

## A new device for studying benthic invertebrate recruitment

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### *Abstract*

An automated larval settlement sampler has been designed and tested in the field to examine the effects of environmental conditions on the recruitment of epifaunal and infaunal benthic invertebrates. The sampler (1) exposes substrates to settling larvae at four discrete levels of naturally varying environmental conditions (e.g. different tidal states, current speeds, light regimes), (2) repeatedly exposes substrates over a period (2–8 weeks) sufficient to accumulate measurable numbers of recruits, and (3) maintains unexposed substrates in an environment that does not result in unnatural levels of mortality. Preliminary field trials indicate that the device was effective in assessing recruitment responses of epifaunal invertebrates to diurnal variations in light level and infaunal invertebrates to variations in tidal state.

The life-cycles of many marine benthic invertebrates inhabiting coastal environments include a planktonic larval phase lasting for minutes to months followed by metamorphosis and settlement to the seafloor. Cues affecting larval settlement have been extensively studied in many types of epifaunal and infaunal invertebrate species (e.g. reviews of Crisp 1974; Woodin 1986; Butman 1987; Pawlik 1992), and it is now recognized that a variety of physical, chemical, and biological factors have the potential to influence the settlement of invertebrate larvae. These factors include (1) the near-bottom hydrodynamic regime (Eckman 1983; Pawlik et al. 1991; Mullineaux and Butman 1991; Jonsson et al. 1991; Snelgrove et al. 1993), (2) substrate texture and type (Crisp and Barnes 1954; Wethey 1986; Snelgrove et al. 1996), (3) physical factors (Korrigna 1949; Thorson 1964; Crisp 1974; Pechenik 1990), (4) chemical attractants and toxins (Dubilier 1988; Davis et al. 1991), and (5) biotic interactions (Grossberg 1991; Woodin 1985; Ölfasson et al. 1994).

Early work on larval settlement generally focused on physical cues (e.g. light, temperature, salinity, substrate type) in a laboratory setting (e.g. reviews of Butman 1987; Young 1990). While these studies advanced our understanding of the proximate factors affecting settlement, they were often conducted under somewhat artificial conditions that neglected the potential contribution of other co-occurring factors on

settlement behavior. For example, we now recognize the influence of hydrodynamic processes on benthic invertebrate settlement behavior and that larval substrate selection can vary with the hydrodynamic regime (Pawlik and Butman 1993; Snelgrove et al. 1993). In addition, field studies of recruitment frequently demonstrate high spatial and temporal variability (Hawkins and Hartnoll 1982; Gaines and Roughgarden 1987). This variation often precludes the ability to rank or develop a hierarchy of factors contributing to settlement patterns (but see Wethey 1986). Lastly, larval settlement cues often co-vary (e.g. gas solubility with temperature; organic content and bacterial abundance with sediment grain size; water velocity with tidal state), further adding complexity to our interpretation and partitioning of the relative importance of each factor in affecting recruitment dynamics of benthic invertebrates.

We have developed a new device for field experimental studies in order to examine recruitment patterns for a broad array of infaunal and epifaunal invertebrates with the goals of establishing general trends and exceptions and determining the influence of specific environmental patterns on natural in situ recruitment patterns. This instrument permits us to control exposure of larval settlement substrates to parameters that naturally vary, expose the substrates under specific conditions for periods up to 8 weeks so that measurable recruitment differences can be determined for an array of species, and maintain unexposed substrates in an environment that does not affect organisms already recruited to the experimental substrates.

Here we describe the design and operational tests of the device and present results of several preliminary experiments on known recruitment patterns of shallow-water benthic invertebrates using either hard substrates (for sessile epifaunal species) or sediment-filled containers (for infaunal species). Specifically, we examined diurnal patterns of epifaunal recruitment since it is well documented that larval release in many species is often affected by variations in light intensity (Ryland 1977; Svane and Young 1989). Our

### *Acknowledgments*

We thank G. Grenier for invaluable assistance in helping to design and construct ELVIS. B. Dziomba and D. Arbige aided with construction and electronics development, respectively. M. Berger, V. J. Cummings, A. Frese, E. Miles, and R. Stankelis assisted with field and laboratory work. D. Cohen and K. Howard-Strobel supplied current meter data. C. A. Butman, L. A. Levin, L. S. Mullineaux, P. V. R. Snelgrove, and two anonymous reviewers provided thoughtful comments of an earlier version of the manuscript. To all: thank you, thank you very much.

This work was supported by NSF grants OCE 91-01815 and OCE 91-23890 and the University of Connecticut Marine Sciences and Technology Center (MSTC).

intent was to verify whether epifaunal recruitment also exhibited diurnal periodicity and to use this experiment as an initial test of the efficacy of the recruitment apparatus. Recruitment patterns of infaunal invertebrates relative to tidal state were also examined. While generally less is known about settlement cues of infaunal species (Woodin 1986), tidal action can influence larval availability for many species (Cronin and Forward 1982; Stancyk and Feller 1986). Several studies have documented tidally induced variations in the larval abundance of infaunal species (Srikrishnadas and Ramamoorthi 1982; Stancyk and Feller 1986; Levin 1986). There are, however, no published field studies that have explicitly examined the relationship of tidal state to patterns of infaunal recruitment.

## Materials and methods

**Recruitment device design**—The design and operation of the electronic larval in situ settlement (ELVIS) device was driven by several basic requirements and constraints. First, we needed to expose substrates to benthic invertebrate recruitment in a variety of shallow-water habitats under known environmental conditions. Second, to collect sufficient numbers of organisms on the recruitment substrates, the device had to repeatedly expose a given substrate or set of substrates under similar conditions for periods up to 60 d. Third, repeated exposure of substrates under similar conditions required sequestering the substrates in a manner that eliminated larval settlement (e.g. larval “contamination”) without affecting the mortality rate of recruits already present on (or in) the substrates. Fourth, exposed substrates needed to be deployed in a manner that minimized hydrodynamic artifacts that could potentially bias or influence larval transport and/or recruitment to the substrates. It is well recognized that recruitment responses of benthic invertebrates to alterations in boundary-layer flow conditions can occur in a wide variety of benthic species (Crisp 1955; Eckman 1983; Keen 1987; Mullineaux and Butman 1990; Walters 1992). Lastly, the device needed to be designed for relative ease of deployment and recovery of experimental substrates under a variety of field conditions.

ELVIS consists of three main components: a rotating circular faceplate, four removable settlement substrate units, and a pressure housing that contains components to control the movement of the faceplate (Fig. 1). The rotating faceplate has four holes that allow one of the four substrates within each of the four substrate units to be exposed to settling larvae. Each settlement substrate unit protects the three unexposed substrates from settling larvae while housing them in an environment as similar as possible to natural field conditions. Finally, electronic components in the pressure housing control the sequence of rotation of the faceplate such that each set of four substrates (one from each of four substrate units) is exposed during a specific set of environmental conditions. The faceplate and cam design, however, can be modified to expose two sets of substrates at eight different times or one substrate at 16 separate intervals depending upon specific needs.

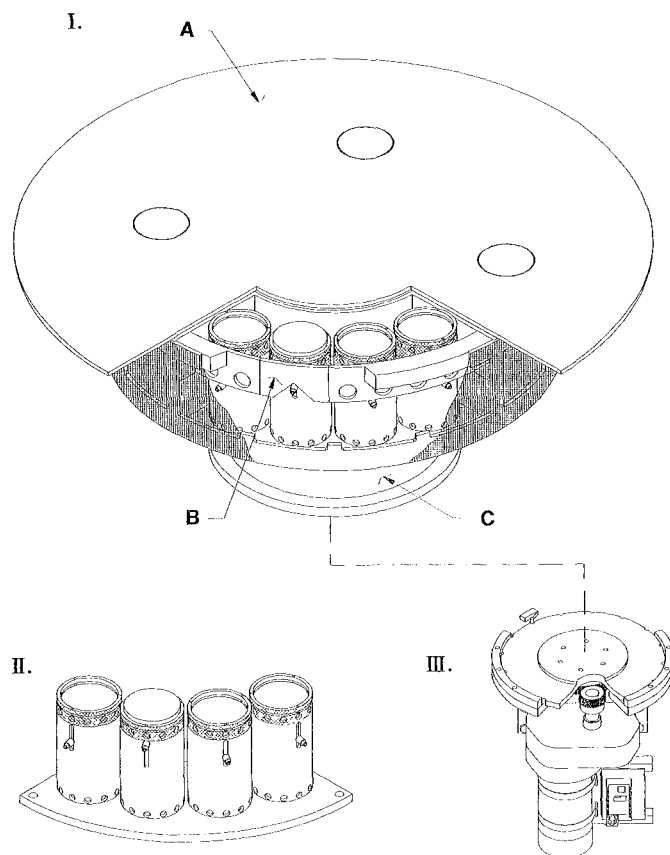


Fig. 1. The new device for studying benthic invertebrate recruitment. I, Cutaway view showing the (A) faceplate and position of four substrate exposure positions, (B) aluminum ring with cam attached to the underside of the faceplate, and (C) pressure housing. Diameters: faceplate, 91.5 cm; circular substrates, 7.8 cm; pressure housing, 32 cm. II, Internal components of the pressure housing showing the (D) microswitch used to align the faceplate over the substrate, (E) gear-drive and faceplate attachment plate, (F) low-speed motor, and (G) microprocessor. III, Outside view of substrate settlement unit. Diameters: PVC pipes, 9.0 cm; holes, 1.1 cm. See text for further description and operation.

**Faceplate**—The faceplate, made of 0.7-cm-thick PVC plate, is 91.5 cm in diameter and has four 8.3-cm-diameter holes that are positioned at equidistant (27 cm) from adjacent holes and 16.5 cm from the edge of the plate. An aluminum ring, attached to the underside of the faceplate, has four cams that are aligned with the faceplate holes (Fig. 1). The faceplate assembly is secured to a circular aluminum frame that can be attached to a stand or suspended in the water column. The faceplate assembly is driven by a  $\frac{1}{15}$ -hp, 12-V DC low-speed (6 rpm) gear-motor mounted inside a 32-cm-diameter, 28-cm-long PVC pipe secured to the aluminum frame. The outer end is sealed with a PVC plate bolted to the pressure housing. The gear-motor is attached to a step-down gear-drive that turns the faceplate at  $\sim 2$  rpm. In this configuration, 2 s are required to index the faceplate from one substrate position to the next.

**Pressure housing**—The pressure housing contains two switches—one for positioning the faceplate holes so they are

precisely aligned over each exposed substrate, the other being used to index the faceplate without interrupting the programmed timing of a low-power microprocessor. The microprocessor (8-bit, 28K RAM Tattle-tale Mark V data logger) is programmed, via an RS-232 serial connection, and all control and data-logging functions are established in software (PBASIC). This configuration permits a high degree of flexibility in the faceplate indexing schedule. When the faceplate is indexed, the microprocessor logs the time along with any possible error flags that can be downloaded at the end of each deployment. The gear motor and microprocessor are powered by a 12 V, 10 A h<sup>-1</sup> sealed rechargeable battery. Under these power constraints, the faceplate can be indexed up to 16 times per day for at least 60 d.

**Settlement units**—Each substrate settlement unit consists of four (9.0-cm-diam., 13.5 cm long) PVC pipes that are attached at one end to a curved section of 0.7-cm PVC sheet (Fig. 1). The other end of each pipe, with a series of 1.1-cm-diameter holes drilled around it, has a 1.0-cm-long PVC ring that moves up and down on four stainless steel pins. The ring is attached to the pipe by gluing an 80- $\mu$ m Nyltex mesh netting around the upper portion of the pipe and ring. Each pipe contains a spring-loaded inner PVC pipe assembly (7.5 cm in diam., 10 cm long) that is kept in position by perpendicularly mounted roller-pins. The settlement units are secured in position on the frame with stainless steel clips. When in position, the four cams allow one substrate from each of the four units to be flush with the outer surface of the faceplate (Fig. 1). The other substrates are depressed  $\sim$ 2 cm below the tops of the pipes, creating a head space of  $\sim$ 93 cm<sup>3</sup> over each unexposed substrate. The upper pipe surfaces containing the unexposed substrates are flush with the underside of the faceplate. Therefore, the only water exchange available to the unexposed substrates is through the plankton netting surrounding the upper portion of each pipe assembly.

The settlement units can be fitted with PVC panels or cups for use in studies of epifaunal or infaunal recruitment, respectively. Circular panels (7.8 cm in diam.), made of roughened PVC sheet with glued threaded stems, are screwed into the inner sleeves of the settlement unit pipes (Fig. 1). Cups (5.5 cm in diam., 2.5 cm deep), machined from solid PVC pipe, are designed to fit snugly into the inner sleeves of the substrate holders. PVC covers, which are clipped on the cups, ensure that any material contained in cups will not be lost or disturbed when the cups are placed on or removed from ELVIS. Unlike the panels, the cups are loaded from the top of ELVIS after the settlement units are secured into position. The switch that overrides the microprocessor (*see above*) moves the faceplate and minimizes disturbance of material in the cups during their deployment and recovery.

**Operational constraints and tests**—When in position, the four exposed substrates are flush with the surface of the faceplate. The purpose of this design was to minimize potential flow-related artifacts in substrate exposure and position that may influence larval transport to, and their retention on, the substrates. At the current stage of development, the substrate exposure method is designed to minimize potential

flow artifacts associated with the leading edge of the faceplate. In laminar flow, vertical advection, shear stress, and turbulence will vary with distance downstream from the leading edge of the faceplate (e.g. Schlichting 1979); and the settlement substrates are located 16.5 cm from the leading edge (aspect ratio  $>20:1$ ) in order to maintain similar flow conditions over each of the exposed substrates. Mullineaux and Butman (1991) demonstrated that for 1.0-cm-thick polycarbonate substrates, advection, shear stress, and turbulence all stabilized at distances 4–10 cm from the leading edge in free-stream velocities of 5 and 10 cm s<sup>-1</sup> in a laboratory flume. At higher flow speeds, advection, shear stress and turbulence will stabilize more quickly in the downstream direction. Importantly, note that in naturally turbulent conditions, flow-related artifacts in larval settlement are likely and should be examined on a case-by-case basis.

Another potential source of flow disturbance on the device is the  $\sim$ 0.5-cm ring of space between each substrate and the faceplate. Field deployments of ELVIS in relatively low-water-flow conditions (e.g. 0–10 cm s<sup>-1</sup> water velocities) have found nonrandom epifaunal recruitment patterns on the surfaces of the substrates; preliminary evidence suggests that the lack of small-scale variations in the boundary-layer flow on the substrates could influence larval transport and retention (e.g. Mullineaux and Butman 1990; Mullineaux and Garland 1993). Deployments of the device in a wider range of conditions are required in order to more fully address this issue. The faceplate design and diameter can be altered to accommodate different types of flow regimes or habitat conditions.

Three different sets of experiments were conducted to test operating conditions and sampling assumptions of the recruitment device. In all of these trials except one (*see below*), ELVIS was deployed faceplate-down from a floating raft moored in 2–3 m of water behind a jetty at Avery Point, Connecticut, eastern Long Island Sound (*see Osman and Whitlatch 1995a,b* for site description). The faceplate was positioned  $\sim$ 1 m below the water surface to collect recruiting sessile epifaunal species. Colonial (*Botryllus schlosseri*, *Botrylloides diagensis*, and *Diplosoma macdonaldi*) and solitary (*Molgula manhattensis*, *Ciona intestinalis*, *Styela clava*, and *Asciidiella aspersa*) ascidians are the most dominant ( $>80\%$  cover) epifaunal species encrusting rocks, jetties, and pilings at the site. Other epifaunal species include spirorbid (*Spirorbis* spp.) and serpulid (*Hydroides dianthus*) polychaetes, barnacles (*Balanus eburneus*, *B. amphitrite*, *Semibalanus balanoides*), bryozoans (*Cryptosula pallasiana*, *Schizoporella errata*, *Bowerbankia gracilis*, *Bugula turrita*, and *Electra* sp.), and hydroids (*Obelia* sp.). All these species have been regularly collected on PVC substrates exposed from 1 d to several months (Osman and Whitlatch 1995b,c). Electromagnetic current meter (InterOcean S4) deployments indicate that water flow at the site is characterized by a tidally driven current (mean peak flows of  $\sim$ 6 cm s<sup>-1</sup>) running predominantly in northeast–southwest and east–west directions. Episodic velocities of 20–30 cm s<sup>-1</sup> have been recorded, and their occurrence correlates well with local wind events (unpubl. data, Dept. Mar. Sci., Univ. Conn., collected  $\sim$ 300 m from the study site).

The first series of ELVIS operation tests determined

whether substrates positioned in an unexposed mode remained isolated from settling larvae. This information was needed to test whether substrates were being contaminated by settling larvae when kept in an unexposed mode. ELVIS was deployed for 7 d on two occasions, 17–24 July and 1–7 August. ELVIS was programmed to expose one set of panels from 0600 to 2100 h (daytime treatment) and another set of panels from 2100 to 0600 h (nighttime treatment). The remaining two sets of panels were exposed for 30 s periods—one at 2100 h (30-s 1 treatment) and one at 0600 h (30-s 2 treatment). At the end of each deployment, substrates were removed from the settlement units and all individuals or colonies of all epifaunal species were counted and identified in the laboratory under a dissecting microscope.

A second set of ELVIS deployments was conducted to assess whether there were differences in the mortality rates of epifaunal recruits attached to exposed and unexposed substrates. These data were necessary to determine whether any observed difference in the number of recruits on a given set of panels was due to differences in the number of settling larvae or to differences in postsettlement mortality of recruits attached to panels with different exposure schedules. ELVIS panels were exposed to competent larvae of the ascidian *Ascidella aspersa* in the laboratory. After 24 h the panels were examined under a dissecting microscope and number of recruits were “gardened” to a known number of individuals (20–40 panel<sup>-1</sup>) by haphazardly removing excess individuals from panel surfaces. Two experiments were conducted—one in the laboratory in order to reduce potential confounding effects of recruitment of conspecifics and (or) other species, the other at the field site when *Ascidella* was not naturally recruiting. In the laboratory, ELVIS was positioned, faceplate upward, in a large (0.91 × 1.36 × 4.38 m) tank supplied by continuously flowing, filtered (10- $\mu$ m nominal size) seawater. Water-flow rates in the tank averaged ~10–12 volume exchanges per day. ELVIS was programmed to repeatedly expose individual sets of four panels at 16-h, 8-h, 1-min, and 30-s intervals over a 7-d period (3–10 November). Two additional sets of four identical substrates were placed in the tank—one with known numbers of *Ascidella* recruits to serve as a reference, the other consisting of blank panels to record the number and type of recruits that may have not been removed by the water filtration system. The field deployment (17–24 November) had a similar panel exposure schedule as the laboratory experiment. Two sets of four additional panels were also deployed at the site in a manner similar to the position of ELVIS—one set with known numbers of *Ascidella* to assess mortality rates of recruits on substrates not attached to the device, another set with blank panels to record the number and type of epifaunal organisms recruiting during the deployment period. At the end of the deployments, substrates were examined as previously described.

The last set of trials assessed recruitment patterns of epifaunal recruits relative to the distance from the leading edge of the faceplate. Evidence of nonrandom recruitment across the ELVIS faceplate may indicate flow-related alterations in larval settlement behavior (Mullineaux and Butman 1990; Mullineaux and Garland 1993). ELVIS was deployed in the field for 7 d, and the numbers of recruits of the colonial

ascidian *Botrylloides diagensis* were counted along four equidistant transects in five consecutive 47.8-cm<sup>2</sup> sampling areas running from the edge to the center of the faceplate. Although other epifaunal species were observed settling on the faceplate, only *Botrylloides* recruits could be recorded by eye because of their relatively large postset size (~1 mm in diam.) and distinctive bright orange color.

*Epifaunal recruitment in relation to photoperiod*—Two ELVIS deployments examined patterns of epifaunal recruitment in relation to photoperiod by repeatedly exposing sets of substrates at intervals of 0600–0900, 0900–1200, 1200–1500, and 1500–0600 h over a 7-d period. The two experiments were conducted from 20 to 27 September and 4 to 11 October. Sunrise/sunset at the start of each experiment were 0605/1941 h and 0701/1810 h, respectively. Current speed and direction, temperature, conductivity, and pressure were monitored at 1 m above the seafloor and 5 m west of the raft with an electromagnetic current meter (InterOcean S4) for the duration of both experiments. All variables were averaged over 1-min intervals recorded every 15 min. At the end of the deployments, panels were examined using a dissecting microscope and all individuals were counted.

*Infaunal recruitment in relation to tidal state*—Two 7-d experiments were conducted to examine patterns of infaunal recruitment in relation to tidal state. ELVIS was deployed faceplate upward in ~1 m (mlw) of water at a site located ~200 m north of the floating raft. Sediments at the site are composed of a silty sand with a 0.6% organic content (ash-free dry wt). The infaunal community at the time of deployment was dominated by spionid (*Streblospio benedicti*, *Polydora cornuta*) and syllid (*Parapionosyllis longicirrata*, *Brania wellfleetensis*, *Streptosyllis arenae*) polychaetes. Other less abundant taxa included bivalves (*Gemma gemma*, *Mya arenaria*, and *Mercenaria mercenaria*) and an unidentified oligochaete. The site is more protected than the breakwater site owing to the existence of a concrete dock positioned to the south and ~100 m from the site.

Before ELVIS was deployed, a hole (~50 cm in diam., 1 m deep) was dug at the site and a plastic ring (26 cm in diam., 80 cm long) was positioned in the hole so that one end of the ring was nearly flush with the sediment surface. ELVIS was placed over the ring so that the faceplate rested parallel to and ~10 cm above the sediment–water interface. Sediment cups were filled with a medium sand (0.3% organic content) previously screened through a 1-mm mesh sieve and defaunated by freezing and thawing. Although it is recognized that placing the faceplate above the sediment surface may limit colonization of the experimental substrates by postsettlement juvenile and adult infauna that may be transported across the seabed (Smith and Brumsickle 1989; Snelgrove et al. 1995; Commito et al. 1995), the primary intent of these initial deployments was to reduce potential confounding interactions of organisms that are transported along the bed from those settling from the water column. Also, comparisons were limited to collections between cups exposed at different times within an ELVIS deployment and not between cups and surrounding ambient sediments.

The sediment exposure schedule for both experiments co-

Table 1. Average abundance ( $\pm 1$  SD) of the total recruits and numbers of the most abundant taxa found on substrates exposed during the day (0600–2100 h), night (2100–0600 h), and two 30-s (30-s 1 and 30-s 2) periods during two 7-d deployment periods. Empty cells indicate that no organisms were found.

Taxon	Exposure period							
	Day		30-s 1		Night		30-s 2	
	17–24 Jul	1–7 Aug	17–24 Jul	1–7 Aug	17–24 Jul	1–7 Aug	17–24 Jul	1–7 Aug
Total recruits	111.2 $\pm$ 16.8	83.3 $\pm$ 16.9	1.2 $\pm$ 0.9	2.0 $\pm$ 0.8	2.5 $\pm$ 3.3	5.3 $\pm$ 2.2	0.5 $\pm$ 0.6	2.5 $\pm$ 2.5
<i>Diplosoma macdonaldi</i>	39.3 $\pm$ 10.4	33.5 $\pm$ 7.0						0.3 $\pm$ 0.5
<i>Botryllus schlosseri</i>	49.5 $\pm$ 9.3	20.7 $\pm$ 4.9	0.3 $\pm$ 0.5		1.5 $\pm$ 1.7	1.3 $\pm$ 1.5		
<i>Asciidiella aspersa</i>	3.8 $\pm$ 2.1	3.5 $\pm$ 3.0	0.5 $\pm$ 1.0		0.5 $\pm$ 1.0	1.8 $\pm$ 1.3		
<i>Botrylloides diagensis</i>	2.3 $\pm$ 1.9	2.3 $\pm$ 0.9						
<i>Mogula manhattensis</i>		1.2 $\pm$ 1.9	0.3 $\pm$ 0.5	0.8 $\pm$ 0.9	0.3 $\pm$ 0.5	0.8 $\pm$ 0.5	0.3 $\pm$ 0.5	0.3 $\pm$ 0.5
<i>Cryptosula pallasiana</i>	5.0 $\pm$ 2.9	4.3 $\pm$ 2.8		0.3 $\pm$ 0.5				0.3 $\pm$ 0.5
<i>Spirorbis</i> spp.	0.5 $\pm$ 1.0	2.8 $\pm$ 2.3	1.0 $\pm$ 0.0					0.5 $\pm$ 0.3
Barnacles	1.3 $\pm$ 0.5	5.8 $\pm$ 2.5				0.8 $\pm$ 0.9		

incided with the following tidal states: 1 h before and after high tide (high tide period); 1 h after high tide to 1 h before low tide (ebb tide period); 1 h before and after low tide (low tide period); and 1 h after low tide to 1 h before high tide (flood tide period). Times for the exposure schedule were derived from a continuously recording tide gauge located ~100 m from the deployment site (unpubl. data, Conn. Dept. Environ. Protection). At the end of each deployment, the cups were capped and removed; cup contents were preserved in 10% buffered Formalin, screened with a 100- $\mu$ m mesh sieve, and transferred to 70% ethanol containing Rose Bengal. Sample residues were sorted under a dissecting microscope and organisms were counted and identified to the lowest possible taxon.

## Results

**ELVIS operation tests**—The experiments assessing the adequacy of the design for isolating unexposed substrates from settling larvae indicated that sets of substrates repeatedly exposed for 30-s daily intervals over the 7-d deployment periods averaged 0.5–2.5 epifauna recruits (Table 1). The few numbers of recruits found on the panels either settled when the substrates were positioned in the unexposed mode or were resulted from larvae trapped in the headspace of water captured above the panels during the daily 30-s periods when the substrates were being indexed from an exposed to an unexposed position. The two trials indicate that the method devised for holding substrates in a unexposed mode was very effective in isolating them from larval contamination. Both deployments also revealed pronounced day/night differences in the abundance of recruiting epifauna (Table 1).

Both the laboratory and field deployments determining differences in mortality rates of recruits attached to exposed and unexposed substrates on ELVIS revealed no natural settlement of *Asciidiella aspersa* and no significant differences in the mortality of *Asciidiella* recruits on substrates exposed on schedules ranging from 30 s to 16 h d<sup>-1</sup>. Also, mortality rates of recruits on ELVIS panels did not differ from recruits attached to identical substrates suspended in the water column adjacent to ELVIS (Fig. 2). *Asciidiella* mortality was slightly higher in the laboratory than in the field, possibly the result of reduced food

availability in the filtered water. These operation tests demonstrated that this epifaunal species can survive on substrates for relatively long periods sequestered in an unexposed mode, and that observed differences in the abundance of recruits on panels with different exposure schedules was not due to exposure-dependent mortality.

In our examination of recruitment of *Botrylloides diagensis* relative to the distance from the edge of the ELVIS, we found significantly lower numbers of recruits in the outermost 7.8 cm of the disc (Fig. 3). No other differences in recruit abundance were found. These data suggest that the positioning of the experimental substrates 16.5 cm from the faceplate edge minimized any potential variations in flow-related recruitment for this species of ascidian.

### Relationship of photoperiod to epifaunal recruitment patterns—ELVIS field deployments examining the presence of

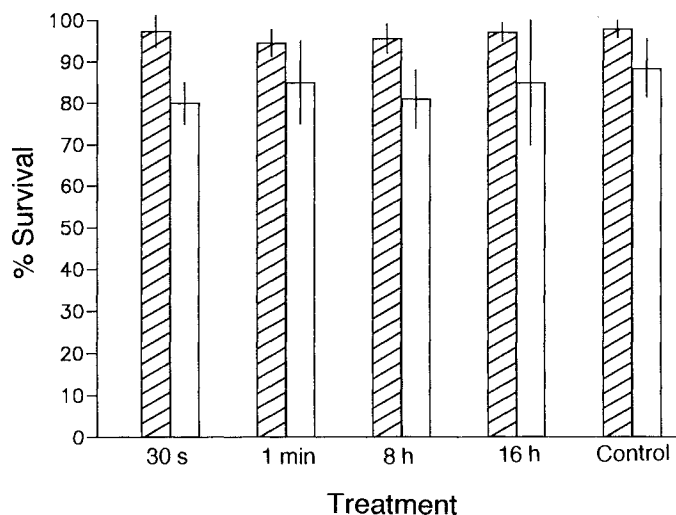


Fig. 2. Effects of ELVIS substrate exposure (30 s, 1 min, and 8 and 16 h) on survival (avg.  $\pm 1$  SD) on recruits of *Asciidiella aspersa* in the laboratory (open histograms) and field (hatched histograms) during a 7-d deployment period. "Control" refers to panels containing *Asciidiella* recruits placed adjacent to ELVIS at the initiation of the experiment.

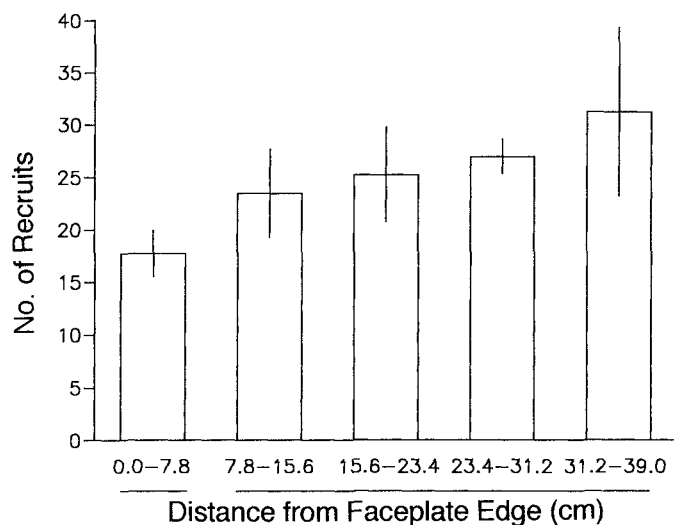


Fig. 3. Recruitment of *Botrylloides diagensis* on ELVIS as a function of distance from the faceplate edge. Average abundance ( $\pm 1$  SD) on 7.8-cm-diameter adjacent samples running from the edge to 39.0 cm from the faceplate edge. Lines connecting sample distances are not significantly different (one-way ANOVA, with data blocked by transect, and Bonferroni-Dunn contrast test;  $P > 0.05$ )

diurnal variations in the recruitment patterns of epifauna are summarized in Fig. 4. Total recruit abundance varied significantly with exposure period; highest numbers were recorded on panels exposed from 0600 to 1200 h, while substrates exposed from 1500 to 0600 h had the fewest recruits. Ascidian (*Diplosoma macdonaldi*, *Asciidiella aspersa*, and *Botrylloides diagensis*) recruitment patterns, the most abundant species recorded in both deployments, were similar to the overall diurnal recruitment patterns. Owing to low individual numbers of species, all bryozoan recruits (*Bugula turrita*, *Crytosula pallasiana*, and *Schizoporella errata*) were combined. Most bryozoan recruits were found on panels exposed in the early morning (0600-0900 h). Spirorbid polychaete (*Spirorbis* spp.) recruits, in contrast, were only found on substrates exposed during the middle of the day (0900-1500 h). Observed diurnal variations in epifauna recruitment generally are consistent with data collected from the day/night exposure deployments (Table 1).

*Infaunal recruitment relative to variations in tidal state*—Unfortunately, relatively few individuals recruited to the sandy substrates in both ELVIS deployments. Of the three species (*Mya arenaria*, *Polydora cornuta*, and an unidentified oligochaete) found in the sediment cups, only *Polydora* recruits averaged more than one individual per exposure period. *Polydora* recruitment varied significantly with tidal state—greater numbers of recruits were found in substrates exposed during the low tide period than during any other tidal states (Fig. 5). The same tidally related recruitment pattern of *Polydora* was found in both deployments.

## Discussion

Experimental tests of the operating assumptions of the benthic invertebrate recruitment device revealed that the de-

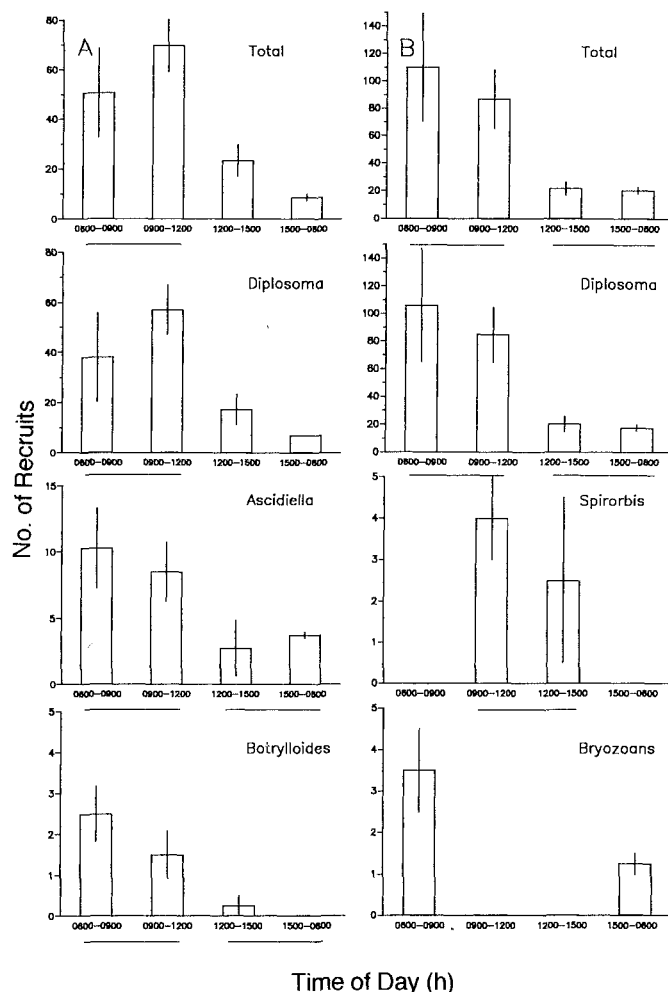


Fig. 4. Total recruits and numbers of the most abundant taxa (*Diplosoma macdonaldi*, *Asciidiella aspersa*, *Botrylloides diagensis*, and bryozoans) found on substrates repeatedly exposed at 0600-0900, 0900-1200, 1200-1500, and 1500-0600 h for a 7-d period. Values are averages ( $\pm 1$  SD) of recruit abundance. Lines connecting treatments are not significantly different from each other (one-way ANOVAs and Bonferroni contrasts tests;  $P > 0.05$ ). A. 20-27 September deployment; B. 4-11 October deployment.

sign could repeatedly expose sets of substrates to a broad array of naturally recruiting infaunal and epifaunal species at repeated, predefined environmental conditions for periods up to 2 weeks. The relatively simple design of substrate exposure and maintenance of organisms on unexposed substrates also indicated that the device was reasonably effective in isolating substrates from larval contamination while not adversely affecting recruits (at least for the test species *Asciidiella aspersa*) previously attached to the sequestered substrates. Users of the device, however, should carefully consider the potential effects of environmental conditions on particular species in enclosed vs. exposed substrates. For instance, using sediment as substratum may lead to possible artifacts from varying exposure times due to differential recruit mortality in the enclosures and sediment scour and re-suspension in the exposed sediment cups. Conditions in which there is a large vertical component to water flow may

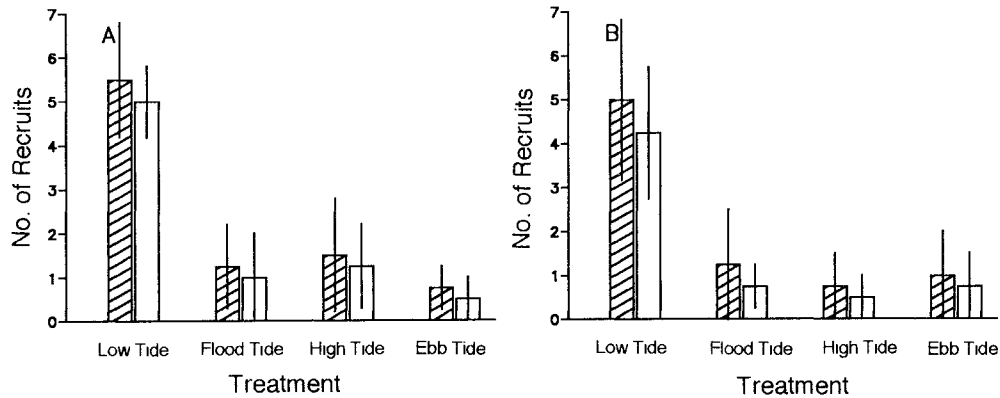


Fig. 5. Average number ( $\pm 1$  SD) of total recruits (hatched histograms) and *Polydora cornuta* recruits (open histograms) found in substrates repeatedly exposed at four different tidal states for two different 7-d deployment intervals (A. 7–14 July; B. 3–10 September).

result in biased larvae transport to, and retention on, the experimental substrates. ELVIS deployments to date have been conducted in relatively low-water-flow conditions. The device is likely most effective in relatively calm conditions, or when deployed several meters below the surface of the water. Habitat- and species-specific operation tests of the device similar to those conducted are recommended in order to fully understand potential artifacts and limitations of the recruitment device.

Preliminary short-term field trials examining recruitment patterns of a variety of epifaunal and infaunal species revealed the device provided temporally repeatable results that were generally consistent with previously published studies. Pronounced diurnal patterns in recruitment were found in five different taxa of sessile epifaunal invertebrates (bryozoans and spirorid polychaetes and three species of ascidians). Previous studies on ascidian and bryozoan reproductive behavior have indicated that larval release can be controlled by light intensity (Ryland 1977; Svane and Young 1989). The nonfeeding ascidian larvae typically reside in the water column for relatively short periods (minutes to hours for colonial forms; days to a week for solitary forms), and several species have been observed to settle during the midday period (Olson 1983; Lindquist et al. 1992).

Although the device found that recruitment of the infaunal polychaete *Polydora cornuta* was related to tidal state, there are conflicting reports regarding the generality of this finding. Levin (1986) reported 10-fold variations in the abundance of polychaete larvae over a tidal cycle and that *Polydora cornuta* (= *ligni*) tended to be most abundant during mid-ebb and late-flood tide periods during the daytime and high and ebb tides during the nighttime. The abundance of competent *Polydora* larvae, however, exhibited no discernable tidally mediated abundance patterns. In contrast, Stancyk and Feller (1986) reported higher numbers of polychaete larvae collected at high slack water than in ebb or flood conditions. The modeling study of Gross et al. (1992) predicted that larval availability near the seabed should be higher during slack water and lower during peak flow and ebb tides because of enhanced mixing with higher flow that mixes larvae away from the bed. Further studies are clearly

required to assess whether there are general patterns of infaunal recruitment relative to tidal state and current velocity.

We have attempted to keep the design and operation of the recruitment device as simple as possible in order to meet our specific needs and basic assumptions. Once we have more fully explored the behavior of the device, we will control its operation using environmental sensors (e.g. light meters, pressure transducers, current meters) interfaced to the microprocessor. This step will allow us to more easily study specific types of environmental conditions and deploy the device in a fully automated mode. Because the recruitment device can repeatedly expose a substrate over an extended period (e.g. up to 60 d), final collections should contain animals of different sizes depending on whether they recruited early or late in the collection period. It may therefore be possible to obtain added information on when the recruits were collected and determine if behavioral patterns change over a given long-term deployment period.

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Received: 31 October 1996

Accepted: 13 August 1997

Amended: 22 September 1997