# The Mechanism of Ultraplanktonic Entrapment in Anuran Larvae

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*ABSTRACT* Tadpoles of several different genera were fed graded suspensions of uniform polystyrene particles to determine the lower size limit of particles that could be ingested. Certain tadpoles can extract suspended particles as small as 0.126 $\mu$ in diameter from the water. In terms of particle size, this is an efficiency comparable to the best mechanical sieves that can currently be produced by man. A mechanism for ultraplanktonic entrapment is proposed on the basis of scanning electron micrographs of the secretory ridges in the branchial food traps of *Rana catesbeiana* before and after feeding.

*Xenopus* tadpoles in yeast suspensions modify their clearance and buccal pumping rates in response to varying food concentrations. This may be an adaptation for maintaining a constant input of food mass to the tissues that extract the food from the water.

Variability in the lower size limit of filterable particles among tadpoles of different genera correlates with the availability of suspe ided matter in the microhabitat where these tadpoles may be found.

The ability of anuran larvae to extract suspended food matter from the surrounding water is well established (Dodd, '50; Savage, '52; DeJongh, '68; Kenny, '69a; Severtzov, '69). However, the efficiency of the mechanism in terms of particle size and clearance rates has never been examined comparatively. Such information is important for understanding the function of the feeding mechanism and the adaptive significance of its diverse morphology in tadpoles of different families, genera and species. Tadpoles of certain families (Pipidae, Rhinophrynidae and Microhylidae) which lack keratinized external mouth parts have been assumed to be more specialized for filter feeding than larvae of other families (Kenny, '69b). Experiments reported here determine the lower size range of particles that may be extracted by certain tadpoles and support the generalization that tadpoles which lack the oral shearing apparatus of most anuran larvae are the most efficient tadpoles in terms of their suspension feeding capabilities. An introductory experiment reveals that clearance rate varies in proportion to varying food concentration. Scanning electron micrographs (S.E.M.) of the tissue presumed to be responsible for ultraplanktonic entrapment in anuran larvae permit new interpretations of the mechanism of food entrapment. Finally factors responsible for variation in clearance rates are considered.

## MATERIALS AND METHODS

Tadpoles of *Ascaphus truei* (Ascaphidae), *Rana pipiens* (Ranidae), *Xenopus laevis* (Pipidae) and *Rhinophrynus dorsalis* (Rhinophrynidae) were kept before and during tests in aerated, deionized water to which a mineral source (Longlife Water Conditioner, Sternco Industries, Inc., Harrison, New Jersey, USA) was added. An eight hour period in this medium was allowed for tadpoles to clear their intestines. Tadpoles were staged (Gosner, '60), weighed to the nearest 0.1 gm and measured (total length to nearest millimeter). They were then transferred to suspensions of latex particles. Tests consisted of placing four tadpoles of each species in four liters of suspension. The tadpoles were selected so as to be nearly identical in size, developmental stage and weight. Control tanks, identical to the experimental tanks but lacking the tadpoles, were used to correct for any clearance due to the slow settling of particles.

The polystyrene latex particles (Diagnostic Products, Dow Chemical Co., Midland, Michigan, 48640 USA) used in the feeding experiments have several advantages over other inert particles used to study suspension feeding (see Jorgensen, '66). They are uniform in size (one std. dev. always $<0.007\ \mu$) and have a density close to that of organisms available as food to a fresh water planktonic feeder (1.05 gm/cc). In concentrations above 50% the particles tend to form irreversible aggregates; because of this property particles removed from suspension by tadpoles precipitate as conspicuous solid fecal strands. Particles with diameters of 0.088 $\mu$, 0.126 $\mu$, 0.264 $\mu$ and 0.557 $\mu$ were used in these experiments.

Scanning electron micrographs (plate 1) of the collecting surfaces in the branchial food traps of two *Rana catesbeiana* larvae (stages 30 and 31 and snout-vent lengths 24 mm and 22 mm, respectively) were made with a Cambridge Stereoscan. One tadpole was kept for 15 minutes in deionized, minerally reconstituted water before being fixed. The other tadpole was kept in a $4.9 \times 10^6$ cells/cc concentration of *Saccharomyces cerevisiae* (approximately 3–5 $\mu$ in diameter when in suspension) for an equal amount of time, then anesthetized in tricaine methane-sulfonate (Sandoz MS 222). Both tadpoles were fixed for 48 hours in 10% formalin buffered near neutral with $Mg(CO_3)_2$, washed in tap water and transferred to 70% ethanol.[1] The tadpoles were dissected in 70% ethanol and tissue samples were dehydrated through an alcohol series, dried at the critical point in Freon (following the method of Cohen, Marlow and Garner, '68), and coated with a thin film of gold in a vacuum evaporator.

## RESULTS

*Ascaphus truei, Rana pipiens* and *Xenopus laevis* all show some ability to ingest 0.557 $\mu$ particles based on the clearing of the suspension and the presence of fecal matter. *Xenopus* was far superior to the others, while *Ascaphus* barely surpassed the control in clearing this suspension. *Ascaphus* could not noticeably extract particles 0.264 $\mu$ in diameter and the ability of *Rana* tadpoles to clear this suspension was greatly diminished. *Xenopus*, on the other hand, effectively removed both the 0.264 $\mu$ and 0.126 $\mu$ particles and *Rhinophrynus dorsalis*, although not tested in suspensions of larger diameter particles, demonstrated clearance abilities comparable to *Xenopus* for the 0.126 $\mu$ suspension. All species failed to show any sign of ingestion with the 0.088 $\mu$ particles. The superiority of both *Xenopus* and *Rhinophrynus* at extracting very small particles is evident; indeed, the smallest particles that these larvae can remove must be in the range of 0.1 $\mu$ in diameter, or smaller than the most minute bacteria.

Absolute clearance rates can be determined in some organisms by indirect spectrophotometric methods (Jorgensen, '43). Using these techniques, an attempt was made to quantify the relative efficiency of tadpoles of different species in clearing suspensions of different particle size. If individual organisms clear the same volume regardless of particle concentration, any decrease of calculated clearance rates in suspensions of diminishing particle size serves as a measure of relative efficiency. Preliminary experiments, using *Xenopus laevis* larvae, failed to validate the necessary condition of constant clearance. In a typical experiment (fig. 1) using six *X. laevis* (stage 36–37, snout-vent lengths 15–18 mm, mean wet weight 0.5 gm) in three liters of suspension, the tadpoles first showed an ability to clear the suspension when the concentration dropped below approximately $4 \times 10^5$ cells/cc. In lesser concentrations the larvae exhibited a rapid, non-linear increase in clearance rate.

Variation in clearance rates may be reflected in buccal pumping since tadpoles do not pump at a constant rate under uniform temperature and oxygen conditions. Pumping rates for a sample of *X. laevis* (N = 12, stage 55–56, snout-vent lengths 16–18 mm, mean wet weight 0.5 gm) plotted against concentration of yeast in aerated, thermally controlled water showed decreases in direct proportion to increasing concentration above the level of $10^6$

[1] An assortment of fixatives was tried but the best results were obtained with neutralized formalin. As this is a standard fixative for museum collections of anuran larvae, much material stored in museums may be available for restudy using S.E.M.

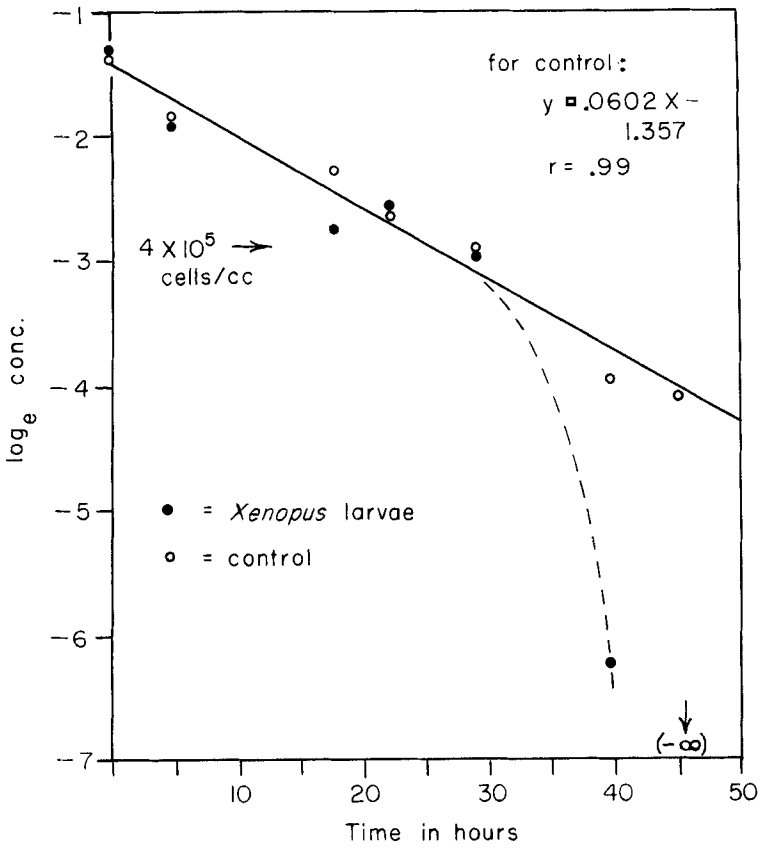


Fig. 1 Clearance rates of *Xenopus laevis* in a suspension of *Saccharomyces cereviseae*. The concentration of yeast was determined using a Bausch & Lomb Spectronic 20 spectrophotometer read at 420 mμ. Spectroscope readings correlate well (r = 0.984) with actual cell counts. Until the concentration drops below 4 × $10^5$, the paired readings for the experimental and control tanks do not differ significantly. Below this concentration the experimental tank, represented by the dashed line, clears rapidly and completely ($-\log_e$ concentration $\rightarrow -\infty$).

cells/cc (fig. 2). Below concentrations of $10^6$ cells/cc pumping rates rose rapidly.

### *The ultraplanktonic entrapment mechanism*

The most impressive of the above observations is the generally high efficiency of tadpoles in extracting ultraplankton. Certain tadpoles are as efficient, in terms of particle size, as the best mechanical sieves designed by man. With the purification of water as a growing environmental health problem all effective methods of filtration are of interest, including those found in nature.

In tadpoles the gill filters proper have a pore size of 5 μ, so other structures may be involved in ultraplanktonic entrapment (Kenny, '69b). Both Savage ('52) and Kenny ('69a,b) have suggested that the structures called the crescentic organs, collecting organs or branchial food traps may directly capture particles on mucus. Kenny ('69a) has published a whole mount photomicrograph of yeast cells on the ventral surface of the anterior filter valve in the branchial food traps of *Phyllomedusa trinitatis* (Hylidae). The photo is focused on the tops of the secretory ridges where the cells appear to be concentrated. While my S.E.M. study substantiates the general idea of mucus capture on this tissue, it also provides grounds for modifying many

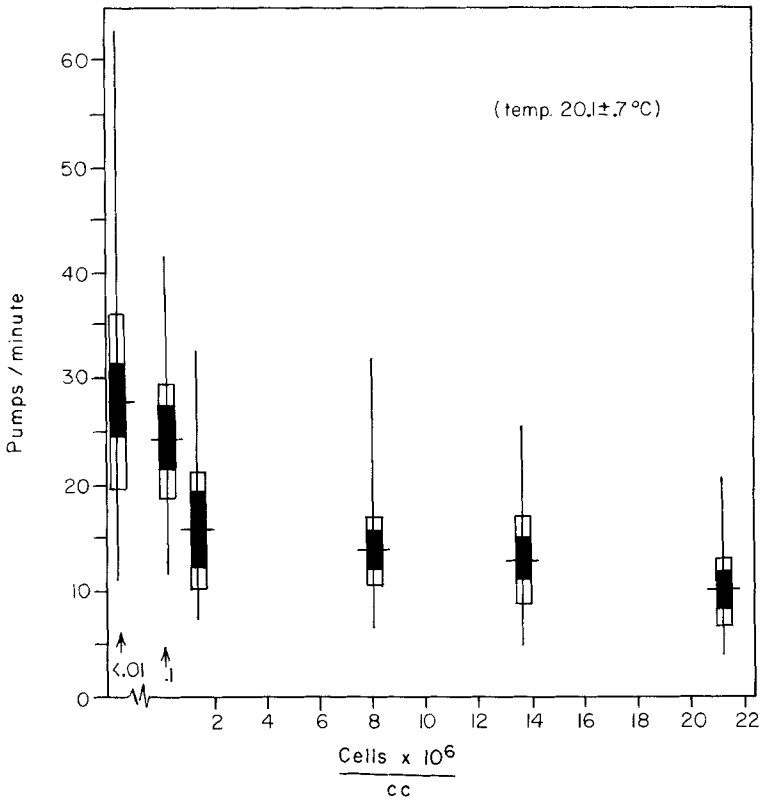


Fig. 2 Buccal pump rates for *Xenopus laevis* in various yeast concentrations. Three measurements each were taken from 12 individuals for each sample (N = 36). Horizontal lines represent means; vertical lines, ranges; largest rectangles, one standard deviation about the means; solid inner rectangles, twice the standard errors about the means.

of the theoretical details previously proposed.

The basic anatomy of the surface being discussed is shown in figure 3. Here the *Rana* tadpole was fixed without being fed and the surface is virtually free of mucus and particulate matter. The ridged form of the tissue is clearly evident. A photomicrograph taken at higher magnification (fig. 4) shows the elevated, secretory zone composed of the narrow clustering microvillous apices of the columnar secretory cells. The sides of the ridges and the troughs are lined with a non-secretory, simple squamous, paving epithelium (compare with the standard light micrographs of Kenny, '69b). The ridges are not perfect, at least in *Rana*, and occasional gaps may be seen (fig. 3) where the secretory zone is discontinuous.

Kenny felt that the mean direction of currents over this surface would be perpendicular to the general axis of the ridges. Eddies would be likely to form on the down current sides of the troughs. This would increase the chances of particles coming in contact with the secretory zone where mucus was presumed to be steadily secreted. It was postulated that particles are entangled in a single mucous string that travels along the crest of the ridge, eventually reaching the ciliary groove and the oesophagus. A major problem, admitted by Kenny, was the movement of the mucuous string along the ridge, for no cilia are present on this tissue in any tadpole.

The scanning electron micrographs (fig. 5, 6) of the tadpole that was fed yeast before being fixed show that the mucus is not in discrete strings. Further, the captured yeast cells are not restricted to the

tops of the ridges. The entrapment process may be hypothesized to act as follows. A particle carried by a local vortex up the back side of a single ridge meets with increased turbulence at the boundary, where textural differences between the squamous and secretory cells disrupt laminar flow. The chance of a particle contacting the surface is high. A particle that hits the surface might stop immediately or be carried a short distance, dragging a mucous strand behind it. Such strands can be seen in the center of figure 5. As more particles make contact with the secretory zone, additional mucus is removed, eventually forming webs such as are seen in figure 6. The strands themselves increase the surface area available for entanglement, thus further increasing the chances of an individual particle being trapped. Such webs as that in figure 6 might readily trap much smaller particles than the yeast cells.

With continuous impact of food particles, clumps of entangled cells are formed (see lower right corner of fig. 5). It is doubtful that clumps of this nature are moved along the ridge, particularly in *Rana* where the secretory zone is occasionally disjunct. It is more likely that as the clumps grow they become more and more an obstacle to the main flow of water and finally get so large as to be swept free of the surface. These clumps could be carried farther back in the mouth by the currents and eventually reach the oesophagus. Thus, this specialized collecting surface functions primarily in causing single small particles to coalesce into larger units. The patchiness of mucus on the collecting surface suggests that localized webs are constantly being created and eroded by this process as the tadpoles feed.

The above mechanism explains why Kenny rarely found mucous strings on the secretory ridges in his specimens, despite his belief that mucus was constantly secreted. In the mechanism proposed here one would not expect to find a uniform field of mucus on the ridges. Unlike static filters, mucous web "filters" are transient, being generated when there is particulate matter to entrap.

If mucous web entrapment is indeed a major component of ultraplanktonic suspension feeding, can some aspects of this mechanism account for differences in the lower size limits of particles that various tadpoles can effectively entrap? Presumably both the chemistry of the mucus and the shape of the secretory zone at the tops of the ridges accounts for the mesh-like form of the mucous strands. If the ridges were not present, the mucus would likely form an adherent film on the adjacent cells. Slight differences in the pattern of the secretory cells and spacing of the ridges could have a profound effect on the structure and strength of the mucous webs that form. Differences in the characteristics of localized webs could, in turn, determine the limits and modality of particle sizes extracted by a given tadpole. While this speculation must await detailed interspecific comparisons and experimental studies, it is consistent with the fact that Kenny ('69b) has noted differences in the collecting surfaces in the branchial food traps of both *Elachistocleis* (Microhylidae) and *Xenopus* as compared with more typical beaked tadpoles.

The chemistry of the mucus presents a special problem. The substance reacts positively with PAS and Sudan Black B, but negatively with Ninhydrin and Mercury-Bromophenol (Kenny, '69b). Kenny concluded from these and other histochemical tests that the mucus was a glycolipid. While lipids in the mucus need not be specifically glycolipids, a high proportion of lipid compounds may be critical to the physical properties of the secretion. The "cord factor" of tuberculosis bacilli, associated with the characteristic tendency of these organisms to aggregate in long cords, is a glycolipid (Law, '60). Lewis ('71) who has studied the richly lipid cutaneous mucus of fish, made the interesting suggestion that the proportion of lipids, specifically phospholipids, "may determine the relative viscosity of mucus and hence its accumulation on the skin or dispersal into water." As with the fish mucus, one would expect the tadpole oral secretion to be relatively adherent and not rapidly lost to the passing currents. A high lipid component could be the anchoring factor.

### *Regulation of ingestion*

The ultraplanktonic entrapment mech-

anism of a tadpole is most efficient at a certain concentration of suspended matter. If the food is too dense, the system may clog, or mucus may not be secreted fast enough to handle the load. Efficiency will drop. It is of advantage to the tadpole to decrease input of suspended matter during these periods. This is presumably the meaning of the variable rates for clearance and buccal pumping noted above. Along these lines it seems significant that as the suspensions clear both clearance rates and buccal pumping activity intensify in the neighborhood of $10^5$–$10^6$ cells/cc; this is a concentration within the upper range of ultraplankton densities in a naturally occurring eutrophic pond (Stráskrabová and Legner, '69). The ability of a tadpole to regulate its pumping rate in response to varying concentrations of available food may be interpreted as an adaptation for maintaining a constant input of food mass in a nutritionally fluctuating environment. Such an interpretation infers the existence of a finely tuned feedback system for assessing food concentration. The superiority of anuran larvae over the static filters of engineers can be seen in this regulatory ability.

Interspecific differences in filtering efficiency deserve a final comment. The proficiency of *Xenopus* and *Rhinophrynus* for suspension feeding is consistent with their planktonic habitus in eutrophic ponds and pools. *Ascaphus* larvae, in contrast, cling to rocks in torrential streams by means of an expanded oral disc modified into a sucker. They have heavily keratinized mouth parts which are used to scrape algae from the rocky substrate. Their comparatively poor ability to extract suspended matter is consistent with their substrate habitus in an oligotrophic environment. Anatomical differences in internal feeding structures among these and other tadpoles are now under investigation.

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PLATE

## PLATE 1

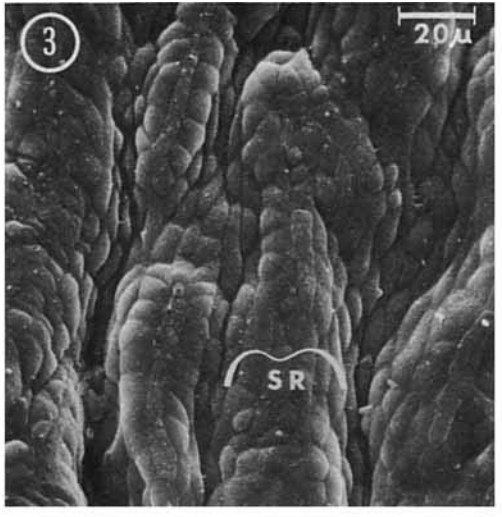
### EXPLANATION OF FIGURES

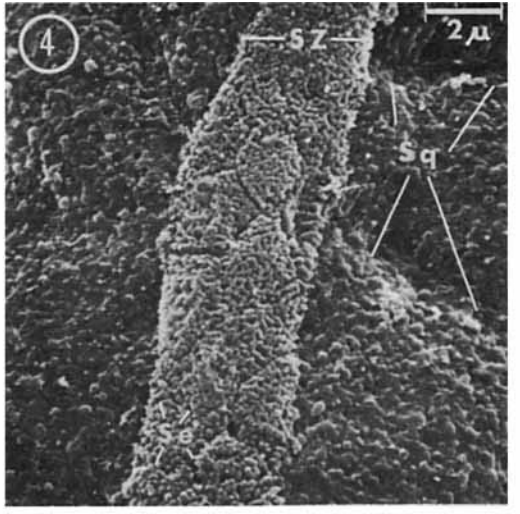
3 S.E.M. photograph of a segment of the ventral surface of the anterior filter valve (branchial food trap) from a *Rana catesbeiana* larva showing the secretory ridges (sr). The tadpole was not fed before being fixed.

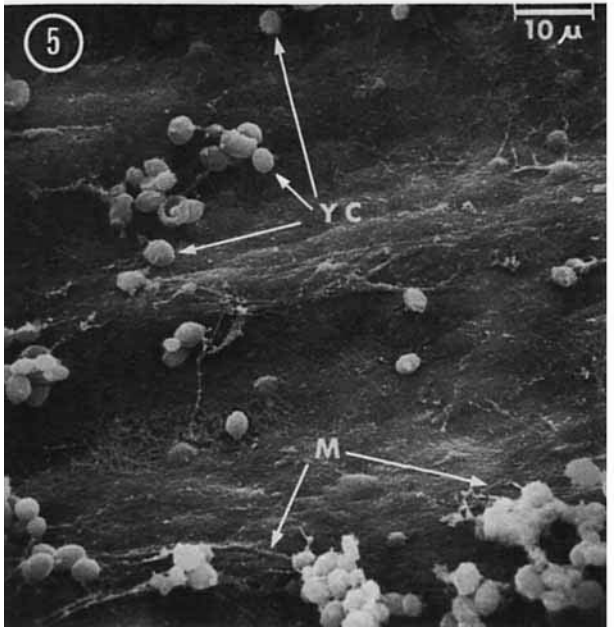
4 S.E.M. photograph of the top of a secretory ridge from the region shown in figure 3. The pustulate apices of individual secretory cells (se) comprise the secretory zone (sz). The secretory zone is bound by simple paving squamous cells (sq).

5 S.E.M. photograph of a segment of the secretory ridges in a *R. catesbeiana* larva kept in a concentrated yeast suspension for 15 minutes before fixing. Yeast cells (yc) are trapped in mucus (m) on the surface.

6 Same specimen as figure 5. S.E.M. photograph showing yeast cells (yc) trapped in a mucus web (m).


3
20μ
SR


4
SZ
2μ
Sq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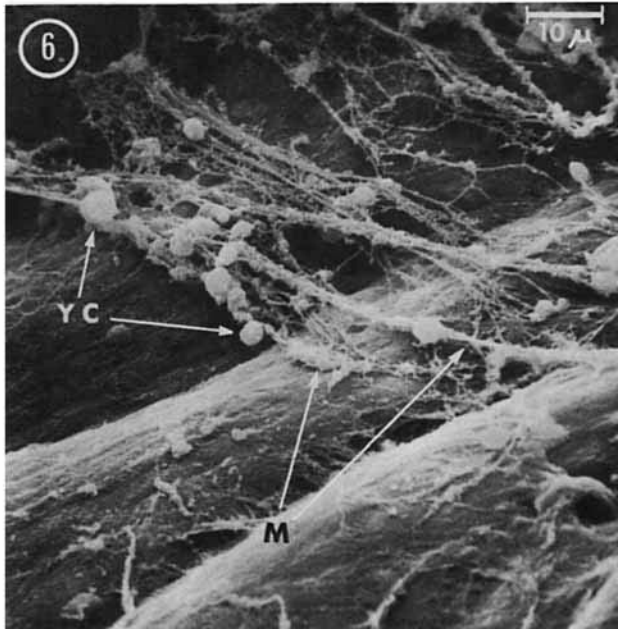
10μ
YC
M


6
10μ
YC
M