LOCOMOTION OF THE TELOTROCH CILIATE *Opisthonecta henneguyi*

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I INTRODUCTION

A. GENERAL DESCRIPTION OF OPISTHONECTA HENNEGUYI

Opisthonecta henneguyi (Fig. 1) is a free-swimming vorticellid inhabiting freshwater. The organism moves constantly and usually rotates on its longitudinal axis as it swims aboral region foremost during the telotroch stage. Neither and attached nor a stalked stage has ever been observed for *Opisthonecta.*

The form of the organism is cylindrical, with the adoral region somewhat truncate, and with the aboral region slightly larger and hemispherical. Encircling the peristomal disc are two rows of adoral cilia (actually membranelles) ten microns long which spiral in counter-clockwise direction one and one-fourth turns before entering the opening of the buccal apparatus. An aboral circlet of membranelles encircles the body one-third of the lenght of the organism from the aboral apex. An epistomal tuft of cilia is described by Fauré-Fremiet as "Comme chez cette espèce egalement, il existe une longue membrane epistomienne indépendante de la frange adorale, et formant gouvernail pendant la natation". Lynch and Noble (1931) say that "Just above the opening into the vestibule is a conspiscuous papilla bearing about a dozen very long, delicate immobile cilia or bristles 23 to 27 microns in lenght ("flamme epistomienne" of Fauré-Fremiet). Bradbury (1965) states that the "epistomal membrane is made up of a row of immobile cillia" and is independent of the adoral membranes.

Opisthonecta varies in length from 81 to 170 microns and in diameter from 69 to 81 microns (Fauré-Fremiet, 1906, 1924; Kofoid and Rosenberg, 1940). Except for adoral cilia, aboral membranelles, and epistomal cilia, the organism is bare and transparent. The body, except for the epistomal disc, is transversed by circular ridges. Under high magnification two water expulsion vesicles (contractile vacuoles), a macronucleus, and infundibulum can be seen. Usually anterior refers to the end of the organism which is foremost during locomotion. The adoral region is sometimes foremost in *Vorticella*, but in *Opisthonecta* the adoral region is always posterior, and the aboral end is always anterior.

The activities of the ciliary regions are divided into two special functions. The adoral cilia provide the water currents useful for gathering food particles, and according to Kofoid and Rosenberg (1940) the "epistomal membrane" with its long cilia seems to deflect food particles into the infundibulum. The aboral membranelles, ranging from 15 to 22 microns in length, are primarily the locomotor organelles, and encircle the organism 20 microns from the aboral apex. Observed aborally the waves of the membranelles progress in a clockwise direction (Kofoid and Rosenberg, 1940).

Reproduction is accomplished by longitudinal cell division, and conjugation also occurs. After the preconjugation division the two organisms can be recognized as a free-swimming macroconjugant and a microconjugant (Rosenberg, 1940). The microconjugant enters the aboral apex of the macroconjugant, and the two are permanently fused. After conjugation is completed, the organism, if not subcultured, will encyst in about 72 hours. Before encystment a mucus-like cyst material is secreted and trails the organism. The organism rotates in a clockwise direction until encystment is complete. The cyst wall is transparent and nearly spherical except at the adoral region where an enlargement is noticeable. Excystment is triggered when cysts are cultured in fresh medium (Rosenberg, 1938).

Ultrastructure of the buccal organelles has been described by Lom (1964), Bradbury (1965), Rosenberg and Grim (1966).

B. PREVIOUS DESCRIPTIONS OF LOCOMOTION OF OPISTHONECTA

No detailed study has been made previously of the locomotion of *Opisthonecta henneguyi*. However, several authors have commented generally as follows:

"*Opisthonecta* is almost continuously in motion, although it is a slow and indeffective swimmer. Its motion is retrograde; i.e.; it swims aboral end forward, and revolves clockwise as seen from the oral end. It moves in a close spiral, the aboral end describing larger circles than the oral end. These circles at times become so large that the animal merely "loops the loop" without progressing. When describing such circles the animal turns toward the ventral aboral "corner"; i.e.; toward the point on the aboral end corresponding to the position of the mouth on the oral end. Often it rests on the substratum, on either the oral or aboral pole, and rotates for a time in one place while describing very small circles". (Lynch and Noble, 1931).

"The epistomal membrane is independent of the aboral membranes. It is not immobile as was stated by Lynch and Noble but is constantly flickering back and forth as though it helped to shunt food particles into the pharynx. The adoral membranes give rise to the spiral rotation of the body. The aboral zone of membranelles is the locomotor mechanism which propels the body forward, i.e., in the aboral direction. Owing to the oblique set of individual membranelles in the zone they probably also assist in spiral rotation". (Kofoid and Rosenberg, 1940).

"*Opisthonecta* is in constant motion, rotating on its longitudinal axis as it swims aboral end first. The adoral ciliature is restricted to a narrow zone of strongly beating cilia which encircles the peristomal disc, a broad round area covering the oral pole. The oral cilia are usually in constant motion setting up vertical currents which sweep bacteria and small particles into the buccal overture. Between the two coils of the adoral spiral and a little distance in front of the buccal overture is the epistomal membrane, which is separate from the adoral ciliary spiral. It is made up of a row of long immobile cilia" (Bradbury, 1965).

C. TAXONOMIC REMARKS

Kent (1880-1882) discovered a peritrichous infusorian which he described as resembling the telotrochal plan explained by Huxley (1877) but with modifications. Emphasizing this similarity to the telotrochal plan as being important, Kent proposed the name *Telotrochidium.* He described this organism as having an anal passage opening at the aboral end of the body, and he cited this as fundamentally and significantly different from the typical *Vorticella* in which food vacuoles empty into the oral vestibulum. Kent defined the Genus *Telotrochidium* to include vorticellids which are always free-swimming, not sessile, without a stalked stage, which divide in the telotroch stage, and have a conspicuous anal structure at the aboral extremity of the body. Kent considered his species of *Telotrochidium* to be the same as O. F. Müller's (1786) *Vorticella crateriformis*, and conferred upon it the specific name *Telotrochidium crateriformis*. Fauré-Fremiet (1906, 1924) described a new genus that he designated as *Opisthonecta*. In this description he stated that *Opisthonecta* was different from Kent's description of *Telotrochidium* because *Opisthonecta* had aboral membranelles; the oral region was oppositely located from that of *Telotrochidium*; and *Telotrochidium* did not have an "epistomal membrane" or an undulating membrane associated with the membranelles. He considered the genera to be separate.

The Genus *Opisthonecta* was synonymized with the Genus *Telotrochidium* by Kahl (1935). In comparing *Opisthonecta* and *Telotrochidium*, Kahl (1935) said that the two genera were similar, but he did not explain how they were similar. Kahl noted that only *Opisthonecta* had an "epistomal membrane" and the *Telotrochidium* had a non-functional scopula. On the basis of similarity Kahl synonymized *Opisthonecta* with *Telotrochidium*. Corliss (1961) cited *Opisthonecta* as a junior synonym of *Telotrochidium* as Kahl had proposed previously.

Fauré-Fremiet (1950) in describing the new species *Telotrochidium johanninae*, pointed out that the genus should include members that were related and not just free-swimming forms which result from progeny of any family of Sessilina. By comparing *Opisthonecta* with *Vorticella vaga* (Römer, 1893), basionym of *Telotrochidium vaga* (Römer, 1893), he defended the preservation of Genus *Opisthonecta* by finding the two genera similar but not identical, with *Opisthonecta* having as ancestor *Epistylis fluitans*. Although *Telotrochidium johannine* is different both from *Opisthonecta henneguyi* and Kent's description of the Genus *Telotrochidium*, Fauré-Fremiet defended placing his new species in the Genus *Telotrochidium* by stating that Genus *Telotrochidium* was of the Family Vorticellidae. This seems to be a violation of the earlier generic description of Fauré-Fremiet (1906, 1924) for *Opisthonecta*.

Lom (1964) pointed out one difference as a reason for retaining the Genus *Opisthonecta*, namely the epistomal membrane which he considered to be particularly significant. He further explained that up until now the descriptions of *Telotrochidium* had not included this structure. In addition, Lom stated that the new species *T. alabama* should be placed in Genus *Opisthonecta* because his new species is different from *Opisthonecta henneguyi*, if only by having a shorter spiraling of the infundibulum and a single contractile vacuole.

Although the nomenclature for *Telotrochidium* and *Opisthonecta* has not been definitely resolved, Fauré-Fremiet's (1906, 1924) description seems appropriate for the organism under study. Therefore, the organism will be referred to as *Opisthonecta henneguyi*, after Fauré-Fremiet, 1906. Furthermore, the description by Kent (1880-1882) does not fit any of the organisms described by more recent investigators, and probably is highly inaccurate.

The name *Opisthonecta* meaning "back swimmer" does not distinguish this organism from the telotrochs of *Vorticella* which also swim with the aboral end forward. However, a stalked *Vorticella*, if broken to its point of attachment to the substrate, will swim with the adoral end forward, because of the absence of aboral membranelles.

II MATERIALS AND METHODS

A. ORGANISM

The strain of *Opisthonecta henneguyi* for this study was made available by Professor Lauren E. Rosenberg, University of California, Davis, who isolated the organism from Strawberry Creek on the campus of the University of California at Berkeley about 1935.

B. MAINTENANCE

Opisthonecta was maintained in 100 to 200 ml of culture medium in covered fingerbowls. Subcultures were made at 24 hour intervals so an abundant supply of vigorous motile organisms was available the following day. In subcultures made every 24 hours there was no encystment. Subculture were made by inoculating fresh medium with 2 or 3 ml of culture containing motile organisms which were swimming rapidly in the upper portion of the medium as shown by microscopical observation.

The culture medium (Osterhout's solution) was prepared with glass distilled water. Composition of the solution was NaCl, 0.105 mg; $MgCl_2$, 0.0085 mg; KCl, 0.0023 mg; $MgSO_4$ 7 H_2O, 0.0040 mg; and $CaCl_2$, 0.0010 mg, per liter. All chemicals were procured from commercial sources. Dehydrated grass leaves (Cerophyl Labs., Inc., Kansas City, Missouri) were used in making a 5 % solution with glass distilled water. Aliquots of 1 to 3 ml of the cerophyl solution were dispensed in 100 to 200 ml of the salt solution.

Storage of *Opisthonecta* is easy and convenient since the organism encysts after 72 hours.

C. TYPES OF OBSERVATIONS

In order to delineate the locomotion accurately, two methods were employed: 1) dark field time-exposure photographs at low magnification; 2) cinemicrographic recording on 16 mm film, at high magnification. For some observations 1.17 μ diameter polystyrene spheres were placed in the medium.

D. MICROSCOPES

1. Freshly excysted organisms were studied by means of a dissecting microscope with 12X ocular and 3X objectives.

2. Streak photographs were made with a Leitz Ortholux microscope equipped with a Heine condenser and

apochromatic phase optics. This system of optics provided transitional changes from either phase or dark field illumination without discontinuity. The light source was an Osram xenon-lamp (XBO-162) operated at 4 to 6 amperes. The magnification most useful for photomicrography was obtained with a 10X ocular and 6X and 10X objectives.

3. Motion pictures were made with a Zeiss phase microscope equiped with apochromatic optics. The light source was a carbon-arc lamp. Magnification for cinemicrography was obtained with 10X ocular and with 16X objective.

E. RECORDING EQUIPMENT

All cameras were mounted on drill press stands, as were the Leitz and Zeiss microscope, to insure that no vibrations affected the photographs.

1. Photomicrography

Mounted on the Leitz microscope was a Graphic Polaroid Back camera (Graflex, Inc., Rochester, New York). Polaroid Land picture roll type No. 47 (Polaroid Corporation, Cambridge, Mass.), a very high speed (3000 ASA equivalent) black and white film, was used.

2. Cinemicrography

On the Zeiss microscope a Hycam 16 mm motion picture camera, Model K1001 (Red Lake Laboratories), was mounted. Kodak Double-X Panchromatic negative film with 100 feet per roll, DX X 430 (Eastman Kodak Company, Rochester, New York) was used at 225 to 700 frames per second. The films were commercially processed.

F. FRAME BY FRAME ANALYSIS

A Kodak Analyst movie projector, 16 mm (Photo-Optical Data Analyzer, Model 224, L-W Photo, Inc.), equipped with a reversible frame counter, was employed in a time-and-motion-study and for screening particular sequences. In conjunction with the projector on the floor, a mirror, 1-1/2' X 2', which reflected upward to a glass top table was used for analysis of the organism’s locomotion. Tracing paper was placed on the glass top table, for drawing directly from the projected film. By employing this combination of equipment, many frames in a series could be drawn directly, and could be superimposed to record progressive movement of the cilia and of the organism. In addition, Polaroid pictures of the frames were taken during this time.

G. PREPARATIONS FOR OBSERVATIONS

For photomicrography and cinemicrography a suspension of polystyrene latex particles (Dow Chemical Company, Midland, Michigan), with diameter of 1.17 μ, and distilled water was made by mixing a drop or two of polystyrene latex spheres with one or two ml of distilled water. One drop of culture of *Opisthonecta* and one drop of polystyrene sphere suspension were placed together on a slide, gently mixed for uniformity of dispersion of spheres, and covered with a cover glass that had petroleum jelly evenly applied along the edges, for sealing to prevent drying. An air bubble was included in the chamber to sustain the living organisms and to help maintain steady pressure. No chemicals such as methyl cellulose were added to slow the organism's locomotion.

III OBSERVATIONS

A. MOVEMENTS OBSERVED BY USING THE DISSECTING MICROSCOPE

Opisthonecta henneguyi always swims with the aboral region foremost, and at no time was the organism observed swimming with the peristome foremost. Rapidly moving organisms appear to prefer the upper portion of the medium while the slower organisms with mucus-like cyst material adorally attached preferred the lower part. The

slowest ones were found to be nearest the bottom of the container just prior to encystment. Small and large aggregates of the organisms were seen throughout the medium. From dim light to intense light there appeared no preference by the organisms for one part of the medium to another. In cultures 24 hours old the organisms were secreting mucus-like material that could be seen trailing, and the organisms were swimming in the lower part of the container. During the process of encystment the organisms rotated clockwise adorally until the completion of encystment. At the end of 72 hours the organisms were encysted on the bottom of the container. The cyst were transparent and nearly spherical except for a slight elevation at the adoral region which is the last area to be covered. Transferring cysts to fresh medium triggered excystment. After excystment there was a flourish or longitudinal cell division within a 24-hour interval. These were the vigorous motile organisms used for subculturing as well as for photographing. Macroconjugants and microconjugants were observed after cell division, and conjugation was observed before secretion of mucus-like material. Observations showed rapidly swimming microconjugants and slower swimming macroconjugants (Rosenberg, 1940). The mucus-like material appeared always to be adorally attacher, but whether the mucus-like material was secreted adorally or by the external surface of the organism will require further investigation. Time-exposure streak photographs were taken of the organism with the mucus-like material training.

B. MOVEMENTS SHOWN BY DARK FIELD 1/2 SECOND TIME-EXPOSURE PHOTOGRAPHS OF ORGANISM'S PATHS

The paths of the organism (Figs. 2-13) indicate the diversity of locomotion of *Opisthonecta*. In Figures 2 and 3 the organisms are following an almost unidirectional course, but in figures 4, 5, and 6 the organisms are following a helical course. In figures 11, 12, and 13, the path is circular. There are combinations of movements shown by figure 7 which is almost unidirectional with turning, and by figure 8, 9, and 10 which are helical with turning.

In figures 11, 12, and 13, the organisms are moving in circular paths which are obviously different from the other two described, but the mechanism is not apparent from the photographs.

From these observations the following types of paths can be identified: 1) almost straight linear; 2) helical; 3) almost straight linear with turning; and 4) helical with turning.

Although the rate of movement was not an objective in this investigation, calculations were made of the rates of speed for unidirectional and helical swimming of the organisms. Calculations for unidirectional swimming were approximately 1000 μ per second (Fig. 2), while the calculations for helical swimming were approximately 1200 μ per second (Fig. 6). The fastest organisms seem to move in a helical path.

C. MOVEMENTS SHOWN BY DARK FIELD 1/5 SECOND TIME-EXPOSURE STREAK PHOTOGRAPHS, WITH SPHERES

To further delineate the locomotion of *Opisthonecta*, dark field 1/5 second time-exposure streak photographs, with polystyrene latex spheres in the medium, were taken to show the currents of water set in motion by the aboral membranelles. The distance moved by a sphere in a given time indicates the rate at which the water is moving. The latex particles are useful in delineating the vertiginous patterns of water currents created by the membranelles and by the locomotion of the organism. Furthermore, if the spheres are trailing the organism, it might be assumed that the spheres are enmeshed in a substance secreted and pulled forward by the organisms.

In the streak photographs, figures 15, 16, and 17, the organisms are swimming unidirectionally. The water currents shown by polystyrene spheres are laterally and adorally (i.e., posteriorly) directed in a broad swirling motion that begins behind the aboral tip. The swirling motion is vortiginous and results in a configuration that resembles turbulent flow. The spheres forced in a vertical pattern are circulated adorally and then become redirected aborally to follow the organism. Figure 27 was drawn from an enlargement of photograph figure 16, and shows the direction of the water currents. The curves with arrows describe the paths of the water currents, and the single arrow denotes the direction of forward motion. These observations demonstrate that the adorally directed water currents are symmetrical when the locomotion of the organisms is unidirectional.

Figures 18, 19, and 20 demonstrate that the vortices may be both anterior and posterior and may appear as two to four vortical sections. Figure 18 has two laterally and adorally directed water currents that swirl into a posterior vortiginous motion, and also a mucus-like trail is dragging polystyrene spheres. In figure 19 here is an anteriorly

directed as well as a posteriorly directed vortex, demonstrable by the four circular patterns. The laterally and adorally directed water currents form the same pattern of circular movement as is described for figures 15, 16, and 17. In the region directly behind the adoral region, polystyrene particles are shown being dragged forward in a trailing position. The adherent particles are distinguished from the vertiginous particles by the straight paths while the vertiginous particles move in curved paths. Figure 20 resembles figure 19 except it has four circular water currents instead of three. The vortiginous water currents have two aborally (anteriorly) and two adorally (posteriorly) directed, and also a trail of spheres adherent to mucus. Figure 28 has been drawn directly from an enlargement of figure 20. The curves with arrows show the directions of the swirling motions of the water currents, and the single arrow depicts the forward direction of the organism. The paths of the adorally dragged adherent particles are shown in a long trail depicted by particle streaks. In brief, the organism of figures 18, 19, and 20 show that the locomotion of the organisms is unidirectional, and that the flow patterns form two to four vortiginous configurations. Each organism has a trail formed by polystyrene latex particles being dragged adorally in the direction of locomotion.

Figures 21, 22, and 23 show that the organisms have initiated water currents laterally and aborally directed in circular motion. The organisms of these figures also have extensive trails of polystyrene spheres being dragged. Figure 29 has been drawn directly from an enlargement of figure 21. The laterally and aborally directed curves with arrows show the circular direction of the water currents with the single arrow denoting the direction of the moving organism. This organism has a very long trail of adherent polystyrene spheres being dragged adorally, and the same is true of the organisms of figures 5 and 6.

The streak patterns of figures 24, 25, and 26 are obviously different from all of the previous ones. The latex particles show that the water currents swirl from the aboral region to the adoral region in a broad sweeping curve, but the swirling motion forms a single laterally and adorally directed vortex *toward* the side of turning. The path of the moving organism also forms a segment of a curve. Figure 30 drawn from an enlargement of figure 25 depicts the adorally directed water currents on the inside of the turn shown by the curve with the arrow, and the central arrow shows the direction of the moving organism. In the area outside the turn the paths of polystyrene particles are small sharply curved arcs. The organism does not have a trail of dragging polystyrene particles. These observations show that as the organism turns the large vortical pattern is formed *only* on the inside of the turn.

From the preceding observations of streak photographs the following types of locomotion can be identified: 1) unidirectional movement with two adorally directed water currents; 2) unidirectional slower movement, with one or two adorally and two aborally directed water currents, and polystyrene trails; 3) unidirectional very slow movement with two aborally directed water currents, and with very long trails of polystyrene particles; 4) turning movement with a single broad swirl of adorally directed water currents on the inside of the turn, and numerous sharply curved arcs on the outside of the turn. The fourth item is the opposite of what might be expected.

D. MOVEMENTS DEMONSTRATED BY CINEMATOGRAPHS

High speed cinemicrographs were taken in order to elucidate the actual mechanism of locomotion. The films show a rotation in the counter-clockwise direction rather than clockwise, which is seen with an ordinary microscope. This is caused by reinversion of the image by a prismatic viewfinder and the camera lens. Consequently, rotation is clockwise, as described by Kofoid and Rosenberg (1940), as viewed through an ordinary microscope. The films were projected singly and from 1 to 16 frames per second. Each series of frames was analyzed to show the motion of the membranelles and the relative position of the organism. Several series of frames were drawn and analyzed.

Figure 31 is a consecutive series of frames drawn directly from the projection of a film one frame at a time. The film was taken at 225 frames per second. The diagram for each frame consists of only the aboral region including the aboral membranelles, and the position of each lateral membranelle in the focal plane is shown in relation to the body. Proceeding from frame to frame, the positions of each lateral membranelle demonstrate a sequence of locomotor activity of the membranelles. In frames 2 to 10 each membranelle has changed position by becoming aborally directed. Frame 10 shows that both of the membranelles have become straightened and pointed aborally (anteriorly). Frames 1 to 9 show a normal return stroke (NR). In frames 10 to 18, positions of the membranelles again become aborally directed. These show a normal power stroke (NP). In brief, the pattern of beat for the consecutive frames of figure 31 may be described as having NR frames 1 to 9; NP frames 10 to 18; NR frames 19 to 30; NP frames 31 to 38; and NR frames 39 to 42. The elapsed time (calculated from 225 frames per second) was found to be approximately 0.18 second for the 42 frames, and the frequency of beat was about 10 per second. When the film was projected at 16 frames per second, the locomotion of the organism was found to be unidirectional with no rotation of the body. The epistomal membrane with its long cilia was immobile, but the adoral cilia were moving, and mucus-like material enmeshed with polystyrene latex particles was training. The polystyrene spheres were

observed being ingested.

In figure 31 the membranelles on the two sides are beating almost in phase. However, this does not mean that the aboral zone of membranelles beats synchronously. The beat is actually sequential and counter-clockwise as seen from the anterior end, and this can be seen clearly in the motion pictures.

Another series of frames drawn directly from the projection of another film one frame at a time is shown by figure 32. This sequence consists of 146 frames taken at 700 frames per second. In frame 1 the positions of the two membranelles in the focal plane are different, with the left one adorally oriented and the right one aborally oriented which is also demonstrated in photograph figure 14. In the following 146 frames movements of the two membranelles are not in phase and the one on the right can be seen to beat in reverse. Power strokes are characterized by being almost straight while return strokes are bent. “I” denotes inactivity.

From figure 32 the following data can be obtained:

Left Membranelle	Right Membranelle
Outside of turn	*Inside of turn*
Number of frames	Number of frames
NR-74	I-60
NP-72	RP-46
	RR-40
Most frames in sequence	Most frames in sequence
NR-13	RP-21
Least frames in sequence	Least frames in sequence
NP-5	RR-10

In this sequence the organism was turning sharply to the right as shown in figure 33. However, the diagrams of figure 32 have been reoriented so that the position of the aboral end is relatively the same for each frame; this permits a study of the position of the membranelles in relation to the body, whereas figure 33 will show the position of the body relative to the observer. When a straightened membranelle is moving it is interpreted as being in a power stroke, regardless of direction. On this basis the sequences with straight cilia moving adorally on the left side depict normal power strokes (NP) while those moving aborally on the right side are reverse power strokes (RP). On the left side frames 1 to 11 are stages in a normal return stroke (NR) which had begun prior to the time of frame 1. Frames 12 to 16 show a normal (and rapid) power stroke. The following sequences are: NR frames 1 to 10; NP frames 11 to 16; NR frames 17 to 31; NP frames 32 to 39; NR frames 40 to 54; NP frames 55 to 63; NR frames 64 to 76; NP frames 77 to 86; NR frames 87 to 95; NP frames 96 to 107; NR frames 108 to 117; NP frames 118 to 129; NR frames 130 to 141; and NP frames 142 to 146. In brief, on the left side the membranelles behave very much like those shown in figure 31.

In figure 33 the positions of the organism used in figure 32 are shown for a few representative frames, as denoted by numbers on the figures. The organism is turning abruptly to the right, and the positions of the left membranelles describe the outer circumference of the turn, and right membranelles the inner circumference. The seven normal power (NP) strokes of the left membranelles are denoted by the seven straight lines, and the two reverse power strokes (RP) of the right membranelles are by the two straight lines. The radius of the turn is slightly less than the diameter of the body. The right side of the aboral end on the inside of the turn is at times moved slightly backward as the left side moves forward, and this causes the apparent abnormality of the return reverse strokes shown in figure 32. This sequence of 146 frames covers a time interval of about 1/5 second, and the turn is about 135°. During this period the "epistomal membrane" was immobile. The adoral membranells beat, and the organism rotated slightly on its long axis, as demonstrated by the position of the irregularly shaped macronucleus.

However, the membranelle on the right behaved very differently. It was held immobile (I) almost directly aborally for the first 20 frames, then underwent what appears to be an abnormal reverse return stroke (frames 21 to 37) (Figure 32). The abnormality is more apparent than real, and is caused by the turning to be discussed later. A

reverse power stroke begins in about frame 38 and continues until the membranelle is again directed aborally in about frame 60 (Fig. 32). This membranelle is then held aborally until a reverse return stroke starts in about frame 80. This reverse return stroke begins with a direction of curvature which would be normal for and RR stroke (frames 81 to 95) and then appears abnormal (96 to 105) because of the sharp turning of the organism. This is followed by a rapid reverse power stroke (106 to about 120), and the membranelle remains directed aborally to about frame 140, after which another reverse return stroke starts but is not completed at frame 146.

IV DISCUSSION

A. GENERAL OBSERVATION OF LOCOMOTION OF OPISTHONECTA

Opisthonecta henneguyi, a free-swimming telotroch, swims constantly and rotates on its longitudinal axis clockwise in forward progression with the aboral region foremost. Other investigators (Fauré-Fremiet, 1906, 1924; Lynch and Noble, 1931; and Bradbury, 1965) have reported these same observations. But Kofoid and Rosenberg (1940) reported that "counter-clockwise rotation may occur for short periods; the actual reversal of ciliary motion has not been observed-divided organism spirals clockwise with occasional counter-clockwise movement". During the present investigation the organism was not observed moving in a counter-clockwise fashion. Occasionally, *Opisthonecta* would rotate clockwise at the bottom of the culture on its aboral pole. This is in agreement with Kofoid and Rosenberg (1940) and Lynch and Noble (1931). Occasionally, the adoral ciliature was contracted momentarily, and the organism continued rotating clockwise. This was not observed by Kofoid and Rosenberg (1940) who stated that "the adoral membranes give rise to the spiral rotation of the body". Apparently, the rotation also can be produced by the oblique beat of the laboral membranelles.

The "epistomal membrane", an independent organelle, was immobile as also observed by Fauré-Fremiet (1906, 1924); Lynch and Noble (1931); and Bradbury (1965). However, Kofoid and Rosenberg (1940) stated that the "epistomal membrane" was "not immobile" but flickered back and forth shunting food into the cytopharynx.

At the preconjugation division, one macroconjugant and one microconjugant are formed. The microconjugant moves more rapidly than the macroconjugant. Conjugation takes place at the aboral ends of the free-swimming macroconjugant and macroconjugant. These phenomena were also described by Rosenberg (1940). Encystment was preceded by the formation of adoral mucus-like trails and by concentration of the organisms in the lower portion of the medium. During encystment the organism rotated clockwise on the bottom of the container. These findings seem not to have been observed previously.

B. PATHS OF LOCOMOTION

Helical pathways have been described for many species of protozoa, probably beginning with the work of Nägeli (1860) on flagellates and spores. Jennings (1906) investigated the helical movement of *Paramecium*. He noted that the helical path was caused by rotation of the organism around its long axis, and that this rotation automatically compensated for any deviation of the organism from a straight line. He stated that there were three possible factors which contribute to helicing. These were an oblique stroke of the cilia, the oblique position of the peristome, and the unsymmetrical form of the body. But by a simple experiment of cutting off the posterior part of *Paramecium* behind the oral groove, he found that this severed portion rotated the same as the whole organism. From this experiment he assumed the contributing factor was the oblique stroke of the cilia. Bullington (1925, 1930) also concluded that "all Infusoria regardless of size, shape, classification, or mutilation" followed a spiral (i.e., helical) path due to the oblique stroke of the body cilia in the same direction. Párducz (1953), on the bassis of fixed preparations, arrived at the same conclusion for *Paramecium*.

Schaeffer (1920) stated that all organisms without orienting organs moved in paths by an automatic mechanism "locked up" in the protoplasm. His experiments with amebas, ciliates, and man pointed to the hypothesis that all organisms are similar because they seem to spiral in orderly paths by an automatic mechanism.

The elical path of *Opisthonecta* probably is caused by a combination of slightly unequal forward propulsion (i.e., a slight turning) plus rotation of the body on its long axis. The chief and probably the only mechanism of forward propulsion is obviously the aboral membranelles, possibly with some opposing force from the adoral membranelles. The inequality of forward movement, i.e., a slight turning is probably caused by non-uniform movement of the

membranelles of the adoral zone and also possibly the slightly truncated shape of the adoral end. Rotation on the longitudinal axis is caused by 1) the oblique beat of the aboral membranelles and 2) the adoral zone of membranelles which beat circumferentially, thereby moving food particles toward the citostome. The latter can be seen clearly in the high speed motion pictures.

The use of photographic methods for the study of helical pathways was introduced by Ferguson (1955, 1957, 1959). The method was also used by Dryl (1958), Sears and Elveback (1961), Gittleson and Sears (1964), Sears and Gittleson (1964), Párducz (1964), and Cooper (1965).

Tamar (1965) has recently reported the locomotion of *Halteria grandinella* as determined by photographic phase microscopy. His observations of locomotion of *Halteria grandinella* are interesting since he found that the organism moved forward and backward in helices and "jumped". A real jump, i.e., a spring or leap from the substrate, seems to be hydrodynamically impossible because the organism probably could not have enough momentum to overcome the viscous drag. Therefore this "jumping" must be a very rapid swimming, which could be photographed.

C. VORTICAL PATTERNS

Posterior (i.e., adoral) vortical patterns of the type present in figures 15-17, superficially resemble true patterns of turbulent flow. However, they do not demonstrate turbulence as the term in ordinarily used in hydrodynamics. Turbulence consists of one or more vortices which are caused by the inertia of the water which has flowed past the object (i.e., relatively). The vortices of figures 15-17 are caused by the action of the membranelles and are easily explained on the basis of Newton's third law of motion, and will be designated as "pseudo-turbulence". Hydrodynamically this pseudo-turbulence is a type of rotation

Existence or non-existence of true turbulence can be deduced from the Reynolds number of any moving object. As defined by Reynolds (1883) the number is the ratio of the retarding force of inertia (F_t) to the retarding force of viscosity (F_n), i.e., $R = F_t / F_n$. Computation of this number can be made if the size and velocity of the object and the viscosity and density of the fluid are known. For a ciliate the value of R is approximately 10^{-2}. This means that the retarding force of inertia is only about 1% of the total. The vortical patterns of true turbulence are not pronounced until R is greater than 1 and actually not until it approaches 10 (Jensen, 1959). Reynolds number for *Opisthonecta* is approximately 0.0004. Therefore, on the basis of Reynolds number alone we may rule out the possibilities that these patterns are evidence of true turbulence.

However, in order for a ciliate to move forward, the cilia must push water backward, in accordance with Newton's third law of motion, as expressed in the Law of Conservation of Momentum, i.e., mass times velocity for the ciliate is equal and opposite to mass times velocity for the water that is pushed backward. If a uniformly ciliated has a blunt posterior end, the water is pushed backward as a hollow cylinder, and the organism moves forward inside the cylinder. The space vacated by the organism must be filled by water, and the nearest available water is that of the surrounding cylinder. Therefore, movement of the water of the cylinder is reversed 180°, and it flows inward and forward to fill the space. The path of the water particles is U-shaped and not rotary as in true turbulence.

In *Opisthonecta* the backward flow is caused, not by a uniformly ciliated field, but by the aboral membranelles. These do not beat directly backward, but backward and outward at an oblique angle. This creates conical flow, expanding posteriorly, to form the vortiginous pattern recorded. This pseudoturbulent pattern differs from true turbulence in that 1) reversal of the direction of the water is only about 180°, and is not rotary, and 2) reversal results directly from Newton's law of motion and is not caused by inertia of the water.

D. MUCUS TRAILS

The mucus trails shown in figure 18-23, and figures 28 and 29, may extend behind the organism for at least the equivalent of a half dozen body lengths. These trails are not part of the U-shaped vertiginous patterns discussed above, but may exist in addition to these patterns (e.g., Figs. 19 and 20).

These trails can not be explained on the basis of hydrodynamic flow around the organism nor on the basis of flow caused directly by the membranelles. However, they can be explained on the basis of the secretion of mucus or of a mucus-like substance by the organism and the pulling of this material forward by the organism.

Rosenberg (1938) stated that cyst material was secreted by the whole surface of *Opisthonecta.* The streak photographs figures 18-23 demonstrate that the mucus-like material is adorally attached. It seems possible that if it were secreted all over the pellicle, some polystyrene particles might adhere to the pellicle of the organism, but this was not detected optically. Adherent particles appear only adorally and trailing, while non-adherent particles are forced by the membranelles in a lateral circular pattern towards the adoral region. Trails of adherent particles have been shown photographically (Jahn, Bovee, Dauber, Winet, and Brown, 1965) to be formed by *Paramecium multimicronucleatum* and *Tetrahymena pyriformis* along the oral groove. Since in *Opisthonecta* the mucus-like material is adorally attached, there is a possibility that *Opisthonecta* secretes the substance along the oral region in the same manner. Mucocysts of *Tetrahymena pyriformis*, are attached to the pellicle, and on stimulation the particles of the mucocysts become amorphous and are discharged through the pellicular membrane (Tokuyosu and Scherbaum, 1965). Since *Tetrahymena pyriformis* has been shown to have very long trails of adherent polystyrene particles, there is the possibility that *Opisthonecta* could have a similar arrangement for discharging mucus-like material. However, until the pellicle of *Opisthonecta* has been investigated, the answer to this problem is relegated to the future.

E. MECHANISM OF TURNING

The turning of *Opisthonecta* is very abrupt, as demonstrated by streak time-exposure photographs and by motion pictures. The radius of a turn may be less than the diameter of the organism. In one sequence which was carefully analyzed (Fig. 33) the turn was about 135°, and this was produced by an unequal bea of aboral membranelles on the two sides. The membranelles on the outside of the turn made seven power strokes, while those on the inside of the turn made only two power strokes, and these were *in reverse*. It is not known what causes this temporary reversal of the membranelles. This mechanism is capable of producing the very abrupt turns, and is even capable of causing a spinning motion of the body about an axis near the membranelles inside of the turn. The action is comparable to that caused by reversing one oar of a simple rowboat without a keel.

F. ACTION OF CILIA VS MEMBRANELLES AND CIRRI

The form of the beat of the aboral membranelles, as shown in figures 31-33, is identical to that described by various authors, in differing degrees of detail, for other membranelles and cirri and also for individual cilia. The general pattern is that of a forth and back movement, with the cilium being almost straight on the power stroke and definitely bent of the return stroke. The literature is reviewed by Sleigh (1962, 1968) and others.

However, this pattern does not hold for the individual cilia of *Paramecium*, as demonstrated by Kuznicki, Jahn, and Fonseca (1968). These investigators found by means of motion pictures that each cilium beats with a *traveling helical wave*, from base to tip, and that ciliary reversal is caused by a change in the *direction of the helix*; rather than by a change in the form of the beat. Isolated individual cilia of *Tetrahymena* also beat with a traveling helical wave (Gibbons, 1965).

These observations indicate a definite difference between the action of individual cilia and of membranelles and cirri. Membranelles and cirri are more easily seen than individual cilia, and the observations on membranelles and cirri seem to have been transferred to cilia without adequate evidence.

This means that the widely accepted description of forth and back movement, as determined by rapid fixation methods (Párducz, review, 1967), must be erroneous, because they do not conform to what can be seen in motion pictures of the living organism.

Furthermore, the mechanism of cilliary reversal for membranelles and individual cilia are very different because of the difference in wave form. The reversal of the locomotory membranelles of *Opisthonecta* is the same as that described in textbooks for both membranelles and cilia. However, the reversal of individual cilia of *Paramecium* involves only a change in direction of the cilium, and without any change in the wave form of the beat (Kuznicki, Jahn and Fonseca, 1968, 1969, 1969a).

V SUMMARY

1. Locomotion of *Opisthonecta henneguyi*, a non-talked vorticellid, was studied by means of 1/5 second time-exposure streak photographs and high speed (225-700 fps) cinemicrography, with and without 1.17 μ polystyrene spheres in the medium.

2. The organism was found to be aborally propelled by an aboral circlet of membranelles. The dark field streak photographs of polystyrene spheres showed patterns of pseudo-turbulent vortices shaped differently for unidirectional and for turning locomotion.

3. This organism changes direction by turning abruptly up to 360° or more, with the outside diameter of the turn being less than two body lengths. During turning the vortices persisted of the inside but not on the outside of the turn. Motion pictures demonstrated that abrupt turning was accomplished by the membranelles on the inside of the turn beating 3 or 4 strokes or more in reverse.

4. Before encystment, the organism decreased in velocity and showed different vortex patterns. A mucus-like material was dragged adorally and formed a trail in which polystyrene spheres were enmeshed.

5. The "epistomal membrane," an organelle of unknown function, by cinematographic frame to frame analysis, failed to show any movement. The body did not always rotate during locomotion, even though the adoral cilia usually exhibited constant activity.

6. The adoral cilia seemed to be used during locomotion for ingestion of food particles, since polystyrene spheres were observed being ingested.

ABSTRACT

Locomotion of *Opisthonecta henneguyi*, a non-stalked vorticellid, was studied by means of 1/5 second time-exposure streak photographs and high speed (225-700 fps) cinemicrography, with and without 1.17 μ polystyrene spheres in the medium. The organism is aborally propelled by an aboral circlet of membranelles. The dark field streak photographs of polystyrene spheres showed patterns of pseudo-turbulent vortices shaped differently for unidirectional and for turning locomotion. This organism may change direction by turning abruptly up to 360° or more, with the outside diameter of the turn being less than two body lengths. During turning the vortices persisted on the inside but not on the outside of the turn. Motion pictures demonstrated that abrupt turning was accomplished by the membranelles beating 3 or 4 strokes or more in reverse on the inside of the turn while those on the outside of the turn beat normally.

Before encystment, the organism decreased in velocity and showed different vortex patterns. A mucus-like material was dragged adorally and formed a trail in which polystyrene spheres were enmeshed.

The "epistomal membrane," an organelle of unknown function, when examined by cinematographic frame to frame analysis, failed to show any movement. The body did not always rotate during locomotion, even though the adoral cilia usually exhibited constant activity. The adoral cilia seemed to be used during locomotion for ingestion of food particles, since polystyrene spheres were observed being ingested. Some of these observations are in contradiction to those of other investigators who used only simple visual methods.

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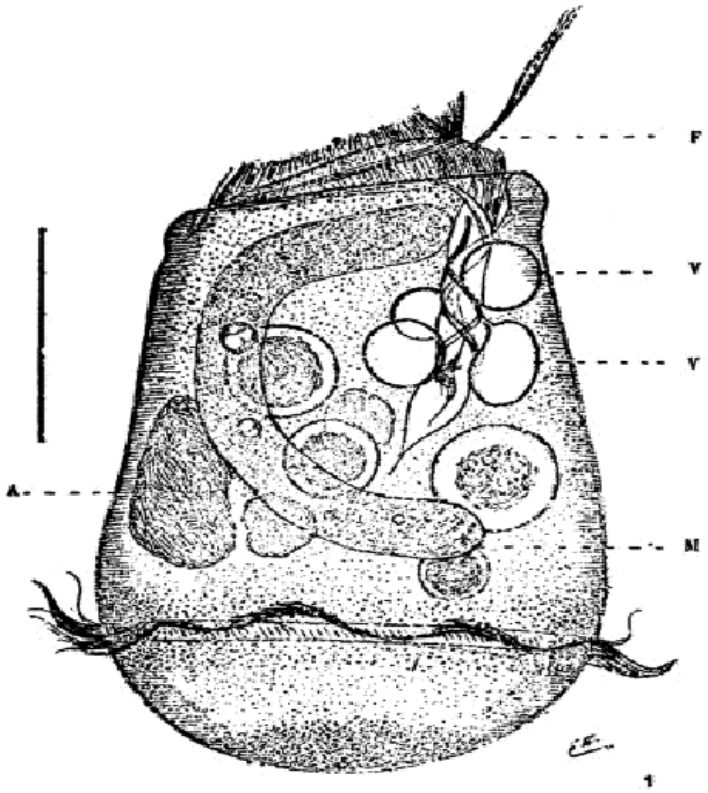
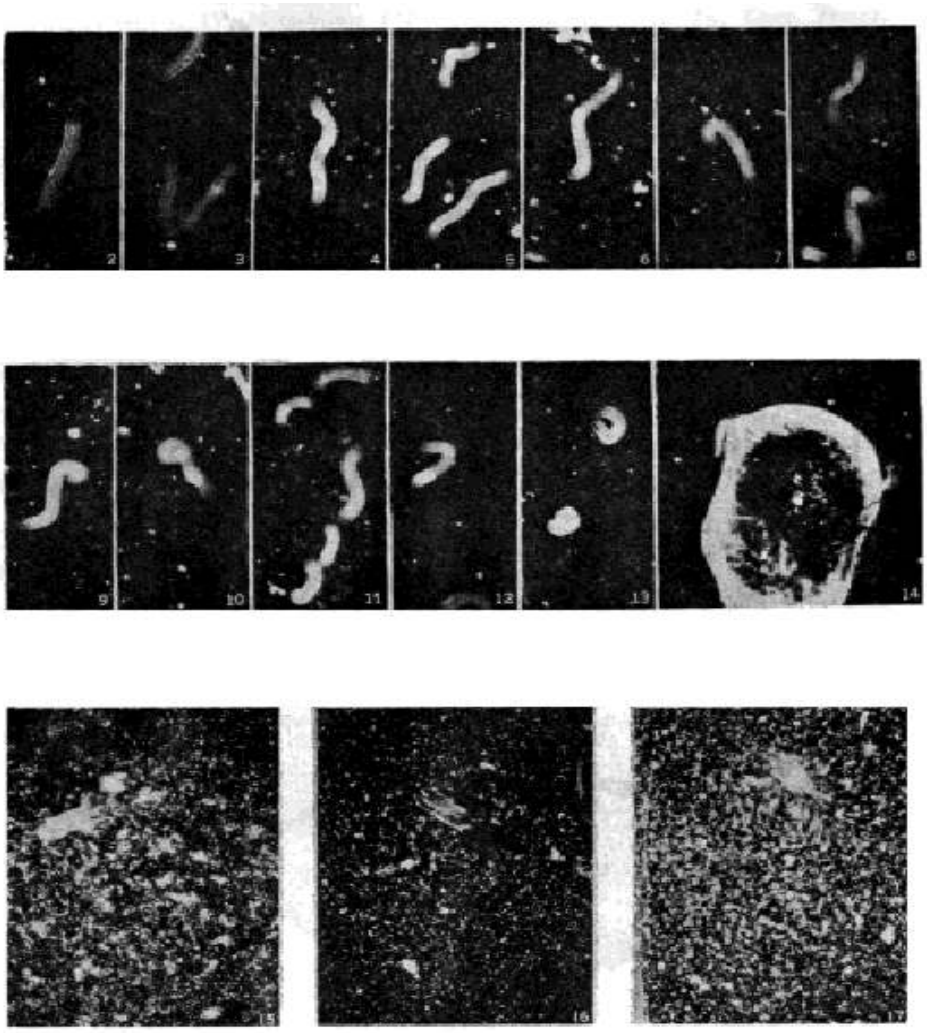
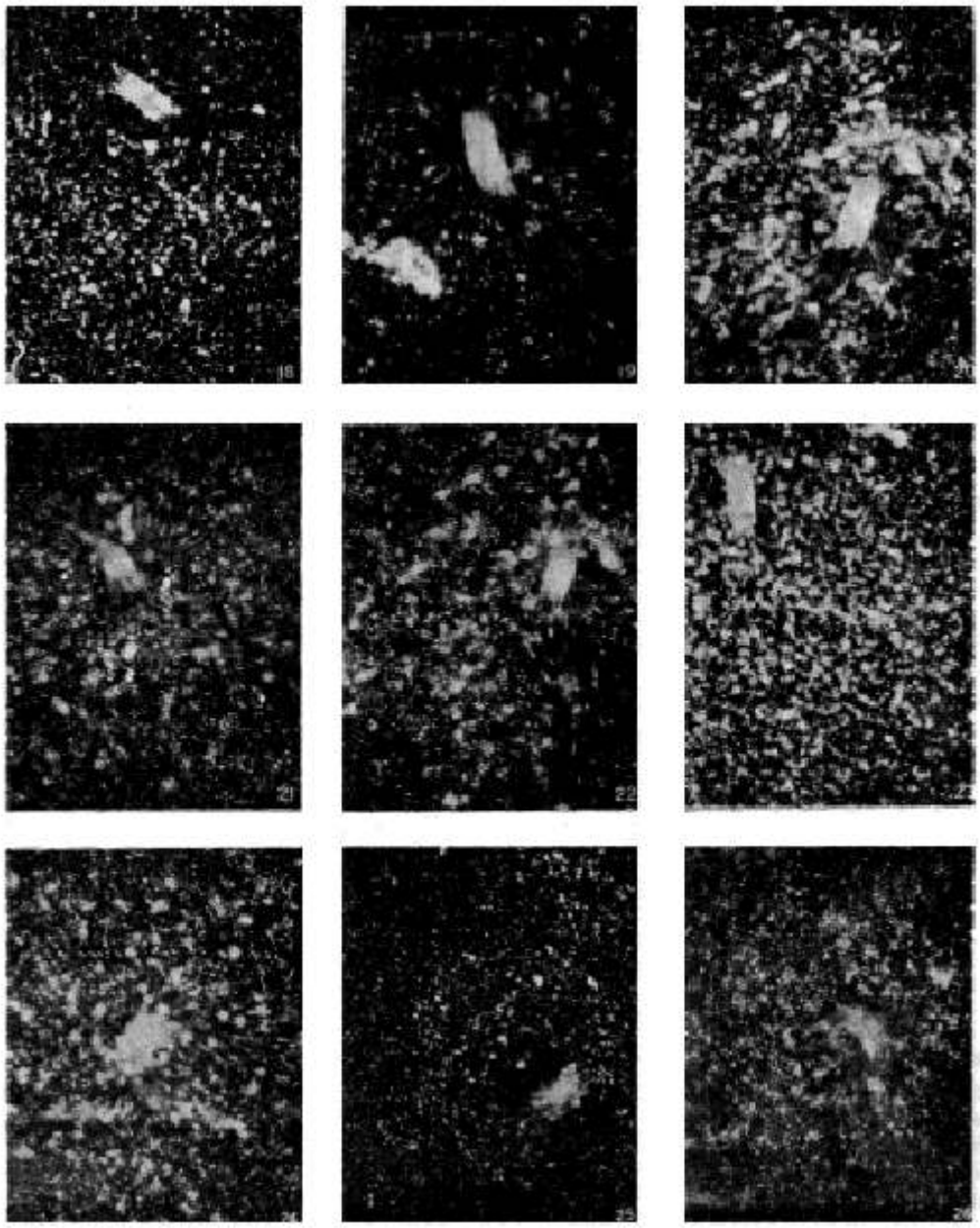


Fig. 1. —*Opisthonecta henneguyi* (after Fauré-Fremiet, 1924). F. Flamme “epistomal membrane”; V. V. Vacuoles; M. Macronucleus; A. Alimentary residue. The adoral region is at the top of organisms as shown, and the membranelles are shown aborally at lower part of organism. As shown here the organism would swim downward.



Figs. 2-17. —For explanation see text.



Figs. 18-26. —Polystyrene Streak Photographs taken with 1/5 second time exposure and with 100x magnification. Figs. 18, 19, and 20.—Intermediate stage of swimming which is slowed by a mucus-like material trailing. The vortices are 2, 3, and 4, respectively, in numbers. Figs. 21, 22 and 23.—Slower swimming with vortices aborally directed and a longer mucus-like trail. Figs. 24, 25, and 26.—Turning with the vortical pattern of water currents on the side of turning with no evidence of a vortex on the opposite side of turning, and no mucus-like trail.

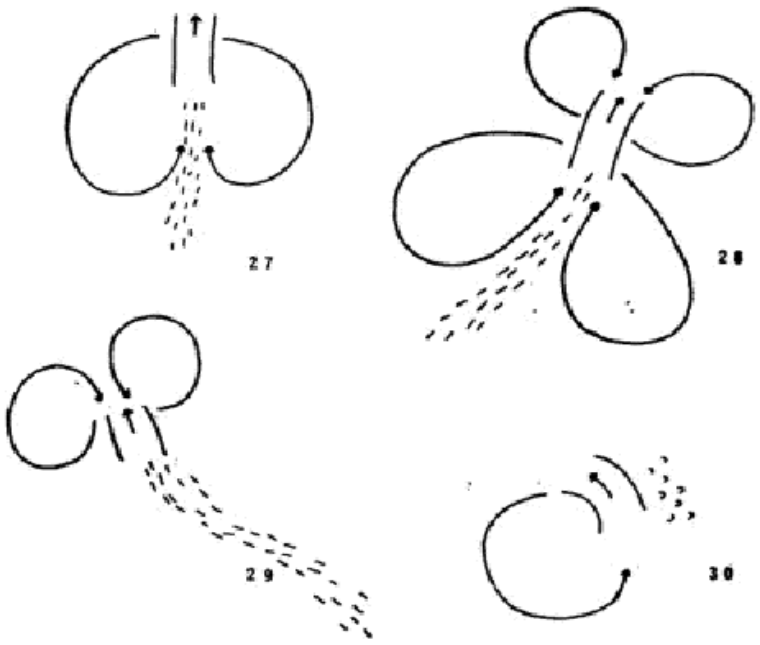


Fig. 27.—Central arrow pointed aborally shows direction of organism. The two curved lines with arrows describe the vortical water currents that are adorally directed. Particles are adorally following by force of drag. Fig. 28.—Central arrow pointed aborally shows direction of organism. The four curved lines with arrows describe the vortical water currents that are aborally and adorally directed. A mucus-like trail is shown adorally attached.

Fig. 29.—Central arrow pointed aborally shows direction of organism. The two curved lines with arrows describe the vortical water currents aborally directed. There is a long mucus-like trail adorally attached. Fig. 30.—Central arrow pointed aborally shows direction of organism. One vortical current of water is on the side of turning, and on the opposite side the particles form U-shaped streaks, open end towards the organism.

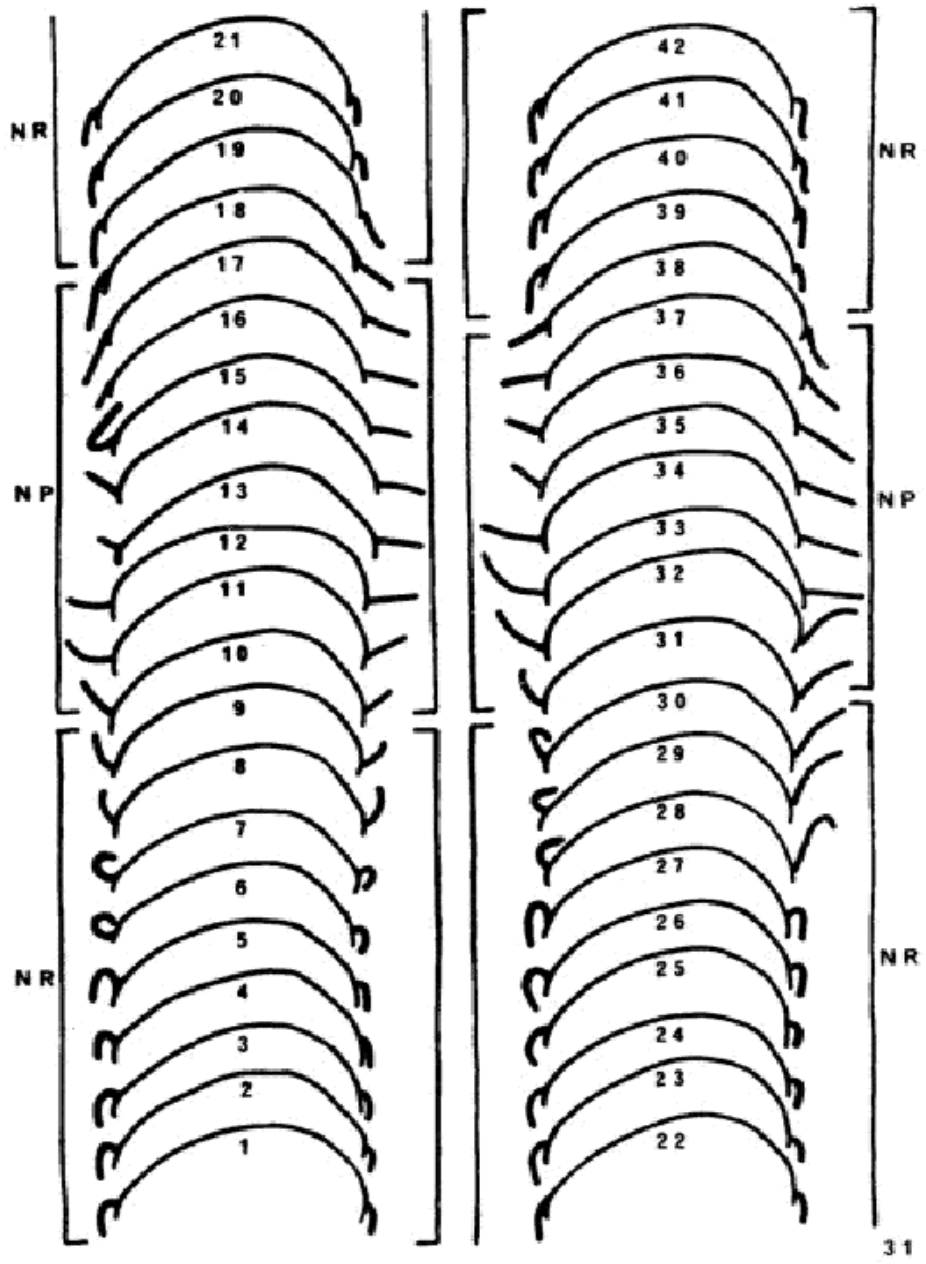


Fig 31.—Frame by frame analysis of normal forward swimming. The aboral region with membranelles was drawn directly from projecting the film one frame at a time. The film series was taken at 225 frames per second. The membranelles have normal power strokes (NP) and normal return strokes (NR), and the direction is unidirectional. The magnification was 160X. The 42 frames in this series includes two full cycles.

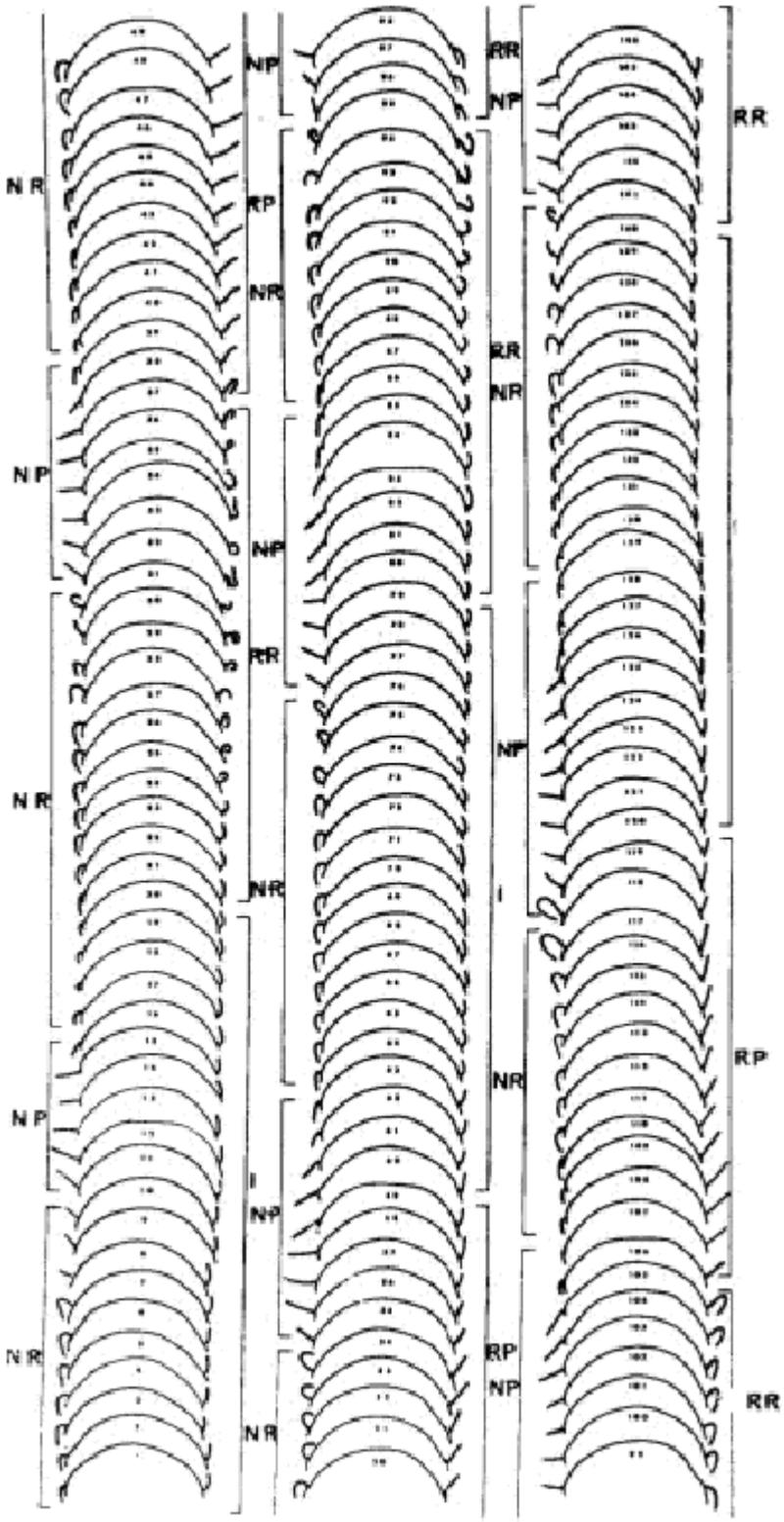


Fig. 32.—Frame by frame analysis during turning movement. The aboral region with membranelles was drawn

from projecting the film one frame at a time. The film series was taken at 700 frames per second and 160X magnification. The membranelle have normal power and normal returns strokes (NP, NR) on the left side, but on the right side the membranelles have reverse power and reverse return strokes (RP, RR).

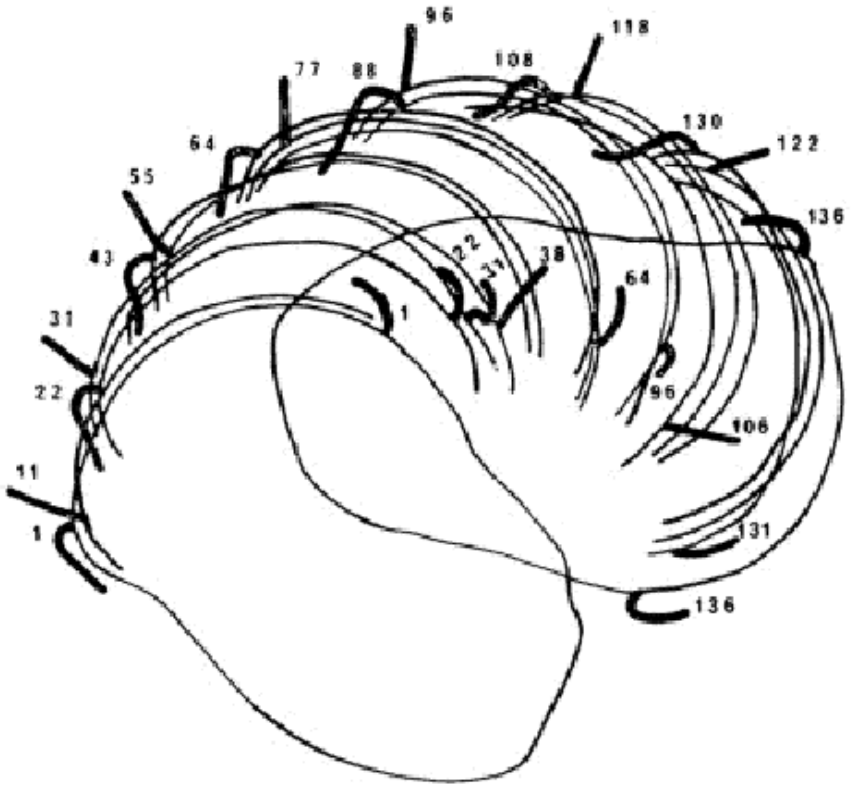


Fig. 33.—Frame by frame analysis during turning-movement. Same data as figure 32 but shown in relation to the observer. The turn was nearly 1350. The outer curve shows the membranelles with NP and NR strokes. The inside curve shows the membranelles with RP and RR strokes. Intermediate changes are not shown, but the changes with distinguishing characteristics are shown. On the outside curve the cycle consists of NP and NR, while on the inside curve the cycle consists of RP and RR. Frames 1, 22, 43, 64, 88, 108, 130, and 136 are earlier return strokes. Frames 11, 31, 77, 118, 122 are the first of the power strokes.