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COMMENTS ON THE DESCRIPTIONS AND EVALUATIONS OF TADPOLE MOUTHPART ANOMALIES

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Abstract.—**Some terms used in published accounts of the interactions of tadpole mouthparts and the chytrid fungus *Batrachochytrium dendrobatidis* are incorrect, inaccurate, or controversial. I suggest means to correct these problems. A first estimate of terms that describe oral anomalies (e.g., malformities versus deformities) in larval amphibians is presented.**

Key Words.—***Batrachochytrium*; chytrid; mouthparts; tadpole**

The sudden appearance of a chytrid fungus, *Batrachochytrium dendrobatidis* (Bd) that attacks amphibian keratin caught all relevant researchers unaware. At that point, and continuing to the present, we did not have sufficient information on many facets of the developmental biology and ecology of tadpoles to understand what was happening. Also, it seems likely that the differences in research perspectives, techniques, and terminologies among disciplines (e.g., pathologists / mycologists - histology, histochemistry, and genetics versus herpetologists - morphology and ecology) may have contributed to the problem. None of the researchers in the two major research camps had previously encountered such a detailed involvement of larval amphibian biology.

In the process, we were poorly prepared, and we have been lax in the usage of available terms. The following is a discussion of this situation. This is not a data paper or a discussion of tadpole development and morphology (see appropriate chapters in McDiarmid and Altig 1999 and citations therein; Altig 2007). The use of a given citation below does not imply it is the only relevant one. Regretfully, permissive usage of terminology is not an uncommon or a new phenomenon (e.g., Engle 1925).

***Keratinization and Chytrid Infections.*—**Berger et al. (1998) wrote that "Bd attacks keratinized structures. . ." This idea needs to be revisited on the grounds of possible larval epidermal keratin (e.g., Miyatani et al. 1986; Suzuki et al. 2001). Based at least on histological sections, we would surely be more accurate to say that Bd attacks keratinizing tissue or those tissues fated to keratinize. Keratinized tissue is dead, while keratinizing tissues are alive and mitotic. Bd cells sometimes become trapped within keratinized jaw sheath tissues, but their number and appearance suggest that these populations are not totally viable.

Several frustrating usages appear in Marantelli et al. (2004). Consider "live zoospores [author's terminology] were in the part of the tooth row closest to the mouth." Based on their figures and text, one sees obviously viable zoospores not in the keratinized teeth, but in the soft tissue of the tooth ridge (= very crudely analogous to mammalian 'gums' but not associated with the jaws) where the keratinized teeth are rooted in living, mitotic tissue. A labial tooth is formed from the activities of one cell, and thus zoospores cannot be 'in' a tooth and not in a transversely linear array of teeth, a tooth row, as stated.

Another poor usage of terms by Marantell et al. (2004) is "The jaws and tooth rows are covered by cornifying epidermal cells that are continually growing…" The use of probable synonyms, 'cornifying' and 'keratinizing,' confusingly implies different activities. Also, because a keratinized labial tooth is never multicellular like a jaw sheath, it is not covered by 'cornified' epidermal cells. The jaw sheaths are formed of many compact layers of keratinized cells formed in the mitotic zone at their bases and thus are not covered by any normal epidermal cells. Dead keratinized cells are not constantly growing; only the basal mitotic cells can do so. Last, the use of 'jaws,' suggesting the jaws of the tadpole (i.e., suprarostral, infrarostral, and Meckel's cartilages) instead of jaw sheaths, is a miscommunication.

The term hyperkerotosis suggests a hyperproduction of keratinized tissues, an action that seems counter to the argument that Bd destroys or disrupts keratin or the process of keratinization. Keratinized skin cells of adults may become more numerous, but is this caused by hyperproduction or a reduced rate of sloughing (i.e., an analog of human skin tanning when irritated by UV light).

The legend of Fig. 5 in Marantelli et al. (2004) notes the presence of hyperkerotosis but the figure lacks a notation for the reader to locate it. If the authors are referring to probable sloughing of keratinized cells, the inclusion of a noninfected control would allow one to differentiate between the proposed pathology and the normal, rapid sloughing that occurs from the jaw sheaths. From my experience, histological processing of these brittle, keratinized tissues often results in cellular disjunctions, probably of tissue about to be sloughed at the time of preparation, that do not imply pathology.

Marantelli et al. (2004) noted that "Keratin also extended a short distance caudally from the jaws along the surfaces of the buccal cavity." First, they meant 'jaw sheaths,' and their implication of hyperkerotosis appears to describe the typical morphology of the buccal face of the upper jaw sheath. The current concept of hyperkerotosis in chytrid-infected tadpoles is tenuous, and a depiction of the sloughing of keratinized cells is not a verification of Bd.

***Pigmentation of Keratinized Mouthparts.*—**A number of papers report depigmentation of keratinized mouthparts (e.g., survey in Knapp and Morgan 2006), although verification of pigmentation and its loss is lacking. Depigmentation says that structure is present but pigment is reduced to absent; artificially bleached hair is an actual analogous case of depigmentation of a keratinized structure - structure is present, pigment is reduced or absent. For example, Fellers et al. (2001) illustrated the presence of pigment at the tips of the jaw sheaths and the tooth rows in a

fairly normal individual and the absence of that pigment at these sites in a severely infected individual. In fact, the keratinized jaw sheaths and teeth are present in the first case (not just pigment) and absent in the second. Histological illustrations in their paper show that structures are missing in the second case, not the presence of depigmented structures. Besides the jaw sheath being absent, the normally V-shaped (in cross-section) mitotic zone that forms the jaw sheath is at least severely compromised if not absent. Knowing if the mitotic zone and thus a sheath can recover from this degree of damage would be informative. What is being interpreted as depigmentation actually represents partial, sometimes localized, or complete absence of structure caused by disruption of basal mitotic tissue, not attacks on keratinized tissues or depigmentation of these structures. Rachowicz (2002) rightfully pointed out that all such anomalies are not associated with Bd.

What are the data that verify that keratinized mouthparts are pigmented? Luckenbill (1965) labeled a few granules in the interior of the formative tissues of the jaw sheaths as melanin, but she never analyzed their composition; these granules seem too large, too rare, and in the wrong histological position to be melanin granules. Furthermore, ectotherms retain the various pigments within organelles inside of chromatophores. This arrangement allows for the intracellular redistribution of pigments that causes metachrosis. In contrast, the endothermic chromatophores extrude the pigment into the surrounding extracellular areas, and thus these animals cannot undergo metachrosis because the pigment cannot be redistributed (Smith 1960). I conclude that labial teeth and jaw sheaths are dark because they are the keratinized (i.e., condensed, pycnotic cells filled with fibrous protein) product of one cell (e.g., labial teeth) or the keratinization of many cells (e.g., jaw sheaths). If mouthparts were pigmented, one would expect the keratinized mouthparts of albino and leucistic tadpoles to be white, but they are dark even with the lack of melanic pigments (Corn 1986; Gradwell 1976; Smith-Gill et al. 1972; pers. obs.).

Tooth Row Gaps.—Tooth row gaps that are part of the normal morphology (e.g., medial gaps in the second upper row and first lower tooth rows common in tadpoles with 2/3 tooth rows) is caused by a consistent, embryologically-produced gap in the tooth ridge, not simply the lack of teeth. Staining with Crystal Violet to add contrast to translucent tissues will show this. Tooth row gaps in atypical positions are caused by the absence of teeth even though the tooth ridge remains intact, not the presence of depigmented teeth (see Fellers et al. 2001: Fig. 1B).

I propose the following scenario to explain a total break in a jaw sheath that is not caused by physical damage. If one scans along a sheath from an area of normal morphology across such a break to normal structure on the other side of the break, one sees a progressive reduction in sheath width without jaw serrations, the break where no visible keratinization exists, and a progressive increase in keratinized tissue without serrations until one returns to a normal sheath structure. In histological sections across such an area, one would likely see this sequence of Bd infection: (1) none; (2) progressive increase in either severity or duration; (3) constant degree of at least some minimum that would cause an absence of keratinization; (4) a progressive decrease in severity or duration as one moved toward normal structure; and (5) none. The absence of jaw serrations in the intermediate areas surely holds information about the mechanisms of sheath formation and thus, the pattern of its disruption.

The examination of the edge of a disrupted jaw sheath that does not involve a total absence of keratinized tissue provides a different scenario. With progression of the intensity or duration of the infection, it may grade into the situation discussed above. Bd cells may be carried to the surface during sheath growth, but I propose that damage to the edge of the sheath, as in the case above, is not caused by an active destruction of the keratin at the point of the visible damage. These areas actually are caused by the slowing down or cessation of basal cell mitoses to a lesser degree than above. This failure, as if one left out some internal bricks in a wall that is continually being increased in height by adding bricks at the bottom, leaves a disrupted area in the sheath once the zone of missing cells reaches the surface; thus, the surfaces appears jagged because keratinized cells have been added too slowly or haphazardly. These kinds of anomalies heal quickly if the infection abates so that cells are again added basally in sufficient numbers, and the rather short lag time for such healing reflects the time it takes for new cells to transit from the mitotic base to the surface (pers. obs.). Whether or not the damage alters mitotic rates is not known.

The term ‘keratinized’ needs to be standardized to distinguish between the viewpoints of various research fields. Molecular weight and chemical determinations of the keratin among species and stages would likely show differences that are reflected in the physical characteristics of the structures. These kinds of variation may also reflect the interspecific differences in Bd susceptibility. Also, histologists can detect either keratin or its precursors chemically well before morphologists can do so structurally. I view keratinization as the production of morphological structure with an implied function, and the nonkeratinized basal cells that propel the process are not of interest to a morphologist in the way that they are to a histopathologist.

Tadpole Ecology.—We need to know a lot more about the growth processes of tadpole mouthparts and tadpole developmental ecophysiology before we can accurately assign cause and effect. For example, some mouthpart anomalies may be associated with temperature (Rachowicz 2002). Whether this concept is causative or correlative is unknown. The same may be true with data in Bresler (1954). I suggest that temperature is only the proximal factor and we need to know either the ultimate factor or ultimate interactions (e.g., physiological or energetic response, cyclic changes in mitotic rates, and photoperiod or light quality). Additional studies like that of Rachowicz (2002) performed with tighter controls are needed to answer many questions. Do tadpoles that are truly starved (i.e., absolutely no food sources, including bacteria and protozoans, and thus difficult to attain in laboratory experiments) reduce or lose mouthparts regardless of temperature? Do tadpoles reared on different food sources, at different densities, at different temperatures under different light conditions reduce or lose mouthparts? Are there fixed or variable patterns of mitosis in the mouthparts and what influences them? How do stored energy reserves respond in all of the above? Rachowicz’s (2002) suggestion that feeding increases the strength of the mouthparts is intriguing but questionable; for this to occur, feeding versus nonfeeding tadpoles would have to produce keratin of different chemical or physical characteristics (perhaps by some sort of energetic trade-off), or keratin would have to change its physical characteristics in response to work. Because we know nothing about the physical or chemical characterizations of the keratin in

tadpole mouthparts, collaborations with materials analysts would surely be fruitful.

Keratinized structures change with time and unknown conditions other than by wear. These changes seem more common in some taxa than others and are common in cultured tadpoles. Taxa with long larval periods or those that overwinter would be prime research targets because of the environmental variations encountered over seasons. For example, assays of oral anomalies in *Ascaphus* tadpoles are of interest. The taxon is basal within anurans, the tadpoles overwinter 1-3 years at different elevations, and they live in cold streams that often are cleaner than many lentic sites. Tadpoles of *Lithobates catesbeianus* that overwinter multiple years, one year, or not at all on a north-south gradient throughout its range are a ready-made experiment; congeners that do not overwinter accompany this species throughout its range. At the moment, we have no real idea what promotes or inhibits keratinization, but citing Bd as the universal causative agent surely is erroneous.

Ancillary Comments.—A number of other situations can be mentioned that influence the study of tadpole anomalies. I know of no data that shows that Bd disrupts the early embryological events that form the tissue topography of the soft mouthparts. As one of many examples, the illustration of the tadpole of *Hyla* [*Hypsiboas*] *freicanecae* (Carnaval and Peixoto 2004) shows a fourth lower tooth row that merges with the marginal papillae, and labial teeth occur on scattered submarginal papillae. The authors noted that these anomalies surely are not within the normal variation of the species. The causes of changes in the topography of the mitotic beds that result in abnormally arranged tooth rows remain unexplained.

Supplying a truly suitable food source for cultured tadpoles is not an easy task because even typical rasping tadpoles not known to be cannibals or carnivores are probably more carnivorous than herbivorous (Schiesari 2004; also Altig et al. 2007). In light that nutrition surely influences growth and thus mouthpart formation or repair, one should avoid feeding cruciferous (Brassicaceae) plant material (Brem 1965). The effects of temperature, stage, and various ecological factors on the activities of hind gut fermenters (Pryor and Bjorndal 2005a–b) should be examined. More data on the developmental processes of tadpole mouthparts and perhaps additional rounds of pathological tests (e.g., Pessier 2002 and citations therein) are needed, and time-course studies (i.e., observations of the same individuals repeatedly through ontogeny) are prime requisites. Momentary snapshots of a dynamic process obtained from observations of preserved specimens may mislead us; conditions that are deemed as severe in a snapshot may be quite transient and inconsequential overall. The common absence of comparisons with uninfected individuals (or internal controls that involve infected and noninfected parts of the same specimen) is dangerous. Common absences of keratinized structures in tadpoles that test negative for Bd should raise an immediate alarm. Also, because of the inherent difficulty of identifying tadpoles and with a hopeful increase in our abilities to diagnose various problems, research specimens should be archived if we wish the data to be useful through time; a second look after additional data are obtained may be quite revealing.

Finally, we must be aware of all presumed deviations from normal morphology. Some may include ecological effects on morphology that are actually within the normal range of variation (Kraft et al. 2006; Relyea and Auld 2004, 2005). One rather commonly finds short sections of bi- or triserial tooth rows in a species that typically has uniserial tooth rows; are these cases malformities, deformities, atavisms, lax developmental controls, damage during development, or none of these? Other structures with presumed high mitotic rates need to be examined (e.g., buccopharyngeal papillae, skin, and viscera). The jaw sheaths, jaw musculature, and labial teeth of cannibalistic *Spea* tadpoles are grossly different from those of their omnivorous siblings (McDiarmid and Altig 1999). The fact that these changes occur postembryologically is astounding (Altig 2006), but such situations are not anomalous only because we understand the biology in this case.

Terminology of Anomalies.—I end with a first estimate for restrictions of definitions. 'Anomaly' is proposed as a generic term applicable to any sort of non-normal morphology when one cannot, or does not, choose to commit to a cause. No implications are made about the occurrence, severity, or duration of an extraneous insult, when the insult occurred, or when the effects of the insult are seen relative to its proposed occurrence. Eventually we would hope to be able to distinguish between proposed anomalies caused by normal ecological factors, injury, and extraneous insults. The immediate (i.e., larval viability) and eventual (i.e., reproductive fitness of adults) effects of any larval anomaly are largely undocumented (see Parris and Beaudoin 2004).

A 'malformity' (i.e., bad + formed) is an anomaly caused by a perturbation of embryological processes; improperly formed structures are not recoverable once embryological processes have finished. Proposed agents (e.g., parasites, xenobiotics, environmental factors, and genetic or developmental components) can qualify the term. Malformities include such cases as multiple limbs and missing eyes (e.g., Meteyer et al. 2000) and perhaps some kinds of soft tissue anomalies (e.g., tooth ridge disruptions; see Carnaval and Peixoto 2004 above).

A 'deformity' (i.e., properly formed embryologically at least to some point and subsequently de + formed) is an anomaly caused by a perturbation of postembryological events. Deformities are produced after development has ceased but not necessarily the growth or subsequent production (e.g., jaw sheaths and labial teeth). Deformities commonly are partially or completely recoverable via future mitoses involved in growth, healing and regeneration (D. Drake, unpubl. data) if the suspected insult is removed or reduced. The oral anomalies of keratinized tissues discussed in this paper are deformities.

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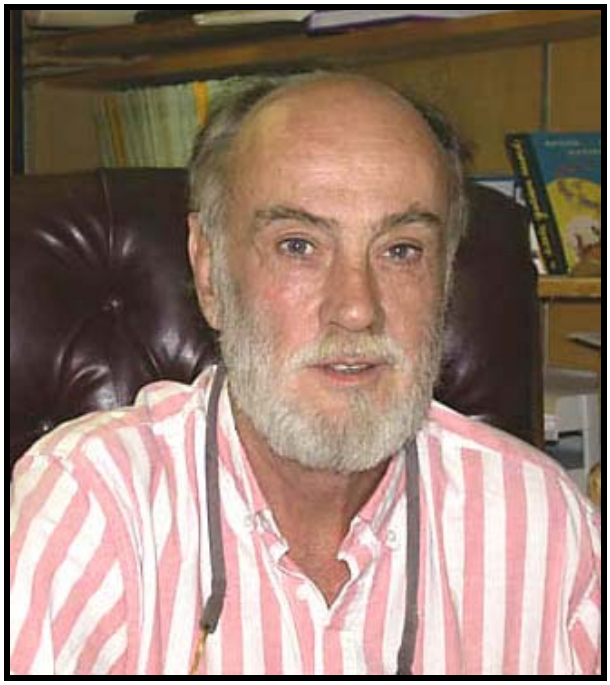

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