

Comparison of the Respiratory Currents Produced by Ephemeropteran Nymphs with Operculate Gills

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ABSTRACT The respiratory currents produced by ephemeropteran nymphs with operculate gills are described for the genera *Tasmanocoenis* Lestage (Caenidae) from Australia, and *Neoephemera* McDunnough (Neoephemeridae) and *Tricorythodes* Ulmer (Tricorythidae) from the United States. Flow pathways were followed by using a suspension of elemental silicon powder (300 µm diameter and finer) in small observation aquaria containing the nymphs. These nymphs are found in silty habitats and the different water paths observed probably assist in keeping the respiratory surfaces of the gills clean.

Introduction

Gill opercula are found in nymphs of several families of mayflies including Tricorythidae, Neoephemeridae and Caenidae. Operculate gills are those which have been modified to form protective covers over more posterior gills; the middle abdominal segments are shortened, decreasing the distance between succeeding gills, allowing the smaller, more posterior gills to be covered by the enlarged, operculate gill.

In mayflies exposed to silt and other debris that could interfere with the functioning of the respiratory surfaces, the operculate gill condition is an important adaptation. Exposed gills are at risk of being damaged or clogged, resulting in a reduced ability to continue their respiratory function. In addition, the more posterior, membranous gills generally have an increased surface area available for respiration permitting these nymphs to inhabit silty waters which may be warmer and with a reduced oxygen content (Brittain 1982; Eriksen and Moeur 1990).

The patterns of water circulation over the gills in three genera (representing three closely related families), *Tasmanocoenis* Lestage (Caenidae), *Neoephemera* McDunnough (Neoephemeridae), and *Tricorythodes* Ulmer (Tricorythidae), are briefly described, as are reactions of the nymphs to various stimuli.

Material and methods

Tasmanocoenis nymphs were collected by the author from Commissioner's Waters, Armidale, New South Wales, Australia on 10 October 1974. Additional material of *Tasmanocoenis* was collected at Lake Zot on the University of New England campus in Armidale on 26 March 1993. The nymphs of *Neoephemera* were collected by Dr G. F. Edmunds Jr. from Rocky Comfort Creek, Gadsden Co., Florida, USA on 30 March 1974, while I collected *Tricorythodes* nymphs from a small stream in Salt Lake City, Utah, USA on 10 September 1973. The nymphs were acclimated in the laboratory (at either the

University of New England or the University of Utah) for 2-3 d in small holding aquaria with substrate from the collecting site provided.

Small observation aquaria with nylon netting in the bottom were constructed from microscope slides. This restricted movement of the nymphs, facilitating observation through the microscope. To follow the water pathways produced by the beating of the gills, very small amounts of elemental silicon powder (300 µm diameter and finer) were added to the water in the observation aquaria containing the nymph. The largest particles quickly settled to the bottom while the lighter particles formed a relatively uniform suspension in the water. The silicon suspension did not colour the water or impede observations of the nymph; the currents produced by the beating of the gills were easily followed by observing the direction of flow of the individual particles. The nymphs were also gently probed on various parts of their body, including the gills, and their reactions observed.

Results Caenidae (Figs 1-2)

Tasmanocoenis nymphs have gills on abdominal segments I-VI. Gill I is reduced to a finger-like structure bearing many hairs and inserted anterolaterally. The operculate gills are found on abdominal segment II. The opercula are more or less quadrate in shape, well sclerotised with slight sculpturing, and covered with long hairs. The gill opercula are attached dorsolaterally and overlap medially; slight elevations occur on the posterior of segment II where the opercula are attached (Fig. 1). The gills on segments III-VI are membranous, and have a fringe of filaments, each bearing two or three branches; these gills become progressively smaller posteriorly. The tracheae radiate from the gill base and can be seen to enter the gill filaments (Fig. 2).

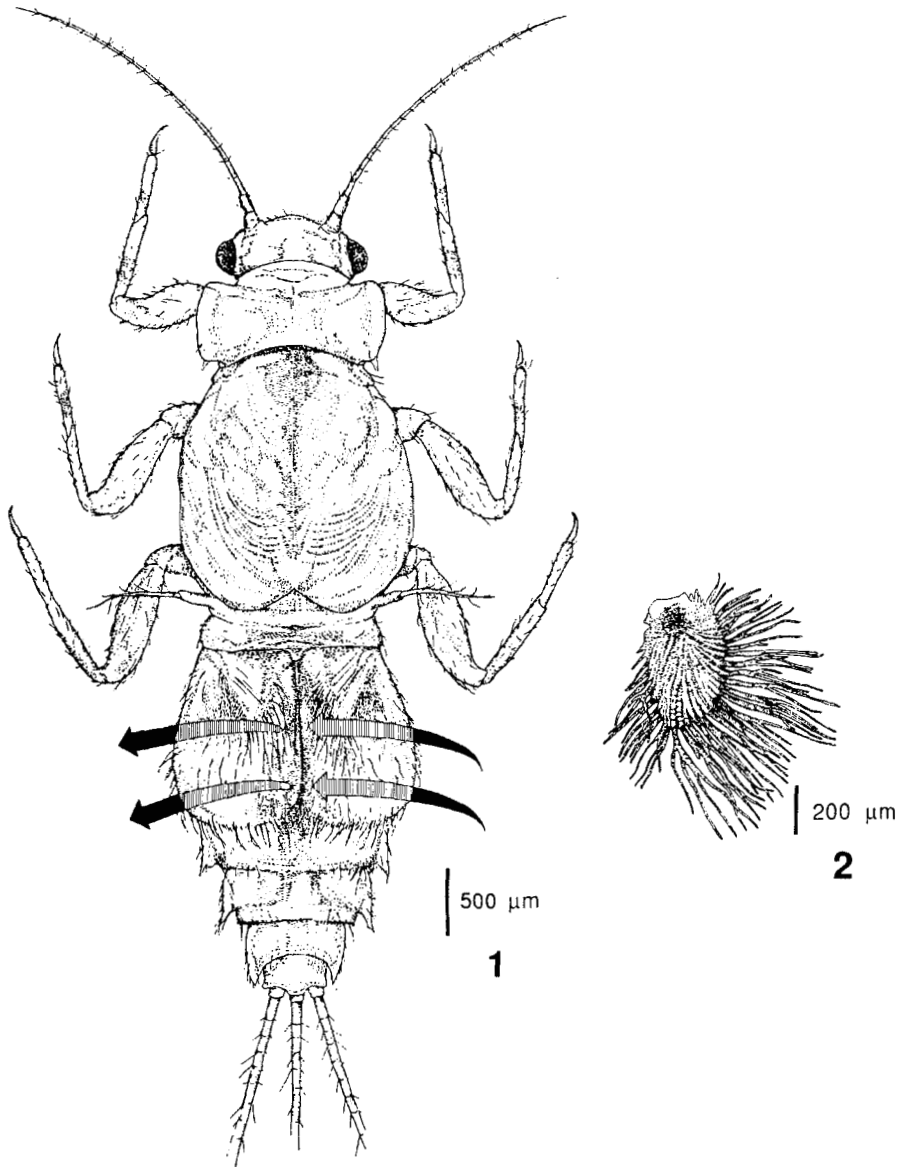
One preserved nymph of *Tasmanocoenis* examined had the right operculate gill II regenerated. The gill was unsclerotised, with the

edges rounded and lacking any fringe of hairs; it was somewhat elongate, covering only segments III and IV and some of segment V. The operculate gill generally extends to the posterior of segment VI.

Eight living nymphs of *Tasmanocoenis* were examined. When placed in the observation dish with netting, the nymphs refused to settle until they were under the netting with the dorsum oriented down. Gill I is held straight up from the body and becomes covered with silt. As the operculate gills on segment II are raised, the more posteriad gills can be seen to beat continuously unless the nymphs are disturbed. Currents of water enter the gill area laterally and posteriorly

on one side and exit out the other side (Fig. 1). Water can be drawn into the gill area from either side, thus reversing the direction of flow. I first thought that the direction might be controlled by how the opercula overlapped. Usually, if the left operculum overlapped the right, currents entered from the right and exited to the left, and vice versa; however, a few exceptions did occur. The only time the gills stop beating and the opercula close down is when the nymphs are probed on the venter or the hairs on the edge of the gill opercula are touched. There is no reaction if gill I is disturbed and gill I always returned to the same, erect position.

The nymphs of *Tasmanocoenis* were collected



Figs 1-2. (1) Mature nymph of *Tasmanocoenis* (Caenidae); arrows indicate path of respiratory current over the gills; (2) Gill IV of *Tasmanocoenis* nymph; note tracheae radiating into the fringe of filaments.

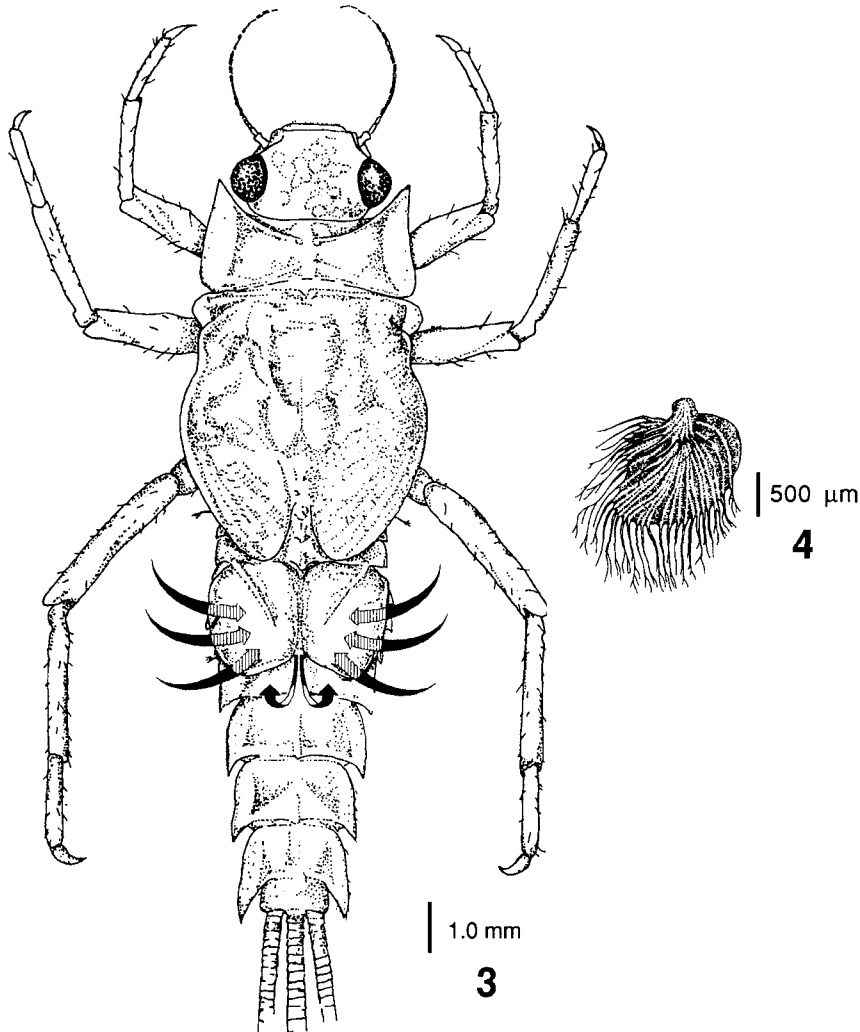
from areas having a flocculent, silty substrate. The abundant, short hairs on the body collected this debris and helped to camouflage the nymphs in their natural habitat. These nymphs were frequently observed spreading the membranous gills on segments III-VI laterally across the setae found on the edges of the abdominal segments, presumably to keep them clean.

Neoephemeridae (Figs 3-4)

In *Neoephemera*, the gills are found on abdominal segments I-VI. Gill I is reduced to a slender filament with many hairs and is attached to the anterolateral area of the segment. The operculate gills are found on segment II; these are heavily sclerotised, ridged structures that are squarish in shape and possess a small ventral tuft (Fig. 3). The two opercula are connected by a thin, delicate

membrane which was broken in some preserved specimens; the venter of the operculum also has a membranous lining. No tracheae are visible in the gill opercula. The gills on segments III-VI are composed of dorsal, fringed lamellae with a small, ventral, fringed flap (Fig. 4); they are somewhat triangular in shape with distinct tracheae. These gills become progressively smaller posteriad.

Observations on five live *Neoephemera* nymphs were made. When the gill opercula are raised the other gills can be seen vibrating underneath; in living specimens these gills have a pink to reddish hue. If the nymph or the surrounding water is disturbed, the opercula are immediately lowered and held close to the body; simultaneously the abdomen is raised slightly, securing an even tighter fit of the opercula to the body. Figure 3 illustrates the normal path of water over the gills. The



Figs 3-4. (3) Mature nymph of *Neoephemera* (*Neoephemeridae*); arrows indicate path of respiratory current over the gills; (4) Ventral view of gill IV of *Neoephemera* nymph; note tracheae radiating into the fringe of filaments and the small ventral flap.

majority of water flows into the gill area laterally and is ejected somewhat forcibly from the medioposterior area and is directed upwards. If the membranous connection between the opercula is cut, the two components still appear to function as a unit. However, some water does enter the gill area anteriorly and also escapes medially between the gills. If one of the opercula is removed, the remaining gills continue to beat, even when probed; however, when the other intact operculum is touched, all gills stop beating.

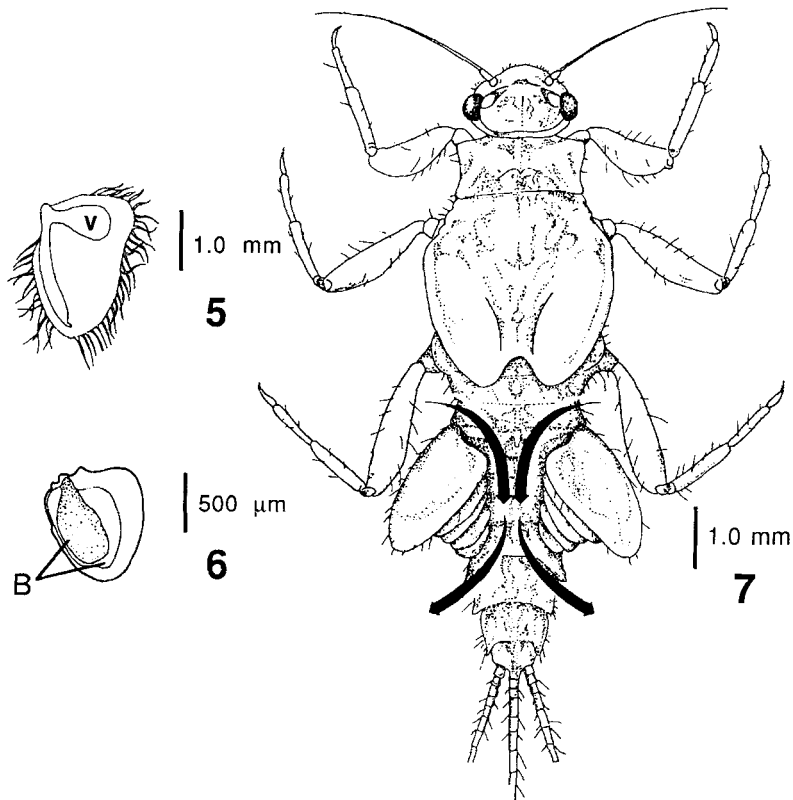
Tricorythidae (Figs 5-7)

The gills of *Tricorythodes* nymphs are found only on abdominal segments II-VI and are comprised of dorsal and ventral lamellae. The operculate gills on segment II are triangular to suboval in shape, sclerotised, with numerous long hairs on the surface; the ventral component is composed of a lateral, elongate portion with an ovate extension that parallels the anterior edge of the dorsal lamella (Fig. 5). Gills III-VI are membranous and consist of a dorsal flap, and a bilobed ventral component (Fig. 6). The gills lie in slight depressions of the abdomen formed by the median portion of the abdomen being raised and the lateral portions being somewhat depressed.

The path of water flow over three living nymphs of *Tricorythodes* was observed (Fig. 7). The operculate gills are raised, extend laterally and are held steady while the gills on segments III-VI beat vigorously. If a gill operculum on one side of the body is touched, that operculum only is held close to the body; if the dorsal or lateral surfaces of the abdomen are touched lightly with a probe, both operculate gills are held close to the body for a very short time and then returned to the extended position. The other gills stop beating if the opercula are held close to the body and resume beating when the opercula are extended. The gill response elicited is variable if the thorax is touched. The gills also close down, i.e., stop beating, when the nymph is moving around actively.

Water flows into the gill area in the anterodorsal region and exits at the posterior; no water was observed to enter or leave the gill area from the lateral or ventral regions (Fig. 7). The suspended particles aggregate on the nymphal body but none adheres to the beating gills or to the abdomen in the region immediately surrounding the gills.

The right gill operculum was removed on one specimen. The other gills were less sensitive to being probed as evidenced by their not closing



Figs 5-7. (5) Ventral view of operculate gill II of *Tricorythodes* nymphs; note the membranous ventral component indicated by V; (6) Ventral view of gill IV of *Tricorythodes* nymphs; the bilobed ventral component (B) of this gill is clearly visible; (7) Mature nymph of *Tricorythodes* nymph; arrows indicate path of respiratory current over the gills.

down as rapidly; the other operculum also appeared somewhat less responsive.

Discussion

Tasmanocoenis nymphs have been collected in slow flowing reaches of streams among pebbles in areas of silt deposition and in lakes and ponds with high amounts of flocculent, silty material and in vegetation (pers. obs.). In these habitats, the gill opercula function to protect the more posterior, membranous gills. The ability to reverse the flow of water through the gill area in *Tasmanocoenis* nymphs is advantageous, as it may assist in keeping the gills free from accumulations of silt or particulate matter.

In *Caenis* Stephens nymphs, Eastham (1932, 1934, 1936) found that water currents can travel across the body in either direction, right to left or left to right; this is similar to that seen in *Tasmanocoenis* in the present study. The gills reversed the current direction against a "proffered mud suspension" (Eastham 1934). If the gills became covered with debris, they were able to cleanse their surfaces by drawing them through the setae that act as lateral gill "guards" to the gill area; these setae also captured particles, preventing their entrance into the gill area (Eastham 1936). This behaviour was also observed in *Tasmanocoenis* nymphs in the current study. In his 1934 paper Eastham describes in detail the principles on which the gills of *Caenis* beat and how they are able to reverse the direction of water flow over the gills; his interpretations are also applicable to *Tasmanocoenis* nymphs.

Neophemera nymphs are found in protected areas of streams with slow to moderate current. They are found amongst debris, plant roots and moss where they grasp hold of the substrate and are difficult to dislodge (Edmunds *et al.* 1976). In areas of debris accumulation, the membranous respiratory gills need protection from damage or interference with gas exchange; the fused, sclerotised opercula of *Neophemera* effectively provide this protection. Neophemeridae nymphs are highly sclerotised and probably rely heavily on the gills to obtain their oxygen requirements.

Tricorythodes nymphs are found in sand, gravel, amid plants or in moss (Edmunds *et al.* 1976); they are often found in silty streams. The water currents produced by the beating of the gills in these nymphs reduces the chances of silt accumulation on these respiratory surfaces, especially as water enters the gill area only from the dorsoanterior region. In preserved nymphs examined, the body surface was often silty but the gills were consistently clean. The gills are able to close down if they come in contact with particulate matter, or if the nymphs are disturbed. Berner (1950) reported that *Tricorythodes* nymphs rhythmically raise the opercula to allow water to flow over the more posterior gills.

The fringe of filaments on the gills in both caenid and neophemerid nymphs substantially increases the surface area exposed to water; in addition silt may be more easily removed from a fringed structure. These fringes are lacking in *Tricorythodes*. In both *Tasmanocoenis* and *Neophemera* nymphs gill I is present and presumably serves some function, perhaps sensory. An examination of these gills for trichoid or campaneiform sensillae would be useful. The hairs on the opercula of *Caenis* nymphs have been demonstrated by Eastham (1936) to be sensory; sensillae are distributed all around the edges of the gill area and these function to detect substances such as silt or grit in the water that can damage the gills or interfere with gaseous exchange. Wichard *et al.* (1972) found that the structures described by Eastham as campaneiform sensillae actually function osmotically and are involved in maintaining chloride ion concentration; they refer to these structures as chloride cells. However, Eastham's work on the trichoid sensillae of these gills has not been disputed.

Gill opercula may also function as a means of predator avoidance. In those nymphs lacking opercula they can often be detected by flashing of gills beating. By hiding the vibrating gills under an operculum they are less easily seen.

Acknowledgments

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