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Locomotion through use of the mouth brushes in the larva of *Culex pipiens* (Diptera: Culicidae)

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The larva of the mosquito *Culex pipiens* is a filter-feeder and is able to use the feeding current generated by its mouth brushes to glide slowly through the water. The hydrodynamics of the mouth brushes, and of gliding, were investigated by visualizing the feeding current using dyes. Unlike the mouth brushes of a sessile filter-feeder such as the blackfly larva, those of *C. pipiens* function more like 'paddles' than 'rakes', a beneficial adaptation to life in still as opposed to running water. Technically, the Froude efficiency of gliding is very low (0.23) because the design of the feeding brushes favours delivery of water into the wake rather than forward momentum to the body. The wider implications of these findings to foraging strategy and other aspects of the behaviour of mosquito larvae are discussed.

Keywords: filter-feeding; mouth brushes; browsing; gliding; hydrodynamics; behaviour

1. INTRODUCTION

A considerable variety of locomotory techniques has been described amongst juvenile dipterans, ranging from telescopic crawling and explosive leaping in maggots (Berrigan & Pepin 1995; Maitland 1992) to sinusoidal and figure-of-eight swimming in ceratopogonid and chironomid larvae, respectively (Nachtigall 1961, 1962), underwater somersaulting in culicid pupae (Nachtigall 1962; Brackenbury 1999*a*) and water-skating in dixid larvae (Brackenbury 1999*b*). No doubt, further mechanisms remain to be elucidated but these examples are sufficient to show that, even within a single taxonomic group, the legless, worm-like body plan is remarkably flexible in its adaptation. Although the tendency in modern studies of invertebrate locomotion is to concentrate on mechanism and control, perhaps with a view to applying these principles to the design of man-made machines (Full 1997), it should also be remembered that locomotion is not an end in itself but a means to attain an end, usually the acquisition of food, a mate, a place of refuge from adverse conditions in the environment or from enemies, or a suitable site in which to deposit eggs. A reading of some of the older literature such as the classic *Aedes aegypti* by Christophers (1960), reminds us how intricately locomotion and other behaviours can be linked even in a simple organism like a mosquito larva. Chironomid larvae, familiar because of their conspicuous figure-of-eight looping through the water, actually possess at least three distinct locomotory styles, each tailored to a different set of environmental circumstances (Brackenbury 2000).

Although movement towards a goal is the primary purpose of locomotion, examples may be cited where locomotion is linked to another equally important role. Locomotory movements in the planktonic crustacea, particularly copepods, are connected with feeding, and a superficial examination of the bristled appendages responsible for producing movement in these and other forms of crustaceans may leave some doubt as to whether these structures are behaving like paddles or sieves (Koehl & Strickler 1981). In any biological situation there is unlikely to be genuine ambiguity of function, although there may well be duality, and for this reason Cheer & Koehl (1987) derived a theoretical model predicting the circumstances in which a bristled appendage would be expected to behave primarily as a paddle or as a rake. Applying this model to one of the best-known insect filter-feeders, the simuliid larva that inhabits fast-flowing streams, suggests that, as expected, the brushes appear to behave more like rakes than paddles (Craig & Chance 1981; Cheer & Koehl 1987). The question therefore arises as to the possible status in such a scheme of a hypothetical filter-feeder, equipped with mouth brushes but inhabiting still water. Logically, assuming that the organism was sessile, the design of the mouth brushes would need to incorporate at least some paddle-like features in order to ensure a continuous flow of water towards and away from the body. On the other hand, if the organism were at liberty to swim, it could simply extend the brushes and passively rake the water going by.

The present study was prompted by observations made during the course of an independent study of the characteristic lashing form of locomotion used by the larva of *Culex pipiens* in which the body progresses by being flexed vigorously from side to side. It was noted that, when undisturbed, the insects frequently adopted a far less conspicuous style of gliding locomotion in which the body was held straight and propelled by the mouth brushes. This form of locomotion was noticed by Christophers (1960), who referred to it as 'browsing'. The objective of this investigation was to quantify the water movements through the mouth brushes and into the wake of the slowly gliding insect, using a simple flow visualization technique. This information was then used to assess (i) the effectiveness of the mouth brushes as 'paddles', and (ii) the Froude efficiency of gliding as a means of locomotion.

2. MATERIAL AND METHODS

Final-stage larvae were collected from local pools in summer and transferred to indoor tanks at water temperatures of 18–22 °C, similar to outdoor temperatures for the time of year. Organic debris from the parent pools was included in the water samples to provide a suitable environment for the growth of the

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micro-organisms on which the larvae continued to feed. Swimming behaviour was recorded in daylight conditions with a Panasonic video camera (Matsushita Electric Industrial Co. Ltd, Osaka, Japan) producing 50 images per second at a shutter speed of 0.001 s. Videotapes were viewed on a cassette recorder with a single field advance facility allowing detailed examination of kinematic and hydrodynamic events. Additional data were obtained with a high-speed video system (frame rate 200 frames s^{-1}) (nac, Inc., Tokyo, Japan). Flow was visualized using a harmless food colouring agent (E122) suspended in water and glycerol (specific gravity 1.012). At the start of each experiment, one or two individuals were transferred from the holding tank to a transparent container measuring 8 cm (height) × 6 cm (frontal width) × 3 cm (front-to-back depth). The camera lens was adjusted so that its field of view coincided with the frontal area of the container, and to enhance image contrast, the insects were viewed against a white card placed behind the container. A thin layer of dye was carefully deposited on the bottom of the container via a syringe, making sure that a clear interface remained between the dyed and the clear water resting above. Normally, undisturbed larvae rest at the surface of the water, attached by their respiratory siphons, but a slight tap to the container initiates rapid downward swimming using the side-to-side lashing technique. Since the larvae are less dense than water, they tend to float back towards the surface as soon as swimming ceases, unless they become engaged in feeding. Underwater feeding, as opposed to feeding while hanging from the surface, is almost always associated with gliding, which is usually horizontal, either just above the bottom of the container or, since feeding can be initiated at any point during upwards flotation, at any level in the water. Further observations were made of insects gliding above a layer of dye deposited on the bottom of a Petri dish, beneath a centimetre or so depth of water. With the camera positioned directly overhead, the traces made by the feeding current as it propelled the body forwards could be clearly seen against a white card held below the Petri dish. In total, approximately 30–40 individuals were recorded in various states of behaviour. In a further eight to ten individuals, freshly killed by crushing the prothoracic region, observations were made of the structure and mechanism of the mouth brushes using a binocular microscope and micro-dissection instruments. Data are presented as mean values ± 1 s.d. (*N*, no. of observations).

3. RESULTS

Details of the structure and mechanism of the mouth brushes are given in figure 1, using as far as possible the nomenclature adopted by Craig & Chance (1981) in their study of the simuliid larval feeding organ and taking into account the description of feeding given by Christophers (1960) in the *Aedes aegypti* larva, which appears to use an identical mechanism to that of *C. pipiens*. Here I prefer to use the terms 'brush' and 'brush hairs', which seem to give a more accurate description of the appearance of the culicid feeding organ than the synonyms 'fan' and 'fan-rays' applied by Craig & Chance to the more two-dimensional simuliid condition. The brushes occupy a recessed area of the labrum dorsal to the mandibles and maxillae and flanking a median, bristle-bearing, tongue-like structure referred to by Christophers as the 'palatum' (figure 1*a*). In the flexed or adducted state the hairs are pressed together, their tips converging to a point near the mouth (figure 1*b*, left). In the extended or abducted state the hairs radiate out with their tips forming a domed surface, indeed like the hairs on a hand-brush (figure 1*b*, right). The hairs are arranged in 20–30 rows, which, as Christophers has noted, raise or lower in strict succession; Christophers estimates the total number of hairs in *Aedes aegypti* to be *ca.* 1000. The hair plaque is dorsally continuous with an area of hairless cuticle termed by Christophers the 'tessellated' membrane. The plaque is effectively hinged at its two ends and can therefore rotate to a limited extent like a bucket handle. This motion is normally relayed to the plaque by a tongue of integument, dubbed the 'scallop' by Christophers, and probably homologous with the 'distal plate' of Craig & Chance, which is in turn connected to an elongated element, Christophers' 'apodeme', or the 'torma' of Craig & Chance (figure 1*c*, left). A tubercle on the ventral surface of the torma provides the insertion for the adductor muscle. Direct manipulation demonstrates that flexion and extension of the brush can be brought about by rotating the torma about its longitudinal axis, either pushing the distal plate against the hair plaque and causing it to extend, or pulling it away, thereby lowering the hairs (figure 1*c*, middle and right). The mechanism is bistable, the brush remaining either in the flexed or the extended position and being unstable in intermediate positions. In living larvae the brushes extend and flex together, and operate rhythmically at a frequency of 11.2 ± 1.4 (13) strokes s^{-1}.

The action of the mouth brushes draws a current from a broad area in front of the head and expels it backwards as a jet, the axis of which lies at an angle of *ca.* 20–30° to the longitudinal axis of the body. Dye taken up by larvae trawling the bottom of the container clearly showed the pulsatile nature of the jet (figure 2), these pulsations having the same frequency as the movements of the mouthparts. An example of gliding behaviour interrupting the upward motion of a passively floating larva is shown in figure 3. Larvae floating up from the bottom of the container usually trailed a column of dye, which allowed the feeding jet to be visualized, as shown in the figure. Usually the body was driven forwards horizontally, rarely upwards and occasionally downwards, and the transition to gliding was accompanied by an alteration in the angle at which the axis of the body was held in the water from 64.7 ± 3.5 (15)° away from the horizontal during flotation to 46.1 ± 6.7 (23)° during propulsion (figure 4*a*). Figure 4*a* also shows the gliding and flotation speeds of the body and the speed of the feeding jet as measured immediately behind the mouth. Gliding was almost always in a straight line, as was clearly indicated by the tracks left by the water jet in the Petri dish experiments. Turning was abrupt and unpredictable and not necessarily related to contact with obstacles.

4. DISCUSSION

The mouth brush mechanism of the *Culex pipiens* larva as presented here is essentially in agreement with Christophers' (1960) account of the feeding organ in the larval *Aedes aegypti* and bears close resemblance to the mode of operation of the simuliid cephalic fans (Craig & Chance 1981). Both of these studies identify rotation of

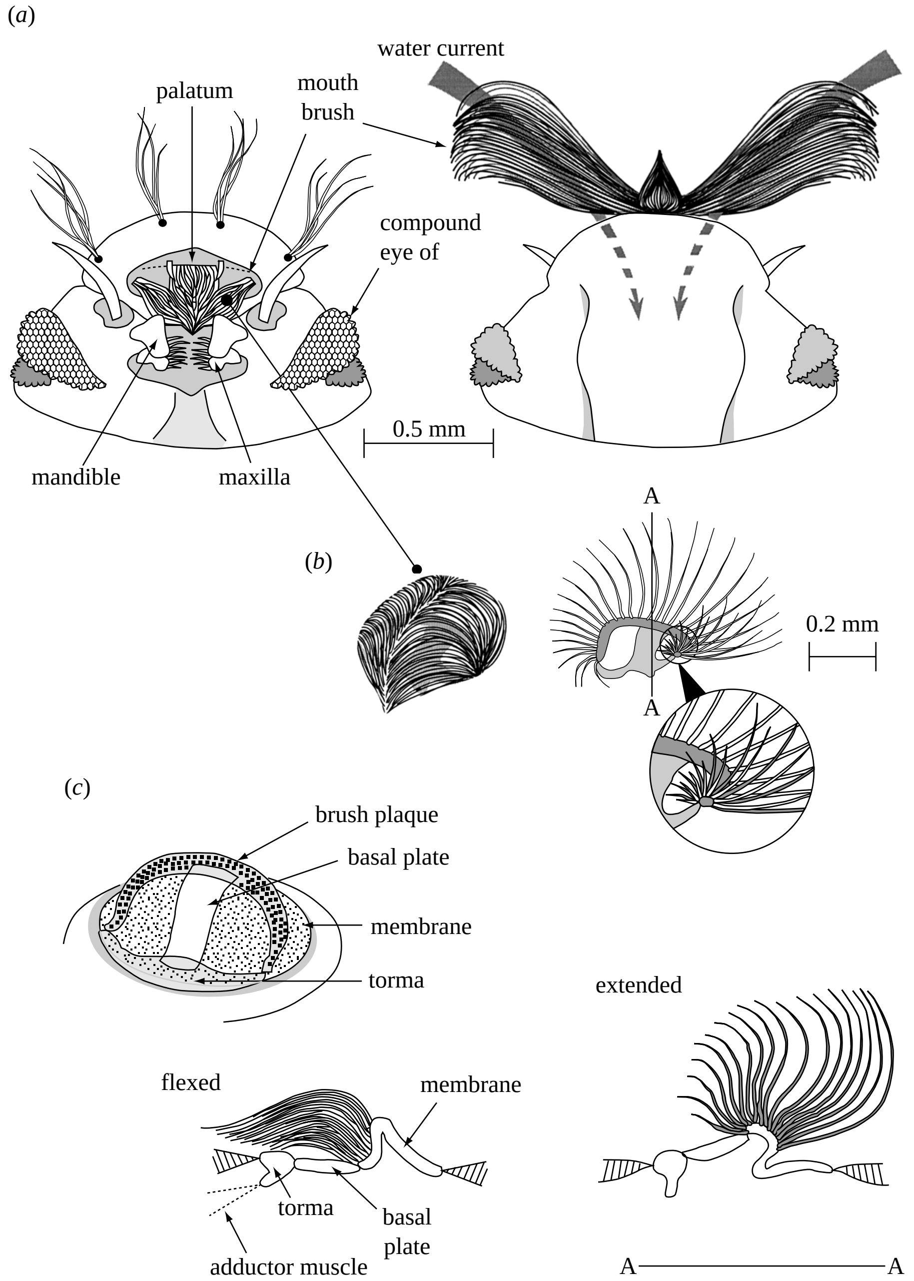

Figure 1. Structure and mechanism of the mouth brushes of final-instar larval *Culex pipiens*. (*a*) Left, ventral view of the head with brushes flexed; right, dorsal view with brushes extended. (*b*) Semi-schematic view of the right brush, seen from below in the flexed and extended states. In the flexed state all the hairs are lowered and come together in two series with a parting down the middle, as shown. The shaded structures below the hairs represent the torma, basal plate and brush plaque, shown more clearly in the extended state (right). In the latter state, the brush hairs are raised into a pin-cushion-like dome made up of rows of units, the tallest units being at the dorsal end of the brush plaque. For convenience, only one of these rows is illustrated in full, the rest of the brush being represented by the tallest units of each row. (*c*) Details of the structure and mechanism of flexion and extension of the brushes. In the upper-left drawing the brush is depicted as though the hairs had been shaved off to the base. The bases are represented by small filled squares and the line of squares nearest the dorsal border of the plaque corresponds to the tallest bristles represented in (*b*), right. The other two drawings represent schematic sections through the brush along the line A–A in (*b*), right, which passes through the basal plate. Downward levering of the torma and distal plate by contraction of the adductor muscle flexes the brush to the flattened state. Upward levering, possibly due to an increased haemolymph pressure, causes the hairs to radiate outwards into a dome-like shape.

the torma as the prime movement, and these authors state that adduction of the mouth brushes is the active phase, there being no abductor or extensor muscle. Christophers highlights perhaps the most important feature in the culicid mechanism, viz that the form of the brush is determined by the curvature of the hair-bearing plaque: when it is concave, as during flexion, the hairs are drawn together; when it is convex, as during extension, the hairs are made to radiate widely. The deployment of the brush is therefore seen to be governed by the elasticity and folding characteristics of the integumentary membrane. An analogy might be made with the configuration of the pile along a fold in a carpet. If the carpet is folded above (i.e. towards the pile), forming a

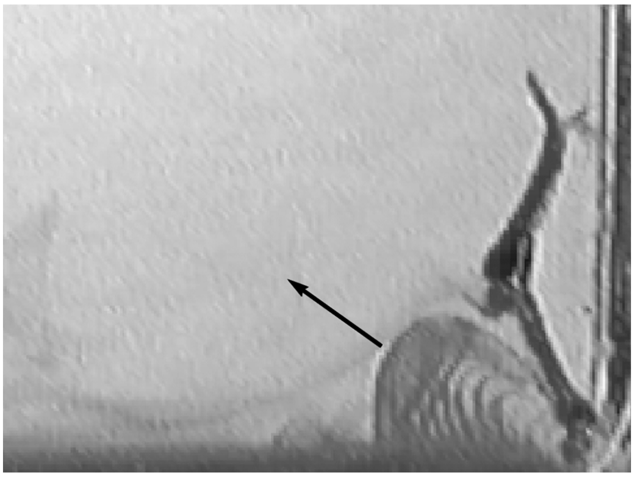

Figure 2. Visualization of the feeding current. Two larvae are seen in the bottom right, the lower of which is generating a feeding current in the direction of the arrow. Note the pulsatile nature of the current, the pulse frequency being the same as that of the flexion and extension of the mouth brushes. The insect was previously gliding from left to right but has now come to rest against the side of the water container.

concave gutter, the hairs along the inner surface of the fold are compressed together. If the carpet is folded beneath, forming a ridge on the pile, the hairs along the ridge are spread wide. Christophers (1960) and Craig & Chance (1981) were also struck by the click-like behaviour of the feeding organ during opening and closure, attributing it to elasticity in the various elements. In the absence of an abductor muscle, Craig & Chance speculated that increased haemolymph pressure might contribute to the normal extension of the feeding brushes.

Some of the bristled appendages identified amongst filter-feeding invertebrates may, according to the model of Cheer & Koehl (1987), operate more like leaky paddles than sieves or rakes. Their model is based on the fact that each unit in a spatial array of bristles imposes a local shear gradient on the flow through the array, which influences its overall 'leakiness'. The leakiness index of a particular array is governed by its geometry, namely the diameter and spacing of the bristles, and by the Reynolds number (*Re*) of the flow. In general, unless the spacing is not much greater than a few bristle diameters, as flow increases and *Re* approach 1 and above, the appendage functions more and more like a rake, and less and less like a paddle. The larval simuliid cephalic fan, with 5 μm diameter units spaced at 30–50 μm and held in currents with characteristic velocities of 0.18–0.8 $\mathrm{m\,s^{-1}}$, proves to be highly leaky. Simuliid larvae are obligate stream-dwellers, and do not need to paddle their own feeding current; they require a stream of sufficient velocity to maintain an adequate supply of particles towards the fans, but not so rapid that the flow over the body and through the fan becomes turbulent (Chance 1970; Kurtak 1978; Craig & Chance 1981).

Filter-feeders such as culicid larvae that inhabit still waters cannot rely on passively raking the water in their immediate vicinity, which would soon become depleted of suspended particles, but must actively generate a continuous particle-bearing current towards and away from the bodies. Assuming that the general structure of the brush is similar in culicid and simuliid larvae, the model of Cheer & Koehl (1987) shows that such an organ could function like a paddle simply by operating at a relatively low *Re*. The *Re* of simuliid fans of various species raking normal stream waters varies from about 1 to 60 (Chance 1970). The velocity of the flow through the culicid brushes is not known exactly, but a crude estimate can be made assuming that the stream is driven primarily by the tallest elements in the array (shown in figure 4*b*, right). These are *ca*. 0.4 mm in length and if they sweep back and forth through 90° (figure 2*c*) at the rate of 11.2 cycles $\mathrm{s^{-1}}$, their mean velocity relative to the head, measured at a point halfway along the shaft, is 0.07 $\mathrm{m\,s^{-1}}$. This analysis assumes that the active sweep phase of the bristles occupies half of the total cycle duration i.e. 45 ms. If the sweep phase is longer, the estimate of the velocity is proportionally reduced. The velocity of the hairs relative to the water depends on the leakiness index, but it cannot be greater than their velocity relative to the head. Although the leakiness index is not known, it is obviously much less than 100% since the brushes manifestly paddle a current. This suggests a maximum *Re* for the brushes of around 0.2–0.4, well within the range where efficient paddling could be expected. The hydrodynamic performance of the culicid larval brushes is unlikely to be affected by the forward motion of the body as a whole since gliding is slow compared with the velocity of the feeding jet. Gliding is a secondary, although beneficial, effect of the thrust created by the feeding jet and, as such, would not necessarily be expected to be highly efficient, in energy cost terms, as a locomotory manoeuvre. The following analysis shows this to be the case. The forces acting on the gliding body are shown in figure 4 and comprise the jet force F_{jet} of the feeding current, the drag

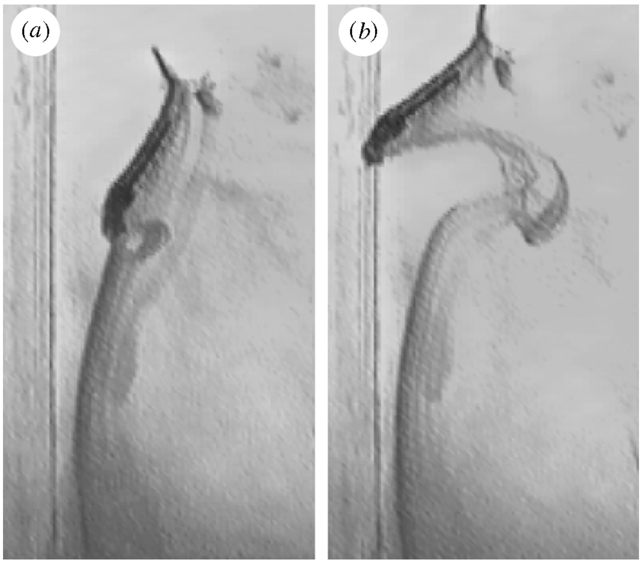

Figure 3. Beginning of gliding locomotion in a larva previously floating to the surface. (*a*) The insect is rising towards the surface, trailing a column of dyed water from the bottom layer of the container. The mouth brushes have just begun to function, initiating a backwards stream. (*b*) A few moments later, flotation has ceased and the body is driven forwards to the left.

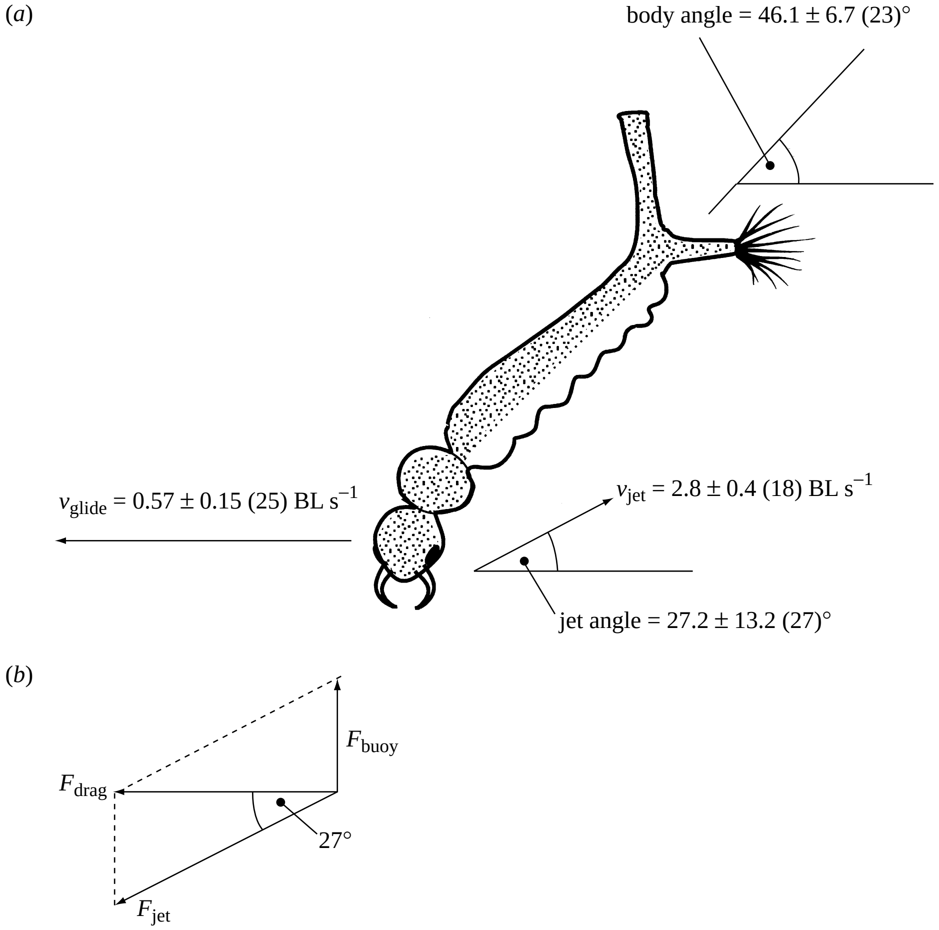

Figure 4. (*a*) Measured parameters during gliding. (*b*) Scheme of forces acting on the body during gliding. See § 4 for details.

force on the body F_{drag} and the upward force of buoyancy F_{buoy}. F_{jet} can be equated with the rate at which momentum is added to the water by the action of the feeding brushes. If the mass flux of water through the brushes per unit time is M_{jet}, the force required to accelerate this to the jet velocity V_{jet} is given by

$$F_{\mathrm{jet}} = M_{\mathrm{jet}} \times V_{\mathrm{jet}}. \qquad (1)$$

Note that this analysis assumes that all the fluid momentum is contained within the visible jet and that there is no added mass in the form of entrained circulations. From figure 4, F_{drag} and F_{buoy} can be expressed, respectively, as

$$F_{\mathrm{drag}} = F_{\mathrm{jet}} \times \cos 27° = 0.89 M_{\mathrm{jet}}\, V_{\mathrm{jet}}, \qquad (2)$$

$$F_{\mathrm{buoy}} = F_{\mathrm{jet}} \times \sin 27° = 0.45\, M_{\mathrm{jet}}\, V_{\mathrm{jet}}. \qquad (3)$$

The work done per unit time (power) in overcoming drag on the body moving at a velocity V_{glide} is given by

$$P_{\mathrm{drag}} = F_{\mathrm{drag}} \times V_{\mathrm{glide}} = 0.89 M_{\mathrm{jet}}\, V_{\mathrm{jet}}\, V_{\mathrm{glide}}. \qquad (4)$$

The work done on the water per unit time is the kinetic energy added to it in the form of the jet:

$$P_{\mathrm{jet}} = 0.5 M_{\mathrm{jet}}\, V_{\mathrm{jet}}^2. \qquad (5)$$

The work done to overcome buoyancy is the same as the work that would need to be done to stop the body floating to the surface at its normal flotation speed V_{buoy}:

$$P_{\mathrm{buoy}} = F_{\mathrm{buoy}} \times V_{\mathrm{buoy}} = 0.45 M_{\mathrm{jet}}\, V_{\mathrm{jet}}\, V_{\mathrm{buoy}}. \qquad (6)$$

The Froude efficiency (FE) of swimming can be defined as the ratio of the useful work done in overcoming the drag on the body and the total mechanical work done:

$$E = \frac{0.89\, M_{\mathrm{jet}}\, V_{\mathrm{jet}}\, V_{\mathrm{glide}}}{0.89 M_{\mathrm{jet}}\, V_{\mathrm{jet}}\, V_{\mathrm{glide}} + 0.45 M_{\mathrm{jet}}\, V_{\mathrm{jet}}\, V_{\mathrm{buoy}} + 0.5 M_{\mathrm{jet}}\, V_{\mathrm{jet}}^2}$$

$$E = \frac{0.89 V_{\mathrm{glide}}}{0.89 V_{\mathrm{glide}} + 0.45 V_{\mathrm{buoy}} + 0.5 V_{\mathrm{jet}}}. \qquad (7)$$

Inserting values of 0.57, 0.75 and 2.8 body lengths s^{-1} for V_{glide}, V_{buoy} and V_{jet} respectively yield an efficiency of 23%. A small amount of the energy generated by the feeding brushes, 15%, is expended in opposing buoyancy, and nearly two-thirds, 62%, is injected into the feeding current. Viewed as a purely locomotory manoeuvre, gliding is extremely inefficient, less than squid jet propulsion (FE = 0.3–0.4; Alexander 1999) and much less than fish swimming (FE = 0.7–0.8; McCutchen 1977; Alexander 1999), but this should not be surprising because the design characteristics of hydrodynamically efficient propulsive systems do not necessarily coincide with those of efficient water delivery systems. The former seek to inject maximum momentum into the water (mass flux × velocity) at minimal cost, by accelerating as large a volume of water as possible to the slowest possible speed. Larger, more-slowly beating mouth brushes would no doubt produce better paddles for propelling the body forwards, but this is not their main function: driving water backwards is more important. In this case using relatively small paddles to produce a high-velocity jet is no disadvantage hydrodynamically and is evidently more practical in terms of the size and deployability of the structures concerned.

So what is the purpose of gliding? Gliding appears to benefit feeding in two ways. First, it provides a highly controllable means of cruising between patches of water of variable nutritional value. Second, gliding is part of an optimized system of foraging of which the principal objective, it could reasonably be speculated, is to avoid re-circulating water back through the mouthparts, thereby avoiding inefficient filtration. The current generated by a filter-feeder is not one-way, but is part of a closed circulation and a sessile feeder in still waters cannot avoid re-circulating the same water through the mouth brushes. The mean recycling time of the water will depend on the particular form that the circulation takes and this is not known, either in the culicid larva or in any other filter-feeder that has been studied. However, if for the sake of argument it is assumed that the jet expelled behind the mouth brushes constitutes the centre of a small-cored ring vortex, the mean velocity of the water re-circulating back towards the head in the ring itself would obviously be many times less than the jet velocity. Therefore, to stay ahead of its own re-circulation, it would only be necessary for the larva to glide forward relatively slowly compared with the speed of the jet. No extra benefit would be obtained from gliding in excess of this minimal speed.

Finally, in addition to being auxiliary to feeding, gliding may serve another behavioural role. Having had to dive from the surface in order to escape from threat, the larva can halt its return to the surface and remain at any chosen level in the water, simply by initiating mouth brush activity. In this way, a running check can be kept on the perceived level of hazard on the surface, either through vision or vibration receptors, which would be impossible during normal swimming because the latter disturb the water and impose large rapid rotations on the body.

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