214

25

High-Speed Silhouette Photography of Live Zooplankton

Harold E. Edgerton
Massachusetts Institute of Technology
Cambridge, Massachusetts

Harold A. Moffitt, II
Florida Atlantic University
Boca Raton, Florida

Marsh J. Youngbluth
Harbor Branch Foundation, Inc.
Fort Pierce, Florida

INTRODUCTION

The ninteenth-century art of shadow photography, as practiced by W. H. Fox-Talbot in 1834, has been revived by the use of a small-diameter electronic flash lamp and fine grain film (Edgerton and Wilson 1977; Edgerton 1977a). One practical application has been the photographic recording of living plankton from freshwater and marine environments (Edgerton 1977b; Ortner *et al.* 1979). Exposures of microsecond duration are made after pouring a plankton sample over a sheet of negative film under darkroom conditions. The resultant one-to-one silhouette images of these organisms are without appreciable edge blur, diffraction, or imperfection due to subject motion and may be identified to genus or species. The technique is nondestructive and several exposures can be made at sea before preserving a sample for other analyses onshore.

The purpose of this paper is to describe technical aspects of high-speed silhouette photography, such as resolution limitations, film types, exposure calculations, light sources, and practical techniques.

THEORY

The resolution of silhouette photography is influenced primarily by three factors: film grain, shadow error, and diffraction.

Film Grain

Grain is a fundamental limitation to the resolution of the emulsion of a film. Suppose the film is rated to resolve n lines per mm. Then the resolution can be given approximately as $1000/n$ microns. Examples of suitable films are (Ortner *et al.* 1979):

Eastman Plus X: $n = 50$ lines/mm, resolution = 20 microns
Eastman 7302: $n = 150$ lines/mm (fine grain positive), resolution = 7 microns
Eastman SO343: $n = 2000$ lines/mm (high resolution), resolution = 0.5 microns

Shadow error

The shadow error (equivalent to the penumbra) of a sharp edge can be estimated from the geometry of an effective point source (Figure 1) by:

$$B = A\frac{d}{D} \tag{1}$$

where B is the error in microns, A is the lamp diameter in microns, d is the subject-to-film distance, and D is the lamp-to-film distance (d and D are in the same units).

Diffraction error

The resolution error caused by diffraction can be calculated from:

$$I = 0.85\sqrt{d\lambda} \tag{2}$$

where I is the resolution error due to diffraction effects in microns, d is the subject-to-film distance in microns, and λ is the wavelength of the light source in microns.

Close spacing of the subject to the film (d) and the use of a light source of short wavelength (λ) both reduce the error caused by diffraction. This diffraction calculation is based upon parallel light for a small-diameter lamp at a relatively great distance from the film, that is, an effective point source.

The relative effects of penumbra and diffraction are shown in Table 1. Note that both penumbra and diffraction errors increase as the subject-to-film distance increases. However, diffraction is the more serious error. For example, if a resolution of 2 microns is desired, the subject-to-film distance (d) should be 14 microns ($I = 2$ microns $= 0.85\ \sqrt{d \times 0.4}$), which is a very short distance. Eastman SO343 film is required for a resolution of this magnitude. Note also that Eastman SO343 demands considerably more incident light than Eastman 7302 film to produce an acceptable negative density of 0.7, as shown by the H & D curves of Plus X, 7302, and SO343 as obtained by use of a xenon flash lamp (Figure 2) (Edgerton 1979).

EXPOSURE CALCULATIONS

Exposure of the film can be calculated from the inverse square law:

$$\mathrm{IT} = \frac{\mathrm{CPS}}{D^2} \tag{3}$$

where IT is the incident exposure on the film in lumen-sec/m^2, CPS is the source output in candela/sec, and D is the lamp-to-film distance in meters (Edgerton 1979).

LIGHT SOURCES

Any light source can be used for silhouette exposures. However, the selection of a light source for the best image resolution is influenced by four factors: penumbra, diffraction, flash duration, and source output.

Penumbra

The penumbra resolution is affected directly by the diameter of the light source. The diameter should be at least ten times less than the lamp-to-film distance. The xenon flash lamps commonly used in silhouette photography have diameters of 3-7 mm.

Diffraction

The diffraction error can be reduced by the use of an effective point source and by a source with an output of short wavelength. Most xenon flash lamps have a large component of near ultraviolet light. One flash lamp

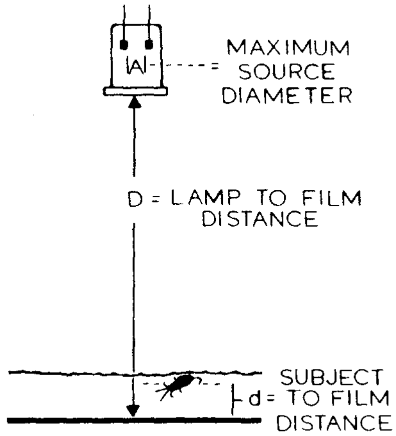


Figure 1. Spatial geometry of silhouette photography.

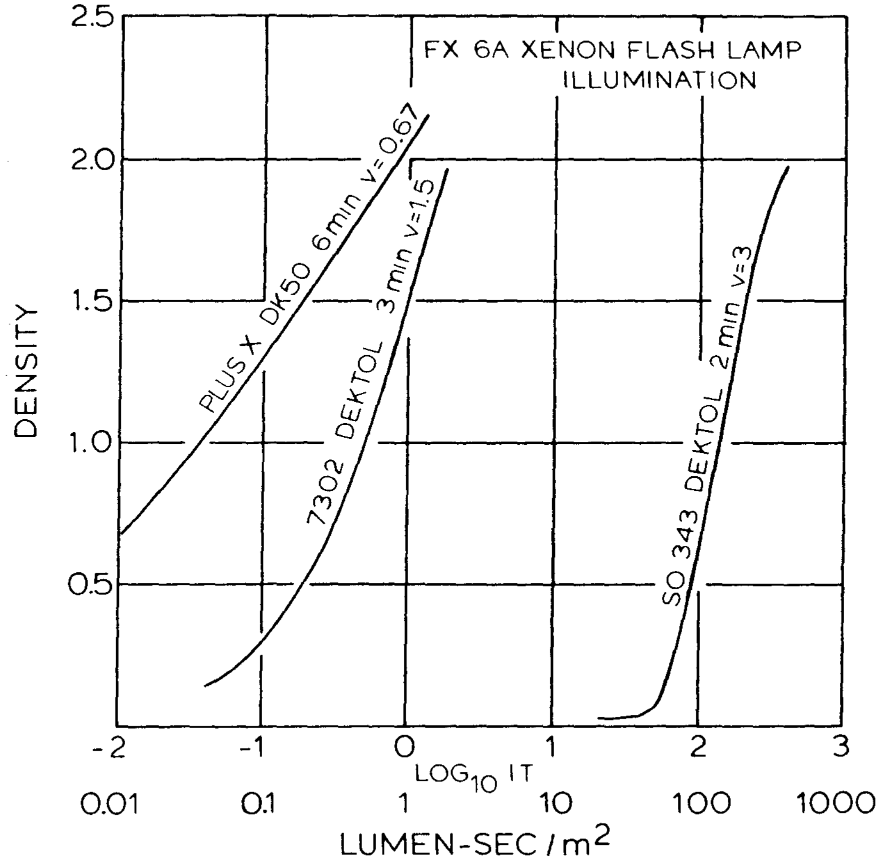


Figure 2. H & D curves for Eastman film types Plus-X, 7302, and SO343.

Table 1. Resolution Error Due to Penumbra and Diffraction for Three Subject-to-Film Distances.

Subject-to-film distance (mm)	*Resolution error*	
	Penumbra (μm)	*Diffraction (μm)*
0.1	1	5.4
1.0	10	17.0
10.0	100	53.7

Note: lamp diameter = 3 mm, lamp-to-film distance = 30 cm, and wavelength of blue light = 0.4 μm.

in particular (EG&G type FX-265) has a special quartz window that transmitts ultraviolet light (EG&G, a).

Flash duration

To stop the motion of any organism effectively, a flash duration on the order of 1 to 10 μsec is required. Flash durations of 3 to 5 μsec are readily obtainable with the xenon flash lamps.

Source output

The source must provide sufficient incident light to the film over the lamp-to-film distance (D) to produce an acceptable density of 0.7. Selected values of CPS for given distances, D, can be estimated from equation 3:

$$\mathrm{CPS} = (\mathrm{IT})D^2$$

The light output of an electronic xenon flash source may be measured directly (Edgerton 1979) or calculated from

$$\mathrm{CPS} = \left(\frac{\mathrm{CE}^2}{2}\right)n \qquad (4)$$

where C is the flash tube storage capacitance in farads, E is the potential of the charged capacitor in volts, n is the efficiency of the flash tube in lumens/watt, and CPS is the calculated output of the source in candela/sec. Typical efficiencies of xenon flash lamps used in silhouette photography are 0.5 to 2.0 cp/watt (EG&G, b).

The EG&G Inc. line of small xenon flash lamps (such as type FX-6A) is particularly well suited to silhouette photography because of its small

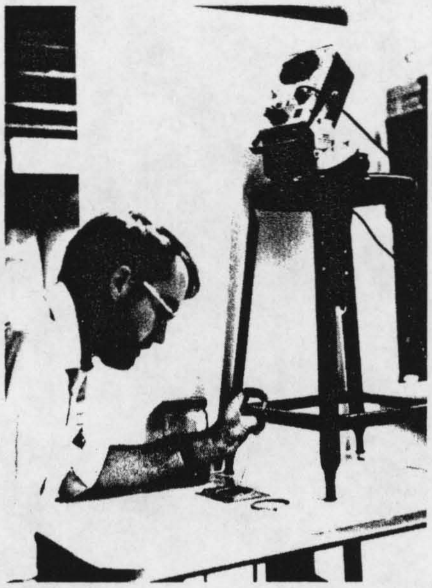


Figure 3. GenRad Strobotac Model 1531AB with reflector removed for use in silhouette photography.

diameter (7 mm) and the near ultraviolet spectral component. This lamp is widely used in the GenRad Company lines of stroboscopic instruments for measuring speed and observing periodic motion (EG&G, c). Examples of models particularly adaptable to silhouette photography are numbers 1531, 1538, and 1539. These instruments have reflectors that can be removed to expose the bare lamp. The dial light of these units must be masked to prevent accidental film exposure (Figure 3). Set on the medium range, the lamp's single flash output is about 0.5 CPS, which works well with Eastman 7302 film at a lamp-to-film distance of about 1 m.

The EG&G FX-6A and FX-265 lamps can be incorporated into a single flash circuit dedicated to silhouette photography. Schematics are shown in Figures 4, 5, and 6. Battery and AC powered units have been field tested (Figures 7 and 8). In order to use Eastman SO343 film, an additional capacitor of 25 μF must be connected in parallel with the 2 μF capacitor. The SO343 lamp-to-film distance is 70 cm.

Another source of small size and ultraviolet output is the EG&G Microflash equipment with a point source adaptor. This unit is commonly used for the silhouette photography of bullets and shock waves (Edgerton and Wilson 1977).

Any photographic strobe lamp can be used if an aperture is placed over the reflector. The exact size of the aperture is dependent upon the particular system. A starting value for the experimental determination of the aperture diameter can be calculated from equation 3. Experimental exposures will determine the optimum aperture size for the desired negative density.

The use of a light source producing a double flash over a known time

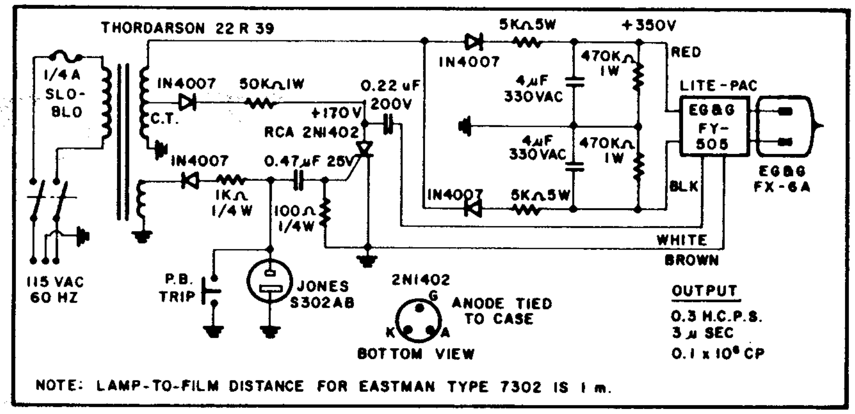


Figure 4. AC line powered electronic schematic for EG&G FX-6A bulb type flashtube.

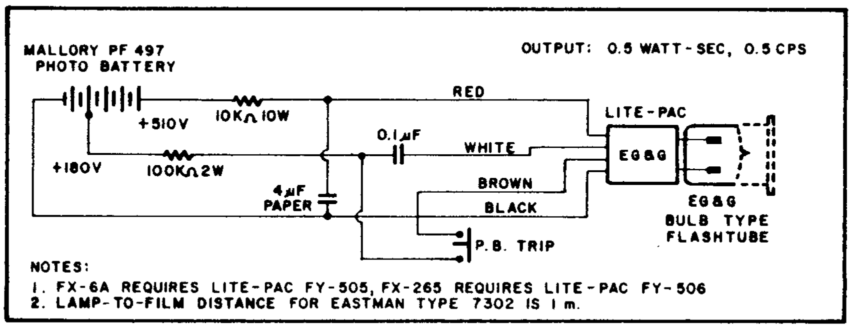


Figure 5. Battery powered electronic schematic for EG&G FX-6A or FX-265 bulb type flashtube. Orient lamp for maximum output.

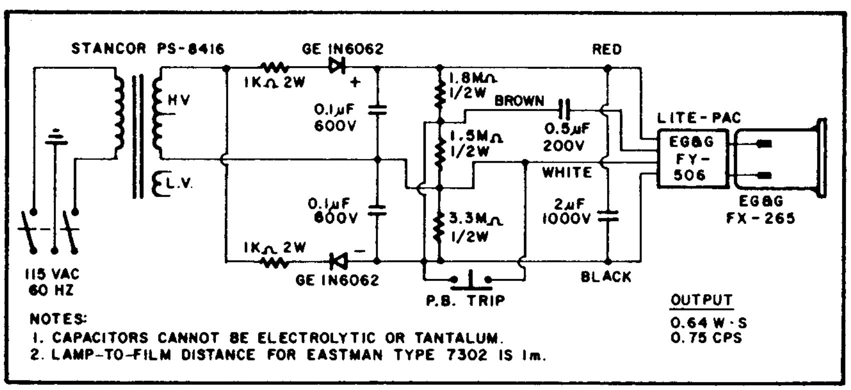


Figure 6. AC Line powered electronic schematic for EG&G FX-265 bulb type flashtube.

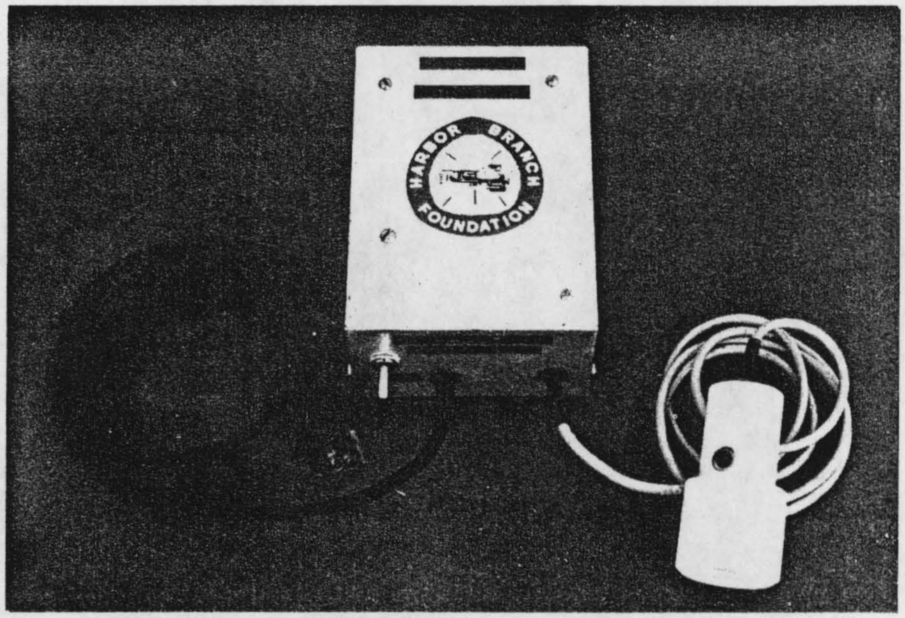


Figure 7. Assembled AC powered FX-265 flash unit.

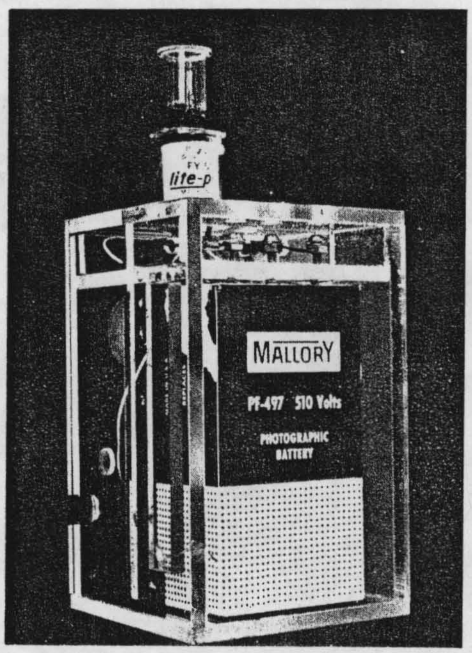


Figure 8. Assembled DC powered FX-265 flash unit.

interval may be used to estimate average swimming velocities by measuring the placement of the subject on the film between exposures (Edgerton and Killian 1979). The duration of each individual flash must be 3 to 5 μsec to stop the motion of the subjects effectively without blurring. Equipment designed to produce a double flash over a very short and known time interval is described by Edgerton (1979) in *Electronic Flash, Strobe.*

PROCEDURE

The silhouette photography of zooplankton is usually conducted in a darkroom or in the field with the use of a suitably sized dark box (Edgerton 1977a). Fine grain negative film is commonly used in sheet format (4 × 5 or 8 × 10 in.). The blue sensitivity of these films responds well to the spectral output of the xenon flash lamp and enables the investigator to use a red safe light during the exposure (Eastman Kodak).

Upon the selection of the film, the lamp-to-film distance can be calculated for a source of known output. For example, to use Eastman 7302 film, the required incident exposure (IT) is 0.2 lumen-sec/m^2. For a source output of 0.3 CPS, the lamp-to-film distance should be

$$\mathrm{IT} = \frac{\mathrm{CPS}}{D^2} \quad \text{or} \quad D = \sqrt{\frac{\mathrm{CPS}}{\mathrm{IT}}} = \sqrt{\frac{.3}{.2}} = 1.2\ \mathrm{m}$$

Note from equation 5 that the actual output of the source and, lamp-to-film distance are directly affected by the lamp efficiency at the energy input level used. The optimum lamp-to-film distance then is determined by experimental exposures based on the calculated distance of 1.2 m.

There are two basic methods for making silhouette exposures. The subjects can be placed directly on the film or they can be put in a sample holder that is placed on the film. With the first or wet method the subject is exposed to chemicals that leach from the film. Some species of gelatinous zooplankton are traumatized with this method (for example, tentacles not relaxed, swimming movements erratic, mucous secretions produced). Whether other zooplankton are similarly stressed is not known. Therefore, if several photographs are taken it is important to keep the water bath fresh.

With the alternative or dry method the subject is isolated from the film by a thin sheet of cellophane (Figure 9).* Care must be taken with

* *Saran Wrap*™ [Trade mark of the Saran Protective Coatings Co., 1342 Gakmain Blvd., Detroit, Michigan 48238. (313)867-4900]. This material is preferred over other brands because it is thicker and has fewer imperfections.

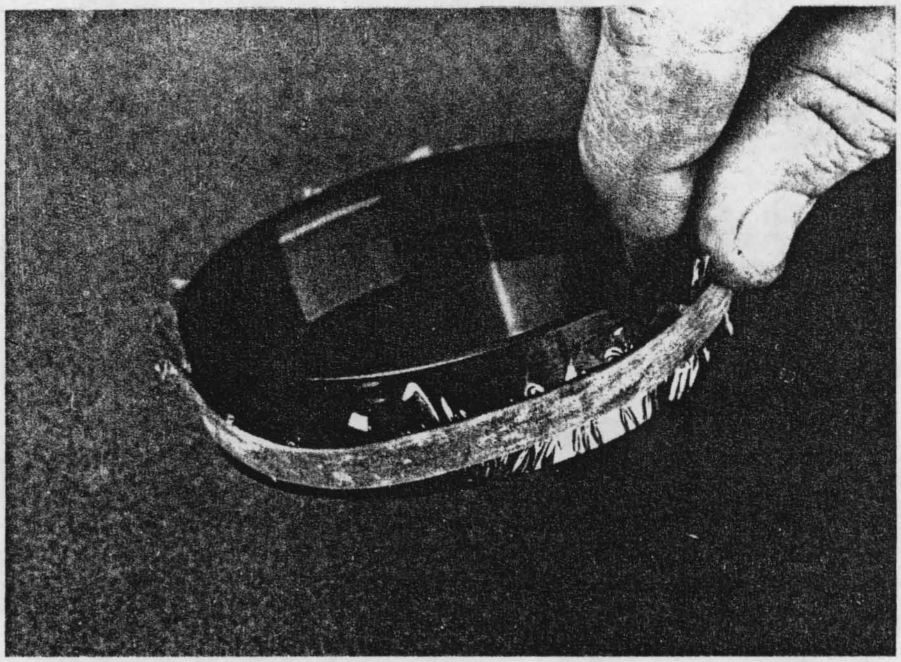


Figure 9. Zooplankton sample holder for silhouette photography.

this material to avoid scratching or creasing its surface and to select a section where variations in thickness are not obvious since any of these imperfections will cast a shadow on the film. The ring of the sample holder should be black plastic or covered with black tape to prevent light scattering from the ring to the emulsion surface.

Dust and other particles present in the air or seawater can reduce the quality of the silhouette image. Most of this contamination can be prevented by placing subjects in 0.2 μ-filtered water and covering the photo chamber until ready for exposure.

The emulsion surface should be cleaned just prior to positioning the sample on the film to remove any extraneous particles. A low pressure jet of clean, dry, oil-free gas such as air, Freon, or nitrogen may be used (Eastman Kodak).

When photographs are taken at sea, the photo chamber should be isolated from vibrations of the ship's machinery since these vibrations ripple the water surface and cast shadows on the negative. This problem can be avoided by setting the photo chamber on a heavy steel plate supported by rubber pads.

Exposures are made under darkroom conditions. The first step is to place the emulsion side of the film under the subject. A red safelight can be used to provide enough illumination to position the specimen over the film if the safelight is kept at least 1 m away. The light source is then positioned at a predetermined distance from the film and with a single flash of the lamp the exposure is made (Figure 1). With both methods, the subject-to-film distance should be kept as small as possible to reduce the penumbra. Image resolution is also improved if the subject is covered with as little water as possible but enough to insure that the subject does not contact the air-water interface.

Development in Dektol or Ektaflo provides a denser negative than the D-76 developer recommended by Kodak for the 7302 film. If the wet emulsion method is used, the film should be rinsed with fresh water prior to processing.

The resultant one-to-one negative image may be studied with a hand lens, enlarged and printed, or mounted and projected. The contrast of a print may be enhanced by the use of Kodak opaque red to mask out areas of the negative surrounding a subject of interest.

EXAMPLE PHOTOGRAPHS

Gelatinous, marine zooplankton (such as ctenophores, siphonophores, and salps) are extremely fragile. As a consequence, the natural appearance of these semitransparent animals is difficult to record and some species cannot be preserved chemically. Silhouette photography of living specimens is one means of surmounting this problem. Examples of gelatinous zooplankton are shown in Figure 10.

Figure 11 illustrates an example of double flash exposure of live crustaceans in a seawater sample. The average velocity for the copepods shown in this example is 0.48 cm/sec (0.19 in./sec).

SUMMARY

The silhouette photographic technique produces a high resolution record without appreciable shadow error, diffraction, or blurring due to animal or ship motions. The resultant one-to-one black-and-white negative image may be studied immediately after development with a hand lens, enlarged and printed, or mounted and projected. Since the technique is nondestructive, a zooplankton sample may be preserved for further analysis onshore.

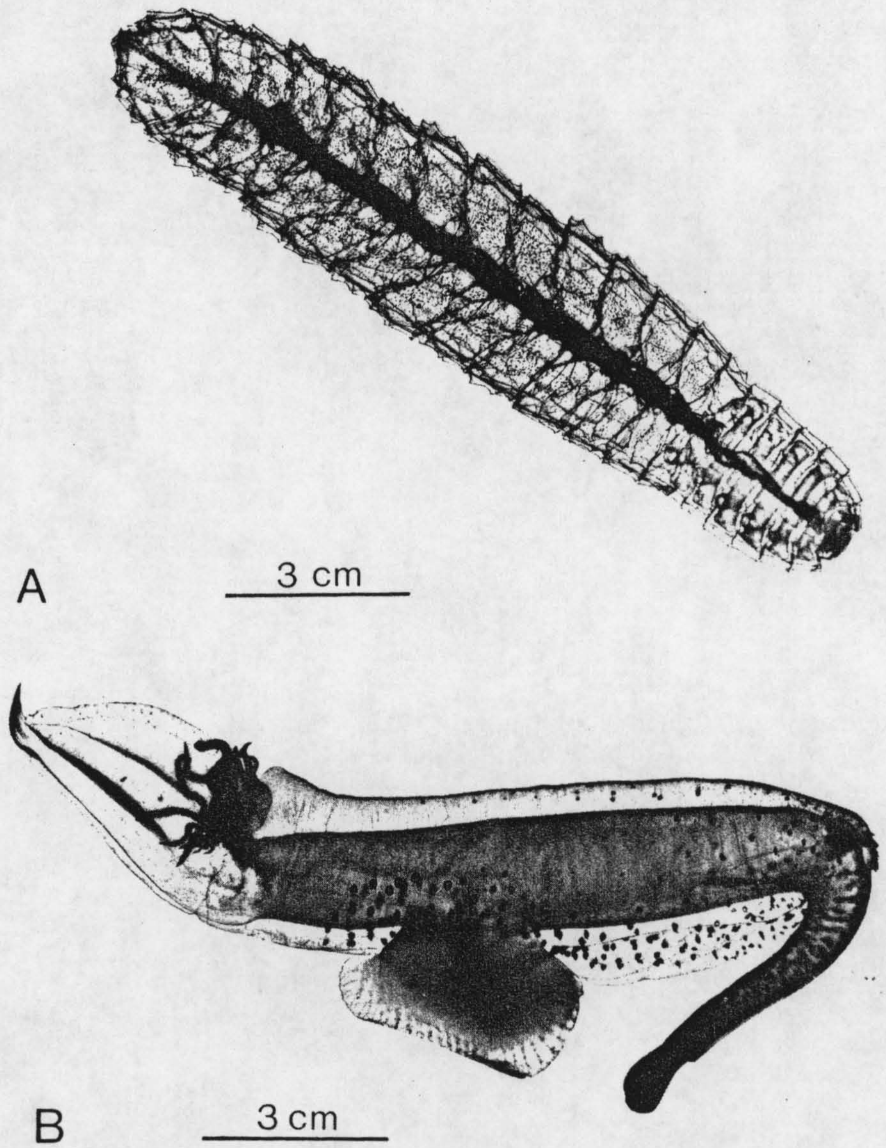
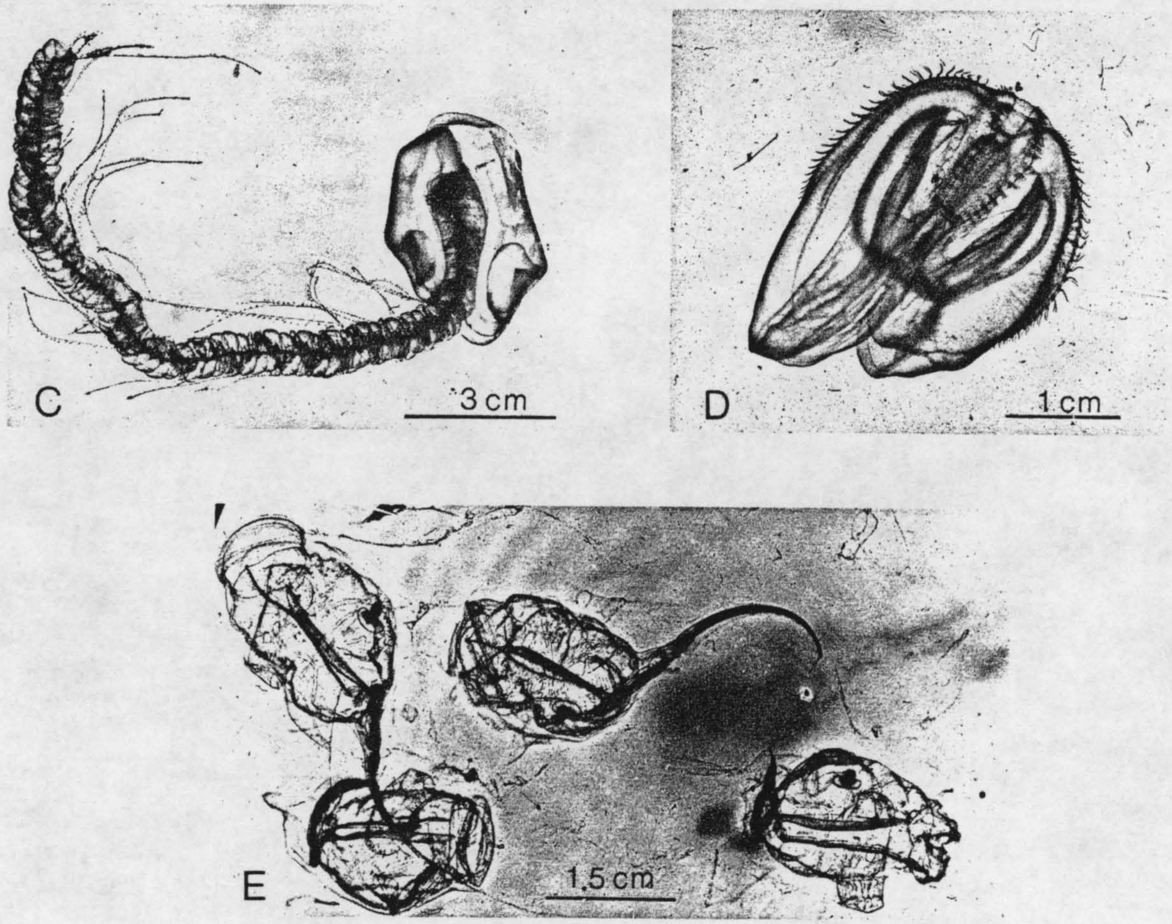


Figure 10. Examples of gelatinous marine zooplankton
(a) Siphonophore, *Agalma okeni* (live, enlarged × 1.2, FX-6A flashtube, negative masked).
(b) Heteropod, *Pterotrachea scutata* (preserved, ×.8, FX-265, masked).
(c) Siphonophore, *Rosacea cymbiformis* (live, ×.67, FX-6A, unmasked).
(d) Ctenophore, *Mnemiopsis mccradyi* (live, ×1.47, FX-6A, unmasked.)
(e) Aggregate forms of the salp, *Helicosalpa virgula* (live, ×1.12, FX-6A, unmasked).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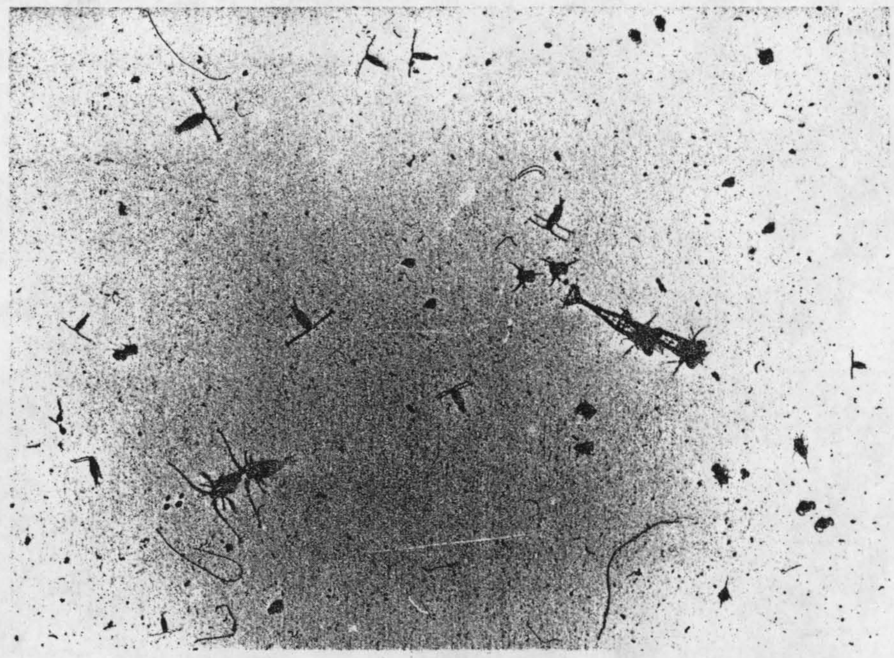

Figure 11. Double flash exposure of crustaceans in a seawater sample (live, ×6, GenRad Strobotac Model 1531, flash interval 0.65 sec, unmasked).

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