VIDEOMACROSCOPY FOR THE STUDY OF EPHEMEROPTERA AND OTHER AQUATIC MACROINVERTEBRATES

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Abstract

Videomacroscopy allows scientists to observe Ephemeroptera and other macroinvertebrates in ways not previously possible. Adapting proper equipment and techniques, researchers can observe activities of several individuals or the movements of microscopic structures. At Purdue University videomacroscopy has been incorporated into studies of mayfly behavior, functional morphology, and ecology. The system is described in terms of electronics, optics, and observational arenas. Two types of video cameras, a number of optical systems, different lighting regimes, and observational arenas are described for studying larvae under naturalistic conditions at magnifications up to 120x (on a 23cm screen).

Introduction

Recent advances in technology afford new opportunities to observe behavior of Ephemeroptera and other aquatic macroinvertebrates by using relatively inexpensive video equipment. The proliferation of consumer video equipment and continuing demands by consumers for increased video capability at low cost is causing manufacturers to produce better equipment at lower prices. Video cameras can be fitted to a number of optical systems permitting viewing organisms of a wide size range, and can often utilize light from outside the human visual spectra, i.e. infrared and ultraviolet light; light wavelengths may be chosen so that the organism under study is unaware of the light, or so that the scientist can evaluate the environment from the perspective of the organism under study. Slow-speed capabilities of cameras and videotape recorders (VTR's) allow the examination of behavioral phenomena that occur too rapidly to be discerned by the human eye. Videotape allows for storage of observation sessions, and for close, repeated study and comparison of behavioral attributes. For the scientist, this means the availability of powerful tools that allow behavior to be observed and recorded for later detailed analysis.

Videomacroscopy

Videomacroscopy is the use of electronic image gathering, processing, and recording devices (video) to observe, analyze, and record moving images at magnifications greater than 1x. Videomacroscopy has existed almost as long as video cameras, but the recent availability of high-quality, low-cost consumer equipment has greatly enhanced its appeal. Videomacroscopy has several advantages over cinematography (which uses photographic film): 1) Costs are much lower for video; whereas the initial investment for video or cinematographic equipment may be similar, videotape is less expensive than film, and there are no processing costs. 2) Videotape can easily be copied; its electronic format makes editing, image processing, adding titles, etc. much simpler. 3) Since the video signal is monitored in real time, the exposure can be corrected immediately with little loss of data; with film, exposure problems are only detected when the film is processed. 4) The video signal is routed electronically through wires, thus the camera operator may be some distance away; this enhances observational flexibility. 5) Video image brightness can be amplified electronically, making observations at low light levels possible. 6) Video image size is also amplified electronically, thus reducing the amount of magnification that must be accomplished by optics.

Video does have drawbacks; its main disadvantages are relatively poor resolution and blurring of single-frame images in comparison to high-speed cinematography. A basic video system (Figs. 1,2) is composed of a camera, monitor, and videotape recorder (VTR). Resolution increases in video with the number of horizontal lines of resolution and ultimately is limited by the lowest resolving component in the video system, generally the VTR. High resolution black and white (B/W) surveillance-type cameras that are commercially available have a resolution of 800 lines, B/W monitors have resolution up to 750 lines, conventional VHS VTR's have resolutions of 240 lines, super VHS (S-VHS) VTR's about 400 lines, and extended definition Beta (ED-Beta) resolve up to 500 lines.

In terms of obtaining clear single-frame images, the main limitation is the scan speed. Video, like cinema film, is made of frames, and a conventional (NTSC) picture changes at a rate of 30 frames per second. Each frame comes up on the screen in the form of two sequential meshed (interlaced) fields. The electron gun in the monitor scans each of these fields in 1/60 of a second, but, since two fields are needed to make a complete frame, the effective speed corresponds to a 1/30 second shutter speed in 35mm film photography. This in turn means that almost any moving image will be blurred to some extent. Unlike 35mm photography, where shutter speeds can be increased, the speed of the standard video camera is fixed by the broadcast standard.

The situations outlined above are being improved. Higher density electronics, such as new charge-coupled devices (CCD's) are increasing resolution in cameras. The development of high definition TV (HDTV) in the future should also improve resolution immensely. High-speed shutters are already available in cameras, and although they require more light, they are able to freeze motion by allowing only a brief exposure of the camera's pickup device to the image. The pickup device is then scanned at the normal 1/30 second, resulting in a picture with less fluid motion, but clear individual frames. Digital technologies, which eliminate the need for interlacing by combining information from an incoming field with the next field, are now beginning to enter the market (Kenny 1989).

The following discussion of the components of videomacroscopy (electronics, optics, and observational arenas) emphasizes the particular system developed in the Aquatic Entomology Laboratory at Purdue University over several years, and in use in 1989. Whereas work in this laboratory pioneered application of videomacroscopy for studying freshwater macroinvertebrates, other laboratories have used or modified our methodology for their own purposes in studying aquatic insects.

Electronics

The primary camera in use at Purdue is a Panasonic WV-1850 surveillance-type camera (Fig. 3a). The WV-1850 video camera and its Extended Red Newvicon pickup tube (S4119) are extremely effective at low light levels (down to 0.1 foot-candles), and have relatively good resolution for video equipment. The basic configuration used by Keltner and McCafferty (1986) for study of burrowing behavior routed the video signal through a Panasonic time-date generator (WV-810) (Fig. 2d) to a VTR (Panasonic NV-8950) (Fig. 2c) and monitors, including a color monitor (Fig. 2a). A second VTR is attached to this basic configuration and is used to copy tapes from the main VTR or the 8mm camcorder. The time-date generator has been modified so that its stopwatch functions are fully enabled and can be operated remotely.

For color observations, and for field work, a Nikon VN-810 color 8mm camcorder is used (Figs. 1a,2e). It is powered by a nickel-cadmium battery pack and contains both a camera and VTR in one unit, thus it can be used in the field without the need for any connecting cables. It can be mounted in an Ikelite underwater housing for field observations underwater. Although overall resolution is not as good as the WV-1850, the availability of a color picture is often an advantage. The camcorder also has a 1/1000 s^{-1} shutter allowing clear still frame photographs to be made.

Transferring images from videotape to film media for publication remains problematic. A Polaroid FreezeFrame unit in use at Purdue does produce 35mm photographs from videotape, but they are not of publication quality. Consumer demands for such devices are strong, and better quality pictures should be possible soon, particularly after the arrival of high definition digital video equipment.

Optics

The primary optical system attached to the Panasonic camera consists of a 50 mm Macro-Switar lens and 75 mm of extension tubes (Fig 3e). The 35x magnification (on a 23cm monitor) is not sufficient for observing mouthpart movement, but is adequate for observations of whole organisms or large structures such as legs. Several different configurations are used to increase magnification. A Fujinon 20-100mm remotely controlled zoom (zoom refers to change of focal length, hence magnification) lens (Fig. 3f) gives acceptable performance only when fitted with close-up lenses or extension tubes, both of which eliminated the remote zoom function. A modified Fujinon 50 mm lens (Fig. 3f), gives performance similar to the Macro-Switar, with the added convenience of remote control of the iris.

An adapter (Fig. 3d) that permits Nikon F mount equipment to be mated to the "C" mount of the videocamera is the single most useful piece of equipment for obtaining high magnifications. Magnifications up to 80x (23cm screen) are possible using the extension tubes, adapter, a bellows unit, and 50 mm Nikon lens (Fig. 3c). The bellows unit is modified by the addition of a remotely controlled electric motor with elastic drive to the rear focusing knob, making remote zooming possible. The high magnification achieved by using this configuration reduces working distance and depth-of-field. At maximum extension, the lens is so close to the observation cell that it is very difficult to get light around the lens to the subject. At the same time, increased light is needed because lens apertures have to be reduced to increase depth-of-field. While adequate light can be obtained for visual-spectrum observations by a difficult arrangement of fiber optics light pipes, infrared illumination is insufficient. A partial solution is achieved by attaching infrared light-emitting diodes (LED's) to the surface of the observation cell to provide spot illumination.

The Nikon to "C" adapter also permits mounting the videocamera on a trinocular-head dissecting microscope via a Nikon AFM photomicrography unit. This requires that the shutter in the photomicrography unit be kept open by setting it on the "bulb" position. The stereomicroscope provides high-magnification views (up to 120x, 23cm screen) with ample working distance, but focusing is difficult since the heavy camera has to be moved. In addition, depth-of-field and observational flexibility are severely limited. Still, it was used with great success in the study of *E. needhami* (McShaffrey 1988).

The Nikon camcorder has an integral zoom lens with close-focussing capabilities. It is not exchangeable, but magnification can be increased by the addition of diopter lenses to the front of the zoom lens.

Light sources are another important component of the optical system. A fiber optics microscope illuminator (Fig. 4a) provides copious light that can easily be concentrated on a small area without conveying excessive heat to the specimens. Such illuminators can also be fitted with simple filters such as Kodak photographic darkroom filters, to provide infrared or near infrared light. Keltner and McCafferty (1986) used near infrared light almost exclusively. Their study involved burrowing Ephemeroptera such as *Pentagenia*, which live in low-light interstitial habitats, and they were concerned that high levels of human visual-spectrum light would affect behavior. There is general agreement that many insects are not sensitive to infrared light, and in particular, aquatic insects would not be expected to sense infrared since it is absorbed quickly in water. Still, there have been few if any studies on the visual spectrum of aquatic insects; the assumptions about their visual spectra are based on extrapolations from other insects such as the honeybee *Apis mellifera*.

Observational arenas

Observational arenas in the laboratory are of two basic types, those designed for studying overall behavior of the organism, and those designed for detailed observations of a portion of that behavior at high magnifications. Both types of arenas must recreate certain critical aspects of the natural habitat such as current or light levels, with arenas designed for detailed behavioral study often sacrificing some natural conditions. Keltner (1983) stated that the key to detailed behavioral observations was confinement of the organism to a small area, and he developed a series of small observation cells (Figure 1, Keltner and McCafferty 1986) for this purpose. These observation cells are not suitable for observations of heptageniid feeding which requires flowing water to make conditions more natural, and the ability to view feeding underneath the head capsule.

These problems were solved by the development of the flow cell (Figs. 3b, 4b; Figure 1, McShaffrey and McCafferty 1986), which essentially simulates the crevice microhabitat of *Stenacron interpunctatum*. The flow cell includes a microscope slide with cultured periphyton which makes up the front wall/viewing port of the observation cell and simulates the rock surface with its biofilm food source.

The flow cell, as used in the study of *S. interpunctatum*, proves useful, with modifications, in studying other organisms. The cell is easily adapted for tests of filtering and gathering functions by adding detritus in suspension and adjusting water flow. Flow cells can be constructed in various sizes and enclosure depths. The bolt spacing can be arranged so that the camera lens can approach the viewing port more closely in high magnification/short working distance situations. The front surface surrounding the viewing port is beveled to allow more light to reach the viewing port.

Flow cells are not suitable at maximum magnifications where the short working distance demands a flush front surface with no protruding bolt heads. Tank cells, aquaria, and artificial streams provide a flush surface, but must contain interior structures to confine the organisms near the viewing surface when working at high magnifications. In addition, large tank cells and aquaria preclude the use of advanced remotely controlled stages

(Fig. 4c) such as those developed by Keltner (Keltner and McCafferty 1986). At lower magnifications, large aquaria are quite useful for overall behavioral studies, since the organisms can be given more room to move around.

For field work, the environment itself is the observational arena, and recordings are made with camcorders enclosed in underwater housings. Such housings are either constructed of rigid materials or flexible plastics (with glass lens ports). The former are bulky but durable, the latter may be used with caution in shallow waters. Magnification in such a system is generally limited. Fiber optics systems can be used in the field for remote sensing of otherwise inaccessible spaces under and between rocks, logs, and other substrates.

Applications

The video equipment and observational arenas described above have been used for a wide variety of studies on Ephemeroptera and other aquatic macroinvertebrates. Burrowing behavior and functional morphology of burrowing structures in *Hexagenia* and *Pentagenia* were based on videomacroscopic techniques (Keltner and McCafferty 1986). Feeding behavior and associated mouthpart functional morphology with respect to ecology have been described in detail for several Heptageniidae (McShaffrey and McCafferty 1986, 1988). In these instances the correlation of videomacroscopy with fine structure scanning electron microscopy proved to be an indispensable combination for generating results.

Studies of the feeding behavior of a number of other Ephemeroptera larvae, including species of *Callibaetis, Ephemerella, Ephoron, Isonychia* and *Potamanthus*, have been conducted at various levels of detail in our laboratory (McShaffrey 1988). Detailed ecological and behavioral studies of *Ephemerella needhami* that have incorporated videomacroscopy are about to be published. Studies of the hydrodynamics of the water penny larva (Coleoptera: Psephenidae) have also been conducted (McShaffrey and McCafferty 1987). Other researchers that have used video techniques to study Ephemeroptera include Braimah (1987a, 1987b), who studied the hydrodynamics of filter feeding in *Isonychia*, and Soluk and Craig (1988), who examined the exploitation of vortices by the filter-feeder *Ametropus*. At Purdue, behavioral data generated from videomacroscopic studies are being applied to systematic studies, particularly with respect to morphological adaptations of head and mouthpart structures.

Conclusions

The decreasing cost and increasing capability of consumer video equipment is good news for Ephemeroptera workers who would like to add behavioral analysis to their research programs. Experience to date, including work with organisms that live in habitats where filming is traditionally difficult (under rocks, in current, in silty water or burrows), indicates that video observation and analysis of behavior is possible for most Ephemeroptera using consumer video equipment. The low cost of such equipment puts it in the range of even underfunded research programs. There is great potential for video techniques to provide valuable data on research problems ranging from behavior to ecology to systematics.

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References

Braimah, S.A. (1987a): Mechanisms of filter-feeding in immature *Simulium bivittatum* Malloch (Diptera: Simuliidae) and *Isonychia campestris* McDunnough (Ephemeroptera: Oligoneuriidae). Canadian J. Zool., 65: 504-513.

Braimah, S.A. (1987b): Pattern of flow around filter-feeding structures of immature *Simulium bivittatum* Malloch (Diptera: Simuliidae) and *Isonychia campestris* McDunnough (Ephemeroptera: Oligoneuriidae). Canadian J. Zool., 65:514-521.

Keltner, J. (1983): A study of functional morphology and aptation, with particular reference to burrowing in the mayfly genera *Hexagenia* and *Pentagenia*, and new methods of observation. M.S. Thesis, Purdue University.

Keltner, J. and W.P. McCafferty (1986): Functional morphology of burrowing in the mayflies *Hexagenia limbata* and *Pentagenia vittigera*. Zool. J. Linn. Soc., 87:139-162.

Kenny, G. (1989): Get the picture. A guide to high technology TV. Stereo Rev., 54:69-73.

McShaffrey, D. (1988): Behavior, functional morphology, and ecology related to feeding in aquatic insects with particular reference to *Stenacron interpunctatum, Rhithrogena pellucida* (Ephemeroptera: Heptageniidae), and *Ephemerella needhami* (Ephemeroptera: Ephemerellidae). Ph.D. Thesis, Purdue University.

McShaffrey, D. and W.P. McCafferty (1986): Feeding behavior of *Stenacron interpunctatum* (Ephemeroptera: Heptageniidae). J. N. American Benthol. Soc., 5:200-210.

McShaffrey, D. and W.P. McCafferty (1987): The behaviour and form of *Psephenus herricki* (deKay) (Coleoptera: Psephenidae) in relation to water flow. Freshwater Biol., 18:319-324.

McShaffrey, D. and W.P. McCafferty (1988): Feeding behavior of *Rhithrogena pellucida* (Ephemeroptera: Heptageniidae). J. N. American Benthol. Soc., 7:87-99.

Soluk, D.A. and D.A. Craig (1988): Vortex-feeding from pits in the sand: a unique method of suspension feeding used by a stream invertebrate. Limnol. Oceanogr., 33:638-645.

Figure 1. Video equipment set-up to film from artificial stream. a) 8mm Camcorder. b) Electronics cart with monitors and video tape recorders (VTR's). c) Artificial stream.

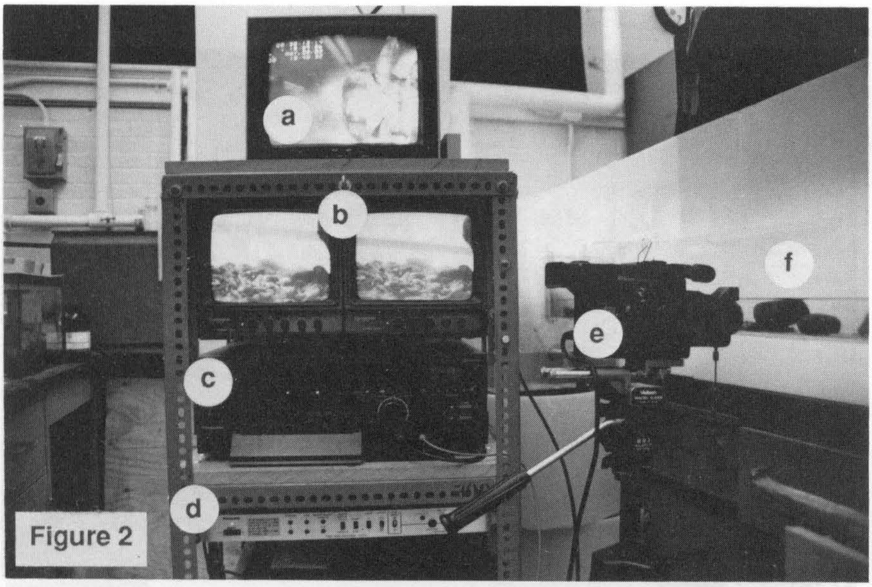

Figure 2

Figure 2. Close-up of Fig. 1. a) Color video monitor. b) High-resolution black-and-white (B/W) video monitors. c) Video tape recorder. d) Time-date generator. e) 8mm Camcorder. f) artificial stream.

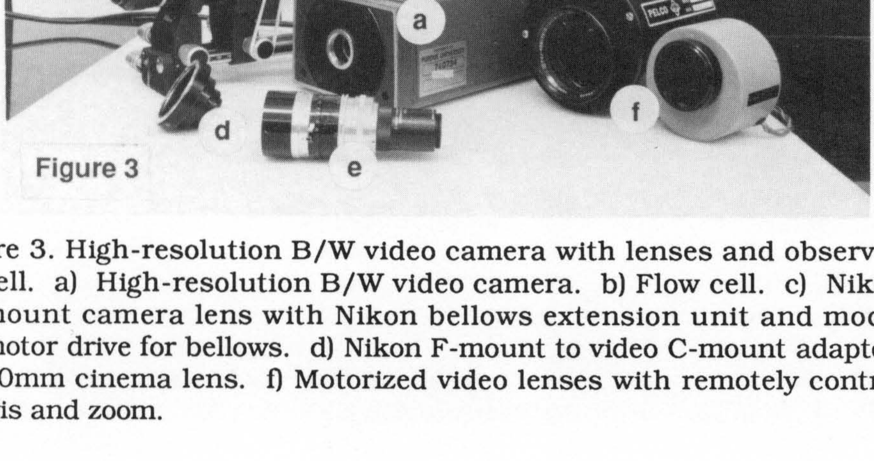

Figure 3

Figure 3. High-resolution B/W video camera with lenses and observation cell. a) High-resolution B/W video camera. b) Flow cell. c) Nikon F-mount camera lens with Nikon bellows extension unit and modified motor drive for bellows. d) Nikon F-mount to video C-mount adapter. e) 50mm cinema lens. f) Motorized video lenses with remotely controlled iris and zoom.

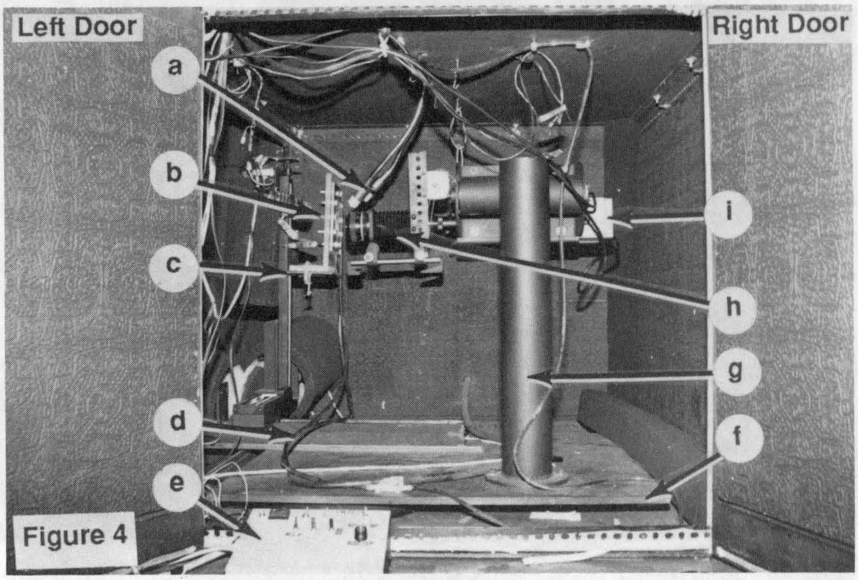

Figure 4. High-magnification observational set-up in "der schwarze Kasten" observational theater. a) Fiber optics light pipes with infrared filters. b) Flow cell (as in Fig. 3b) c) Remotely controlled movable stage. d) Water supply/drain tubing for observation cell. e) Remote control box for movable stage (remains outside theater when doors closed). f) Pneumatically supported base plate. g) Camera mount. h) Nikon F-mount camera lens with modified Nikon bellows unit (as in Fig. 3c). i) High-resolution B/W video camera (as in Fig. 3a).