micropterous morphotypes would not even be placed in Sciaridae. All structures of the head are reduced, especially the antennae, compound eyes, ocelli, maxillary palpi, and proboscis. The thoracic sclerites are greatly reduced in size and are modified to the degree that homologies with those of typical adult Sciaridae, including the macropterous morphotype of the same species and sex, are obscure. The wings, halters, and legs are much reduced and lack most normal structural features. The abdomen is enlarged but discrete sclerites are absent. The genitalia are similar in both morphotypes of each sex. The rare intermediate forms are either macropterous morphotypes with brachypterous wings or micropterous morphotypes with functional legs.

The behavioral differences between the two morphotypes were extreme. The female macropterous morphotype oviposited on the surface of the substrate or between the substrate and the thin layer of organic debris. Larvae generally fed on the surface. Late fourth instar larvae constructed individual pupal chambers near or on the surface of the agar substrate. Pupae forced their way out of the pupal chambers before adult emergence. The adults courted and mated as described by Steffan (*4*).

The female micropterous morphotype oviposited within the pupal chamber. Larvae usually burrowed, fed, and remained beneath the surface of the substrate. Late fourth instar larvae constructed communal pupal chambers, each containing at least one male and one female. Larvae pupated within the chamber, and after eclosion adults mated and females oviposited and died within the chamber. The first instar larvae consumed the dead adults and left the pupal chamber.

Micropterous morphotypes were always produced when an individual pupal chamber containing one or more gravid micropterous females was transferred to fresh agar plates. When a gravid macropterous female was isolated in a fresh agar plate or vial, the F_1 generation also was micropterous (*5*). The macropterous morphotypes were produced only when colonies were maintained in the same plate for more than one generation.

The evolutionary significance of the unusual degree of polymorphism in *P. perniciosa* is related to the apparent ability of this species to take advantage of a favorable microhabitat. The micropterous adult is protected from the danger of predation, inability to find a



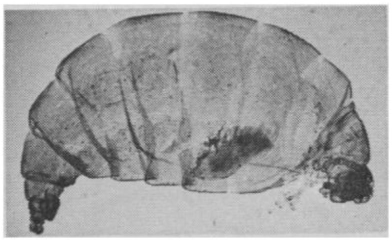


Fig. 1 (left). Macropterous female morphotype of *P. perniciosa*; body length, 1.49 mm; wing length, 1.12 mm. Fig. 2 (right). Micropterous female morphotype of *P. perniciosa*. Excepting the terminalia, note modification of all parts of the body, especially the wings, halters, and legs; body length, 1.76 mm; wing length, 0.15 mm.