Identification of Eastern Oyster *Crassostrea virginica* Larvae Using Polarized Light Microscopy in a Mesoaline Region of Chesapeake Bay

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IDENTIFICATION OF EASTERN OYSTER *CRASSOSTREA VIRGINICA* LARVAE USING POLARIZED LIGHT MICROSCOPY IN A MESOHALINE REGION OF CHESAPEAKE BAY

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**ABSTRACT** Understanding the population dynamics and complete life cycle of bivalves is important for effective management of these commercially and ecologically important organisms. Most of the literature and research on bivalves to date has focused on juvenile and adult bivalves, but much less is known about larvae. The larval stage has been difficult to study due to the lack of a rapid automated approach for identifying the species. A new technique, called ShellBi, utilizes color patterns on the larval shell under polarized light to identify bivalve larvae. The objective of our research was to review the scientific basis for ShellBi and to apply it to bivalve larvae from the Choptank River in mesohaline Chesapeake Bay, with the goal of distinguishing larvae of the eastern oyster (*Crassostrea virginica*) from seven other species that spawn at the same time. A digital camera and polarized light microscope was used to capture images of the larval shells of the eight species under standard and cross-polarized light. Images of *C. virginica* were distinguishable from those of other species based on color patterns, especially at later stages of development. These images could serve as a visual guide to distinguish larvae of *C. virginica* from other bivalves inhabiting mesohaline tributaries of Chesapeake Bay.

**KEY WORDS:** oyster, *Crassostrea virginica*, larvae, identification, polarized light

**INTRODUCTION**

Many bivalves have a complex life cycle with a pelagic larval stage (Gosling 2015). Their population dynamics are based on birth, mortality, immigration, and emigration (Gotelli 2001). Understanding larval ecology is crucial for understanding population dynamics because immigration, emigration, and mortality occur during the larval stage (Kennedy 1996). Many species of bivalves are ecologically and economically important, yet little is known about their larval stage due to the difficulty in distinguishing species of larvae (Garland & Zimmer 2002). Use of cross-polarized light is a new method that has potential for identifying bivalve larvae to species. It utilizes the color patterns from larval shells under polarized light (Tiwari & Gallagher 2003). The aim of our research was to apply this new approach to eight species of bivalve larvae that spawn during summer in the Choptank River of mesohaline Chesapeake Bay, with the goal of distinguishing the larvae of eastern oyster *Crassostrea virginica* (Gmelin, 1791) from the other species whose larvae are in the plankton at the same time.

*Development and Shell Formation in Bivalve Larvae*

The bivalve larval stage is part of a complex life cycle that involves growth, ripening of gametes, spawning, and gonad redevelopment (Gosling 2015). Bivalves reproduce sexually by external fertilization to produce planktonic larvae that develop a shell (Kennedy 1996, Gosling 2015). Bivalves progress through several stages of shell development, including prodissocoench I, prodissocoench II, and dissoconch stages (Carriker 1996). The planktonic larval stages include prodissocoench I and II, with the dissoconch stage occurring after settlement (Carriker 1996).

Bivalve larvae mineralize their shells. Mineralization is the process through which an organic substance becomes impregnated by inorganic substances (IUPAC 2012). Larval shells are made mostly of aragonite, a crystal form of calcium carbonate, rather than the less-soluble calcite that makes up the shells of adult bivalves (Carriker 1996). Shell mineralization occurs in an organic aragonite matrix formed in the shell field, an area of ectodermal cells in the dorsal region of a developing embryo that secretes the embryonic shell (Carriker 1996). The first appearance of the shell field occurs in early embryogenesis (Moor 1983) when the shell field invaginates to form a “shell field invagination” (Eyster 1983). At this moment, organic shell material is secreted by the cells of the shell field externally and the epithelial cells in the shell field spread over the embryonic surface before mineralization (Carriker 1996).

The mineralization of a bivalve larval shell first takes place during the late stages of embryonic shell development. The matrix that forms in the shell field constitutes a brick-wall-patterned biocomposite of biominalerized aragonite platelets surrounded by organic matter known as nacre (Checa et al. 2006). The platelets are arranged in terraces that grow simultaneously (Schmidt 1924, Wada 1972). Within the terraces are three crystallographic axes of crystals (called *a*, *b*, and *c*-axes) with the *c*-axis perpendicular to the nacre surface and the other two axes parallel to the local growth direction of the shell margin (Wada 1972). After the initial mineralization, the larvae are often called trochophores and the new mineralized shell is homogenous and composed mostly of calcium carbonate (Eyster 1986).

More recent studies conducted on adult *Crassostrea virginica* shells have shown that granulocytic hemocytes could be directly involved in shell crystal production in addition to the previously described process of extracellular shell field invagination (Mount et al. 2004). It is not known if this process begins at the larval stage. It is thought that there is only a single shell field invagination in bivalves, but the intricate process is still not fully understood (Carriker 1996, Mount et al. 2004).

Mineralization marks the end of the embryonic stage and the beginning of the prodissocoench I stage which for.
several species lasts between 24 and 30 h at summer temperatures (Andrews 1979). Mineralization in two species, *Mercenaria mercenaria* (Linnaeus, 1758) and *Crassostrea gigas* (Thunberg, 1793), was found to begin with a precursor of aqueous calcium carbonate followed by a crystalline aragonitic phase (Weiss et al. 2002). Aragonite has the same chemical structure as calcite but has a different crystalline structure (Chang et al. 1996). Weiss et al. (2002) postulated that other bivalve larvae would have the same developmental properties as the two species they studied. The prodissoconch I larvae in the crystalline aragonitic phase look like the letter “D” and are often called “D-stage larvae”. Toward the end of the prodissoconch I stage, two equal-length aragonite valves form (Carriker & Palmer 1979) and can be noted by conspicuous punctate-stellate patterns on the surface of each valve when viewed under scanning electronic microscopy (Carriker 1996).

During prodissoconch II, the next shell development stage, the left valve grows considerably wider than the right valve (i.e., more convex) and an umbo (the rounded elevated part of each valve at the anterior end of the bivalve) begins to form (Carriker 1996). As shell secretion continues, the valves become heavier (Carriker 1996). By the end of prodissoconch II, most oyster larvae are over 150 μm and both adductor muscles that close the shell are nearly equal in size (Carriker 1996). At this point, some bivalve species exhibit growth striae that are visible in bands between aragonite layers (Miliar 1968, Siddall 1980).

The prodissoconch II stage ends with the formation of an actively crawling foot as the larva begins searching for a settling place (Nelson 1924, Carriker 1986, 1996). The settling behavior of several bivalves has been studied and some respond to chemical and biological cues that stimulate settlement (Carriker 1996, Kennedy 1996). The planktonic larval development ends after settlement, and shell secretion changes from aragonite to calcite to begin the adult dissoconch stage (Carriker & Palmer 1979).

**Larval Shells Under Polarized Light**

Polarized light as a tool for microscopy has been used in geology and was first applied to the shells of bivalve larvae in the early 1900s (Schmidt 1924). Recent advances in the methodology and machine learning software have made polarized light microscopy useful for rapidly identifying bivalve larvae (Gallager & Tiwari 2008). The shells of bivalve larvae contain anisotropic aragonite crystals with different orientations and are thus birefringent. Birefringent materials (e.g., calcite, mica, cellophane) have two different indices of refraction, that is, light passes through in two directions. When placed between crossed polarizers, birefringent materials produce interference colors (colors that differ from those under normal light) (Murphy et al. 2013). The crystalline shells of bivalve larvae are birefringent and form rainbow-like interference colors that are easily discernable in plankton samples subjected to polarized light (Fig. 1).

Visualizing patterns formed by the crystal structures of bivalve shells under polarized light was first conducted in the early 1900s on a river mussel species in Germany (Schmidt 1924). The use of these patterns to distinguish species is a technique called ShellBi and is based on the fact that the optical orientation of the crystals contained in the nacre of each bivalve species appears to be distinct (Tiwari & Gallager 2003). It is extremely difficult, at best, to visualize the crystal patterns or infer orientation under standard microscopy (Fig. 2A). As described by Tiwari and Gallager (2003), under cross-polarized light, the light that is not in the plane of the polarizer refracts off the shell and a dark cross of light extinction becomes visible in the plane of the two polarization plates (Fig. 2B). With the addition of a full-wave compensation plate (or λ plate), distinct colored interference patterns are produced as the polarized light refracts off the crystals (Fig. 2C). The patterns appear to be species specific, likely because the protein complement of the shell matrix and the axial rotation of the crystals could differ between species of bivalves (Tiwari & Gallager 2003). Thus, the resulting patterns would be distinct if the orientation of crystals that are laid down during shell formation differs among species. Species-specific patterns can be discerned, by eye, under polarized light microscopy with a full-wave (λ) compensation plate or using pattern recognition software to identify larvae to species (Gallager & Tiwari 2008, Thompson et al. 2012, Goodwin et al. 2014). The objective of our research was to capture birefringent images of different size classes of bivalve larvae that are spawned at the same time as larval *Crassostrea virginica* in the Choptank River, a subestuary of mesohaline Chesapeake Bay, and to compare the images by eye to ascertain if species-specific patterns were apparent that might enable *C. virginica* larvae to be distinguished without the use of pattern recognition software in this system.

**BIVALVES OF THE CHOPTANK RIVER**

Although there are more than eight species of bivalves in the Choptank River, this research focuses on seven that spawn at
the same as Crassostrea virginica. Those species are the following: Ischadium recurvum (Rafinesque, 1820) (Chanley 1970), Geukensia demissa (Dillwyn, 1817) (Borrero 1987), Macoma mitchelli (Dall, 1895) (Blundon & Kennedy 1982), Mytilopsis leucophaeata (Conrad, 1831) (Kennedy 2011a), Mulinia lateralis (Say, 1822) (Calabrese 1969), Rangia cuneata (G. B. Sowerby I, 1831) (Sundberg & Kennedy 1993), and Tagelus plebeius (Lightfoot, 1786) (Chanley & Castagna 1971). The following section reviews each species' life history and larval ecology. Table 1 provides a summary of spawning conditions, larval salinity tolerances, and pelagic larval durations for these species.

**Eastern Oyster Crassostrea virginica**

This species ranges along the Western Atlantic from the Gulf of St. Lawrence to Brazil and Argentina and has been introduced to the West Coast of the United States (Carriker & Gaffney 1996). Salinity tolerance of adults varies from 5 to 40 and optimal salinity ranges can vary by geographic location (Galtsoff 1964). When water temperatures reach ~25°C in Chesapeake Bay, Crassostrea virginica spawn and the larvae produced may be present in the water column for up to 2–3 wk (Shumway 1996). Optimal temperatures and salinity for the larvae may vary by geographic location. Larvae from Long Island Sound grow well in temperatures of 17.5°C and salinities of 15–27 (Davis & Calabrese 1964). Spat settlement, however, has been observed in salinities as low as 1.4 and tolerance of larvae may vary by geographic location (Shumway 1996). Native C. virginica populations are economically important as a food source for humans and are important ecologically (Wells 1961, Rodney & Paynter 2006, Fulford et al. 2010) by providing habitat and food for other organisms (Rodney & Paynter 2006) and by filtering algae from the water column (Newell 2004). Eastern oyster populations are declining (Beck et al. 2011), with the abundance of C. virginica populations less than 1% of historical levels in Chesapeake Bay (Wilberg et al. 2011).

**TABLE 1.** Spawning temperature and period, larval salinity tolerance, and larval development time for eight species of bivalves from the mesohaline region of Chesapeake Bay.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Spawning temperature, °C (reference)</th>
<th>Larval salinity tolerance (reference)</th>
<th>Larval development time, days</th>
<th>Spawning period (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Crassostrea virginica</em></td>
<td>28–30 (Shumway 1996)</td>
<td>5–27 (Shumway 1996)</td>
<td>16</td>
<td>Summer to fall (Kennedy 1996)</td>
</tr>
<tr>
<td><em>Macoma mitchelli</em></td>
<td>26–30 (Kennedy et al. 1989)</td>
<td>5–18 (Kennedy et al. 1989)</td>
<td>7</td>
<td>Year round (Blundon &amp; Kennedy 1982)</td>
</tr>
<tr>
<td><em>Mytilopsis leucophaeata</em></td>
<td>30 (Kennedy 2011b)</td>
<td>0.5–18 (Kennedy 2011b)</td>
<td>13</td>
<td>Summer to fall (Kennedy 2011a)</td>
</tr>
<tr>
<td><em>Rangia cuneata</em></td>
<td>30 (Sundberg &amp; Kennedy 1992)</td>
<td>&lt;15 (Sundberg &amp; Kennedy 1992)</td>
<td>8</td>
<td>Late spring to early fall (Sundberg &amp; Kennedy 1993)</td>
</tr>
<tr>
<td><em>Geukensia demissa</em></td>
<td>27 (Cape Shore Laboratory, unpublished data)</td>
<td>12–22.5 (Cape Shore Laboratory, unpublished data)</td>
<td>7–21</td>
<td>Early summer to fall (Borrero 1987)</td>
</tr>
</tbody>
</table>

All species except *Crassostrea virginica* and *Geukensia demissa* were successfully spawned and reared in laboratory conditions of ~23°C and salinities between 11 and 13. The larval development times for all bivalves were observed in the laboratory for all species except *C. virginica* (HPL hatchery data) and *G. demissa* (Cape Shore Laboratory).
Hooked Mussel *Ischadium recurvum*

The range of *Ischadium recurvum* stretches from Cape Cod through the Gulf of Mexico and the West Indies (Allen 1962). Their larvae have salinity and temperature requirements similar to *Crassostrea virginica* (salinities 6–20 and temperatures 25–30°C). This species is often found attached byssally to oyster reefs in Chesapeake Bay (Allen 1962, Shaw 1965, Chanley 1970) where it can affect the growth habits of oysters (Lipcius & Burke 2003). Settlement has been observed in the Choptank River from April through December (Shaw 1965). Although little is known about their pelagic larval duration (Lipcius & Burke 2006), settlement has been observed in the Chesapeake Bay and may reach up to 18 mm in length (Chanley & Castagna 1971). Their gonads are mature from June through December but most spawning occurs in late June and July (Verween et al. 2007). Larvae can survive in temperatures of 22.5–27.5°C and salinities from 20 to 35°C (Calabrese 1969). Temperature has the greatest influence on the duration of the larval stage and growth is “satisfactory” at temperatures from 22.5 to 27.5°C and salinities from 20 to 35°C (Calabrese 1969). Although this clam is not important commercially, its short generation time and high fecundity make it a perfect candidate for studies of pollution effects (Calabrese & Rhodes 1974). Waterfowl of the Chesapeake Bay eat *Mulinia lateralis* (Harmon 1962, Berlin 2008).

Marsh or Ribbed Mussel *Geukensia demissa*

The geographical range of *Geukensia demissa* is from the Gulf of Maine to Florida, although the species was also introduced in San Francisco Bay (Franz 2001). There is usually one annual spawning event between June and September, timing of which depends on the region (Borrero 1987). The larvae are found from early summer to the beginning of fall, including in Chesapeake Bay (Borrero 1987). Baker and Mann (2003) observed later-stage pediveliger larvae of the marsh mussel in surface waters during nonstratified conditions in one tributary of Chesapeake Bay. Populations of *G. demissa* are important because they affect the nutrient dynamics of marshes and estuaries (Jordan & Valiela 1982). Kuenzler (1961) found that the mussels can remove a third of the suspended particulate phosphorus and deposit it on the mud surface. They can also alter the structure of microbiota (Kemp et al. 1990) and are one of the few bivalves able to forage on small-sized bacterioplankton (Newell & Krambeck 1995). Sometimes *G. demissa* can form dense aggregates, altering the physical structure of the marsh and stimulating the growth of *Spartina alterniflora* (Bertness & Grosholz 1985). These mussels are also food for diamondback terrapins *Malaclemys terrapin* (Schoepf, 1793; Whitelaw & Zajac 2002).

Atlantic Rangia Clam *Rangia cuneata*

Atlantic rangia clams are found from the upper Chesapeake Bay to areas in the Gulf of Mexico that have salinities less than 15 (Hopkins et al. 1973, Cain 1975). Gametogenesis begins in this species at water temperatures exceeding 15°C and salinities less than 15 (Hopkins & Andrews 1970). Planktonic larval duration for *Rangia cuneata* is about 1 wk before settlement and the larvae grow to shell heights of 160 and 300 μm as pediveligers (Sundberg & Kennedy 1992). The larvae can develop successfully at temperatures of 23–26°C and salinities of 8–10°C (Sundberg & Kennedy 1992). Although this species is not commercially harvested, it is a nonselective filter feeder turning plant detritus and phytoplankton into clam biomass that animals can eat (Darnell 1958). In some areas of the country, mainly Texas, their shells are economically valuable (Hopkins & Andrews 1970).
August and September (Chanley & Castagna 1971). Larvae of *Tagelus plebeius* have a length of 90–170 μm and complete their development at a smaller size than many other bivalve larvae (Chanley & Castagna 1971). The pelagic larval duration is usually between 8 and 13 days (Chanley & Castagna 1971). Larvae of *T. plebeius* have been successfully reared at temperatures of 22–25°C and salinities of 11–30 (Chanley & Castagna 1971, Table 1). This clam is becoming increasingly important commercially for use as bait in commercial crab and eel traps (Dungan et al. 2002). It is eaten by diamondback terrapins (Whitelaw & Zajac 2002) and most likely other species in the Bay.

These eight species of larvae have diverse size ranges and pelagic larval durations, and play various ecological roles within mesohaline Chesapeake Bay; however, a rapid way to identify these larvae in plankton samples is needed for understanding larval transport and population connectivity, especially for oyster larvae, and for advancing knowledge of the population dynamics of the other ecologically important shellfish. Image analysis with polarized light can help fill this knowledge gap by allowing for improved identification and enumeration of bivalve larvae in plankton samples. The objective of this research is to document the color patterns under polarized light of these eight species of bivalve larvae in the Choptank River and to create a visual identification guide for distinguishing *Crassostrea virginica* from the other cohabiting species by using these patterns.

**MATERIALS AND METHODS**

To create a visual guide and key for identifying bivalve larvae under polarized light, adults of the eight species were spawned and their larvae reared. Images of the larvae at different stages of growth were captured under polarized light for all species. Finally, a visual guide and a key were created that could help distinguish *Crassostrea virginica* from the other species.

**Spawning and Rearing**

Adults of six of the eight species were collected from the Choptank River in summer and spawned and reared in the laboratory (Table 1). Larvae of *Crassostrea virginica* were obtained from the Horn Point Hatchery and of *Geukensia demissa* were obtained from the Rutgers Cape Shore Laboratory. Customary procedures used for spawning (using temperature fluctuation) and rearing larvae were consistent with summer conditions in Choptank River and were explained in detail (Goodwin et al. 2014) for all species with the exception of *G. demissa*. The *G. demissa* larvae were reared in conditions similar to Delaware Bay at a temperature of 24.9°C at a salinity of 22.5 and fed *Isochrysis galbana*, *Pavlova lutheri*, and *Chaetoceros calcitrans*.

**Imaging**

Images used for this guide were captured under standard microscopy and with cross-polarized light with a full-wave (λ) compensation plate. Specimens of three age groups (from 2 to 13 days old) from each species were imaged to discern the differences in birefringence patterns over three larval development stages (D-stage, early Prodissoconch II, and late Prodissoconch II). All bivalve larvae were imaged using an Omax M837PL trinocular inverted polarizing microscope. The microscope was equipped with an automatic stage and had a 5X ocular with an objective lens of 20X. The magnification was calculated to be 7X. An Infinity (model 2–3C) 8-megapixel digital microscope camera was used to image both polarized light and standard light images.

The software program used to capture images with the camera was Lumenera Infinity Analyze Software version 3.1. The camera software settings used to capture images were: exposure (151.0), gain (10.6), gamma (0.82), light source setting (fluorescent), saturation (1.31), brightness (4), contrast (4), red (1.0), blue (1.0), green (1.0), averaging (1), and subsampling (1). Birefringent images and standard light images were captured under these settings.

To prepare larval shells for imaging, they were broken apart and their tissue digested by immersing them in a solution of 40% bleach buffered with sodium borate in de-ionized water. Then, clean shells were pipetted into buffer de-ionized water on a Sedgewick-Rafter slide. Birefringent images were captured using cross-polarized light with a full-wave (λ) compensation plate. Once an image of a shell was captured, the polarizer and the (λ) plate were removed. A standard green glass filter was then placed over the light source and another image was captured under standard microscopy conditions.

A key was created from an image library that was taken with an automated image acquisition system (Goodwin 2015), using the same procedures mentioned above except for different software settings of the camera: exposure (151), gain (10.6), gamma (0.82), light source setting (fluorescent), saturation (1.31), brightness (4), contrast (4), red (1.0), blue (1.0), green (1.0), averaging (1), and subsampling (1). This online library includes 1,000 images of larvae in each of the bivalve Orders Ostreoida, Veneroida, and Mytiloida that spawn during summer in the Choptank River (species listed above) for a total of 3,000 images. The set of images that was used to make this key was called COM1000, is available online (http://northweb.hpl.umces.edu/TRANSPORT/home.htm), and was used as a training set for computer-assisted classification of bivalve larvae (Goodwin 2015).

**RESULTS**

Images of larvae that were captured under polarized light had similar color patterns at the taxonomic level of order: Ostreoida (*Crassostrea virginica* (Fig. 3A–F), *Mytiloida* (*Ischadium recurvum* (Fig. 4A–F) and *Geukensia demissa* (Fig. 5A–F)), and Veneroida (*Mya arenaria* (Fig. 6A–F), *Mulinia lateralis* (Fig. 7A–F), *Mytilopsis leucophaeata* (Fig. 8A–F), *Rangia cuneata* (Fig. 9A–F), and *Tagelus plebeius* (Fig. 10A–F)). A unique pattern of yellow coloration at the posterior and anterior edges of the D-stage shell (Fig. 3D) helped distinguish *C. virginica* from clams and mussels at the D-stage. Patterns on the D-stage shells of mussels and clams varied but were harder to distinguish (panel D in Figs. 4 and 6–10) due to similarity in colors and patterns.

The taxonomic groups were more clearly distinguishable at the prodissoconch II stage. The images of *Crassostrea virginica* (Fig. 3E, F) and mussel species (panels E, F in Figs. 4 and 5) had more yellow in their shell patterns than did Veneroida. Prodissoconch II mussels had the brightest patterns of yellow on their shells (Fig. 4E, F). The dark banding patterns of the oyster and the mussel *Ischadium recurvum* were similar (Figs. 3F and 4F) but the species were distinguishable because the mussel had a more circular shell with a less pronounced umbo. The birefringent images of Veneroida species had a majority of
red and blue coloration (panels E, F in Figs. 6–9) with little yellow, making it difficult to distinguish among Veneroids, but useful for distinguishing them from mussels and *C. virginica*. One exception is later-stage larvae of *Tagelus plebeius* (Fig. 10E, F), which had some yellow coloration but still less than 20% of the shell. The more circular shape of *T. plebeius* helped distinguish these images from *C. virginica* and mussels (Fig. 10).

An identification key was created to help distinguish *Crassostrea virginica* larvae from other species that spawn at the same time as *C. virginica* (Fig. 11). The key identifies larvae based on stage (e.g., D-stage, umbo). Larger *C. virginica* larvae were easier to distinguish because of their color (yellow and orange) and shape (pronounced umbo). Smaller *C. virginica* D-stage larvae were distinguishable...
because they were generally dull in color compared with other D-stage larvae.

**DISCUSSION**

Results of this research indicate that *Crassostrea virginica* larvae can be distinguished with polarized light microscopy from larvae of seven other bivalve species in the Choptank River and elsewhere where these mesohaline species cohabit. This work is built on previous works that used pattern recognition software (ShellBi) to identify bivalve larvae using birefringent patterns under polarized light (Gallager & Tiwari 2008, Thompson et al. 2012, Goodwin et al. 2014). A visual

![Figure 5](image_url)  
**Figure 5.** Images of *Geukensia demissa* larvae captured under (A–C) standard and (D–F) polarized light at a magnification of 7×. Larvae are (A, D) 3-, (B, E) 6-, and (C, F) 13-day-olds. Shell heights are listed in panels D–F.

![Figure 6](image_url)  
**Figure 6.** Images of *Macoma mitchelli* larvae captured under (A–C) standard and (D–F) polarized light at a magnification of 7×. Larvae are (A, D) 2-, (B, E) 8-, and (C, F) 10-day-olds. Shell heights are listed in panels D–F.
guide coupled with a digital library of multiple images for each species/stage could be used for identification with a polarized microscope and a digital camera in systems where there are bivalves with distinct interference patterns. Manual imaging takes considerably longer than bivalve larval classification software. Classification software is available with the system “LIHDAT” from coastaloceanvision.com.

The pattern interference colors on the shells of bivalve larvae under polarized light were sensitive to camera settings and some settings may be better than others at distinguishing different species (Goodwin 2015). Although useful in the mesohaline Choptank River, larval identification by eye likely will not work in other systems that contain many bivalve species which spawn at the same time. For example, in Waquoit

Figure 7. Images of *Mulinia lateralis* larvae captured under (A–C) standard and (D–F) polarized light at a magnification of 7×. Larvae are (A, D) 4-, (B, E) 10-, and (C, F) 13-day-olds. Shell heights are listed in panels D–F.

Figure 8. Images of *Mytilopsis leucophaeata* larvae captured under (A–C) standard and (D–F) polarized light at a magnification of 7×. Larvae are (A, D) 2-, (B, E) 6-, and (C, F) 8-day-olds. Shell heights are listed in panels D–F.
Bay it was necessary to use the classification software (LIHDAT) to identify larvae because they were indistinguishable by eye (Thompson et al. 2012). The settings used in our images were optimized to distinguish *Crassostrea virginica* larvae. If another species was being targeted, different settings may perform better. For example, under these settings, it was difficult to distinguish species in the Order Veneroida from each other (*Macoma mitchelli*, *Mulinia lateralis*, *Mytilopsis leucophaeata*, and *Rangia cuneata*), but altering the software settings of the camera could bring out species-specific differences among them. The key (Fig. 11) was created to target *C. virginica* and would be best used with images and a reference.

**Figure 9.** Images of *Rangia cuneata* larvae captured under (A–C) standard and (D–F) polarized light at a magnification of 7×. Larvae are (A, D) 2-, (B, E) 4-, and (C, F) 8-day-olds. Shell heights are listed in panels D–F.

**Figure 10.** Images of *Tagelus plebeius* larvae captured under (A–C) standard and (D–F) polarized light at a magnification of 7×. Larvae are (A, D) 2-, (B, E) 4-, and (C, F) 8-day-olds. Shell heights are listed in panels D–F.
image library created with the same camera settings described in this study.

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LITERATURE CITED


