

RESPONSE OF SETTLING OYSTER LARVAE, *CRASSOSTREA VIRGINICA*, TO SPECIFIC PORTIONS OF THE VISIBLE LIGHT SPECTRUM¹

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ABSTRACT Settlement site choice was used to test the ability of competent-to-settle oyster (*Crassostrea virginica*) larvae to detect specific portions of the visible light spectrum. Larvae were permitted to settle on illuminated or shaded sides of vertically oriented settlement substrates. Five light treatments were used, including white light (400–700 nm), three fractions of white light; red light (600–700 nm), green light (450–575 nm, peak at 525 nm), blue light (400–500 nm, peak at 425 nm); and total darkness. In total darkness, no settlement preference for either side of the substrates was detected. In all light treatments, larvae settled in significantly higher numbers onto shaded surfaces than illuminated surfaces. *Crassostrea virginica* larvae respond to most portions of the visible light spectrum, unlike many previously studied marine invertebrate larvae. This ability may reflect the diverse light conditions in the largely estuarine habitat of this species.

KEY WORDS: *Crassostrea virginica*, larvae, settlement, light

INTRODUCTION

Phototactic behavior in marine larvae can contribute to site selection during the settlement process even though the presence or absence of light is not required for settlement (Baker 1997). Pediveliger (competent-to-settle) larvae of the American oyster, *Crassostrea virginica* (Gmelin), settled primarily on shaded surfaces of substrates in laboratory trials (Ritchie and Menzel 1969). The pediveliger larva of some bivalve mollusks, including oysters (Ostreidae), has a distinct pigmented region termed the *eyespot*, and believed to be photosensitive. Cole (1938) initially described the eyespot and its presumed function for the larvae of the oyster *Ostrea edulis*, although Thompson et al. (1996) point out that research clarifying "eyespot" function is still needed.

Coastal planktonic invertebrates are usually unresponsive, or only weakly responsive, to long visible light wavelengths (orange and red). Serpulid polychaete larvae, which are negatively phototactic when swimming, are responsive to blue and green light (400–550 nm), but are indifferent or respond weakly to orange and red light (>600 nm) (Young and Chia 1982, Marsden 1986, 1988, 1990). Barnacle (*Balanus improvisus* Darwin) nauplii, which are positively phototactic, respond strongly to blue and green light, and also to long-wave ultraviolet (350 nm), but show a marked decrease in response to wavelengths above 600 nm (Lang et al. 1979). The larvae of some estuarine brachyuran crabs, including *Sesarma reticulatum* and *Uca minax*, appear to be most sensitive to light wavelengths of 500–600 nm (green to orange), but sensitivity of most species declines sharply above 600 nm (Forward and Cronin 1979). This phenomenon is not limited to larvae; coastal planktonic adult arrow worms, *Sagitta hispida* Conant, are most sensitive to blue and green light (400–540 nm) but much less sensitive to wavelengths above 520 nm (Sweatt and Forward 1985). The above phototactic responses correlate with spectral attenuation pattern in coastal oceanic waters in which red light (>600 nm) is strongly attenuated, whereas violet to yellow light (400–600 nm) is attenuated the least (Austin and Petzold 1984).

Crassostrea virginica is not primarily an oceanic species, like

most of the above examples, but occurs in greatest abundance in estuaries. The adult and larval life history of *C. virginica* has been reviewed by Stanley and Sellers (1986). In the estuarine environment of the Chesapeake Bay, Virginia, the attenuation of visible light is greatest for short wavelengths (<500 nm), and greatest during the summer when *C. virginica* larvae are most abundant. Yellow and orange light (550–650 nm) generally have the greatest irradiance (transmission) through the water column (Champ et al. 1980, van Tine 1987). The attenuation coefficient (a natural log scale of light reduction with depth) in the Chesapeake Bay during summer is about 3.1 at 400 nm, 1.6 at 500 nm, 1.1 at 600 nm, and 1.4 at 700 nm (van Tine 1987). At a depth of 1 meter, less than 5% of the surface violet light (400 nm) penetrates, but about 30% of the yellow and orange light (550–650 nm) is still present. Water quality, including inorganic and organic particulate matter, strongly affects light quality in estuarine waters (Pierce et al. 1986). It is probable, therefore, that *C. virginica* larvae experience a wide range of light quality within a single estuary.

If oysters are unresponsive to certain portions of the visible light spectrum, as are the larval species discussed above, then water quality parameters that affect light quality (Pierce et al. 1986) could affect the ability of oyster larvae to use light as a settlement cue. If oyster larvae do not respond to red and orange light wavelengths, then much of the Chesapeake Bay would be a light-poor environment to larval oysters (van Tine 1987). This study addresses the question: Do larvae of oysters (*C. virginica*) respond to different portions of the visible light spectrum, as observed by settlement site choice?

MATERIALS AND METHODS

Oyster larval settlement chambers were constructed from 1/8 inch (3 mm) thick black acrylic Plexiglas. One side was made of clear acrylic to permit light entry. Each chamber had internal dimensions of 7.5 cm in height, 7.5 cm in width, and 2.5 cm in depth, and a volume of about 60 ml. A clear acrylic bracket was placed in the bottom of each chamber, at least 2 cm from the sides of the chamber (Fig. 1), to hold a settlement substrate plate in a vertical position. The vertical orientation of the settlement plate was used to eliminate geotaxis (Pires and Woollacott 1983, Baker 1997) and barokinesis (Crisp and Ghobashy 1971, Mann and Wolf 1983) as confounding settlement cues.

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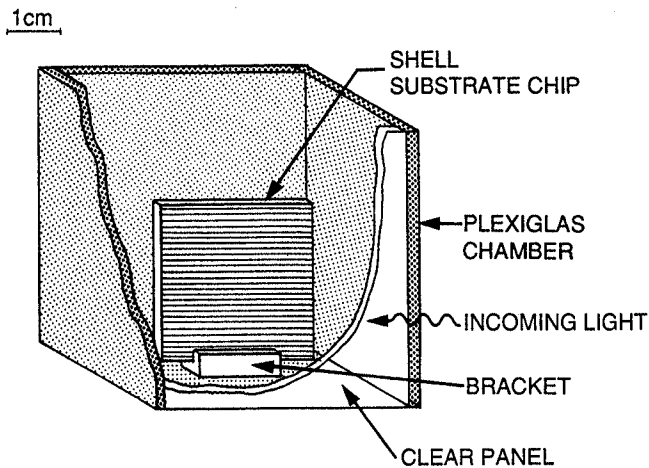


Figure 1. Diagram of a settlement chamber.

Settlement substrate plates were cut from adult oyster (*Crassostrea virginica*) shells, 3 × 3 cm square by 2 mm thick, and ground to equal roughness on both sides. Settlement plates were placed in seawater two days prior to use, to allow bacterial growth on the shell surfaces; water-soluble compounds from oyster shell and some levels of bacterial colonization induce settlement in *Crassostrea* (Hidu et al. 1978, Fitt et al. 1990). The tops of each chamber were covered with a black acrylic plate to reduce evaporation, but were not airtight.

The light source used for this study was a pair of new 48-inch Philips cool white fluorescent tubes. About 95% of the light output of the fluorescent lamp was between 400 and 700 nm, based on specifications provided by the manufacturer. The light source was fixed at 25 cm from the settlement chambers (chambers were arranged in rows facing the fluorescent tubes). Total visible light flux from the fluorescent lamp was measured with a Li-Cor radiometer-photometer, Model LI-185A, using an on-deck light sensor. Neutral density black scrim was used to regulate light intensity. Light entering the settlement chambers was 2.4–2.6 microEinsteins ($\mu\text{E} \cdot \text{m}^{-2} \cdot \text{m}^{-1}$), after all filters were in place. This range is equivalent to midsummer light intensity at about a 5 m depth in the lower Chesapeake Bay, using a light attenuation coefficient (k) of 1.1, and assuming surface insolation of $500 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (Wetzel and Neckles 1986). Light levels used in similar trials by Ritchie and Menzel (1969) were 25–50 foot candles (≈ 5.2 – $10.4 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$).

Five light treatments were used: white, red, green, blue, and total darkness. White light was unfiltered from the fluorescent lamp. The total darkness treatment consisted of settlement chambers made entirely of black acrylic, which blocked all light, with no clear acrylic panel. For red, green, and blue light, theatrical light filters (Rosco and Lee brands) were used. Transmittance spectra of filters were measured with a Shimadzu spectrophotometer, Model UV 160. The red filter was Roscolux Medium Red overlaid with Lee Orange; this combination transmitted wavelengths only above 600 nm. The green filter was Roscolux Dark Yellow Green (470–600 nm, peak at 525 nm), and the blue filter was Lee Deep Blue (380–525 nm, peak at 425 nm). Both green and blue filters transmitted some far red (above 740 nm) light, but these wavelengths were mostly above the fluorescent lamp output. Light filters and black scrim were attached directly to the outside of the settlement chambers.

Competent-to-settle pediveliger larvae of *Crassostrea virginica* were reared at 20 ppt salinity in the Virginia Institute of Marine Science oyster hatchery. Approximately 500 larvae, in 55 ml of 20 micron-filtered seawater (20 ppt), were pipetted into each settlement chamber. A fan was used to circulate air past the settlement chambers, and temperature within the chambers remained at 25–28°C throughout the trial.

All settlement trials ran simultaneously for 24 h at constant light conditions. There were six settlement chambers for each color treatment, and each series of trials (five treatments) was run twice, for a total of 12 replicates per treatment. At the end of each trial, settled juveniles (spat) on the illuminated (“front”) and shaded (“back”) surfaces of each substrate plate were recorded. Settlement on each side (illuminated and shaded, or front and back in the case of the no-light treatment), was expressed as a proportion of total settlement for each substrate plate. The difference between proportional settlement onto shaded and illuminated surfaces was calculated for each replicate substrate.

Paired-sample t -tests were used to test the null hypothesis that the mean proportional difference between illuminated and shaded (front and back) for each light treatment was equal to zero (Zar 1996). Prior to analysis, the absolute of each difference was transformed using the arcsine-square root transformation (Zar 1996), and then converted back to its original sign.

RESULTS

In total darkness, no significant settlement difference between front and back surfaces of the settlement substrate plates was detected. In all other treatments, proportional settlement of *Crassostrea virginica* was significantly higher onto shaded sides of settlement plates. Results are summarized in Table 1.

Larval mortality was less than 1% in any treatment, and larvae that had not settled were still swimming. About 10–12% of the larvae in each trial settled, which was typical for hatchery-reared *C. virginica* larvae at that time (Baker 1994).

DISCUSSION

Competent-to-settle larvae of *Crassostrea virginica* respond to red, green, and blue portions of the visible light spectrum in the same manner as they do to white light. In this regard, they are unlike many previously studied coastal larvae, which appear to be unable to use orange or red light (Lang et al. 1979, Young and

TABLE 1.

Summary of differences of settled *Crassostrea virginica* between back (shaded) and front (illuminated) surfaces. Values are given for mean proportion of larvae settled on shaded (Back) surfaces; the means (Δ Mean) and standard deviations (Δ STDS) of the differences between proportions on shaded and illuminated surfaces, and type I error probabilities (p) from one-sample t -tests (based on arcsine-square root data transformations).

Treatment	Total Darkness	Blue Light	Green Light	Red Light	White Light
Back	0.483	0.783	0.792	0.711	0.743
Δ Mean	–0.035	0.565	0.583	0.422	0.485
Δ STDS	0.513	0.265	0.346	0.267	0.309
p	0.9467	<0.0001	0.0001	<0.0001	0.0001

Chia 1982, Marsden 1986, 1988, 1990). Coastal waters that transmit little usable light to other species still transmit sufficient light in the longer wavelengths to be a settlement cue for *C. virginica* larvae.

The breadth of the spectral responsivity of *C. virginica* larvae reflects the range of water column conditions this species encounters. The spectral transmittance of sea water is strongly modified by dissolved and particulate terrigenous matter, and thus varies between and within estuaries (Pierce et al. 1986). The adult habitat of *C. virginica*, and thus the habitat of competent-to-settle larvae, ranges from oceanic to seasonal salinities as low as 5‰ (Wells and Gray 1960, Stanley and Sellers 1986). A broad spectral responsivity would be a beneficial adaptation for a species occupying this range of habitats. The brine shrimp, *Artemia salina*, also has a broad spectral sensitivity, and also occupies highly variable water column conditions (temporary ponds), although peak sensitivity appears to be below 600 nm (Aiken and Hailman 1978).

On the other hand, light is not a required settlement cue for *C. virginica*. In both this study and others (Richie and Menzel 1969, Baker 1997), *C. virginica* larvae settled in the total absence of light. Gravity appears to be a strong settlement cue by itself. Either light avoidance or geotaxis could permit larvae to settle on lower

surfaces of adult oyster shells in the field, but the settlement patterns that result are equally marked in darkness (Baker 1997), indicating gravity as a sufficient cue. Gravity is a constant, light is not. Furthermore, other cues are also available: *C. virginica* has been shown to settle in response to water-borne chemicals from conspecifics (Hidu et al. 1978), and *C. gigas*, a similar species, settles in response to chemicals produced by certain bacterial conditions on the substrate (Fitt et al. 1990).

The question then arises: Why has *C. virginica* evolved a phototactic response during settlement? Assuming that the eyespot is, in fact, a photosensory organ (Thompson et al. 1996), why does it develop only in the competent-to-settle larvae?

One possibility is that phototaxis permits a "fine-tuning" of the settlement response. Oysters, unlike most bivalve mollusks, cement permanently to the substrate immediately upon settlement, and cannot subsequently adjust their habitat choice (Kennedy 1996). Laboratory studies deliberately reduce variables and provide larvae with clear choices (up-down, light-dark), but natural ecosystems are likely to be more complex. It is, therefore, probably of selective advantage to invest in additional sensory systems to gain as much information as possible about a potential permanent home.

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