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Evaluation Of In-Situ Silhouette Photography In Investigations Of Estuarine Zooplankton And Ichthyoplankton

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EVALUATION OF IN SITU SILHOUETTE PHOTOGRAPHY IN INVESTIGATIONS OF ESTUARINE ZOOPLANKTON AND ICHTHYOPLANKTON

John E. Olney and Edward D. Houde

ABSTRACT

Trials of a paired-net towing frame fitted with a submersible 35-mm camera system were conducted in Chesapeake Bay in 1989–1990 to 1) demonstrate the use and efficiency of in situ silhouette photography in studies of estuarine zooplankton and ichthyoplankton; 2) determine the taxonomic potential of in situ silhouette photography in several estuarine habitats; and 3) compare estimates of plankton density from in situ silhouette photographs with concurrent preserved net collections. Time required to split, sort and enumerate plankton in preserved samples was 2–75 h per sample longer than the time required to view and enumerate silhouettes on film. Ninety-four taxa (or different ontogenetic stages of the same taxon) were identified on film. Of these, 55% were classified to genus or species. The camera-net system failed to detect 16 of 31 rare or uncommon categories of zooplankton at one or more stations. Abundance estimates of two gelatinous forms (ctenophores and doliolids) were provided by the camera but these taxa could not be counted or were absent in paired, preserved collections. There were no detectable differences in estimates of abundance provided by the camera and the paired, preserved collection for hydromedusae, polychaete larvae, marine and some freshwater Cladocera, cyprid stages of barnacles, larval stomatopods, caridean and some brachyuran zoeae, megalopae, and fish eggs. Differences in plankton density estimated by the camera and the paired net were significant for some freshwater cladocera, gastropod larvae, some brachyuran zoeae, mysids, and chaetognaths. Statistical results were mixed, depending upon locality, for copepods, naupliar stages of barnacles, and fish larvae. For all categories of planktonic taxa that differed significantly from net collections, in situ photography provided lower density estimates than the net. Underestimation was partly attributed to the poor photographic qualities of some taxa and stalling of plankton along the sides of the camera net.

Silhouette photography (Edgerton, 1977; Ortner et al., 1979), employed in a towed system that concentrates and photographs live plankton as material passes through the codend of a net, yields 35-mm film rolls containing serial photographs (Ortner et al., 1981). Deployment of the camera system together with hydrographic probes and biological sensors has the potential to produce a highly dimensional and integrated data set resulting from a single cast. To date, the technique has been employed in studies of abundance and vertical distribution of pollock (*Thelagra chalcogramma*) eggs and larvae in the Pacific northeast (Reed et al., 1988) and community analysis of functional groups of zooplankton in the Gulf of Mexico (Ortner et al., 1989).

Prompted by the objectives to reduce cost associated with laboratory analysis of preserved samples and to increase sampling frequency on time and space scales relevant to larval fish survival, Houde et al. (1989) conducted six trial deployments of a camera-net system (Ortner et al., 1981) in the Patuxent River and mid-Chesapeake Bay in July 1982. Silhouette images of fish eggs, fish larvae, copepods, and mysids were easily identified, and analysis of photographs suggested that bay anchovy (*Anchoa mitchilli*) eggs were heterogeneously distributed on scales of about 2 m. Comparison tows by a conventional plankton net that was concurrently deployed yielded similar egg abundance estimates. Fish larvae, which were un-

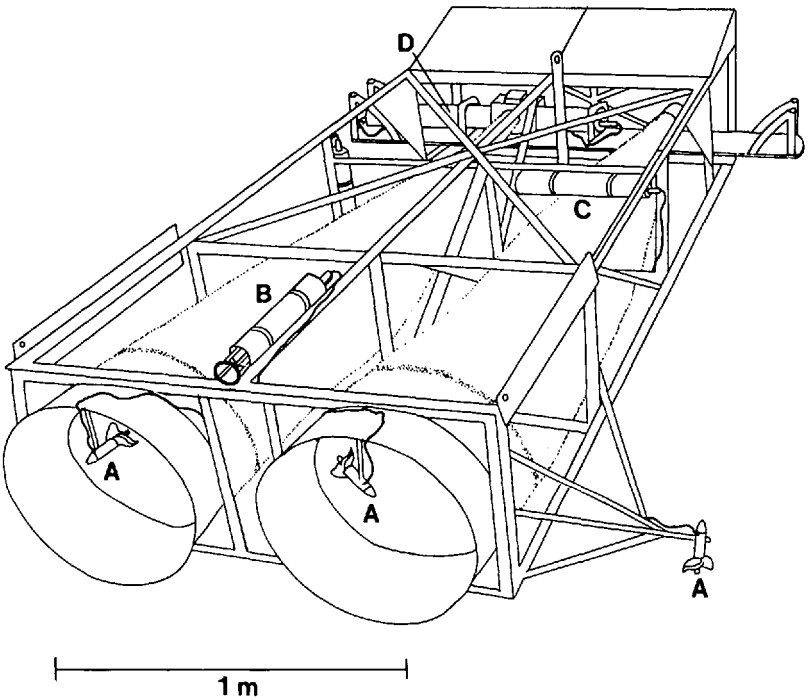

Figure 1. Diagram of the paired-net towing frame. Labelled instruments are: A) electronic flowmeters; B) conductivity-time-depth (CTD) probe; C) inclinometer; D) plankton camera.

common in the preserved samples, apparently were undersampled by the camera (Houde et al., 1989).

Encouraged by these preliminary results, we constructed a new paired-net towing frame, and conducted extensive trials of the camera-net system in 1989. Our interest was to determine its potential in turbid estuarine environments such as Chesapeake Bay where decreased water clarity might alter silhouette image quality. Furthermore, we wanted to evaluate the camera in an environment where seasonally high plankton densities and low species diversity might prove amenable to optical sensing techniques. Our specific goals were to: 1) demonstrate the use and efficiency of in situ silhouette photography in studies of estuarine zooplankton and ichthyoplankton; 2) determine the taxonomic potential of in situ silhouette photography in several estuarine habitats; and 3) compare estimates of plankton density from in situ silhouette photographs with concurrent preserved net collections.

Materials and Methods

Gear Description.—The paired-net system (Fig. 1) was constructed from aluminum and fitted with two 335-μm mesh nets (60-cm diam). Our estimate of the open-area ratio (Omori and Ikeda, 1984) of these nets was $R = 4.7$, given a mesh porosity of 46%, a mouth area of 0.3 m^2, and a total net area of about 2.9 m^2. The bridled frame (3.6 m long, 1.4 m wide) was equipped with a conductivity-temperature-depth sensor (CTD), two inclinometers and three flowmeters (one mounted in the mouth of each net and one mounted on an extended arm). These instruments relayed data on hydrography, flow, horizontal and vertical inclination through a data cable tethered to the frame. Data were recorded at 1-s intervals and the output was monitored and stored by shipboard computer.

The camera system (Model 373 submersible camera, Benthos, Inc.), which was described by Ortner et al. (1981) and Houde et al. (1989), consists of a 35-mm camera and a high intensity flash unit

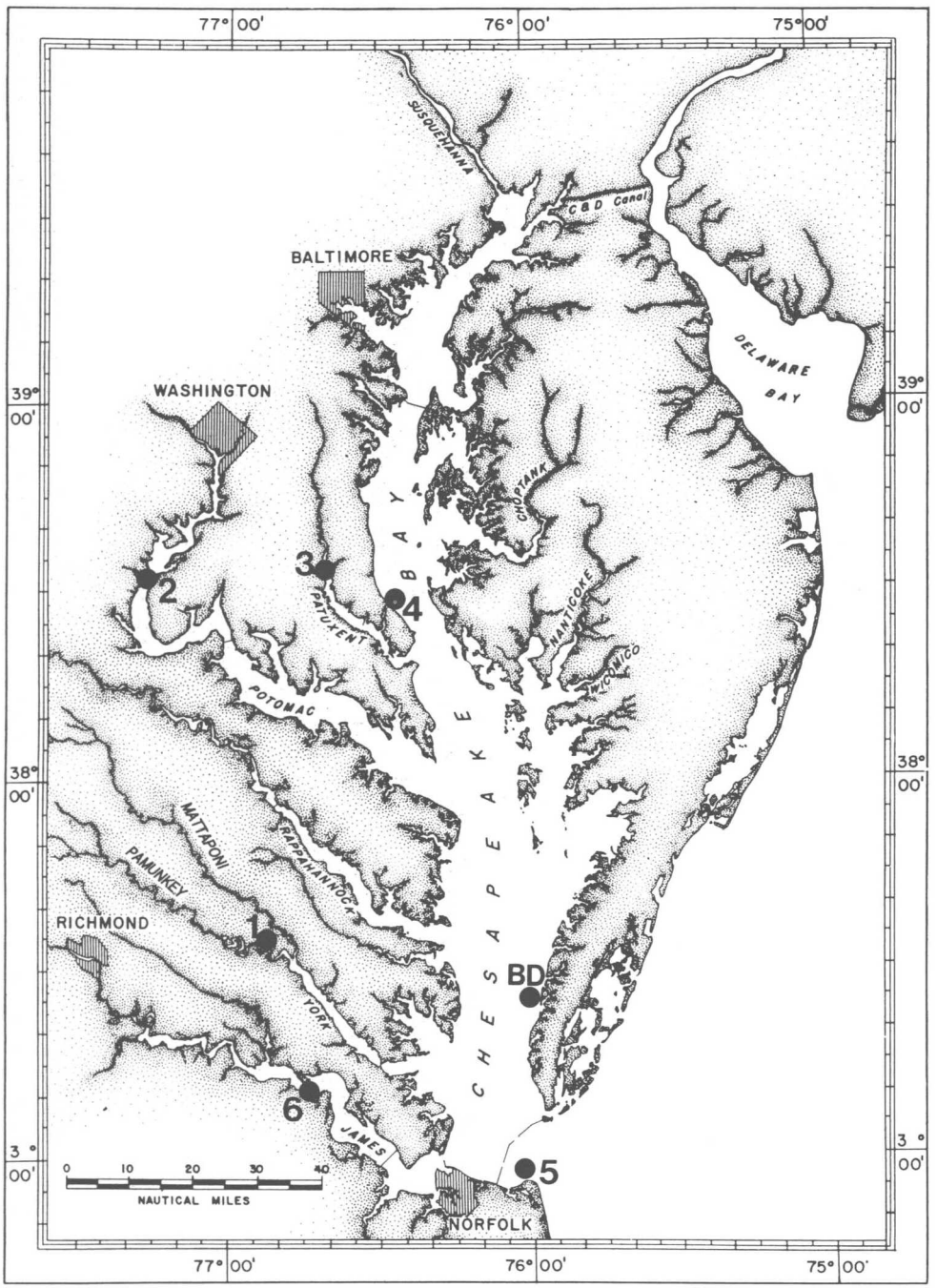

Figure 2. Locations of stations in the Chesapeake Bay where the camera-net system was deployed: (1) the Pamunkey River near West Point, Virginia; (2) the Potomac River at Quantico, Virginia; (3) the Patuxent River at Chalk Point, Maryland; (4) the upper Chesapeake Bay at Calvert Cliffs, Maryland; (5) the lower Chesapeake Bay near Cape Henry, Virginia; (6) the James River at Surry, Virginia and (BD) the lower Chesapeake Bay near Cape Charles City, Virginia.

Table 1. Summary of collection data, film selection, camera settings and hydrographic variables at sampling locations in the Chesapeake Bay region. Abbreviations are: Pan-X, Kodak Panatomic-X film, ASA 32; Pos-Rel, Eastman Fine Grain Positive Release Film 5302, ASA 4; °C, extremes of mean water column temperature (degrees centigrade) in two oblique casts; ‰, extremes of mean water column salinity (parts per thousand) in two oblique casts

Station	Dates of collection	Total deploy-ments	Film	F-stop	Total frames	Temperature (°C)	Salinity (‰)
1	26 Apr 1989	9	Pan-X	5.6	890*	17.6	<0.1
2	4 May 1989	8	Pan-X	5.6	2,162	18.4	<0.1
3	18 Jul 1989	8	Pos-Rel	8	1,956†	26.3–26.4	1.8–1.9
4	28 Jul 1989	8	Pos-Rel	11	1,234‡	26.2–26.3	7.5–7.8
5	14 Aug 1989	8	Pos-Rel	8–11	2,255	24.7–24.8	18.3–28.5
6	18 Sept 1989	8	Pan-X	5.6	2,365	28.7–29.1	1.6–1.7

Notations: * Intermittent failures of power supply to strobe reduced total exposures.
† Over-exposed films in surface tows were not readable.
‡ Films in both surface and first bottom tow were not readable because of condensation on camera lens.

powered by a 510-V battery. The components are contained within two watertight housings capable of sustaining pressures at depths to about 300 m. In our system, the housings were bolted to a rectangular photographic chamber (3.8 cm in width, 10.2 cm in height and 25.5 cm in length; for a diagrammatic view see Ortner et al., 1981: fig. 1A), constructed of polyvinyl chloride (PVC) stock. The camera rested at the rear of the aluminum frame on a platform below two flotation wedges (Fig. 1). The codend of one net was clamped to the photographic chamber, and the concentrated plankton was photographed as it passed through the cylindrical window. The photographic field was 7.7 cm in diameter and 3.8 cm in height, with a volume of 177.9 cm^3. The ratio of the mouth area (2,827 cm^2) to the codend area (81 cm^2) of the net was 34.9, the concentration factor. We calculated that the volume "sampled" by each photograph, 6.2 liters, was the product of the concentration factor and the cylinder volume.

A shipboard sensor monitored strobe flashes, and the camera could be remotely activated or turned off during deployment. We modified the camera to increase film advance rate to one frame per second. Thus, each standard deployment of 5 min at a vessel speed of 85 cm s^{-1} yielded a film strip containing about 300 frames taken at <1-m intervals and a preserved plankton sample collected in the codend of the paired net (Fig. 1).

Field Sampling.—The camera-net system was deployed at six locations in the Chesapeake Bay region in 1989 (Fig. 2). The stations were: (1) the Pamunkey River near West Point, Virginia; (2) the Potomac River at Quantico, Virginia; (3) the Patuxent River at Chalk Point, Maryland; (4) the upper Chesapeake Bay at Calvert Cliffs, Maryland; (5) the lower Chesapeake Bay near Cape Henry, Virginia; and (6) the James River at Surry, Virginia. Stations 2–4 and 6 were near electrical generating plants operated by utility corporations in Maryland and Virginia, and cruise dates were set to allow sampling of specific meroplanktonic taxa that may be affected by entrainment. These taxa included eggs and larvae of the striped bass (*Morone saxatilis*) and the bay anchovy (*Anchoa mitchilli*), zoeal and megalopal stages of brachyuran crabs, larval barnacles, and larval bivalves. Collection data are summarized in Table 1.

At each station, at least eight deployments of the camera-net system were made during daylight hours. At the beginning of each tow, the CTD was initialized at time = 0 and the camera was not activated. When the net mouths were completely submerged, the camera was activated and CTD time recorded. When the gear breached the surface at the end of the tow, the camera was turned off and final CTD time noted. While the camera was operating, a data chamber photographically recorded frame time (minutes : seconds) at 1-s intervals to match individual frames on film with discrete sets of hydrographic and flow data. Two stepped oblique tows from near bottom to the surface were followed by horizontal tows (two each) at the surface, midwater, and near bottom. At each station, several short film strips were developed on board to examine image quality. Camera aperture settings or film selection (Panatomic-X, TMAX 100 or Eastman 5302 Positive Release) were sometimes changed as a result of these inspections. The films with the greatest light sensitivity (Panatomic-X, TMAX 100) were used only at stations where increased turbidity prevented the use of Eastman 5302 Positive Release, the film recommended for use with the camera system (Ortner et al., 1981). Our intention was not to compare the performance of these products. At each station, deployments yielded eight film rolls and eight plankton samples that were preserved in 5–8% buffered formalin. A total of 48 film rolls and 48 preserved samples were obtained.

In addition to our 1989 sampling at stations 1–6, trial tows (stepped-oblique) of the camera-net system were made at station 5 in September 1988. We also deployed the gear at several locations near station 1 on striped bass spawning grounds (Grant and Olney, 1991) in the Pamunkey River, Virginia

in spring, 1989 and 1990. In May 1990, several deployments were conducted at station "BD" near Cape Charles City harbor (Fig. 2).

Laboratory Processing.—Film rolls and formalin-preserved samples from 1989 deployments at stations 1–6 were processed with stereomicroscopes, each equipped with a darkfield/lightfield base. Only film rolls were examined in the additional deployments in 1988–1990. Film was viewed with transmitted light (lightfield setting), and magnified silhouette images of taxa were photographed with an Olympus OM4-T 35 mm camera attached to a phototube. In situ films lacked size bars, and although we were unable to scale images to exact size, relative size of individual targets usually could be judged based on comparison with other taxa (e.g., an adult *Acartia* sp. copepod approximately 1 mm in total length). The photographs (Plates 1–18) subsequently were used as an identification aid by film readers. [In Plates 1–18, each photograph is identified by tow number (values beginning with "Z") and frame time.] Our taxonomic experience varied among groups and some invertebrate taxa were identified only to higher levels of classification. For most invertebrates, we followed the nomenclature and identification keys of Gosner (1971), the descriptions of larvae provided by Smith (1977), and sought help from other experts (see Acknowledgments). Comparison with preserved material in the paired sample helped to interpret silhouette images. Three individuals viewed, identified and enumerated images on film and eight individuals sorted, identified and enumerated taxa in preserved samples. All recorded time required to complete each sample or film. In preserved collections, infrequent taxa (e.g., isopods, stomatopods, parasitic copepods, fish larvae) were sorted and enumerated in whole samples. More abundant forms (e.g., decapod larvae, barnacle nauplii, fish eggs, cladocera, copepods) were sorted and enumerated from aliquots following routine sample splitting procedures (Burrell et al., 1974). With the exception of copepods, all taxa were enumerated in each frame of a film roll. Identifications and counts of taxa were recorded by frame number. When copepod images were abundant, 60 frames were randomly selected from each film for copepod enumeration. After all samples were processed, nine film rolls and nine preserved samples were randomly selected for re-examination to evaluate counting error. Only copepods were recounted by an individual other than the original viewer in selected film rolls. In the re-inspected preserved collections, samples were split to the aliquot size in which copepods originally were sorted and then recounted.

Data Analysis.—The difference between abundance estimates (organisms m^{-3}) obtained from films and preserved samples was the primary data set used in statistical analyses. Negative differences indicated that density estimates from the preserved samples were greater. Initially, we grouped differences by taxon or ontogenetic stage for each sampling method (e.g., we pooled 38 differences between paired estimates of copepod density from all tows at stations 1–6). Two-way analysis of variance (ANOVA, $P < 0.01$) was used to screen these data sets for significant variability between station and tow type (oblique, surface, midwater, bottom). When homogeneity of variance was violated (Fmax test, $P < 0.05$) in these analyses or if data were not normally distributed (Shapiro-Wilk statistic, alpha = 0.01), data were transformed [$\log_{10}(x + 1)$]. For some data sets, transformed data remained heterogeneous and we used nonparametric procedures (one-way analysis of variance of ranked data, the Kruskal-Wallis k-sample test). When ANOVA results indicated no significant variability due to station or tow type, we pooled untransformed data by station (e.g., 20 differences between paired estimates of copepod abundance at stations 3, 4 and 6; 18 differences at stations 1, 2 and 5), and again tested for normality with the Shapiro-Wilk statistic (alpha = 0.01). A paired *t*-test was carried out on the pooled data sets and differences reported as significant ($P < 0.05$) or highly significant ($P < 0.01$). The following data sets were omitted from this final analysis: those failing the test of normality before and after transformation [$\log_{10}(x + 1)$]; those for which five or fewer paired observations were available; those for which maximum camera or preserved sample density estimates were below 0.8 organisms m^{-3}; and ctenophore abundance estimates (see Results section for explanation). A paired *t*-test (alpha = 0.05) also was used to determine if there were differences between the repeated counts of copepods in film rolls and preserved samples.

RESULTS

Taxonomy of Silhouette Images.—A total of 94 taxa (or different ontogenetic stages of the same taxa) was identified in film rolls (Plates 1–18). Of this total, 42 were not identified to genus or species, six were eggs and larvae of the same fish taxon, two were developmental stages of barnacles, and two were naupliar stages of copepods. In addition, three were unknown invertebrates: a large arthropod with a distinct urosome and a bifurcate terminal segment (Plate 7); a slender "Amphioxus-like" form that superficially resembled a chaetognath but lacked the distinctive transparent body (Plate 14); and a small "pluteus"-like individual

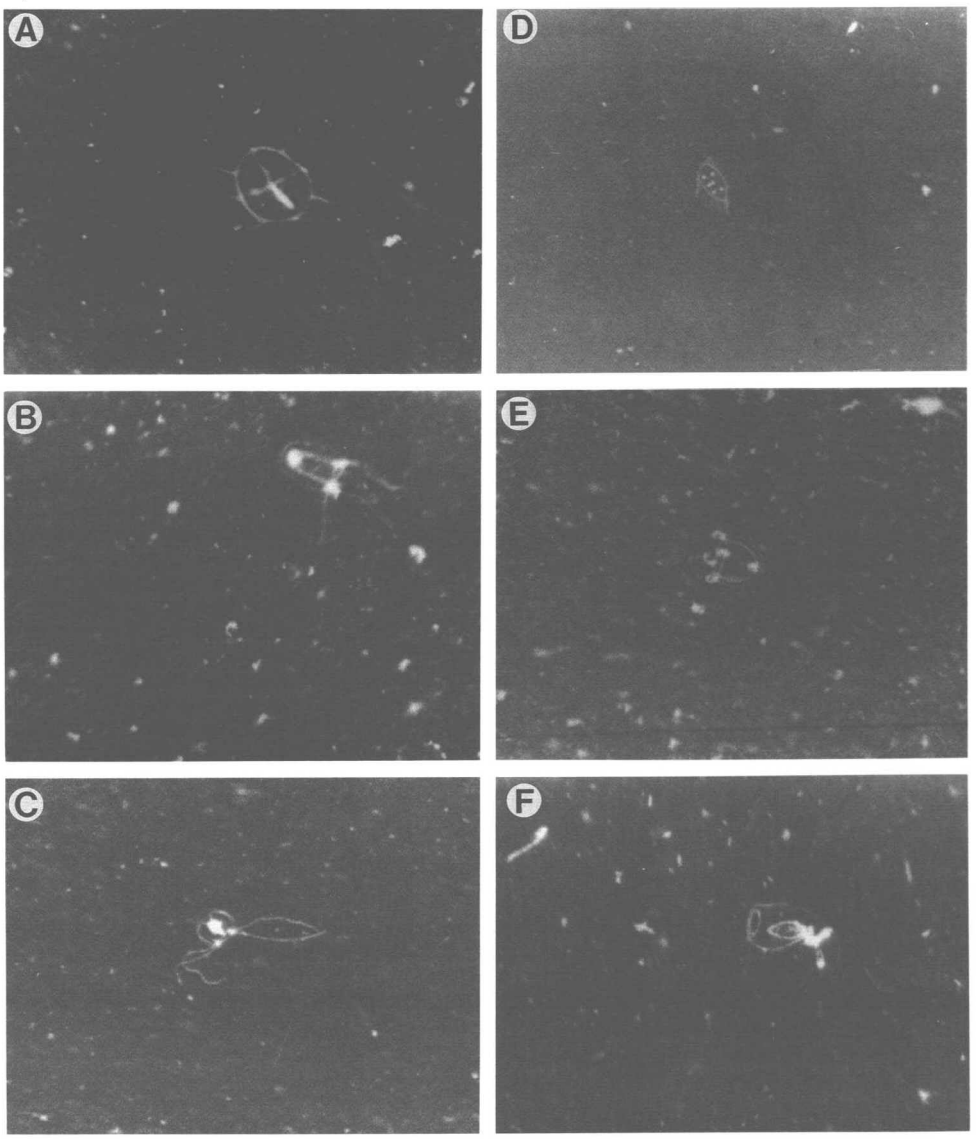

Plate 1. In situ silhouette images of Chesapeake Bay zooplankton. A) young ?*Sarsia,* Z88-214, 01: 31; B) type-1 anthomedusa, Z89-384, 10:46; C) *Podocoryne minima,* Z89-427, 40:19; D) *Obelia* sp., Z90-242, 34:03; E) type-2 anthomedusa, Z89-484, 09:09; F) siphonophore colony, Z89-429, 09:10.

(Plate 14). Whole specimens (and occasionally, fragments) of three species of ctenophores (Plate 3) were photographed, but we were unable to confidently identify images of larval ctenophores since cydippid larvae of all ctenophore genera have tentacles and are spherical (Barnes, 1980). However, information on seasonal succession and relative abundances of taxa in Chesapeake Bay may be useful in delimiting these forms. Most of our photographs of cydippids were taken in summer when *M. leidyi* predominates.

Efficiency and Repeated Counts Comparisons.—Film rolls (N = 44) and preserved samples (N = 44) required a total processing time of 148.6 h (18.6 man-days)

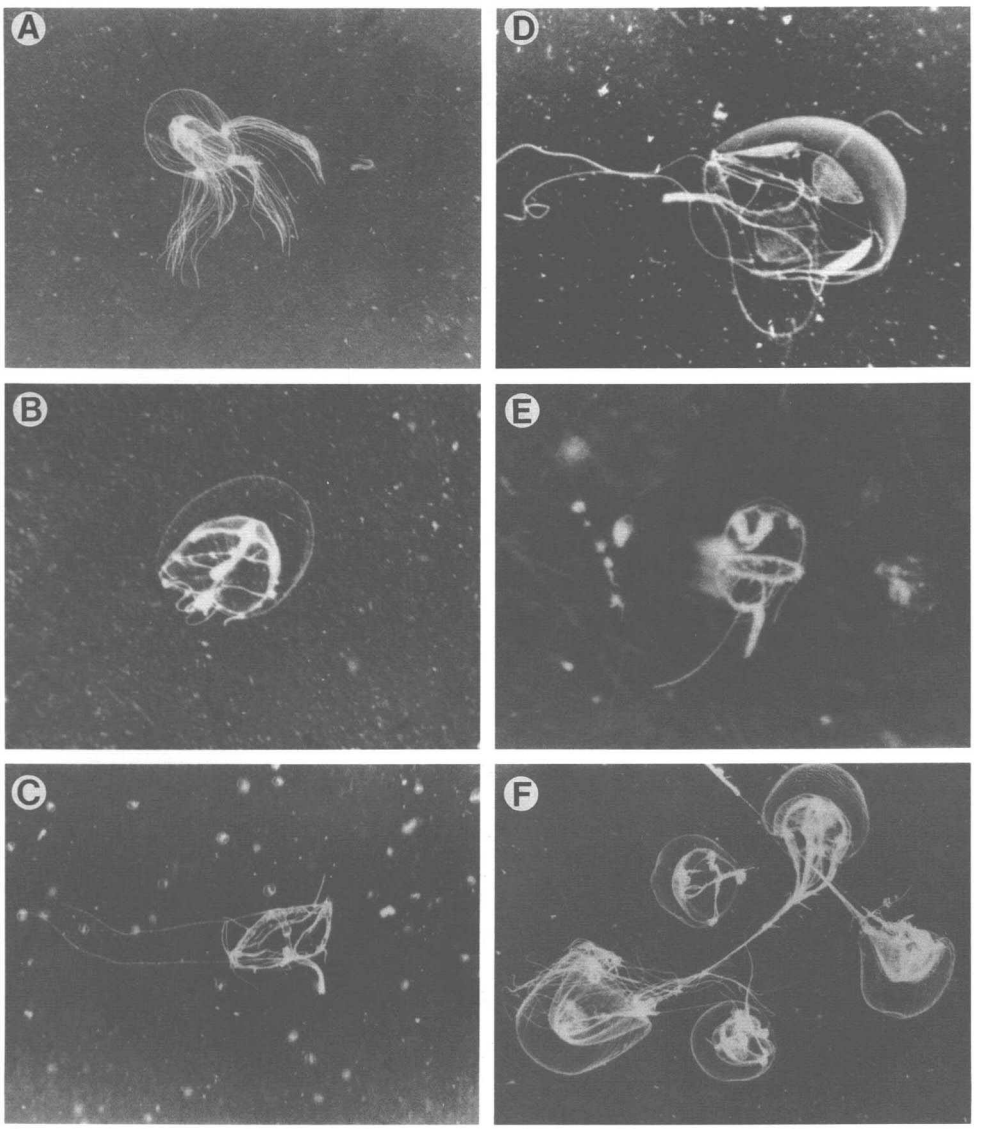

Plate 2. In situ silhouette images of Chesapeake Bay zooplankton. A) *Bougainvillia carolinensis,* Z90-272, 47:43; B) *Moerisia lyonsi,* Z89-390, 31:35; C) adult *Sarsia tubulosa,* Z88-214, 01:05; D) *Liriope tetraphylla,* Z89-437, 08:13; E) "many-tentacle hydromedusa with pointed bell," Z88-212, 52:50; F) *Nemopsis bachei* tangle, Z90-272, 48:19.

and 1304.4 h (163.1 man-days), respectively. The time required to view, identify and enumerate silhouette images on film was always less than the time required to sort, identify and enumerate preserved organisms in the paired sample. On average, preserved samples required about three working days (24.6 h) longer to process (Table 2). Time to process film rolls ranged from 0.8 to 6.7 h, the greatest effort expended on film taken at station 5 near the Chesapeake Bay mouth where targets were numerous (Table 2). Times to process film rolls at station 1 in the Pamunkey River were low because only eggs, larvae, and copepods were counted

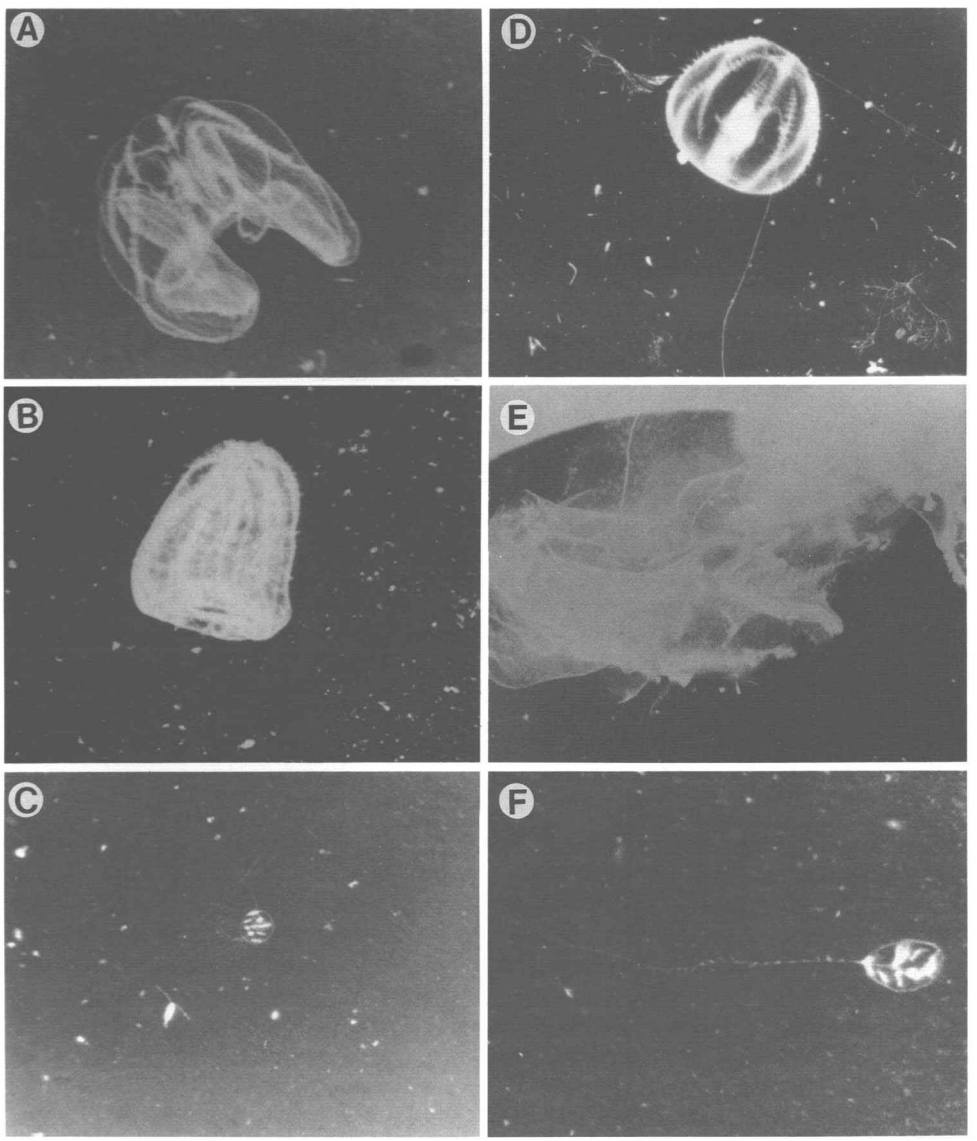

Plate 3. In situ silhouette images of Chesapeake Bay zooplankton. A) *Mnemiopsis leidyi,* Z90-272, 47:50; B) *Beroe ovata,* Z89-433, 25:45; C) unidentified cydippid larva with *Acartia,* Z89-408, 25:53; D) *Pleurobrachei pileus,* Z90-080, 57:42; E) large *Beroe* fragment, Z89-427, 43:36; F) unidentified cydippid larva, Z89-433, 23:50.

and most film rolls had few frames. The greatest difference was recorded at station 6 in the James River where detritus loads in preserved samples were high (Table 2).

In nine preserved collections selected for re-counting, copepods were enumerated in sample aliquots ranging from 1/64th to 1/4,096th of the original sample. Total copepods ranged from 126–907 in the original count and 79–867 in the repeated count. Re-counts of the same aliquot yielded a mean difference of 26.1 copepods. Differences ranged from −152 to 161 (the negative difference indicating

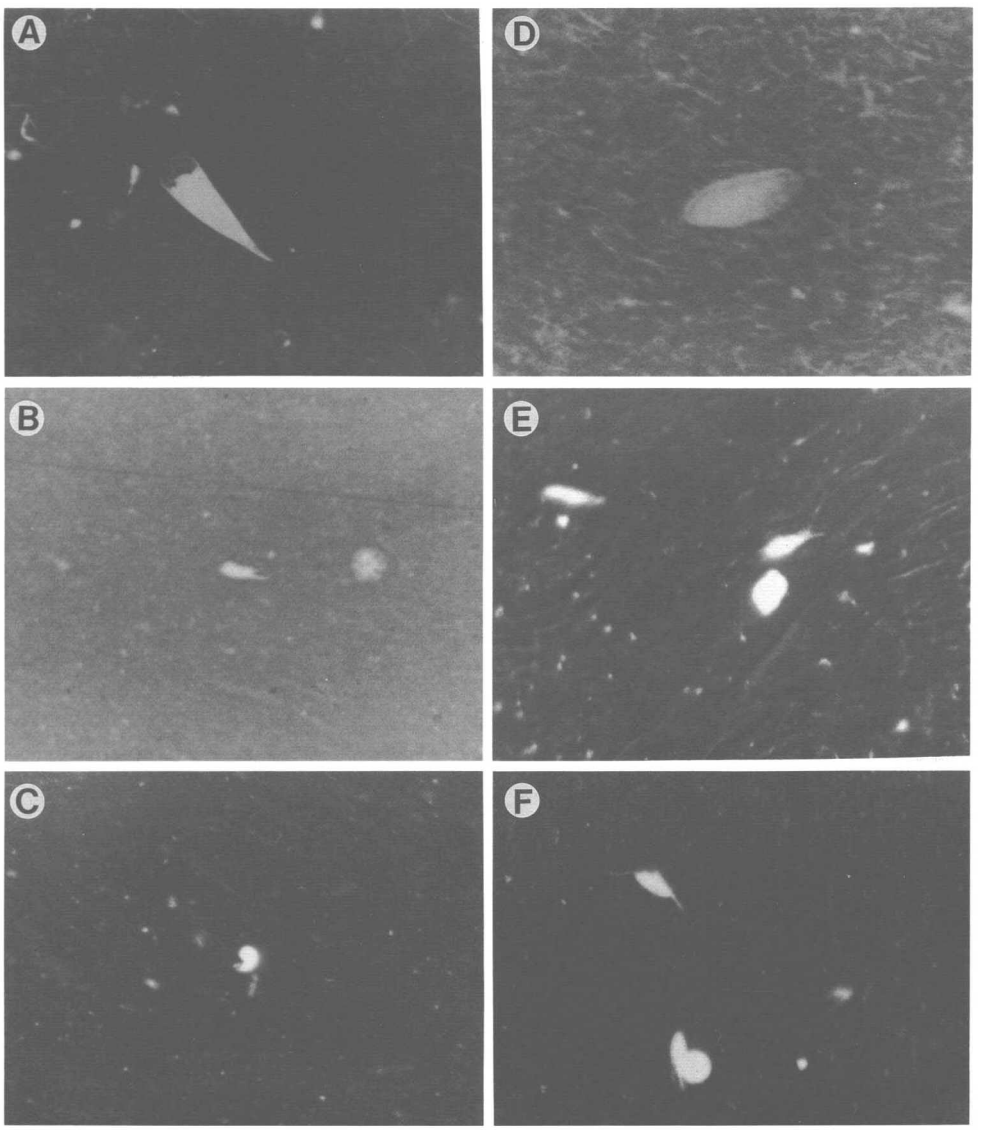

Plate 4. In situ silhouette images of Chesapeake Bay zooplankton. A) *Creseis conica*, Z88-212, 51:08; B) gastropod larva with lenticular shell and clear chambers, Z89-218, 58:42; C) type-1 gastropod larva with lenticular shell, Z89-429, 10:20; D) late larval pelecypod (possibly *Tagelus*), Z90-223, 30:31; E) gastropod larva with turbinate shell, Z89-433, 23:26; F) type-2 gastropod larva with lenticular shell, Z89-425, 03:16.

that the second count was higher) and relative differences (each difference expressed as a percent of its paired original count) were sometimes high (from 4.4% to 54.4%). Total copepods in original and a second count of randomly selected frames in nine films ranged from 58–1,761 and 52–1,894, respectively. Second counts yielded a mean difference of −9.6 copepods. Differences ranged from −133 to 65 but relative differences were lower for films than repeated counts of preserved samples, ranging from 2.4–24.5%. No significant differences between the repeated

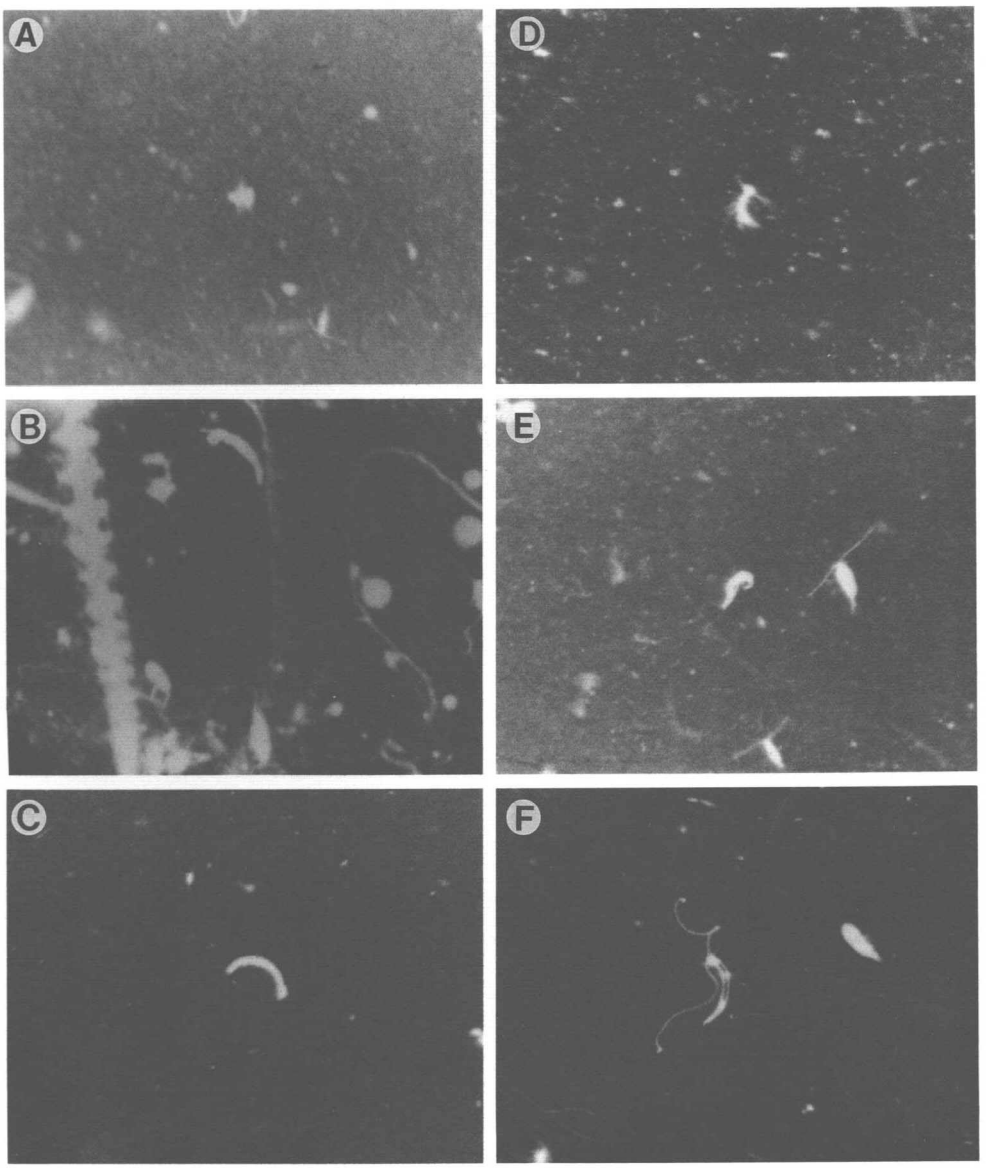

Plate 5. In situ silhouette images of Chesapeake Bay zooplankton. A) type-1 spionid polychaete larva, Z89-427, 41:19; B) terribellid polychaete larvae in a ctenophore fragment, Z89-425, 05:52; C) ?terribellid polychaete larva, Z89-445, 11:48; D) type-2 spionid polychaete larva, Z89-429, 08:41; E) terribellid polychaete larva, Z89-427, 41:22; F) chaetopterid polychaete larva, Z89-445, 12:46.

counts of preserved sample aliquots or second counts of film frames were detectable.

Occurrence and Abundance Comparisons.—We examined maximum observed abundance of 31 categories of planktonic taxa that were photographed by the camera or captured in the paired net (Table 3, Fig. 3). In situ silhouette photography failed to detect the presence of 16 of these 31 categories at one or more

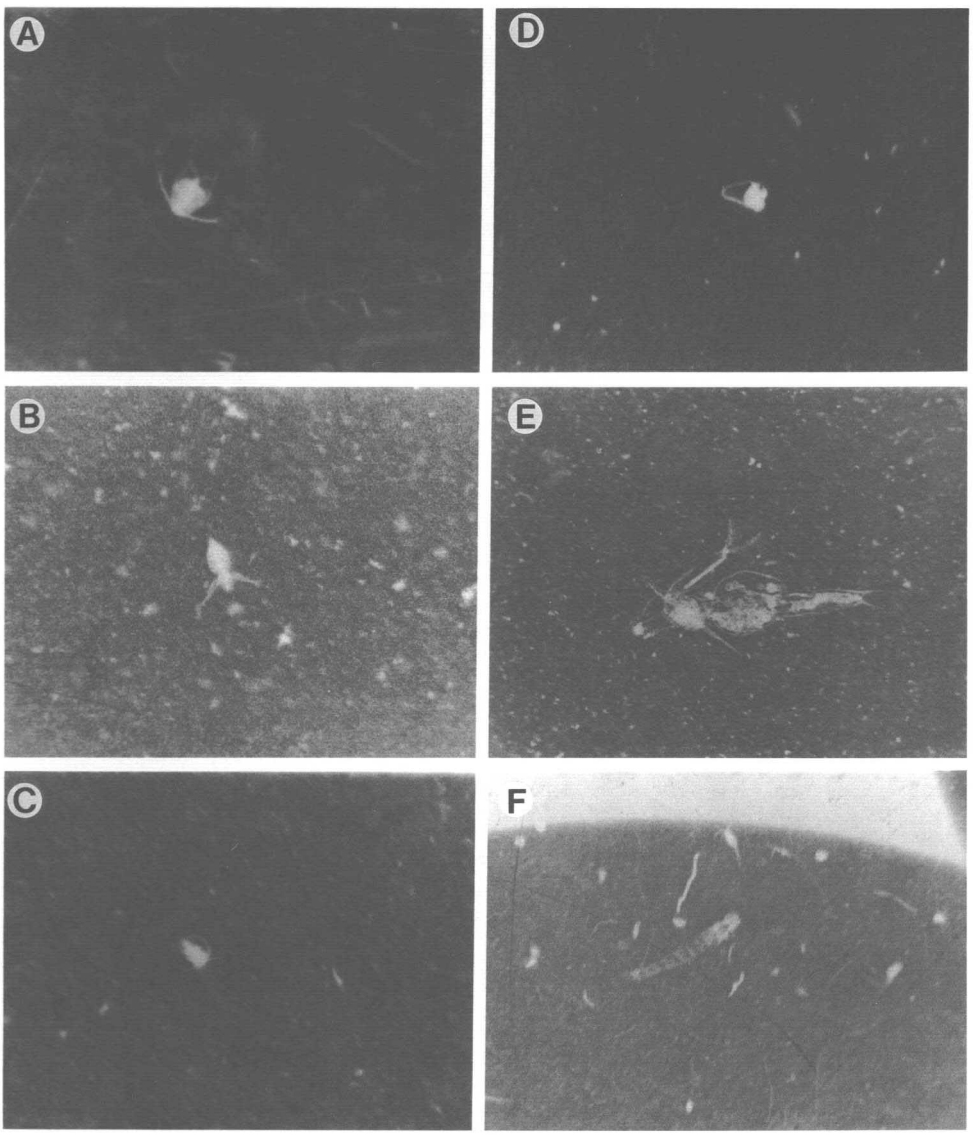

Plate 6. In situ silhouette images of Chesapeake Bay zooplankton. A) *Penilia avirostris*, Z88-212, 01:08; B) ?*Diaphanasoma*, Z89-482, 50:23; C) ?*Bosmina*, Z89-212, 01:51; D) *Evadne tergestina*, Z89-459, 21:11; E) *Leptodora kindti*, Z89-127, 26:56; F) unidentified insect larva, Z89-218, 59:04.

stations (in 25 of 94 paired occurrences or 27%; Table 3). These taxa were: gastropod larvae (at one of four stations or 1/4); bivalve larvae (2/3); marine cladocerans (1/2); ostracods (4/5); parasitic copepods (1/4); stomatopods (1/2); caridean zoea (1/3); brachyuran zoea (1/4); megalopae (1/2); isopods (2/4); amphipods (4/5); mysids (2/4); cumaceans (1/1); insects (1/5); chaetognaths (1/2); and fish larvae (1/6). Most of these undetected taxa were uncommon (maximum density <1.0 m^{-3}) in preserved collections (19 of 25 paired occurrences or 76%). However, gastropod larvae (at station 3), marine cladocera (station 2), ostracods

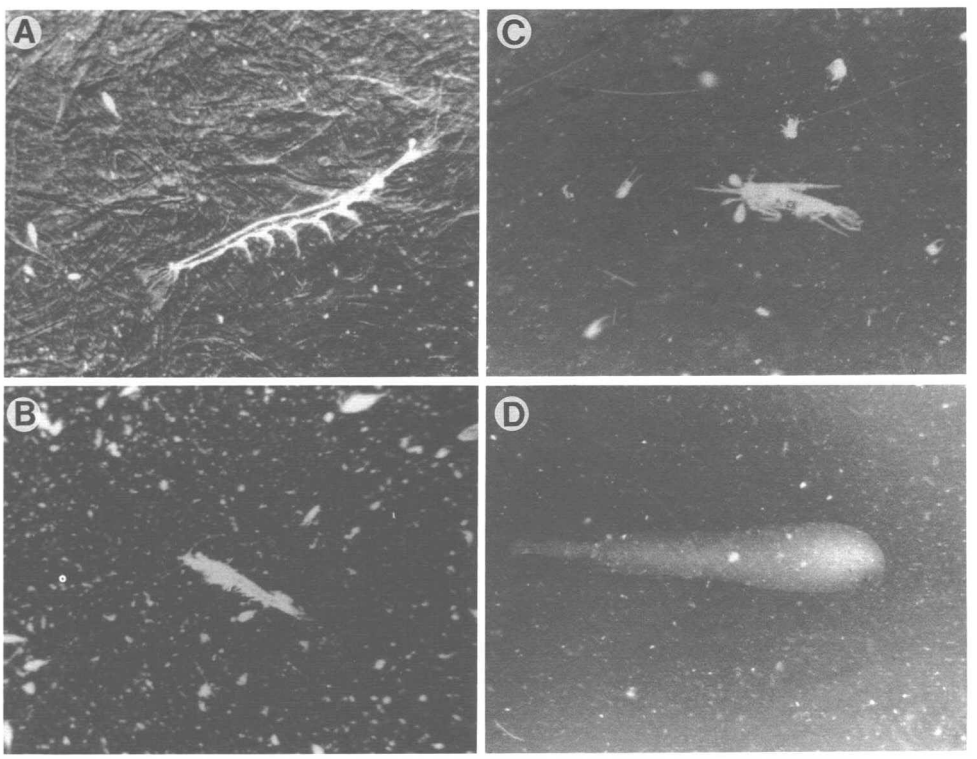

Plate 7. In situ silhouette images of Chesapeake Bay zooplankton. A) *Lucifer faxoni,* Z89-433, 23: 25; B) *Neomysis americana,* Z89-433, 23:13; C) larval *Squilla,* Z88-197, 81:05; D) arthropod unknown, Z89-433, 21:41.

(station 6), amphipods (station 6) and fish larvae (station 4) were missed by the camera in tows where maximum densities in paired, preserved samples exceeded 1.0 m^{-3} (Table 3).

The occurrence of two gelatinous forms, ctenophores and doliolids, was detected by in situ photography but these taxa could not be counted or were absent in paired, preserved collections. Detection of total taxa by the camera matched or exceeded traditional plankton methods at three stations; however, total taxa identified in preserved samples were moderately to severely underestimated by the camera at stations 3, 4 and 6 (Fig. 3).

Table 2. Average time (h) to read films or sort samples and mean difference (h) for 44 pairs of film and preserved samples collected at six stations in Chesapeake Bay, 1989 (the abbreviation N is number of pairs at each station)

Station	N	Film roll	Preserved sample	Mean difference
1	9	0.8	27.2	−26.4
2	8	4.0	6.1	−2.0
3	6	2.4	14.6	−12.2
4	5	3.2	12.4	−9.2
5	8	6.7	29.6	−22.9
6	8	3.1	78.1	−74.9
	Means:	3.4	28.0	−24.6

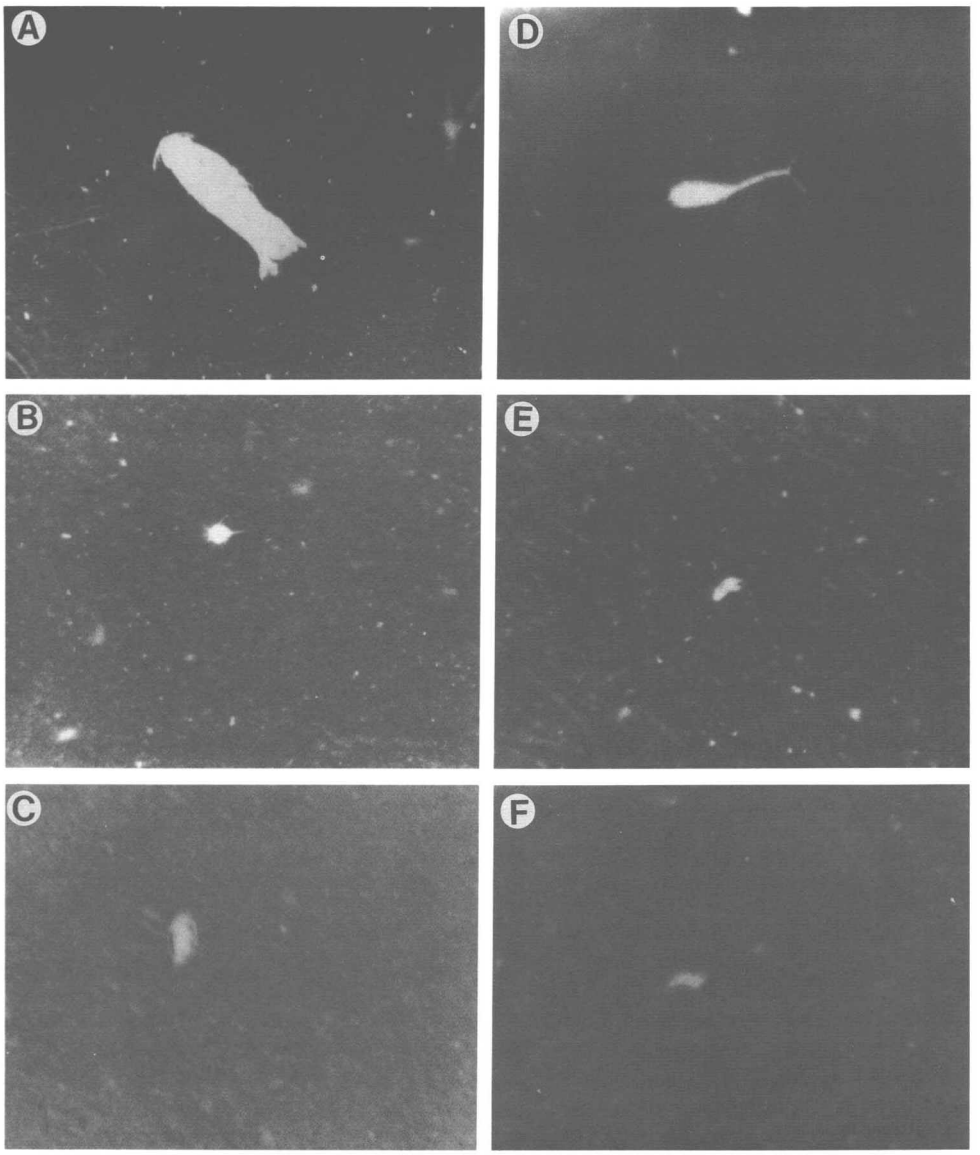

Plate 8. In situ silhouette images of Chesapeake Bay zooplankton. A) cymothoid isopod, Z89-435, 43:15; B) cirriped nauplius, Z89-427, 41:41; C) ostracod, Z89-218, 59:33; D) cumacean, Z90-080, 59:08; E) cirriped cyprid larva, Z89-437, 10:59; F) ostracod, Z89-216, 40:38.

Differences between camera and paired-net estimates of abundance for 19 zooplankton taxa were subjected to statistical analysis (Table 4). Among these taxa, significant differences in abundance estimates by the camera and paired net were not detected for: hydromedusae; polychaete larvae; marine Cladocera; the freshwater cladoceran *Leptodora kindti*; copepods at stations 1, 2 and 5; naupliar stages of barnacles at stations 4 and 5; cyprid larval stages of barnacles; larval stomatopods; caridean zoea; *Porcellana* zoea; megalopae; striped bass (*Morone saxatilis*) eggs; other fish eggs (mixed species, but mostly those of *Anchoa mitchilli*); and fish larvae at station 6. For these taxa, the number of film samples providing

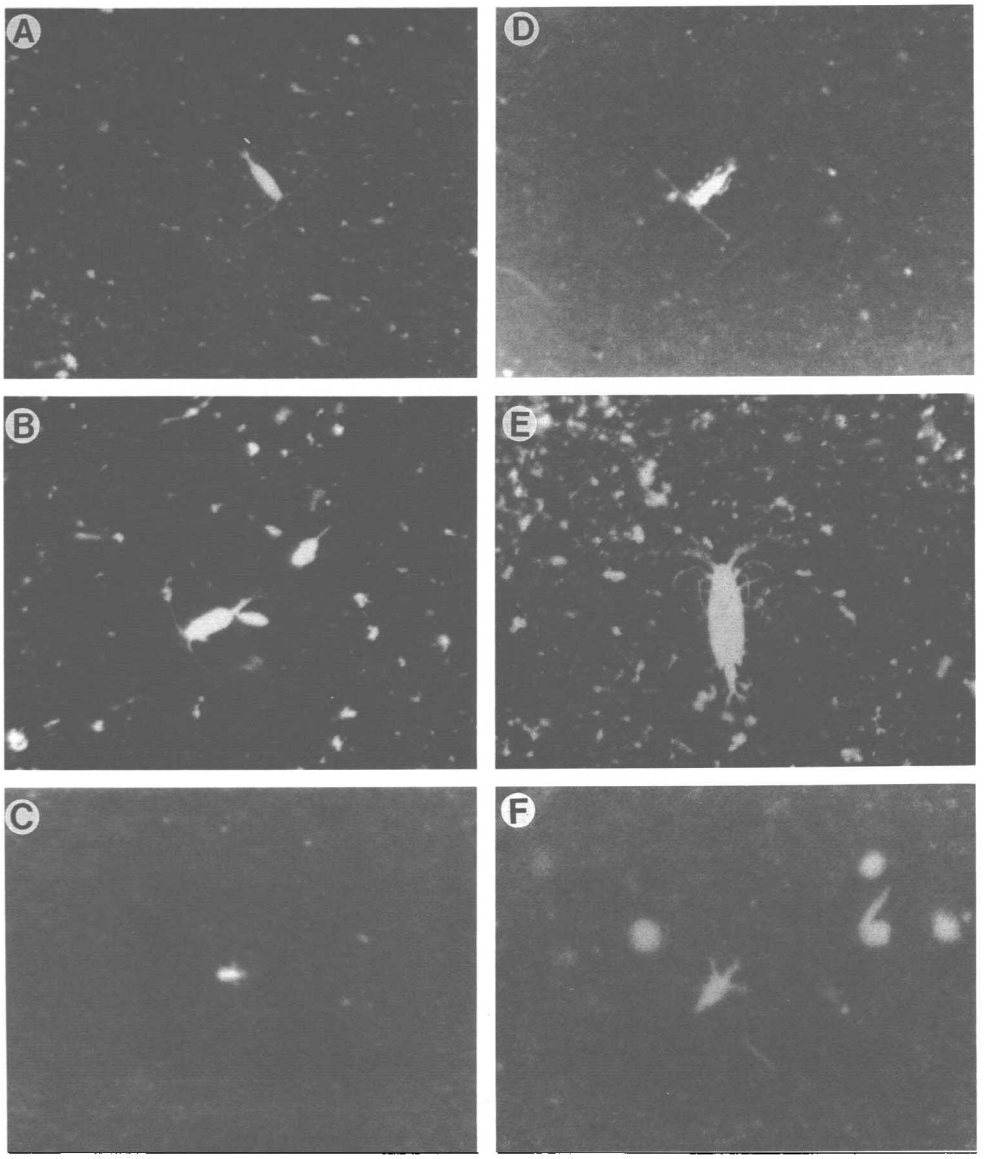

Plate 9. In situ silhouette images of Chesapeake Bay zooplankton. A) *Acartia* sp., Z89-384, 11:50; B) *Pseudodiatomus* sp. (lower left) and ?*Paracalanus* (upper right), Z89-433, 25:07; C) type-1 copepod nauplius, Z89-418, 44:12; D) *Acartia* sp. parasitized by ciliates, Z89-427, 42:24; E) *Labidocera* sp., Z89-433, 23:09; F) type-2 copepod nauplius, Z89-425, 05:48.

larger estimates of abundance than the paired net (expressed as a percent of all positive tows) were: hydromedusae (50.0%, N = 20); polychaete larvae (51.7%, N = 29); marine Cladocera (25%, N = 12); *Leptodora kindti* (50.0%, N = 8); copepods at stations 1, 2 and 5 (50.0%, N = 18); naupliar stages of barnacles (30.7%, N = 13); cyprid larvae (33.3%, N = 18); stomatopods (12.5%, N = 8); caridean zoea (28.6%, N = 14); *Porcellana* zoea (33.3%, N = 6); megalopae (40.0%, N = 10); *Morone saxatilis* eggs (64.7%, N = 17); other fish eggs (53.8%, N = 13) and fish larvae at station 6 (25.0%, N = 8).

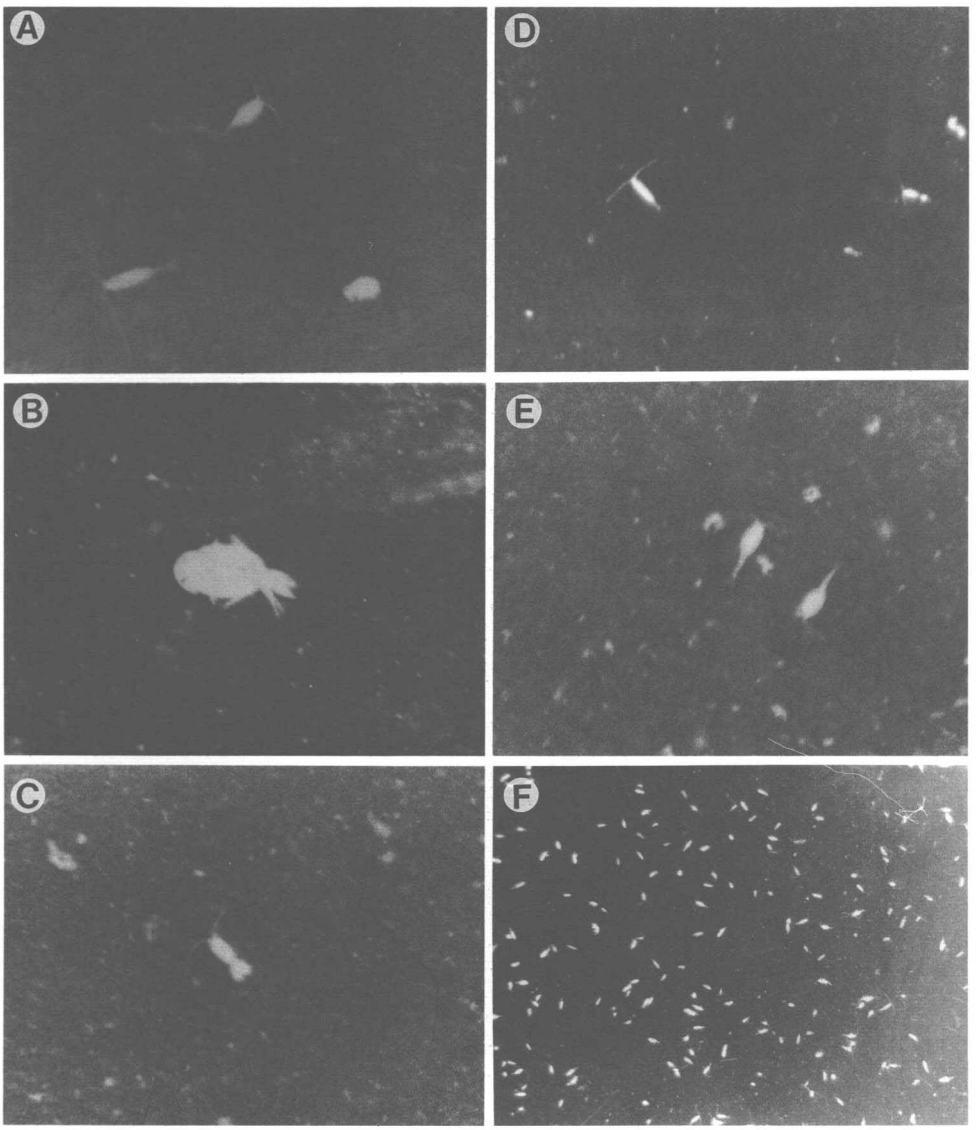

Plate 10. In situ silhouette images of Chesapeake Bay zooplankton. A) long and short antenna copepods, Z89-216, 40:33; B) ?*Caligus*, Z89-392, 54:07; C) ovigerous *Eurytemora*, Z89-486, 30:13; D) ovigerous small calanoid and *Acartia* sp., Z89-416, 18:22; E) two cyclopoids, Z89-486, 29:30; F) large concentration of mainly *Acartia* sp., Z89-427, 40:43.

Differences were significant ($P < 0.05$) for freshwater cladocera, mysids, and chaetognaths (Table 4). Differences were highly significant ($P < 0.01$) for gastropod larvae, copepods (at stations 3, 4 and 6), naupliar stages of barnacles (at stations 3 and 6), brachyuran zoea, and fish larvae (at all but station 6). For all categories of planktonic taxa that yielded significant or highly significant results (Table 4), in situ photography provided lower density estimates than traditional plankton net methods. Highly significant mean differences (for pooled data) ranged from -0.8 m^{-3} (brachyuran zoea at stations 4 and 6) to $-15{,}151.6$ m^{-3} (copepods at

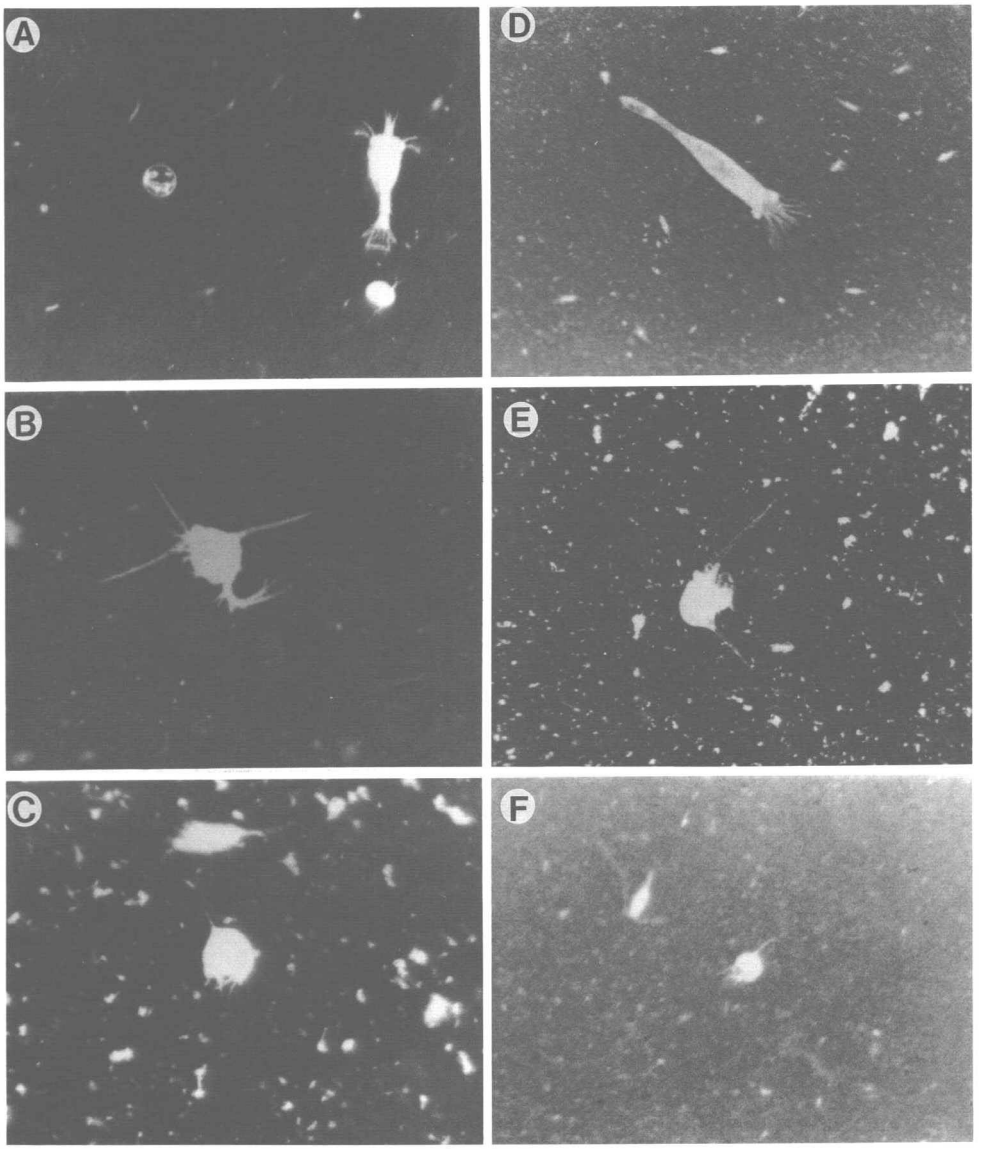

Plate 11. In situ silhouette images of Chesapeake Bay zooplankton. A) natant zoea (with fish egg), Z89-459, 21:27; B) type-1 brachyuran zoea, ?*Callinectes*, Z88-214, 03:27; C) type-2 brachyuran zoea, Z89-433, 22:57; D) *Crangon* juvenile, Z89-482, 51:43; E) type-3 brachyuran zoea, Z89-433, 23:05; F) type-4 brachyuran zoea, ?*Emerita*, Z89-427, 42:28.

stations 3, 4 and 6). The greatest single difference recorded for any taxon between camera and preserved density estimates was in an oblique tow at station 6 in the James River ($-48{,}025.9$ copepods m^{-3}).

Because ctenophores dissolve in formalin-preserved samples (Gosner, 1971), we were unable to calculate abundance estimates. As a result, apparent mean differences at stations 3, 4 and 5 (1.1–81.6 ctenophores m^{-3}) were not analyzed statistically. Furthermore, nine planktonic taxa failed to meet criteria for statistical analysis. Fifteen or more observations were available for five of these categories

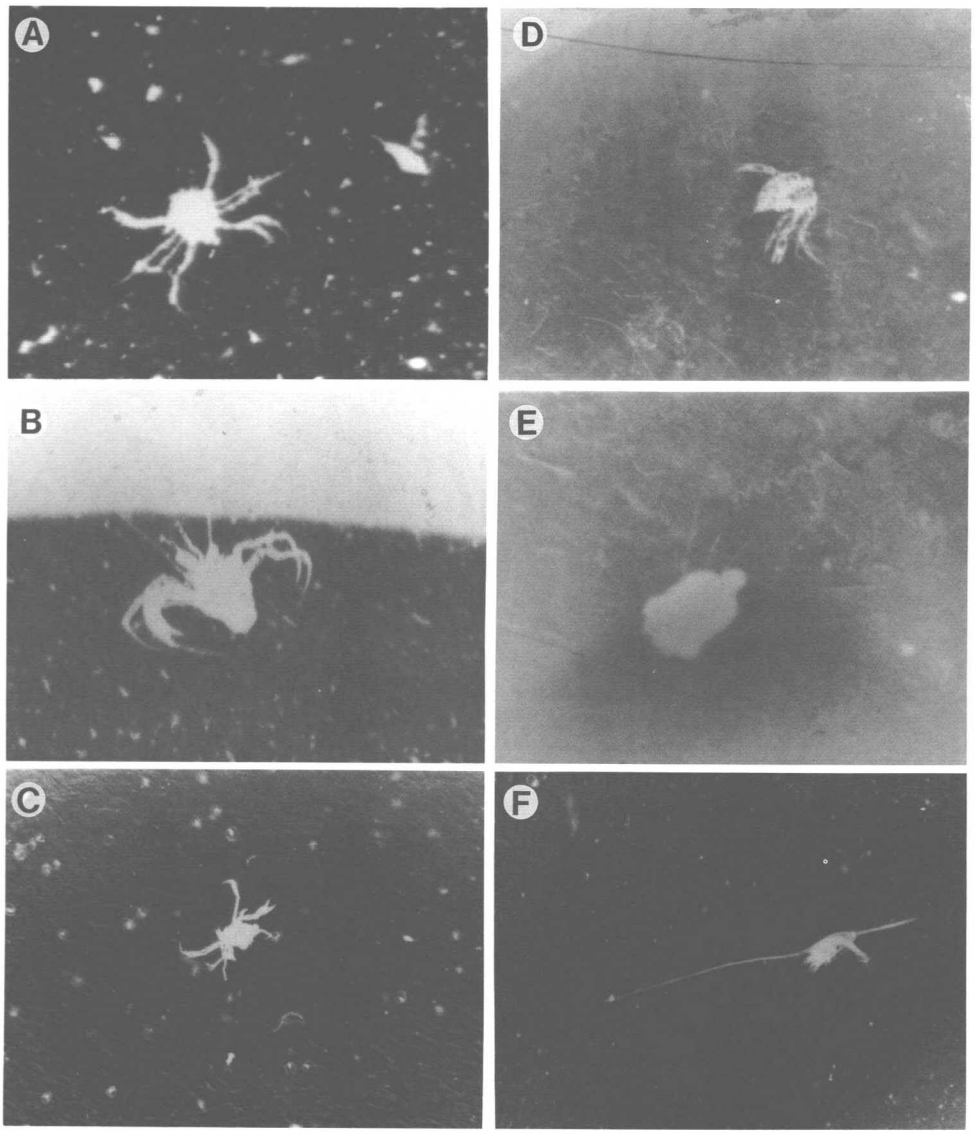

Plate 12. In situ silhouette images of Chesapeake Bay zooplankton. A) type-1 megalopa, Z89-433, 25:24; B) pagurid megalopa, Z89-433, 23:21; C) type-2 megalopa, Z88-214, 01:10; D) type-3 megalopa, Z88-212, 02:02; E) type-4 megalopa, ?*Callinectes*, Z88-212, 52:06; F) *Porcellana* zoea, Z88-214, 01:40.

and the number of film rolls containing images of these taxa (expressed as a percent of those paired collections containing preserved examples) were: parasitic copepods (13.6%, N = 22); isopods (11.1%, N = 18); amphipods (10.5%, N = 19); mysids (13.3%, N = 15); and insects (32.0%, N = 25).

Discussion

In general, transparent taxa (e.g., cnidarians, ctenophores, fish eggs), large taxa with conspicuous morphological features (e.g., stomatopods, sergestids, chaeto-

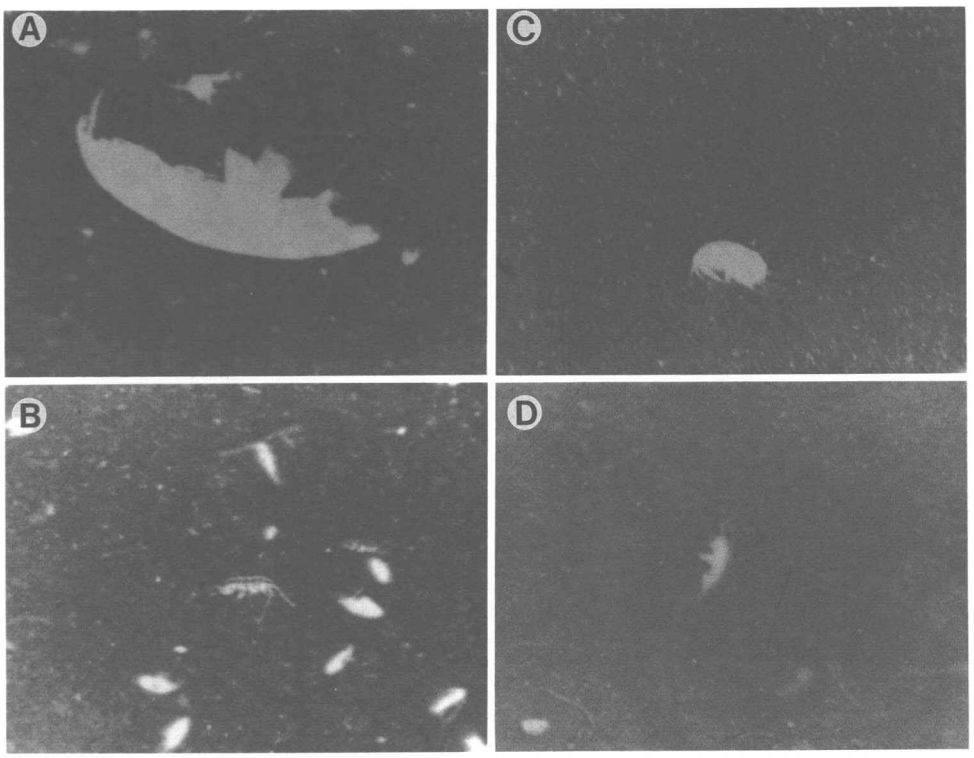

Plate 13. In situ silhouette images of Chesapeake Bay zooplankton. A) type-1 amphipod, Z89-425, 05:15; B) type-2 amphipod, Z89-427, 40:43; C) type-3 amphipod, Z89-218, 00:02; D) type-4 amphipod, Z89-129, 58:49.

gnaths, megalopae) and taxa with low diversity in Chesapeake Bay offered the best opportunity for species level identification in silhouette photographs. Some large targets, such as fish larvae, were difficult to classify since important characters (pigmentation and usually meristics) could not be determined in silhouette. For example, larvae of striped bass and white perch (*Morone saxatilis* and *M. americana*) were tentatively identified (Plate 18) based on comparison with preserved material in paired collections; however, we were not confident that these images could be distinguished from those of larval yellow perch, *Perca flavescens*. In other cases, fin rays or myomeres could be enumerated which aided identification of menhaden (*Brevoortia tyrannus*) and bay anchovy (*Anchoa mitchilli*) larvae (Plates 16, 18). Small zooplankton species that were opaque in silhouette (e.g., copepods, cirriped nauplii, brachyuran zoea) presented identification problems since delimiting characters (e.g., appendage number, segmentation and spination; carapace spine number and position) could not be seen. Some targets were out of focus.

Overall, we were able to identify only about 70% of all observed taxa (Plates 1–18) to the level of genus or species. Since all preserved specimens were not identified to genus or species, we were unable to make direct comparisons of the diversity of taxa recorded by the camera to that of preserved samples. However, previous zooplankton surveys using traditional methods have described the species composition of Chesapeake Bay zooplankton and ichthyoplankton. For example, Grant and Olney (1983) recorded 175 taxa of zooplankton and fish larvae

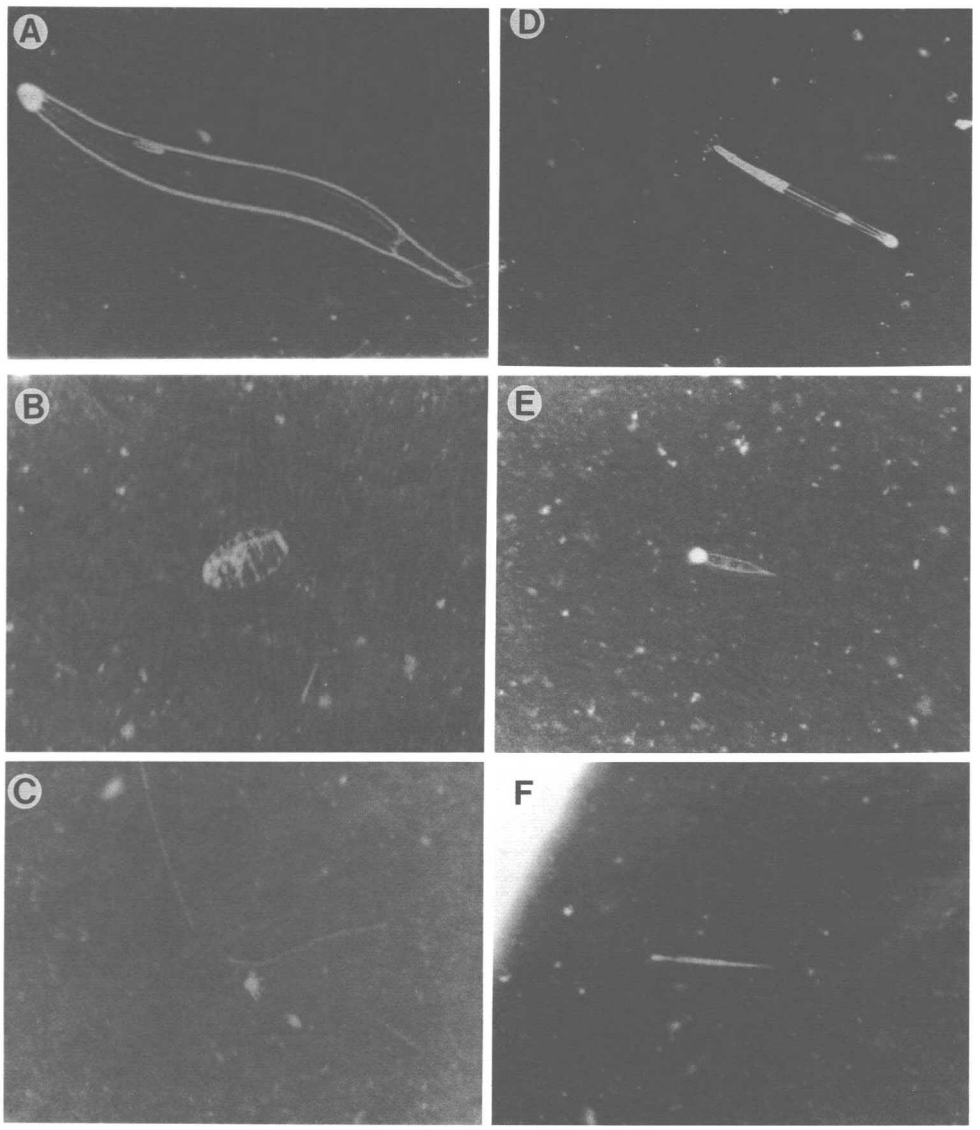

Plate 14. In situ silhouette images of Chesapeake Bay zooplankton. A) *Flaccisagitta enflata,* Z89-441, 55:45; B) unidentified doliolid, Z89-429, 08:37; C) "pluteus" unknown, Z89-441, 57:32; D) *Sagitta tenuis,* Z88-214, 00:36; E) larvacean, Z89-429, 11:16; F) "amphioxus" unknown, Z89-439, 36:21.

in a single August survey of the lower bay, and Olney (1983) identified about 35 kinds of fish eggs and larvae during a two-year sampling period. Although the present and past studies are not directly comparable, it is clear that the taxonomic constraints of in situ silhouette photography in estuarine systems are formidable, and detailed faunal lists for most taxa cannot be obtained with this technique.

Comparisons of data on the occurrence and abundance of plankton obtained from the camera and paired net produced mixed and somewhat surprising results. In standard deployments of the system, an average film roll of 300 frames rep-

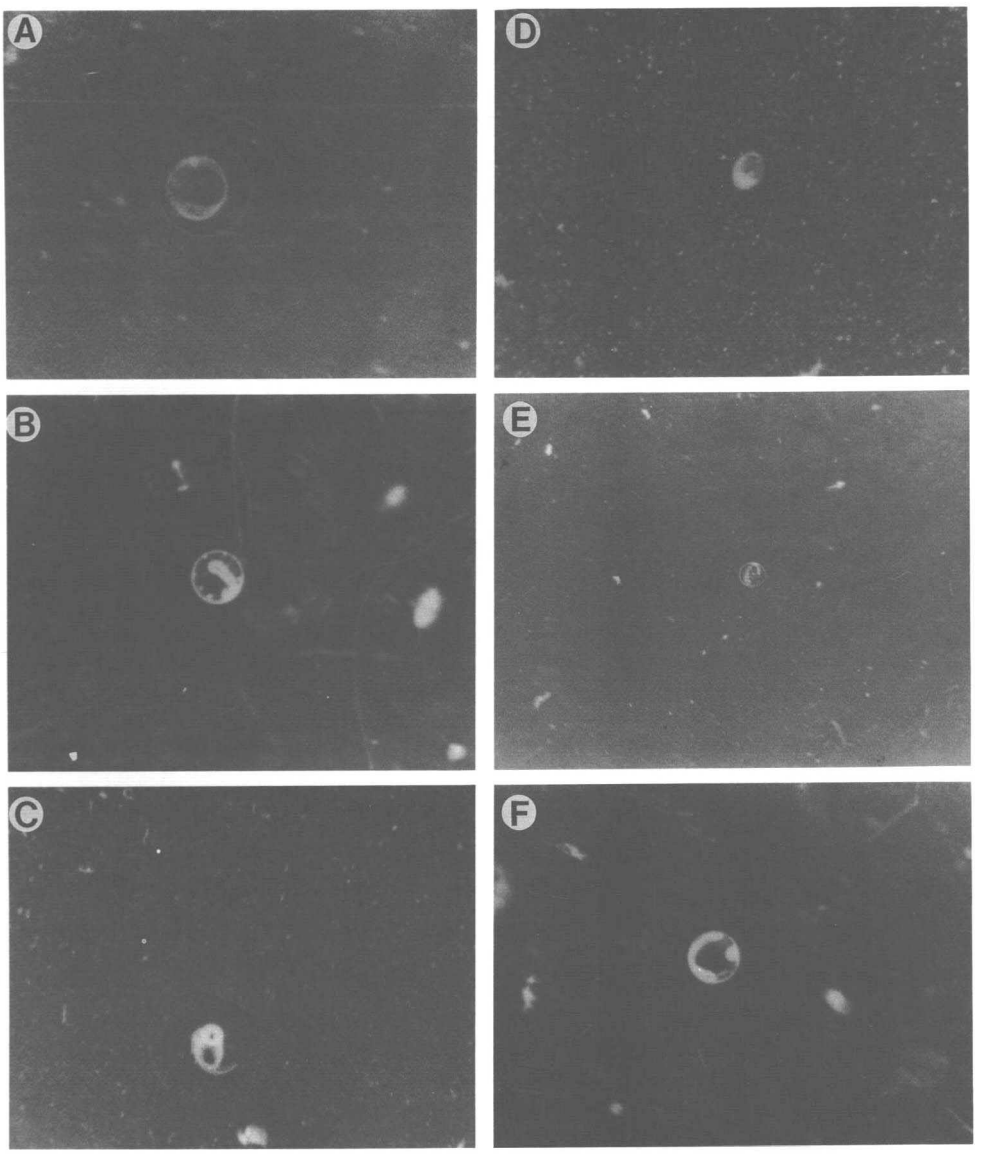

Plate 15. In situ silhouette images of Chesapeake Bay ichthyoplankton, fish eggs. A) *Brevoortia tyrannus*, Z90-250, 41:18; B) ?*Symphurus plagiusa*, Z88-187, 12:54; C) *Morone saxatilis*, Z90-138, 33:49; D) *Alosa sapidissima*, Z89-135, 53:01; E) ?*Tautoga onitis*, Z90-242, 33:36; F) scieanidae, Z88-212, 50:46.

resented a total volume "sampled" of 1.9 m^{-3}, a small fraction (about 5%) of the volume of water that passed through the photographic chamber. Thus, it was expected that ambient density and fine-scale distribution of a zooplankton category would be important attributes in determining potential for detection and accurate abundance estimation by the camera (Houde et al., 1989). In most cases, it is likely that taxa were not detected by the camera simply due to rarity. On the

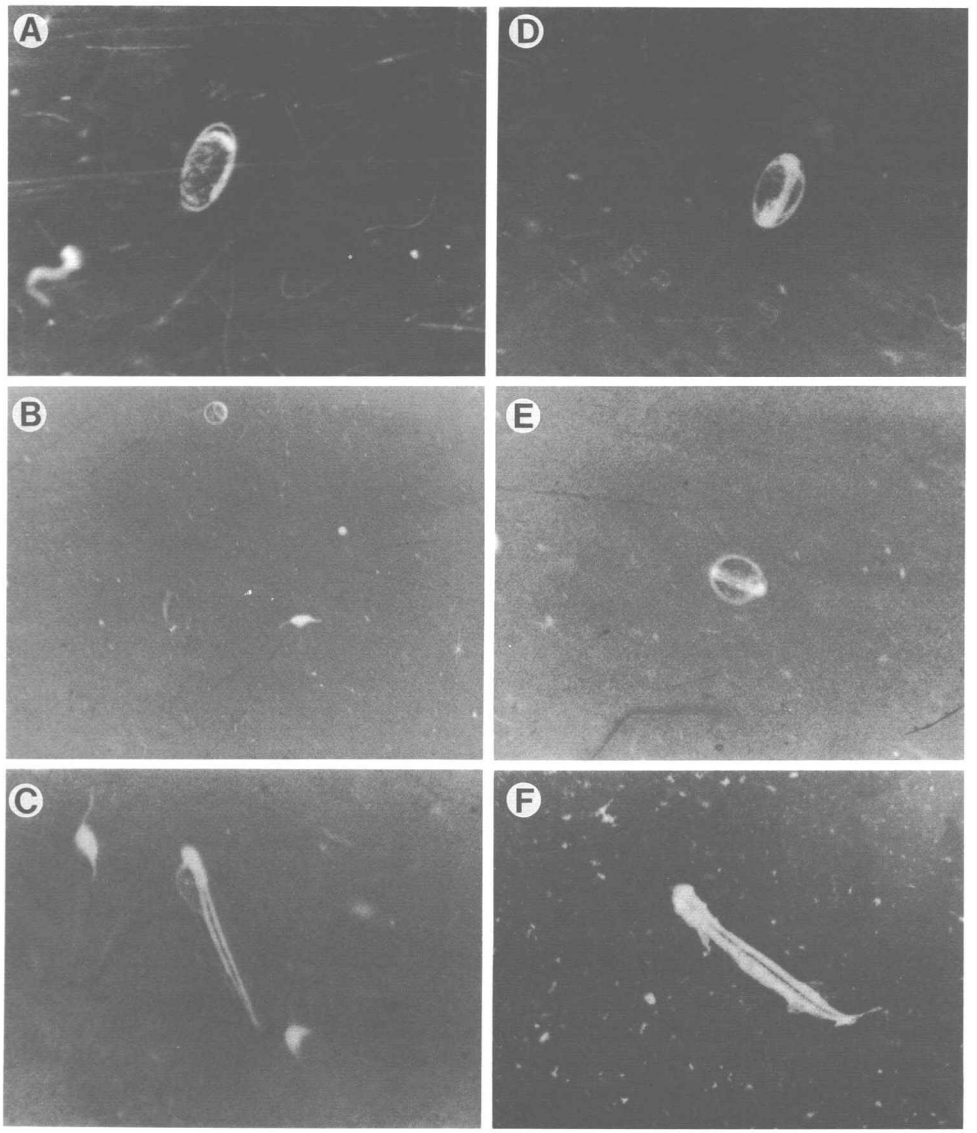

Plate 16. In situ silhouette images of Chesapeake Bay ichthyoplankton, anchovy eggs and larvae. A) *Anchoa hepsetus,* late-early stage, Z88-180, 25:52; B) *A. mitchilli,* early stage, Z90-242, 35:31; C) *Anchoa* sp., early yolksac, Z90-242, 35:20; D) *A. hepsetus,* middle stage, Z88-187, 15:06; E) *A. mitchilli,* middle stage, Z90-242, 35:06; F) *A. mitchilli,* Z89-478, 09:42.

other hand, densities of some categories of zooplankton that were very abundant in preserved samples were severely underestimated by the camera. Laboratory processing error may account for some of these discrepancies, but, overall, recounts of one of these categories (copepods) resulted in small differences. Individuals of some taxa (copepods, barnacle nauplii, gastropod larvae) are small (<1 mm in length) and their images sometimes were blurred or out of focus on film.

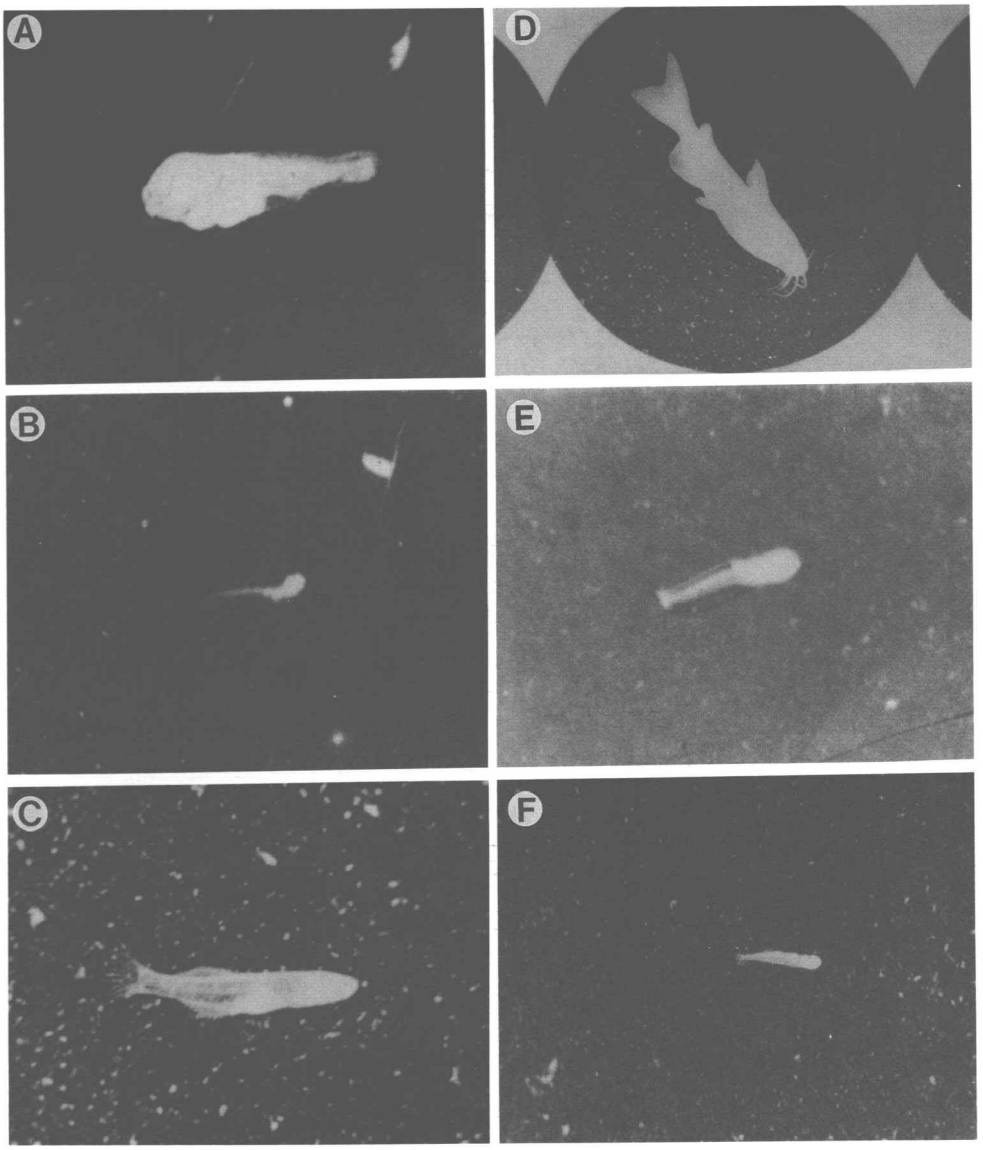

Plate 17. In situ silhouette images of Chesapeake Bay ichthyoplankton, fish larvae. A) unidentified sciaenid, Z89-459, 19:48; B) ?*Symphurus plagiusa,* Z89-425, 05:50; C) unidentified gobiid, Z89-433, 22:46; D) *Ictalurus punctatus,* Z90-128, 32:23; E) unidentified blenniid, Z89-441, 57:44; F) unidentified gobiid, Z89-384, 12:55.

Sorters were asked to look for specific identifying characteristics before counting a target. If these characteristics were blurred, out of focus or otherwise difficult to see, the target would not be enumerated. Thus, the "photogenic" attributes of a zooplankton taxon were important, and may have accounted for a significant part of the underestimation of density of some taxa by the camera.

Abundances of brachyuran zoeae, chaetognaths and fish larvae also were underestimated by the camera. These differences probably were not related to the

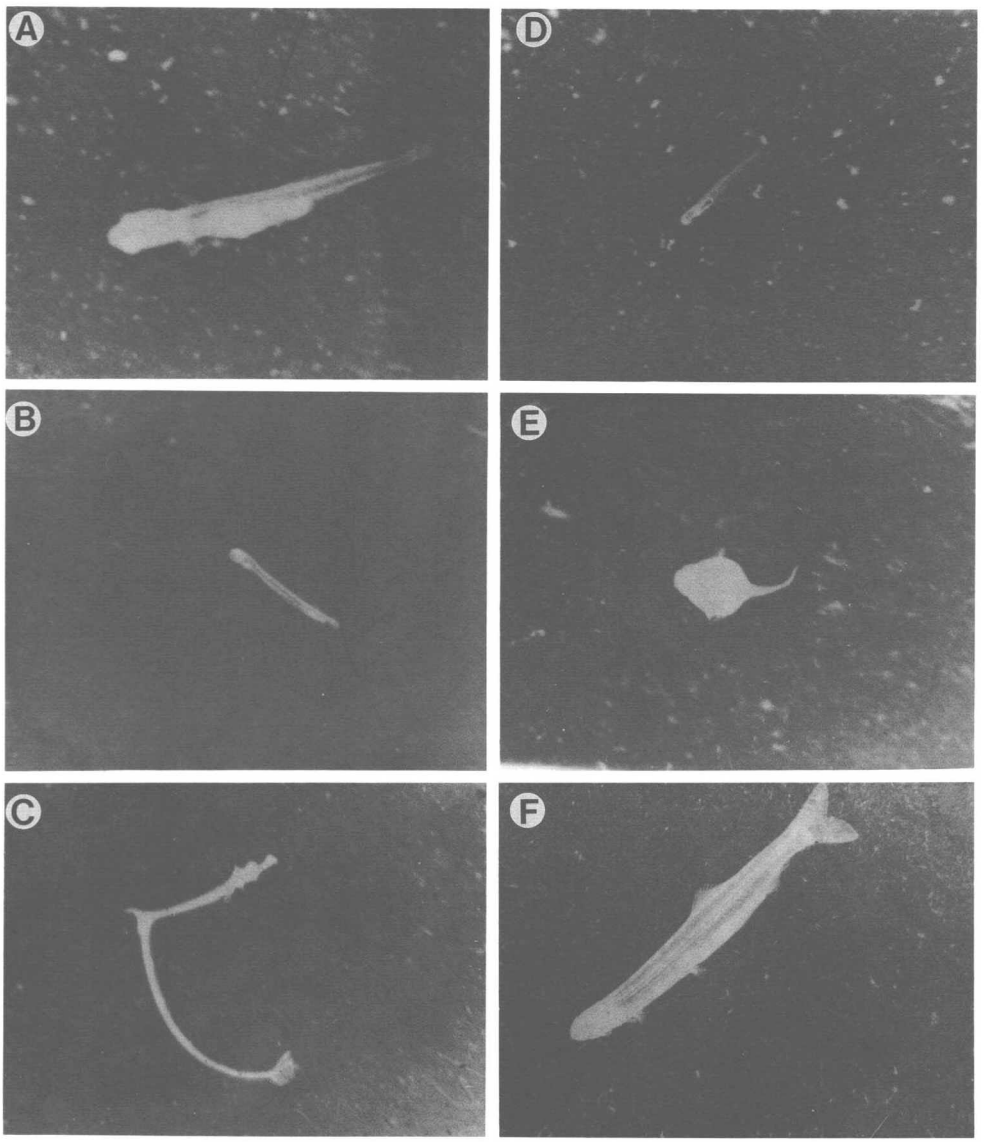

Plate 18. In situ silhouette images of Chesapeake Bay ichthyoplankton, fish larvae. A) ?*Morone saxatillis*, Z89-127, 22:43; B) *Alosa* sp., Z89-218, 00:13; C) *Syngnathus* sp., Z90-332, 06:38 D) ?*M. americana*, Z89-212, 59:46; E) *Sphoeroides maculatus*, Z90-368, 44:58; F) *Brevoortia tyrannus*, Z90-092, 07:44.

photographic qualities of the targets or to rarity in the plankton since all of these taxa produced easily recognized silhouette images and were moderately abundant. Depending on plankton and detritus volumes, we observed (but did not quantify) what we believe was moderate to severe stalling of plankton along the sides of both nets. Stalling did not affect the numbers of organisms enumerated in preserved collections because material was washed down into the codend prior to

Table 3. Maximum abundance (organisms m^{-3}) of 31 categories of planktonic taxa estimated by in situ silhouette photography (upper value) and a paired plankton net (lower value) at stations 1–6 in Chesapeake Bay, 1989. Missing data indicate taxon was not present at the station except at station 1 where only fish eggs, larvae and copepods were enumerated. Dashed lines (camera) or asterisks (paired net) indicate that taxon was present but not sampled by respective gears

Taxon	Stations					
	1	2	3	4	5	6
Hydromedusae			3.3		5.7	0.6
			1.3		7.2	1.3
Ctenophores			1.1	120.8	64.5	
			*	*	*	
Gastropods			—	0.6	4.5	0.5
			14.9	*	48.1	1.5
Bivalve larvae		—			0.6	—
		0.1			292.3	0.6
Polychaetes		3.4	1.1	41.6	8.4	2.2
		0.1	31.1	33.3	7.5	4.5
Cladocera (marine forms)				—	206.4	
				2.0	218.4	
Cladocera (freshwater forms)		644.5	2.7			16.8
		637.6	508.5			10,611.7
Leptodora kindti		8.9				
		5.9				
Ostracods		4.9	—	—	—	—
		0.1	0.1	<0.1	<0.1	1.1
Copepods	5.0	352.3	1,237.9	2,520.2	4,611.8	4,977.5
	5.5	565.9	32,254.8	24,911.4	3,737.4	53,003.4
Parasitic copepods			0.6	—	0.6	0.6
			0.4	0.2	0.2	0.8
Cirriped nauplii			1.9	2.3	17.0	1.1
			132.6	30.4	136.3	253.3
Cirriped cyprids			1.1	0.7	14.9	1.1
			0.1	3.3	9.9	147.8
Stomatopods					0.8	—
					1.5	<0.1
Sergestids					1.7	
					0.9	
Caridean zoea			1.1	—	13.4	
			0.8	0.2	20.5	
Crangon young						0.5
						0.2
Porcellana zoea					2.2	
					3.8	
Brachyuran zoea			20.1	—	49.2	1.1
			207.1	1.0	122.0	2.5
Megalopae					3.4	—
					2.1	<0.1
Isopods			0.5	—	0.6	—
			<0.1	0.1	0.2	0.3
Amphipods		—	—	—	—	1.1
		0.5	0.8	<0.1	<0.1	3.9
Mysids			0.5	—	1.7	—
			0.4	0.2	3.2	0.2
Cumaceans				—		
			<0.1			
Insects		1.2	0.6	—	0.6	1.1
		0.1	0.5	0.2	<0.1	1.7
Chaetognaths					22.4	—
					59.1	<0.1
Doliolids					3.8	
					*	

Table 3. Continued

Taxon	Stations					
	1	2	3	4	5	6
Larvaceans					6.1	
					<0.1	
Morone saxatilis eggs	19.7	1.1				
	16.1	1.1				
Fish eggs (mostly anchovy)				2.6	11.5	
				5.1	7.3	
Fish larvae	1.4	10.3	0.6	—	2.8	1.1
	13.6	17.3	5.3	2.9	7.2	0.8

preservation. Stalling of material on the camera net, however, would interfere with its passage through the photographic chamber and reduce the probability of being photographed. We speculate that zoeae, chaetognaths and fish larvae may have been subject to these conditions.

The efficiency of the camera compared to traditional plankton techniques and its capacity to provide reliable density estimates make in situ silhouette photography a desirable sampling method for certain planktonic taxa in estuarine systems. Among these taxa are important zooplankton and ichthyoplankton predators and young stages of commercially and ecologically important crustaceans

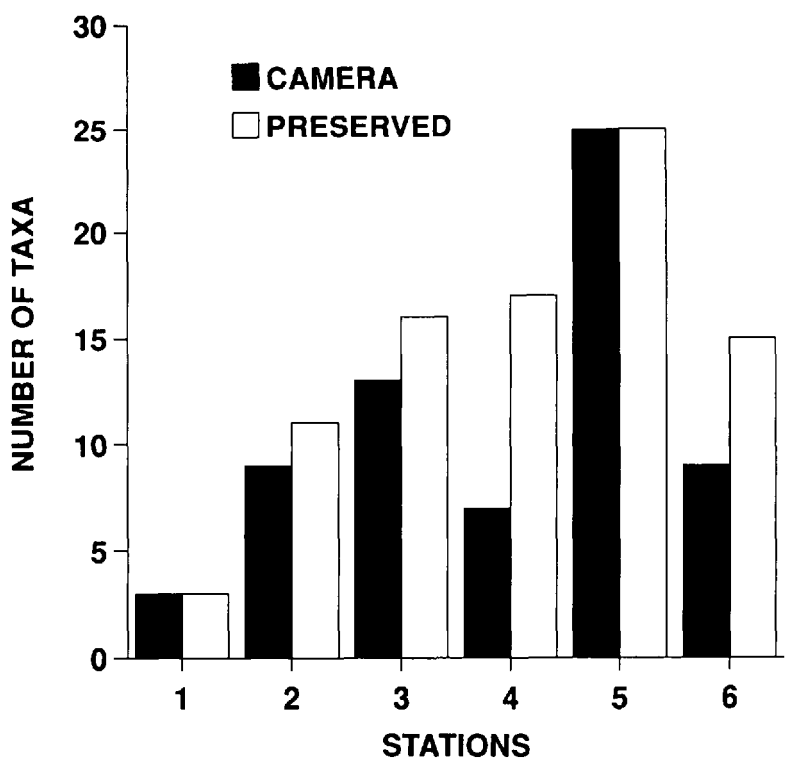

Figure 3. Total planktonic taxa enumerated in preserved samples and in situ silhouette photographs at six stations in the Chesapeake Bay.

Table 4. Mean difference (organisms m^{-3}) and probability values for pairs of film and preserved sample estimates of abundance of 28 categories of planktonic taxa at stations 1–6 in Chesapeake Bay, 1989. Statistical analysis was not performed on differences in some taxonomic categories. Missing values indicate taxon was not observed at the station except at station 1 where only fish eggs, larvae and copepods were enumerated. Negative values indicate that preserved sample estimates were greater. Values in parentheses indicate stations for which differences and probability values are exclusively reported. Values in brackets are single observations not used in statistical analysis. Abbreviations and symbols are: N, number of paired observations; NC, data omitted from analysis; M, marine species; F, freshwater forms; *, differences are significant; **, differences are highly significant; #, data were not transformed for statistical analysis

Taxon	N	Stations						Probability
		1	2	3	4	5	6	
Hydromedusae	20#			0.1		0.5	−0.2	0.53
Ctenophores	14			1.1	81.6	32.9		—
Gastropods	13			−6.4	[0.6]	−13.2	−0.3	0.01**
Polychaetes	29		1.0	−4.9	10.3	0.6	−0.5	0.21
Cladocera (M)	12#				−0.6	−5.9		0.26
Cladocera (F)	8		−53.5	−160.1			NC	0.04*
Leptodora kindti	8#		−0.1					0.90
Ostracods	5		4.7	−0.1	−0.1	<−0.1	−0.1	—
Copepods (3, 4, 6)	20			−10,718.2	−11,037.0		−21,602.4	0.00**
Copepods (1, 2, 5)	18#	1.0	−32.8			−515.7		0.28
Parasitic copepods	22			−0.1	−0.1	<−0.1	−0.3	—
Cirriped nauplii (3, 6)	14			−60.9			−87.5	0.00**
Cirriped nauplii (4, 5)	13				−7.7	−42.2		0.06
Cirriped cyprids	18			0.2	−1.6	−0.5	NR	0.73
Stomatopods	8#					−0.2	[<−0.1]	0.33
Sergestids	5					0.2		—
Caridean zoea	14			0.1	NC	−0.9		0.72
Crangon young	7						<−0.1	—
Porcellana zoea	6#					−1.3		0.12
Brachyuran zoea (4, 6)	12#				−0.3		−1.1	0.00**
Brachyuran zoea (3, 5)	15			−68.7		−36.9		0.00**
Megalopae	10					0.4	<−0.1	0.15
Isopods	18			<0.1	<−0.1	<0.1	0.1	—
Amphipods	19		−0.2	−0.3	<−0.1	0.5	−0.9	—
Mysids	7			<−0.1	−0.1	−0.8	<−0.1	0.04*
Insects	25		0.3	<−0.1	−0.1	0.2	<−0.1	—
Chaetognaths	7					−0.4	[<−0.1]	0.02*
Larvaceans	4					1.9		—
Morone saxatilis eggs	17	2.6	0.4					0.12
Fish eggs	13#				−0.2	1.3		0.25
Fish larvae (1, 2)	17#	−5.7	−4.0					0.00**
Fish larvae (3, 4, 5)	20#			−2.8	−1.2	−1.5		0.00**
Fish larvae (6)	8#						<−0.1	0.46

and fishes. Furthermore, in situ photography offers a non-destructive means of sampling fragile taxa (such as some species of ctenophores) which are difficult or impossible to collect and enumerate with traditional methods. The capability of the camera to detect and estimate abundance of cydippid larvae of ctenophores offers new opportunities in investigations of ctenophore biology and population dynamics. The capability to identify and stage pelagic fish eggs in silhouette photographs offers opportunities for a variety of studies, including egg production and field assessment of egg mortality and viability (Olney et al., 1991). As use of this system and other automated recording devices (i.e., video camera systems such as described by Welsch et al., 1991) continues, it is probable that the list of potential applications of in situ photographic techniques in studies of estuarine plankton will expand.

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