Guide

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GUIDE TO SAMPLING AND IDENTIFYING LARVAE OF SPECIES OF MARICULTURAL INTEREST 2004

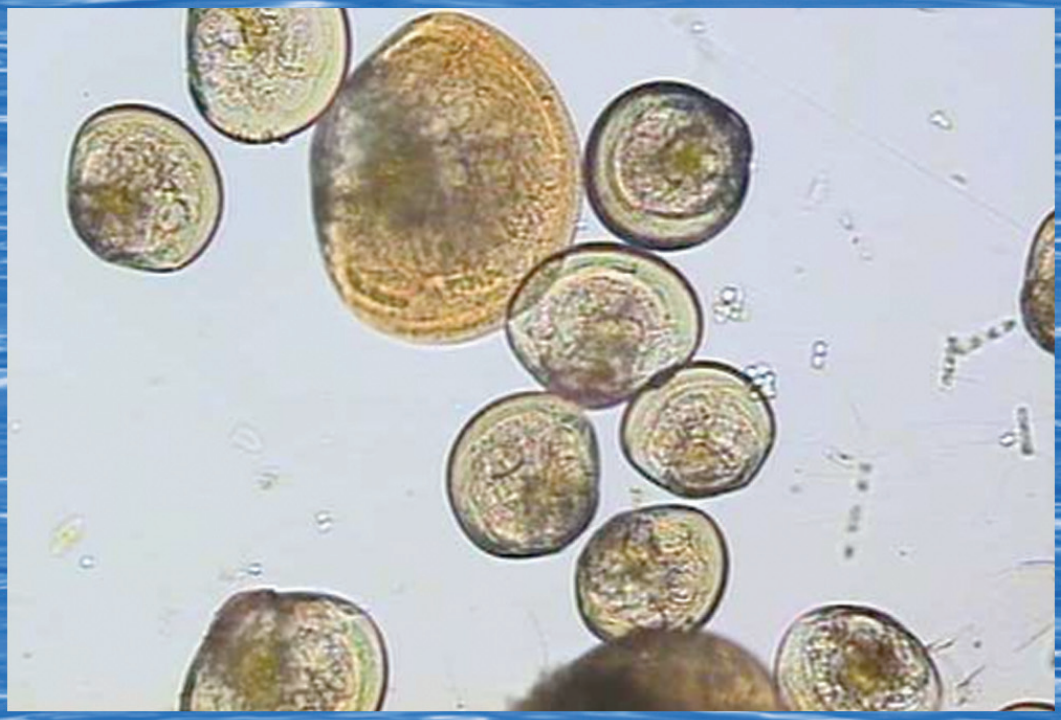

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

The success of a bivalve aquaculture enterprise depends to a large extent on a regular supply of juveniles (spat) from the natural environment. Consequently, a good understanding of the larvae found at the collection sites is of definite advantage to both producers and researchers. Such information makes it possible to ensure better management of maricultural operations. For example, problems associated with the fouling of collection structures by undesirable species can be minimized if the collectors are set out at the most appropriate time.

When larval samplings are conducted, various species may be observed, including ones of interest for maricultural purposes: blue mussel (*Mytilus edulis*), sea scallop (*Placopecten magellanicus*), and soft-shell clam (*Mya arenaria*). There are also larvae of potential predators of cultured species, such as the northern sea star (*Asterias vulgaris*), or larvae of competitors such as the northern rock borer (*Hiatella arctica*) and the jingle shell (*Anomia sp.*). Despite the various reference documents available, it is sometimes difficult to distinguish one species from another when attempting to identify larvae: meticulous identification requires a certain amount of experience on the part of the observer. The acquisition of such expertise will be aided by appropriate training, the reading of simple, non-technical reference guides, with representative photographs, and laboratory work sessions under the supervision of experienced staff.

Intended to facilitate the laboratory identification of larvae, this modest guide offers a compilation of data collected during scientific mariculture projects conducted by the Station technologique maricole des Îles-de-la-Madeleine over the past 10 years or more. These data were combined with the results of work carried out at the experimental hatchery of the Aquarium and Marine Centre in Shippagan, New Brunswick. Reference documents by Bourne (1964), Chanley and Andrews (1971), Culliney (1974), Le Pennec (1980), Loosanoff et al. (1966), Lutz et al. (1982), Medcof (1939), de

Schweinitz and Lutz (1976), Stafford (1912), Sullivan (1948), and Tremblay et al. (1987) made it possible to fill in certain gaps.

Besides providing information about larval biology, the guide sets out a work protocol for collection and laboratory analysis. For each species considered relevant, a series of photographs (six in most cases) corresponding to the different larval stages is presented. These are accompanied by a brief description of the larval stages, making it possible to distinguish the species from one another.

2. LARVAL REPRODUCTION AND DEVELOPMENT

2.1 Reproduction

In most bivalves, the sexes are separate. At the time of reproduction, the male and female gametes are released into the water and carried by the currents. The reproductive period varies by species. For example, the blue mussel and the soft-shell clam generally reproduce between May and June in the Magdalen Islands area. The common razor clam, the surf clam, the northern quahog, and the Arctic rock borer spawn around June. In the case of the sea scallop, the Iceland scallop, the horse mussel, the eastern oyster, and the jingle shell, spawning occurs in mid-August.

Although the cycle shown in Figure 1 is specific to the mussel, it is representative of most bivalve species. The size of a bivalve egg (oocyte) (Figure 1B) varies between 65 and 80 µm. There is always a larger quantity of spermatozoa than of eggs. For instance, a female eastern oyster 76 mm long can release more than 80 million eggs, while a male of the same size will release billions of spermatozoa.

Reproduction can be triggered by a series of factors, of which water temperature is among the most important. In New Brunswick, for example, oysters start spawning as soon as the water temperature reaches 20°C, which generally occurs in mid-June in Bouctouche Bay and in early July in Caraquet Bay. Stressful conditions, such as churning of the water caused by wind, waves, or currents, can also induce spawning, as can the presence of gametes or phytoplankton in the water.

Fertilization takes place in the water. Once released, the oocytes are fertilized by the sperm of males of the same species. After fertilization, the first cell division takes place (Figure 1C). Further divisions result in a mobile larva called a “trochophore” (Figure 1D), which thanks to its apical cilium, moves by spinning round.

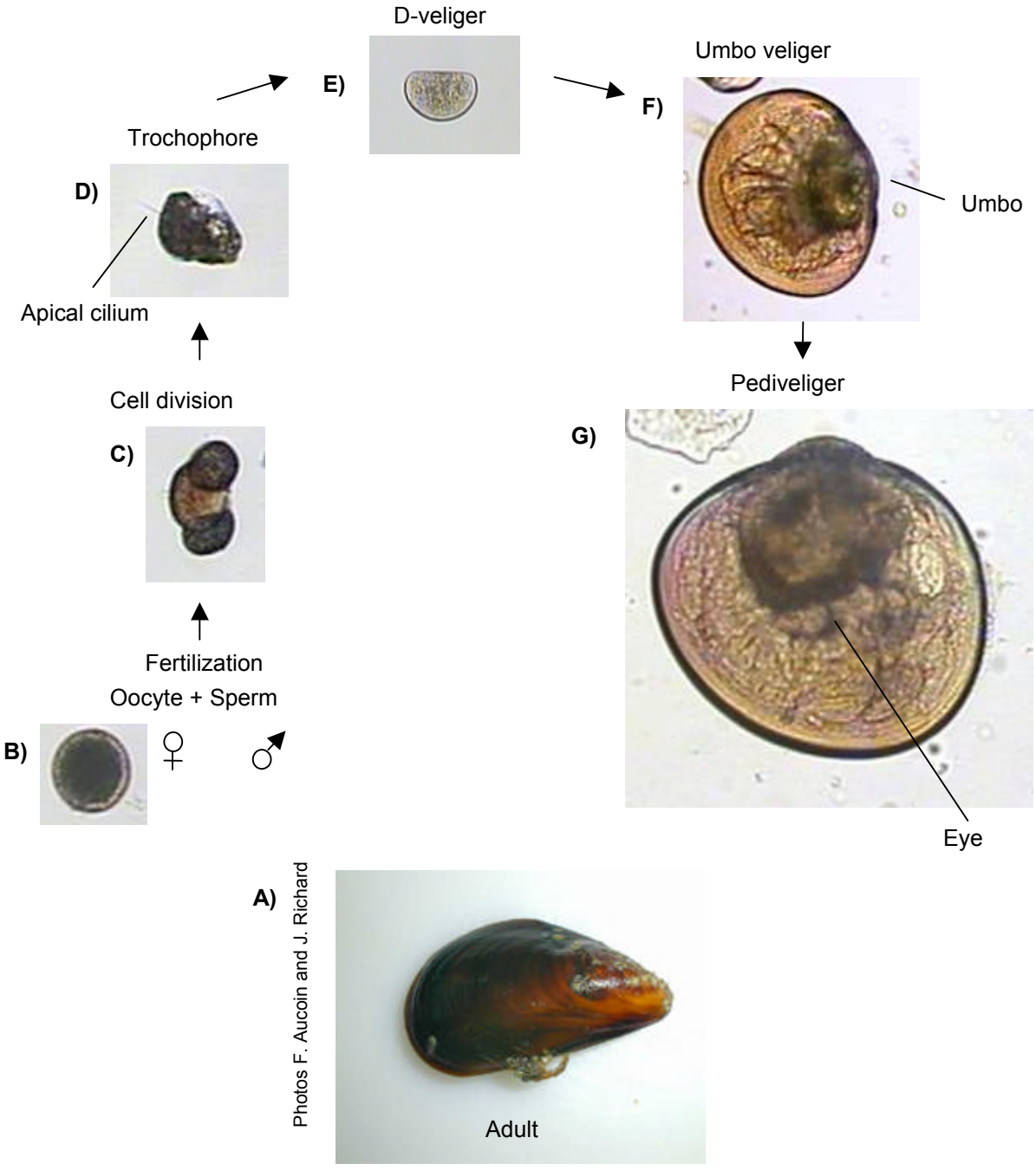


Figure 1. Reproductive cycle of the blue mussel (*Mytilus edulis*).

2.2 Larval Stages

D-veliger: After one to three days of life, the larva becomes D-shaped (Figure 1E), and the apical cilium present at the trochophore stage disappears. The larva moves through the water using its velum, a ciliated locomotive organ.

Umbo veliger: The umbo is the area of the shell located near the hinge where it is most curved. It can take a number of shapes depending on the species and the stage of development (Figure 2 and Table 1). The shape of the umbo can also depend on the position of the larva when it is being observed.

Pediveliger: The appearance of the foot and a black dot called the “eye” is characteristic of this stage. The larva is now ready to settle to the substrate (Figures 1G and 3). To observe the eye under a microscope, the image must be adjusted using the micrometer screw. The other organs will then look blurry.

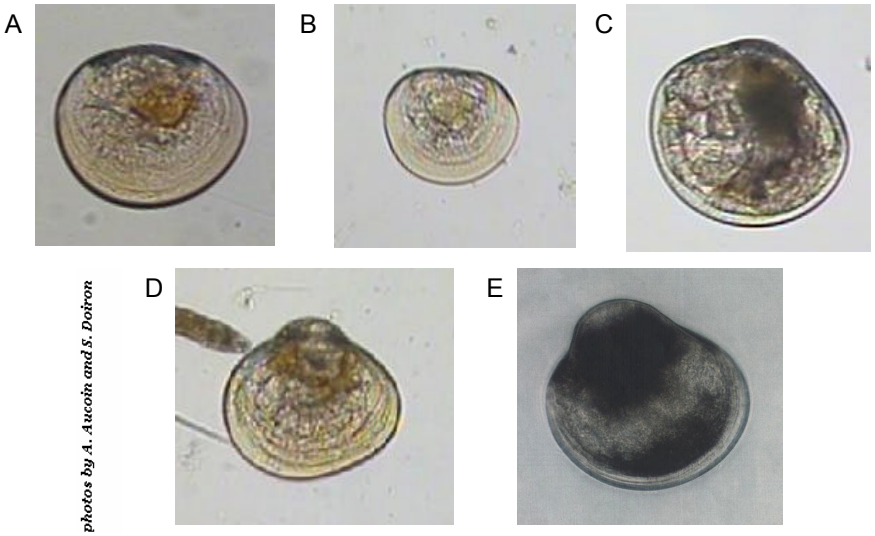


Figure 2. Umbo shapes: a) indistinct (blue mussel, 217 µm); b) flattened (common razor clam, 153 µm); c) angular (soft-shell clam, 226 µm); d) protuberant centred (jingle shell, 198 µm); e) protuberant off-centred (eastern oyster, 192 µm).

Table 1. Umbo shape by larva size and species (length in µm).

Species	Indistinct	Flattened	Angular	Protuberant centered	Protuberant Off-centered
Jingle shell	90-120			90-215	
Common razor clam	135-195	200-275	200-275		
Eastern oyster	85-110			115-150	140-320
Blue mussel	150-260			260-305	
Horse mussel	140-165			170-350	
Soft-shell clam	110-200		170-210		
Atlantic flat lepton		124-250		250-350	
Sea scallop	105-270				
Artic rock borer			135-340		
Northern dwarf tellin	90-135			135-250	210-340
Common shipworm		105-130	115-200		

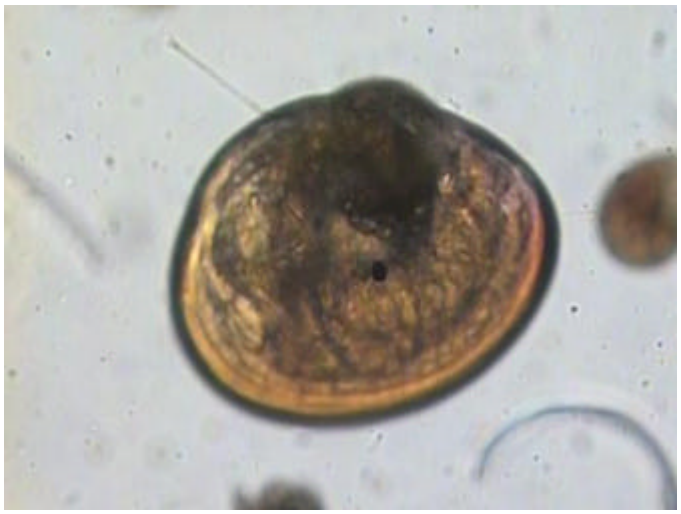


Photo F. Aucoin

Figure 3. Eyed blue mussel larva, 395 µm.

2.3 Feeding

Bivalve larvae filter water through their gills to feed on species of minuscule plants and animals that live in suspension in the water, as well as on pieces of larger plants and animals. The colour of the larvae can vary depending on the food that is eaten. For instance, larvae produced in a hatchery are often paler than those found in the natural environment (Figure 4).

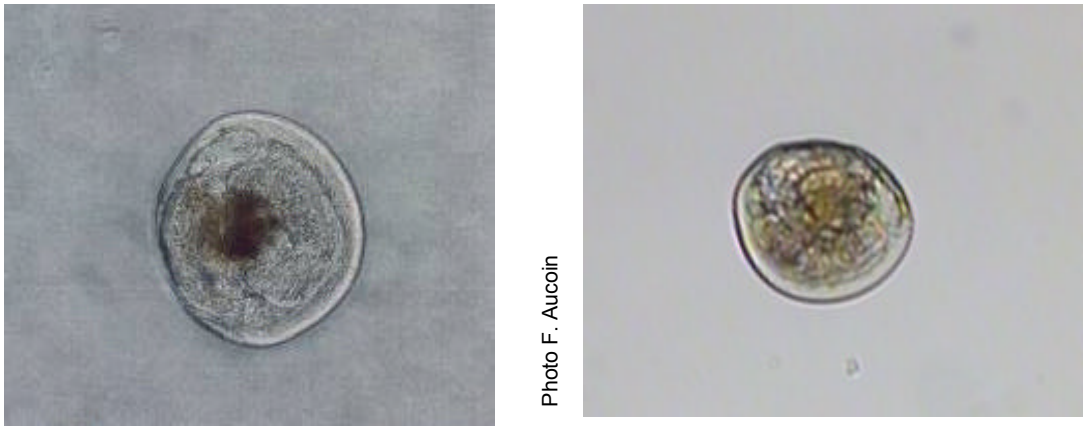


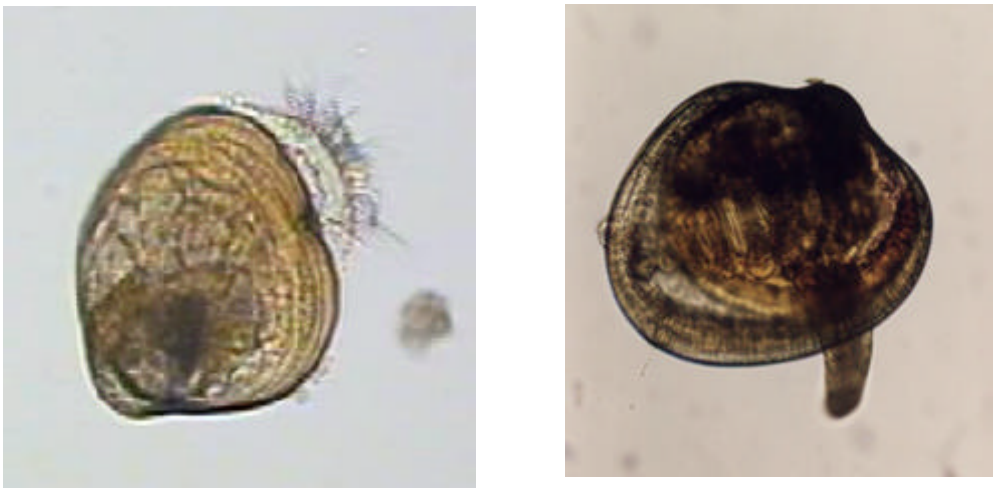
Photo S. Doiron

Photo F. Aucoin

Figure 4. Coloration of soft-shell clam larvae from a) a hatchery (146 µm), and b) a natural environment (145 µm).

2.4 Movement

Larvae are planktonic. Using their velum, they swim vertically in the water column (Figure 5A). Larvae generally stay near the surface of the water at night, descending to a deeper level during the day. The prevailing currents transport them horizontally. Larvae are sometimes carried over distances of several kilometres. After metamorphosis into a postlarva, the velum disappears, and the foot enables the mollusc to move from one settling site to another (Figure 5B).



Photos F. Aucoin

Figure 5. Organs of locomotion: a) jingle shell with velum (212 µm), and b) blue mussel with foot (indeterminate size).

2.5 Metamorphosis and Settling

At the end of the pediveliger stage, mollusc larvae look for a solid substrate where they can settle and metamorphose into postlarvae. The rate of growth during the larval period varies by species and according to such factors as water temperature and food availability. The blue mussel, for example, is ready to settle after about 30 days, whereas the soft-shell clam is ready in 14 days (Table 2). In a mariculture operation, the collection structures must be put in the water before the target species reaches the settling stage.

A pediveliger larva can delay metamorphosis and continue to grow until it finds a suitable substrate. Maximum larval size therefore depends on the availability of substrates in the environment. Metamorphosis takes place quickly once settling has occurred. Depending on the species, a larva may settle temporarily or permanently.

Table 2. Size and approximate age of pediveliger larvae of certain species of maricultural interest.

Species	**Larval duration (days)**	**Size (µm)**
Eastern oyster	16-20	320
Blue mussel	25-30	260
Soft-shell clam	12-16	220
Sea scallop	25-30	220

3. LARVAL SAMPLING METHODS

There are various methods for sampling larvae. The techniques described below – the plankton net and the pumping system – are among the most common.

3.1 Plankton Net

The plankton net is used for vertical, horizontal, and oblique larval tows. Vertical tows make it possible to evaluate the zooplankton throughout the water column. With horizontal tows, larval content at a specific water level can be evaluated. Lastly, oblique tows are used to evaluate larval content in a given space. The first two towing methods are explained below.

3.1.1 Description of Net

This system generally consists of a conical Nytex net (80-µm mesh size) mounted on a stainless steel ring 1.5 cm in diameter. The ring maintains a net mouth diameter of 1 metre (Figure 6). At the base of the cone is a collection bucket in the form of a short tube with holes in it, lined with Nytex. A net of this size, with a total length of 3 metres, is recommended for samples taken at great depths. For shallow tows, it is better to use a smaller net. Nets and

Photo J. Richard

Figure 6. Plankton net for vertical tows.

buckets are available from specialized dealers.

For horizontal tows, three cables attached at equal distances around the mouth ring meet at a point towards the top of the net, enabling the net to be attached to a winch. A swivel and a depressor weight are installed at this junction as well. A flowmeter attached to the mid-point of the net mouth by means of three metal rods measures the volume of filtered water.

For vertical tows, three cables attached at equal distances around the mouth ring run down towards the bottom of the net to hold a 25 kg weight. A cord connects the bucket to the weight to prevent the bucket from rising as the net is lowered. The volume of the water column filtered by the net can be calculated using a flowmeter or the formula $v = \pi R^2 h$ (π: 3.1416; R: mouth radius of net; h: maximum depth of net).

3.1.2 Sampling Method

For each tow, data such as date, time, location of site, and sampled depth must be recorded. If applicable, the initial reading of the flowmeter's counter must be noted as well.

For a vertical tow, the boat must remain stationary. The net is lowered slowly to the required depth, then raised using a winch, at a speed of approximately 6 cm/s. For a horizontal tow, the net is set at the desired depth and then towed by the boat at a slow speed (2 to 4 knots) over a predetermined distance. The net is then raised.

Once the net is on board, the final reading of the flowmeter's counter is recorded. The sides of the net are then rinsed with salt water to remove the plankton and flush it into the bucket. When the bucket is three-quarters drained, it is unscrewed from the net, and the contents are poured into a series of superimposed sieves. The mesh sizes of the sieves vary depending on the size of the larvae being collected. For example, for bivalve larvae, the mesh size of the upper sieve is 350 µm and of the bottom sieve, 53 µm. The first sieve, which

normally retains only larger particles or organisms, is set aside. A bottle filled with salt water is used to wash the entire contents of the 53 µm sieve to one side for transfer to a sample container that has been clearly identified beforehand (sampling site and date). Salt water can be added to the container to keep the plankton under water. The samples are kept chilled in a cooler for transport to the laboratory.

3.2 Plankton Pump

3.2.1 Description of Pumping System

A submersible pump (the R14 model - 3700 gph is suggested) is used in the assembly. It is hooked up to a 12 volt battery enclosed in a case. A flexible hose 6 m in length and about 6 cm in diameter is connected to the pump. A water meter (equipped with a dial indicating the volume of water pumped) and a flow-control valve (Figure 7) are attached to the hose. An elbow pipe fitted to the water outlet makes it easier to pour water over the sieves.

Photo J. Richard

Figure 7. Pumping system.

A series of superimposed sieves filter the pumped water. Generally, for bivalve larvae, the first sieve is 350 µm and the second, 53 µm, as indicated in section 3.1.2. The sieves have a cover with a hole in it for the feed hose. The screening system is installed on the side of the

boat by means of an aluminum frame that holds a large bucket with perforations in it allowing the filtered water to pour out.

3.2.2 Sampling Method

On the sampling site, sampling time, site position, and water temperature and salinity are recorded. The submersible pump is then set about 2 m from the surface and connected to the poles of the battery. While the water circulates through the pumping system, the bottom of the large bucket serving as the base of the filtration system frame is filled such that the larvae collected remain in suspension at the bottom of the last sieve.

Before the filtration operation is started, the water meter reading must be noted. Next, the elbow at the end of the feed hose is inserted in the hole in the sieve cover, and the flow valve is opened.

During filtration, the pump is moved in the surface water column to a depth of 2 m using a regular to-and-fro motion in order to collect a representative sample of the larvae on the site. When the counter indicates that 1,000 litres of water have been filtered, the flow-control valve is shut off, the pump is disconnected, and the filtration system is removed from its frame.

The filtration system's superimposed sieves are then dismantled. The first sieve is set aside. The larvae collected in the bottom sieve are recovered using a wash bottle filled with seawater. The plankton are washed to one side of the sieve and placed in a sample container that has been clearly identified beforehand (sampling site and date).

Once the sieve has been cleaned, the sample container is securely closed and kept cool. When the containers arrive at the laboratory, they are stored in a cool place, at temperatures ranging from 2 to 4°C. Larval quality can be maintained in this manner for about two days. If the analysis cannot be done within that time frame, the larvae will have to be stored in 70 to 85% ethanol.

4. LABORATORY ANALYSIS OF LARVAE

Various steps precede the actual analysis, as described below.

4.1 Treatment of Sample

Before the microscopic examination of the larvae begins, it is important to homogenize the solution containing the plankton. To do that, the contents of the container are poured into a beaker. If the sample is fresh, filtered seawater is then added until a known volume is obtained. Usually, for abundant larval species, such as the blue mussel, the volume used is 500 mL. For less abundant species, such as the sea scallop, 200 mL is used. If the larvae have been preserved in ethanol, the contents of the container must be put through a 53µm sieve, rinsed with fresh water, and then transferred to a beaker. Fresh water is used to treat this type of sample.

After the solution is mixed, five 1 mL subsamples are taken using a pipette and placed in a watch glass 100 mm in diameter. A rotational movement of the watch glass concentrates the larvae in the solution at the bottom of the glass; the algae in suspension and the supernatant can then be removed with a pipette. After successive rinsings with fresh water or seawater and removal of the supernatant with a pipette, only the larvae remain for microscopic examination.

4.2. Observation Methods and Measurements

4.2.1 Use of Microscope

For the microscopic analysis, a small amount of subsample is taken from the watch glass with a pipette and placed in a well glass slide with a 0.1-mL well. A cover is then placed over the well. This step is repeated until there is no more subsample in the watch glass. Measurements are generally taken with a 10x objective and a 10x ocular, giving a 100x enlargement.

4.2.2 Calculation of Density and Measurement of Size of Larvae

The densities of the species found in a given sample vary depending on the site and the time of year. Generally, density is expressed in number of larvae per litre or per cubic metre. To obtain this value, the number of larvae in the 5-mL sample in the watch glass is used to calculate the number of larvae for all of the known volume (see section 4.1) Density is then calculated on the basis of the volume of water pumped or filtered when the sample was taken in the natural environment.

Example of calculation

Six mussel larvae were counted in a 5-mL subsample from a known volume of 500 mL. For this sample, 75 litres of water were pumped from a lagoon.

6 larvae in the subsample = 5 mL
600 larvae in the sample = 500 mL

600 larvae in the sample = 75 litres of pumped water
8 larvae/litre or
8000 larvae/m3 (1000 litres) in the lagoon

The size of the larvae, expressed in µm (micron, or 0.001 mm), is measured using a micrometer in the ocular of the microscope. The distribution of size frequency is generally calculated on the basis of larval length, i.e., the longest distance parallel to the hinge (Figure 8).

It is easier to count and measure sizes using image analysis software (for example, Leica Q500, Image Pro, Optimas) connected to the microscope by a computer. With this type of system, it is also possible to take photographs (in fact that is how the photographs in this guide were taken).

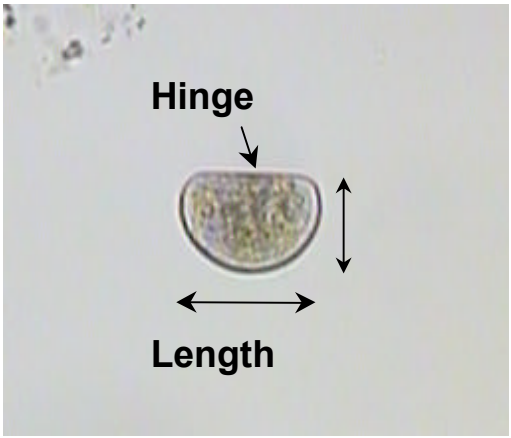


Photo F. Aucoin

Figure 8. Measurement of size.

4.3 Identification of Larvae

Larvae are identified on the basis of both the morphological characteristics specific to each species and the periods during which they occur in the water column. The morphological characteristics of a typical larva are shown in Figure 9. The characteristics specific to each species are presented in section 5.

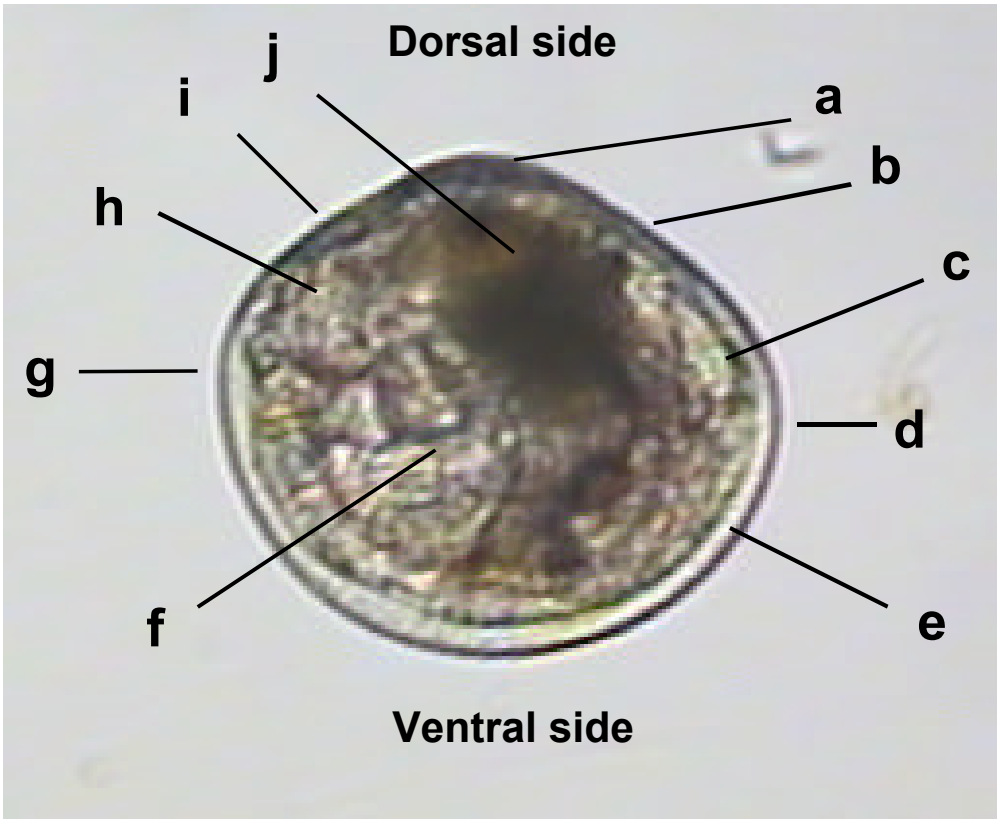


Photo F. Aucoin

Figure 9. Morphological characteristics of a typical umbo-veliger larva: a) umbo; b) anterior shoulder; c) anterior muscle; d) anterior side; e) space between visceral mass and shell edge; f) visceral mass; g) posterior side; h) posterior adductor muscle; i) posterior shoulder; j) digestive gland.

Tables 3A and B present the seasonal variations in the abundance of various species recorded during samplings in lagoons and off the coast in the Magdalen

Islands area between 1995 and 2000. With this information, it is possible to identify certain species during analysis.

Table 3. Abundance of larvae of various species in the samples taken (A) in lagoons, from May to August 1995-2000, and (B) at sea, from mid-August to October 1998-2000, Magdalen Islands.

Lagoon Species	MAY	JUNE	JULY	AUGUST
Jingle shell				
Common razor clam				
Surf clam				
Blue mussel				
Horse mussel				
Soft-shell clam				
Flat lepton				
Arctic rock borer				
Common shipworm				
Northern dwarf tellin				

Sea Species	AUG.	SEPTEMBER	OCTOBER
Jingle shell			
Common razor clam			
Surf clam			
Blue mussel			
Horse mussel			
Flat lepton			
Sea scallop			
Arctic rock borer			
Common shipworm			
Northern dwarf tellin			

High abundance
Abundant
Present
Absent

Using the results in Table 3, certain species that are present only at certain times of the year can be eliminated right away during the larval identification process. For example, a sample taken in June should not contain horse mussel larvae. However, weather conditions (temperatures, storms) can cause variations in the period during which species occur.

4.3.1 Steps in Larval Identification

The chronological sequence of the steps in larval identification differ from one observer to the next. In this guide, it is suggested that sampling dates be used first to eliminate species that should not be present at certain times of the year (Table 3). Next, larval size and umbo shape (Table 1) are used to narrow down the spectrum of possibilities. Lastly, the secondary characteristics described in section 5, combined with larval size, will make it possible to refine species identification. In case of doubt, the observer can refer to section 5.3, in which the larvae of the species mostly likely to be confused are compared.

5. CHARACTERISTICS OF LARVAE

This chapter provides a brief description of various species likely to be found in a larvae sample. The photographs of larvae are from samples collected in the lagoons and off the coast of the Magdalen Islands in connection with projects carried out by the Station Technologique Maricole of the Quebec Department of Agriculture, Fisheries and Food, as well as projects at the experimental hatchery of the Aquarium and Marine Centre in Shippagan, New Brunswick. The first part looks at species of maricultural interest and the second, at associated species that can be observed in the sample.

The approximate size of the adults of the different species is represented by a line under the photograph of the specimen. Size is measured along the animal's longest axis. For large species, the line may be divided into two or more sections.

5.1. Cultured Species

The blue mussel, the sea scallop, the Iceland scallop, the soft-shell clam, the eastern oyster, the northern quahog, and the surf clam are some of the species with maricultural potential. Unfortunately, it was impossible to include photographs and relevant information for Iceland scallop larvae in this guide. A supplementary leaflet concerning this species will therefore have to be prepared as soon as possible.

5.1.1 Blue Mussel (*Mytilus edulis*)

Photo J.

In the lagoons of the Magdalen Islands, larvae density is high towards the end of May (Table 3). A second peak may be noted towards mid-July. Sizes range from 110 x 76 µm to 424 x 343 µm (Figure 10). At 110 µm, the larvae, pale yellow in colour and granular in appearance, are in the shape of a flattened D and much longer than they are wide. The hinge is long and straight at this time. Around 120 µm, the yellow appears darker, and the two ends of the hinge become blacker. At about 130 µm, the larvae are still D-shaped, and the hinge line thickens. The umbo, low and rounded, appears below the hinge. Around 160 µm, the umbo is projected above the hinge line, which becomes curved, rounding out at the ends. The larva's asymmetrical shape and its colour become more pronounced as growth continues. The anterior side remains pointed, while the other side becomes wider. When the larva reaches 260 µm, its foot and a black dot (the eye) can be seen, this being representative of the pediveliger stage. At about 350 µm, the centred umbo becomes protuberant; in certain cases, a purple coloration appears near the anterior adductor muscle. Postlarva are sometimes found in the samples (Figure 10H).

A

110 x 76 µm

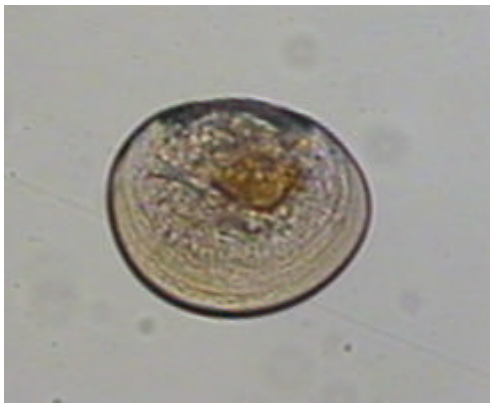
D

163 x 126 µm

B

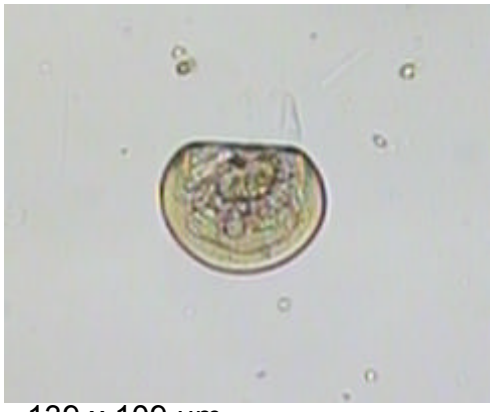
126 x 100 µm

E



216 x 187 µm

C



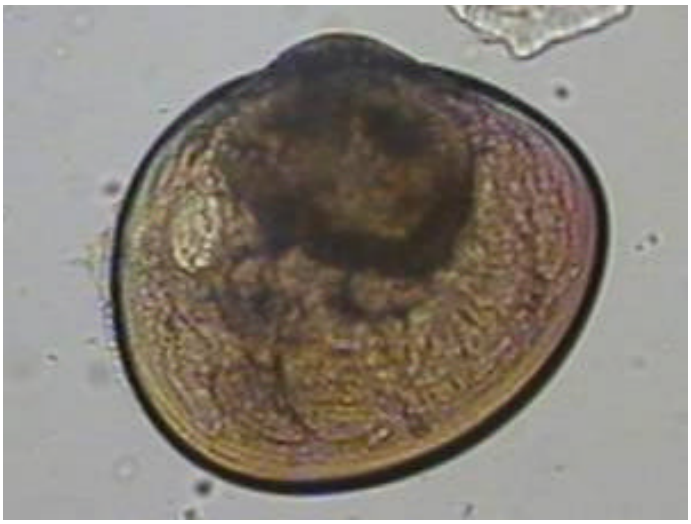
139 x 109 µm

F

260 x 225 µm

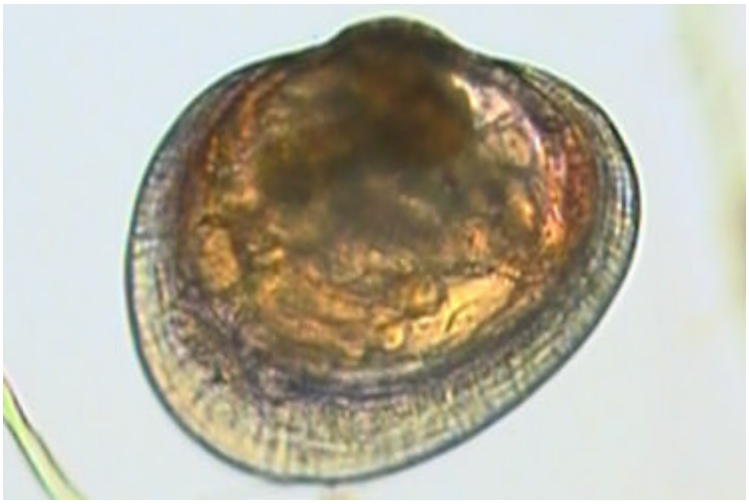
Figure 10. Larvae of the blue mussel (*Mytilus edulis*).

G



350 x 308 µm

H



Photos F. Aucoin

424 x 343 µm

Figure 10 (contd.). Larvae of the blue mussel (*Mytilus edulis*).

5.1.2 Sea Scallop (*Placopecten magellanicus*)

Photo J. Richard

Off the coast of the Magdalen Islands, the larvae appear towards the end of August and remain there until late fall (Table 3). The sizes observed range from 110 x 88 µm to 340 x 307 µm (Figure 11). The delicate looking D-veliger larvae are yellow and very pale green in colour. Like most larvae, they darken as they develop. At around 110 µm in length, the hinge line is very fine but darker at the ends. When the larva reaches 124 µm, the indistinct umbo remains under the hinge. At 135 µm, the central part where the viscera are located becomes darker, but the larva still remains relatively transparent. At this stage, the hinge has thickened, and the umbo remains under the hinge. At about 200 µm, the umbo projects a short distance. At this stage, the larva is egg-shaped. The anterior part is pointed, while the posterior part is rather broad and quite flat. The pediveliger stage may be reached at 220 µm. Figure 11H shows a postlarva.

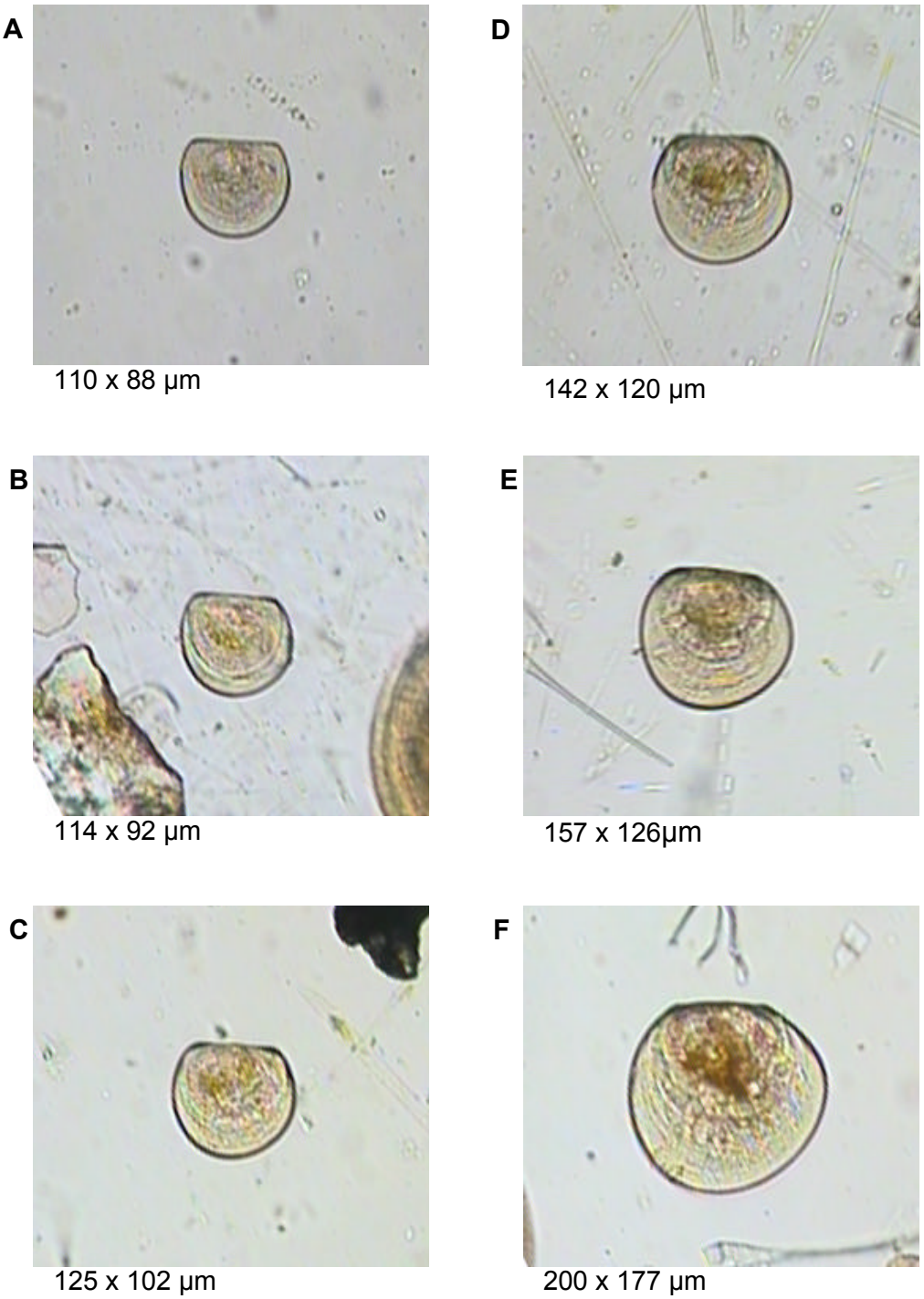
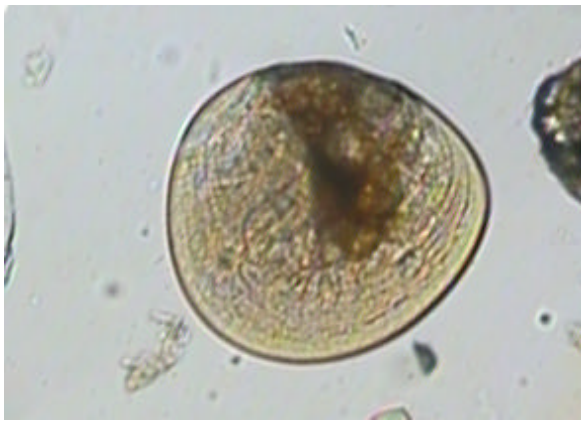


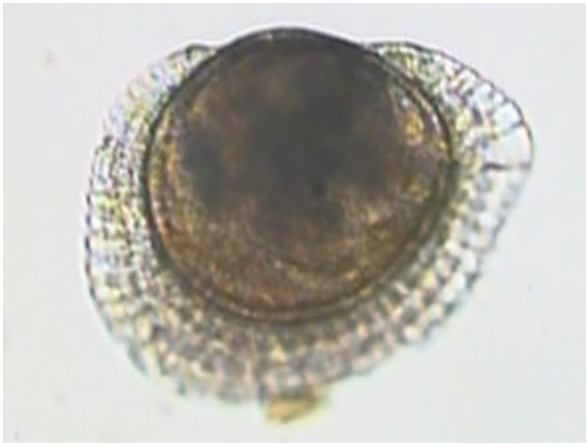
Figure 11. Larvae of the sea scallop (*Placopecten magellanicus*).

G



260 x 236 µm

H



Photos F. Aucoin

340 x 307 µm

Figure 11 (contd.). Larvae of the sea scallop (*Placopecten magellanicus*).

5.1.3 Soft-shell Clam (*Mya arenaria*)

Photo J. Richard

In the lagoons of the Magdalen Islands, soft-shell clam larvae appear early in May and remain until late August (Table 3). Sizes range from 105 x 90 µm to 380 x 310 µm (Figure 12). Initially, the larvae have a rounded D-shape, are delicate in appearance, and are filled with transparent granules. The indistinct umbo is visible under the hinge line. The ends of the hinge curve back on themselves as growth continues, exposing the shoulders. Pale grey and greenish coloration appears and becomes darker as the larva develops. At about 140 µm, a clear ring parallel to the shell can be seen; it separates the visceral mass from the edge of the shell. This ring remains present until the end of the larval stage. When the larva reaches about 155 µm, the angular umbo is projected above the hinge and juts out over two almost equal slopes on either side. In the last stage, brownish pigmentation may help with identification. Figure 12G shows a postlarva.

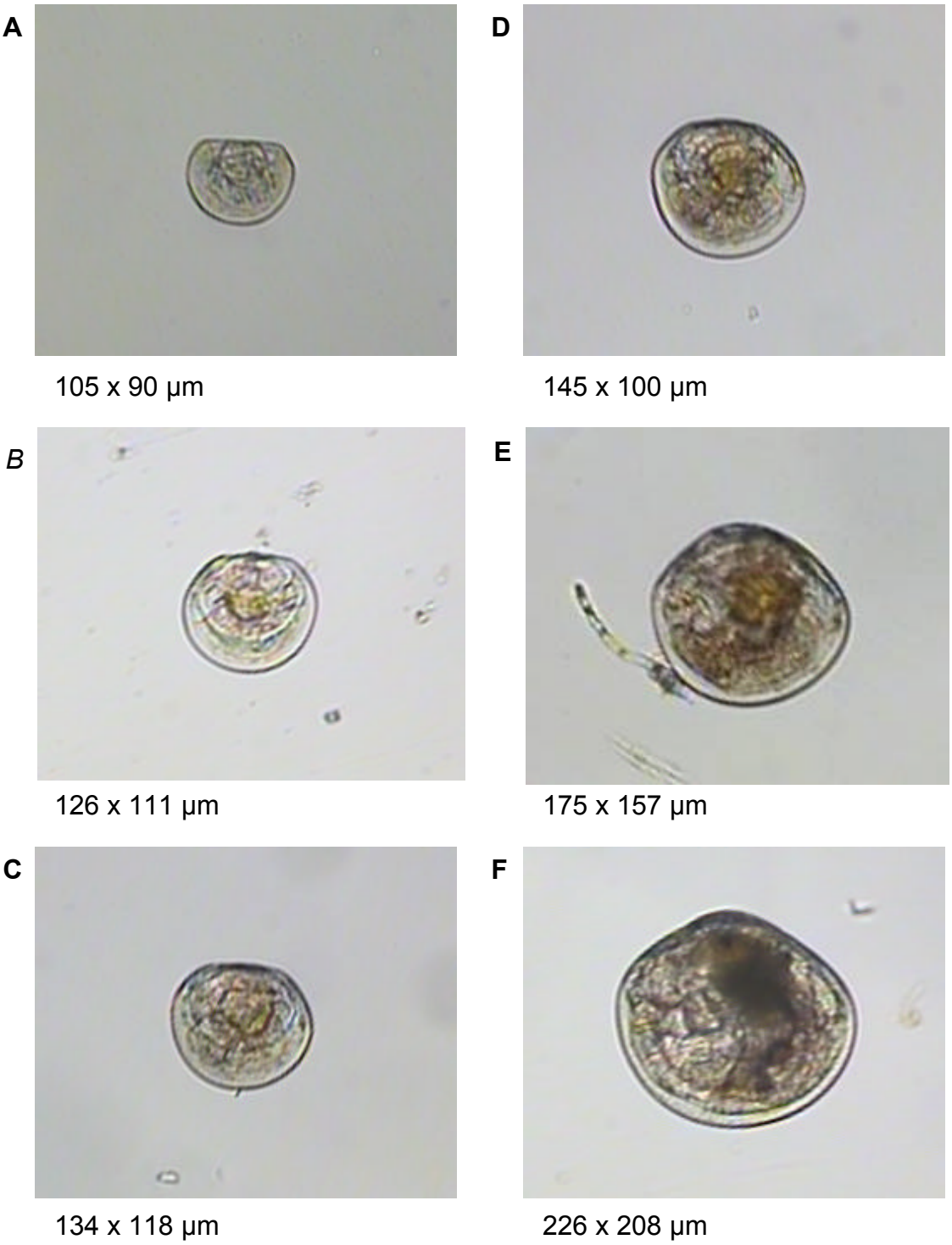
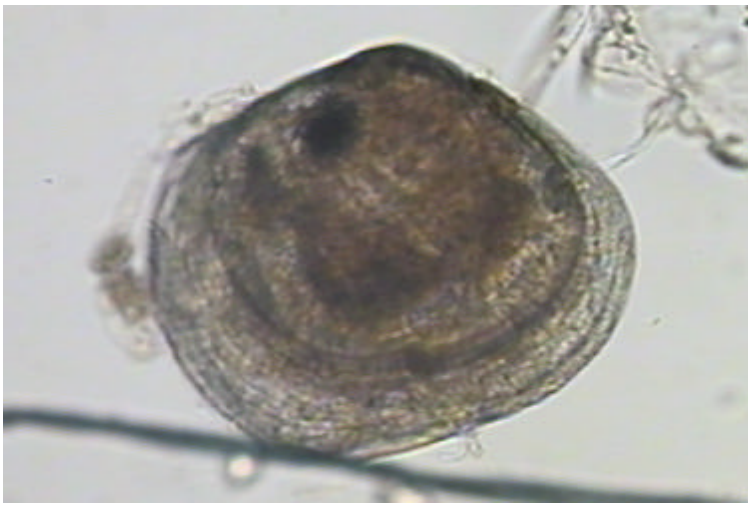


Figure 12. Larvae of the soft-shell clam *(Mya arenaria).*

G



Photos F. Aucoin

381 x 311 µm

Figure 12 (contd.). Larvae of the soft-shell clam (*Mya arenaria*).

5.1.4 Eastern Oyster (*Crassostrea virginica*)

Photo S. Doiron

Sullivan (1948) reports the presence of oyster larvae in Malpeque Bay, Prince Edward Island, from early July until late August, at sizes ranging from 70 x 80 µm to 335 x 350 µm. Still according to Sullivan, the D-veliger larva is pinkish in colour, becoming darker as growth continues. The umbo, indistinct initially, develops rapidly and appears in the centre of the dorsal part (Figure 13). Later, when the larva reaches 115 µm, the umbo moves to the side, becoming off-centred and protuberant. Bilateral asymmetry develops gradually. The thickness of the umbo makes it more difficult to take pictures, which explains the blurriness of the photographs in Figure 13.

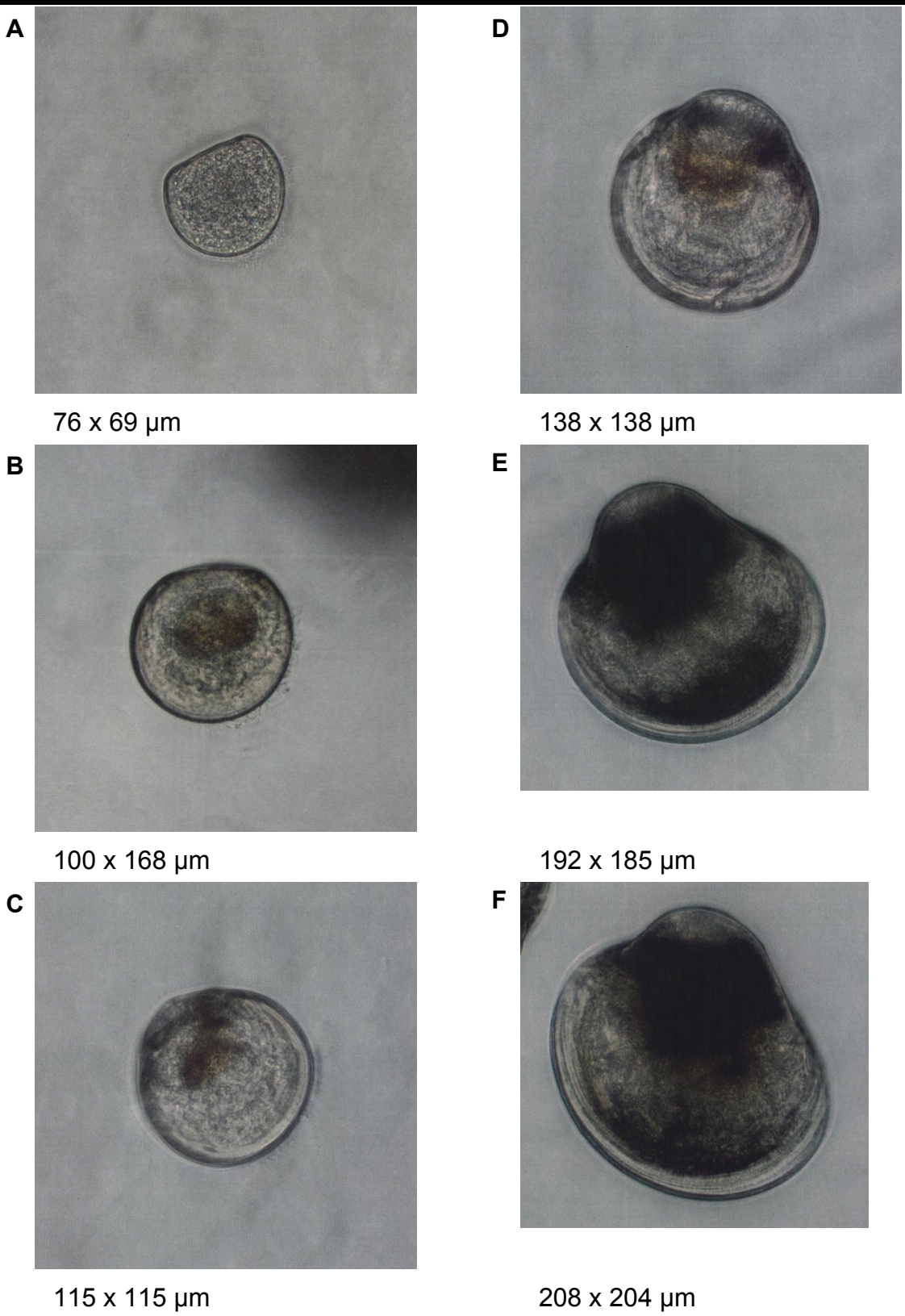
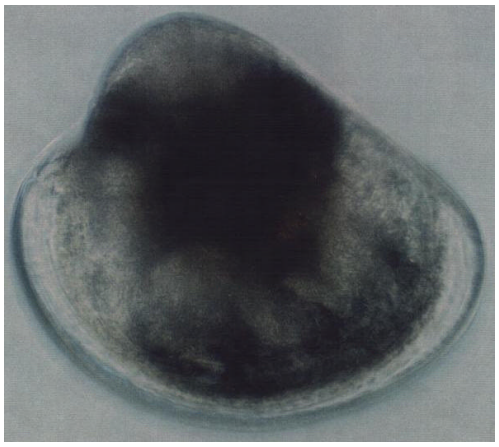


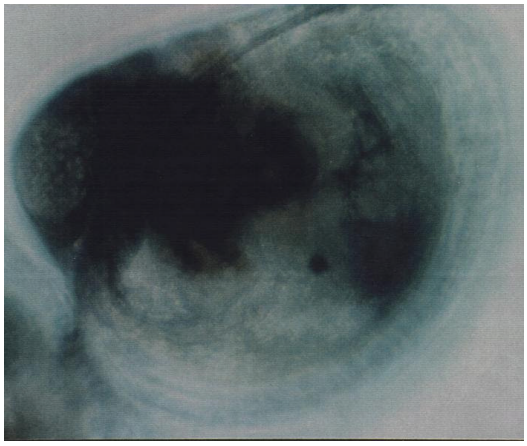
Figure 13. Larvae of the eastern oyster (*Crassostrea virginica*).

G



258 x 242 µm

H



Photos S. Doiron

319 x 300 µm

Figure 13 (contd.). Larvae of the eastern oyster (*Crassostrea virginica*).

5.1.5 Northern Quahog (*Mercenaria mercenaria*)

Photo S. Doiron

In Malpeque Bay, Prince Edward Island, the first larvae appear early in July and remain until mid-August (Sullivan, 1948). Their size can range from 115 x 100 µm to 270 x 255 µm (Doiron, unpublished data). The larva is pale yellow initially, becoming more greyish as it grows. At around 150 µm, the umbo appears thick below the fine hinge line (Figure 14). Between 160 and 200 µm, the umbo is raised above the hinge line, and the shoulders are flat on either side of the crest. When the larva reaches 200 µm, it becomes pointed with an angular umbo.

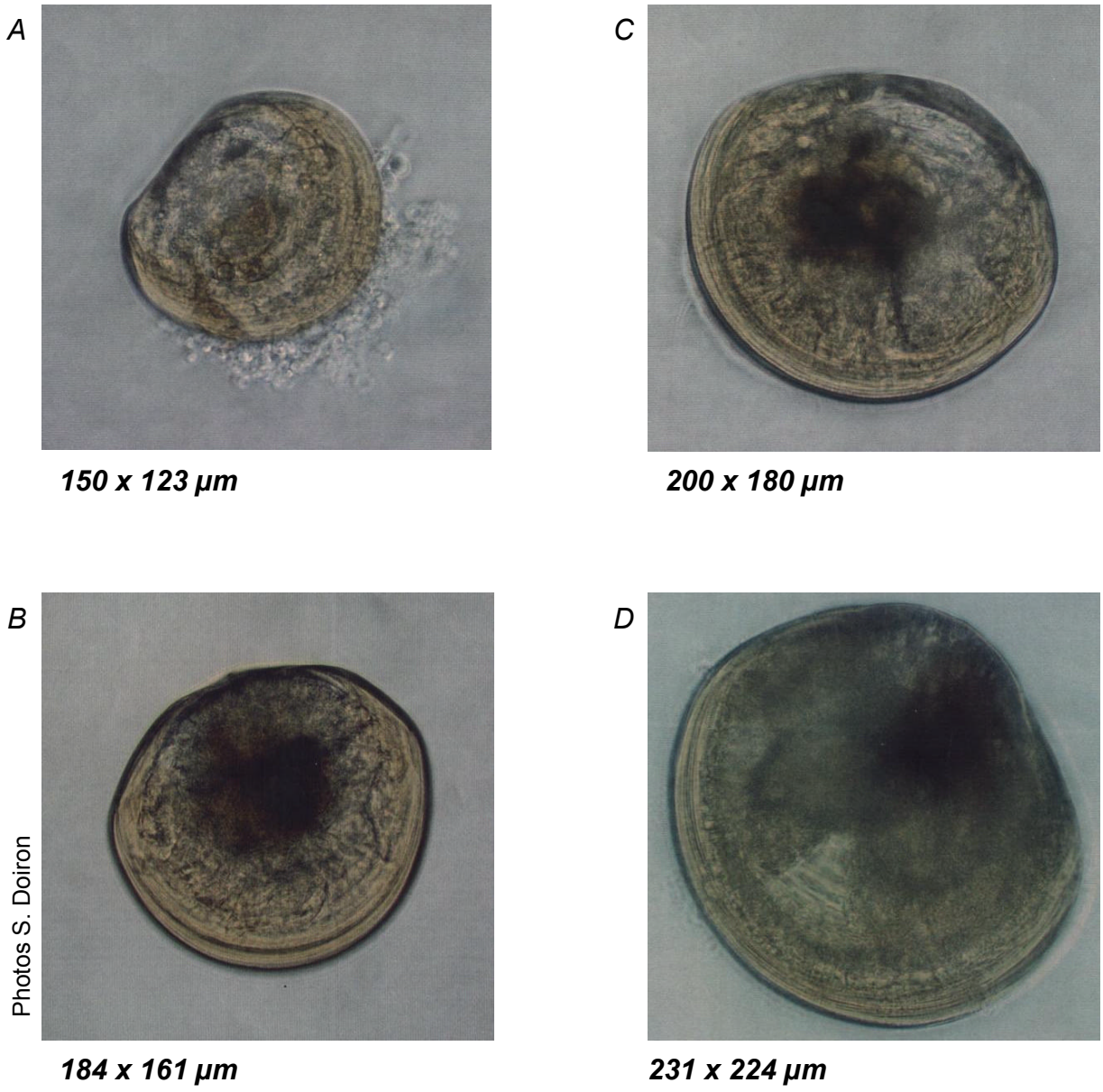


Figure 14. Larvae of the northern quahog (*Mercenaria mercenaria*).

5.1.6 Surf clam (*Spisula solidissima*)

Photo S. Doiron

According to Sullivan (1948), these larvae are found in Malpeque Bay from mid-June to mid-July and sometimes until late August. Their size ranges from 80 to 270 µm. In the early stages, between 90 and 140 µm, a thick, pale-coloured umbo is present under the hinge line. From about 140 µm, the umbo is projected above the hinge and becomes angular. The angles of the anterior and posterior sides are high and acute. The slope of the posterior side is shorter than that of the anterior side (Figure 15).

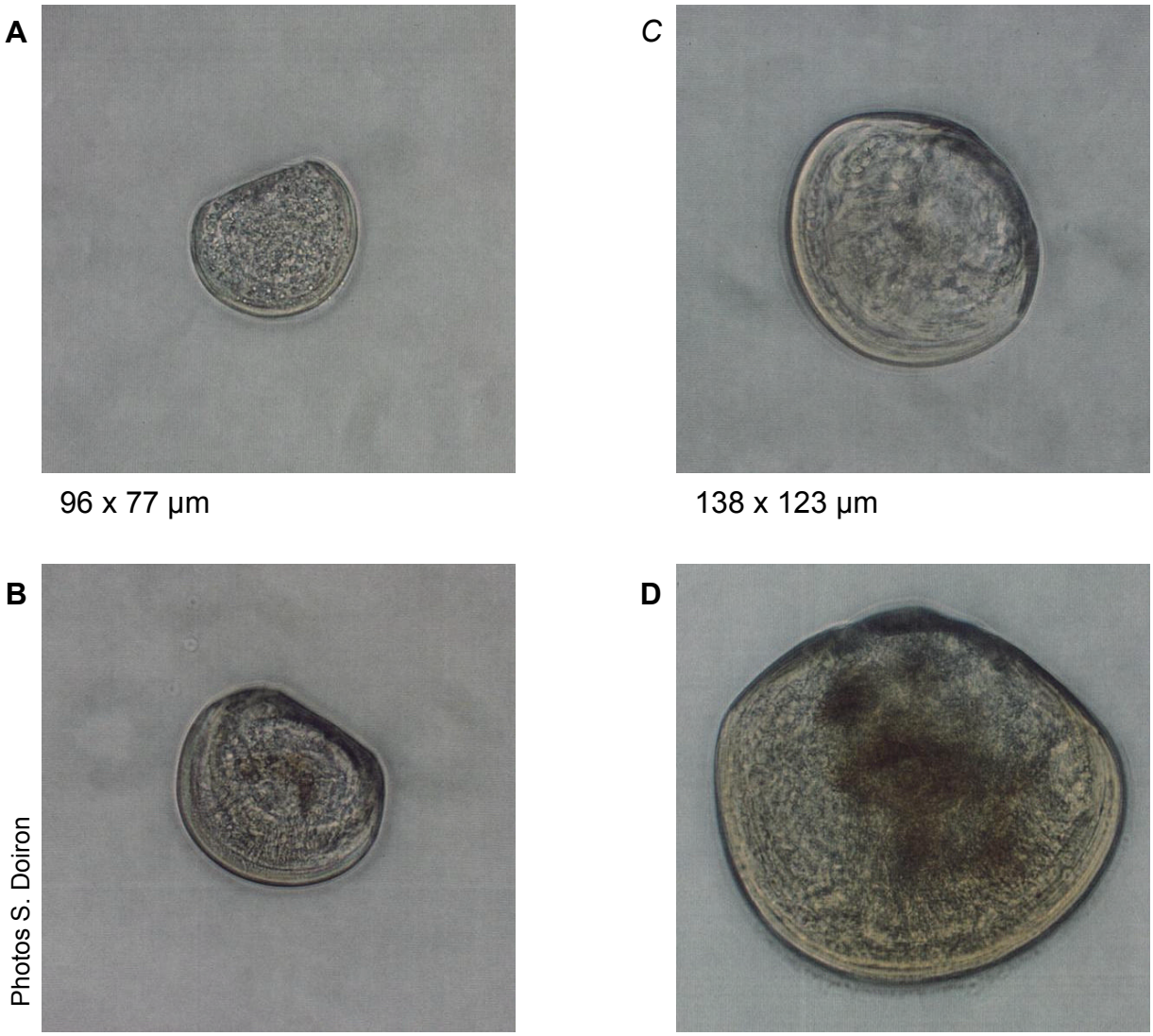


Figure 15. Larvae of the surf clam (*Spisula solidissima*).

5.2 Associated Species

5.2.1 Arctic Rock Borer (*Hiatella arctica*)

Photo J. Richard

Off the coast of the Magdalen Islands, the larvae are observed until early September (Table 3). It was not possible to take representative photographs of the early larval stages during the sampling period at sea, from August to September (Figure 16). Larvae from the sea are sometimes observed in the lagoons, but when they are, they are already at an advanced stage. According to Sullivan (1948), sizes can range from 120 x 130 µm to 310 x 345 µm. From 120 to 140 µm, the larvae are grey or pale brown, their colour becoming darker as growth continues. When the larvae reach this size, the umbo looks like a round protuberance hiding the straight hinge line. Larvae 130 to 210 µm long have a low, round umbo, and their sides form a continuous line with the shoulder. Larvae larger than 210 µm have a more protuberant umbo, and their sides form obtuse angles with the shoulders. The posterior slope is shorter than the anterior slope. When the larvae reach the settling stage, they become longer.

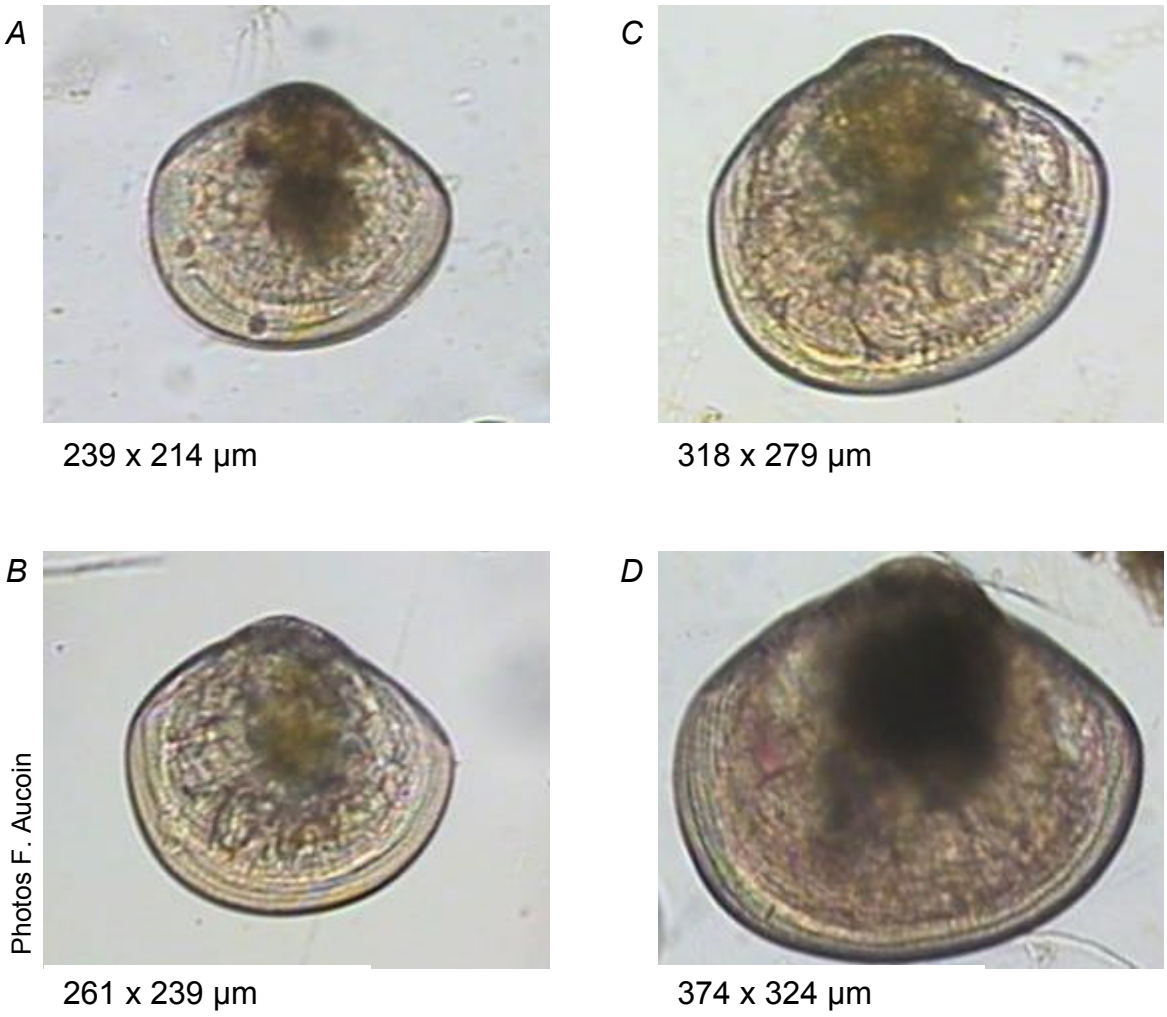


Figure 16. Larvae of the Arctic rock borer (*Hiatella arctica*).

5.2.2 Common Razor Clam (*Ensis directus*)

Photo J. Richard

The larvae appear in the lagoons of the Magdalen Islands around mid-May and may remain there until late August (Table 3). Their size varies from 145 x 120 µm to more than 300 x 260 µm (Figures 17 and 18). The larva is pale yellow throughout its development, but it darkens as it prepares to settle. The indistinct umbo can be seen under the hinge line at the D-veliger stage. At about 150 µm, the umbo is low and rounded. When the larva reaches 200 µm, it may look different depending on its position. If the larva is on the left side, i.e., with the posterior part on the left (Figure 17), the flat umbo appears under the hinge, and the shoulders extend past it. If the larva is on the right side, i.e., with the posterior part on the right (Figure 18), the umbo appears above the hinge, giving an angular shape. At over 220 µm, the hinge ligament appears above the posterior shoulder (hook-shaped black line). When the larva is larger, around 260 µm, there is no longer any difference between the left and the right sides. The umbo then develops quickly and always remains in the centre of the dorsal part. On either side of the umbo, the shoulders are dark (black), a bit purplish, and of equal size.

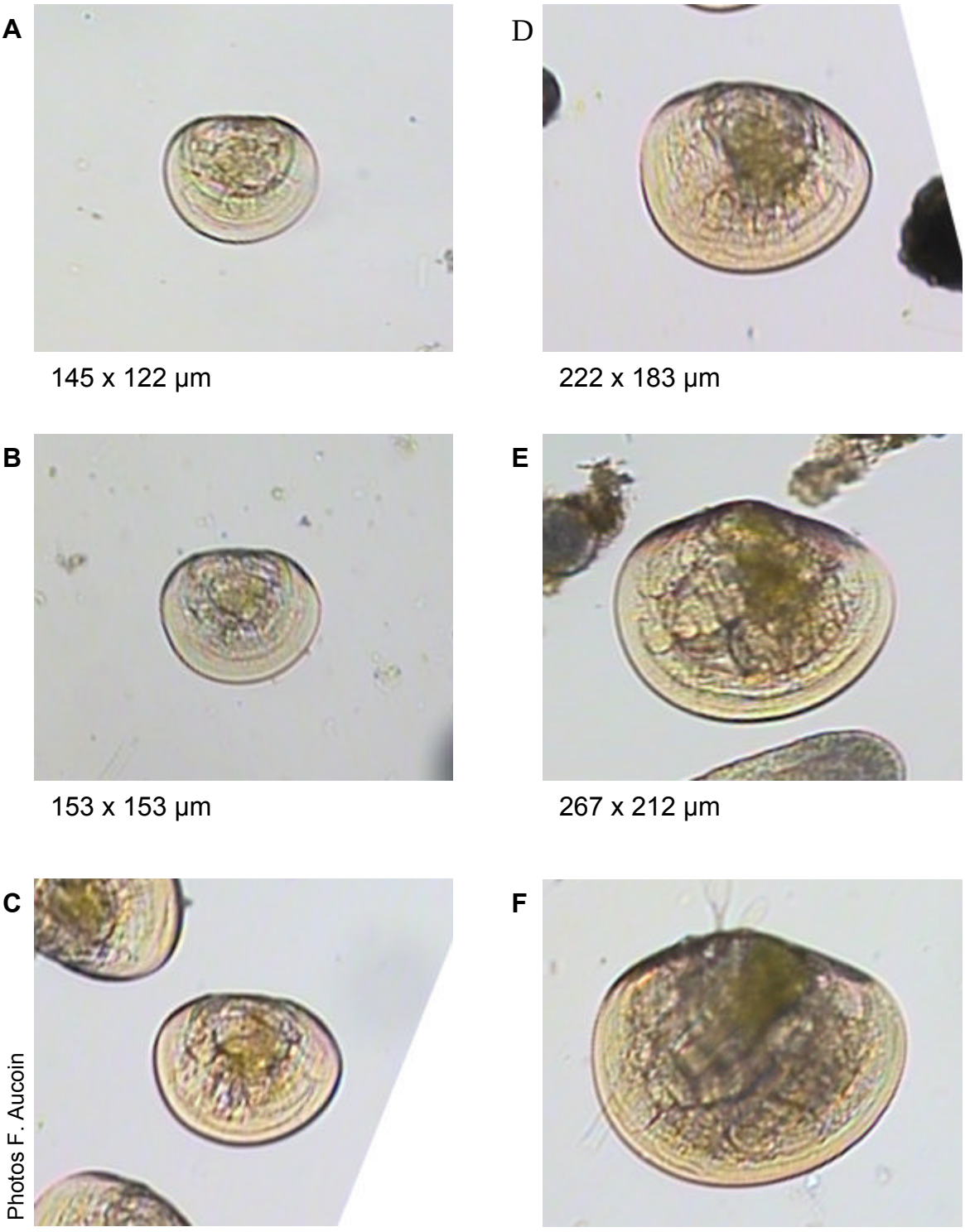


Figure 17. Larvae of the common razor clam (Ensis directus), left side.

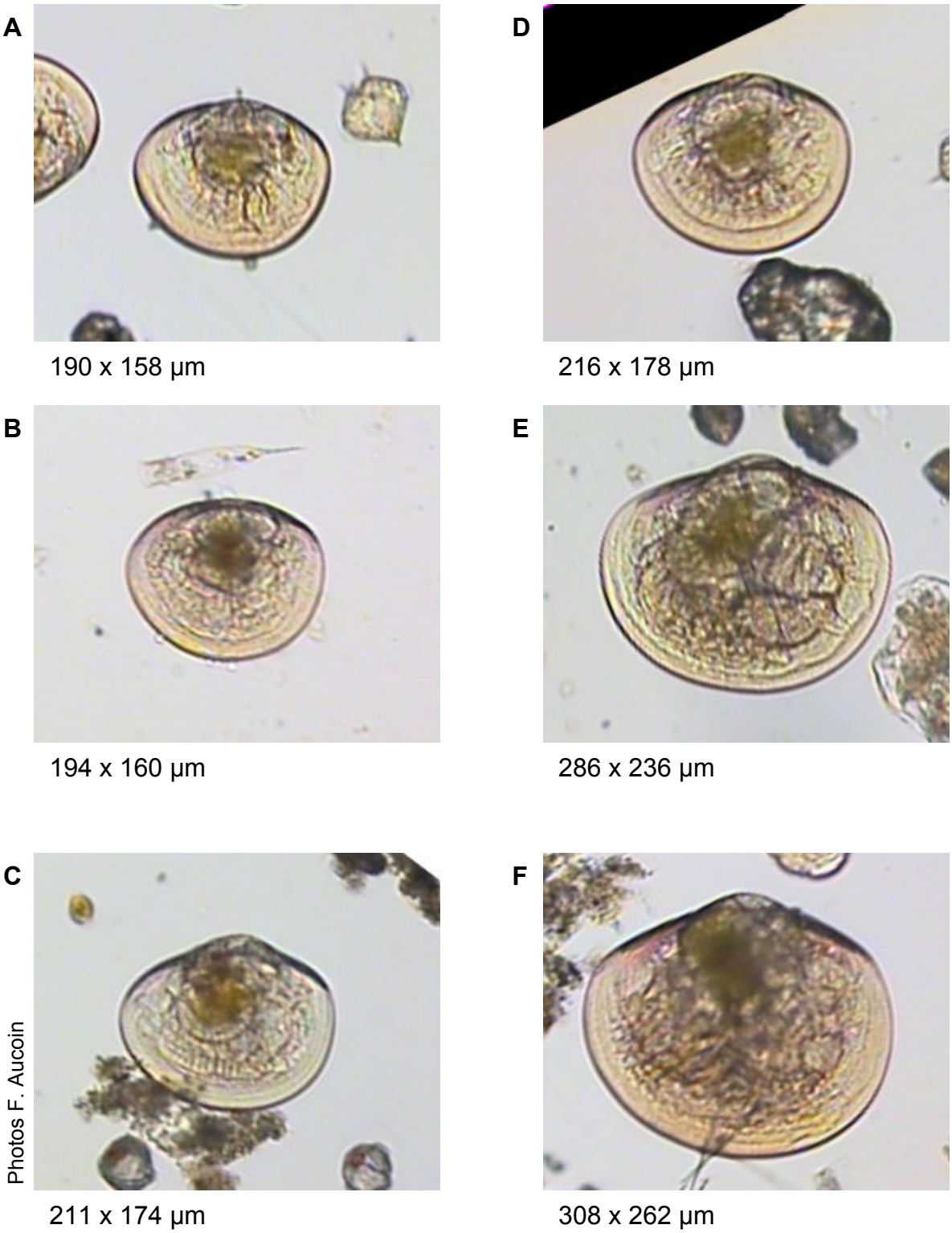


Figure 18. Larvae of the common razor clam (*Ensis directus*), right side.

5.2.3 Horse Mussel (*Modiolus modiolus*)

Photo J. Richard

In the Magdalen Islands area, the larvae appear in lagoons and at sea around mid-August (Table 3). According to Schweinitz and Lutz (1976), the size of the larvae ranges from 128 x 103 µm to 340 x 315 µm. At around 140 µm in size, the D-veliger larva is dark brown in colour with some yellow in the visceral part. From 140 to 300 µm, the shape becomes rounder and the yellow coloration, deeper. The umbo is projected above the hinge, which becomes curved, rounding out at the ends (Figure 19). Over the course of development, the umbo becomes thicker and protuberant.

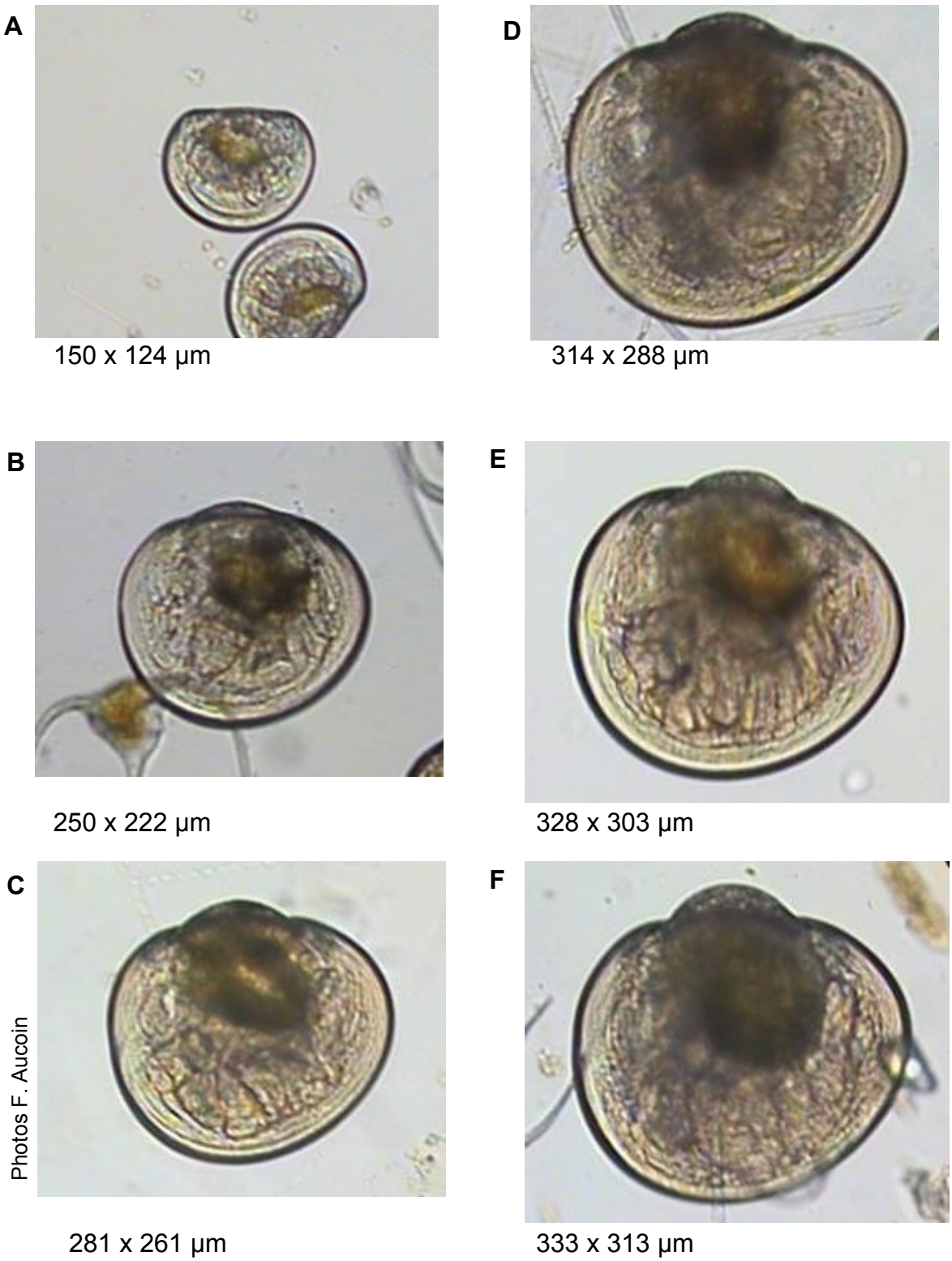


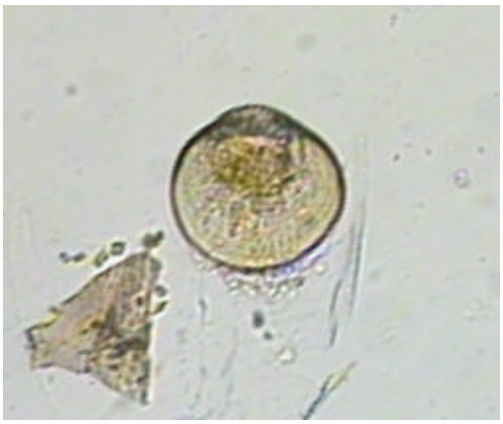
Figure 19. Larvae of the horse mussel (*Modiolus modiolus*).

5.2.4 Common Jingle Shell (*Anomia simplex*) and Prickly Jingle Shell (*Anomia aculeata*)

Photo J. Richard

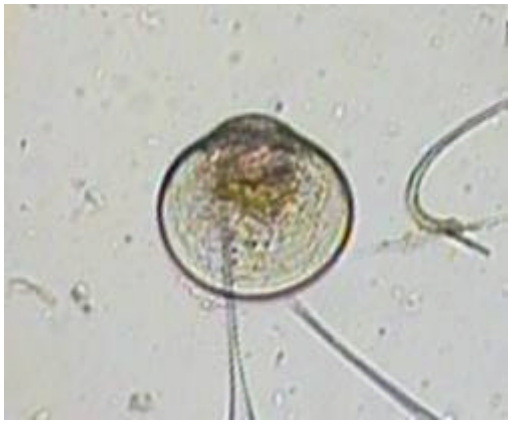
In the Magdalen Islands area, the larvae are observed in lagoons beginning in mid-August and at sea throughout the sampling period, from August to October (Table 3). According to Sullivan (1948), their size can range from 100 x 110 µm to 280 x 285 µm. In the earliest stages, the umbo is below the hinge, and the larva is pale yellow in colour. This coloration deepens over time. Bilateral asymmetry appears at the D-veliger stage and becomes very pronounced as the larva develops. When it reaches around 160 µm in size, the shell's anteroventral margin becomes flat, and gradually, a notch takes shape (Figure 20).

A



137 x 130 µm

B

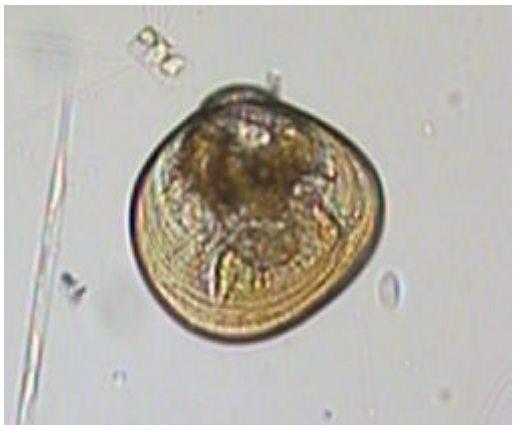


154 x 146 µm

C

198 x 193 µm

D



202 x 196 µm

E

225 x 214 µm

F

258 x 257 µm

Photos F. Aucoin

Figure 20. Larvae of the jingle shell (*Anomia sp*.).

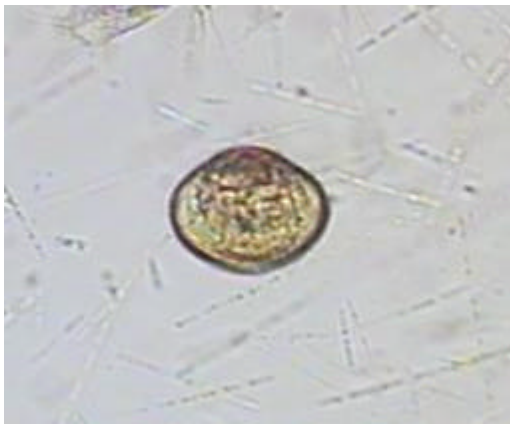
5.2.5 Northern Dwarf Tellin (*Tellina agilis*)

Photo F. Aucoin

←→

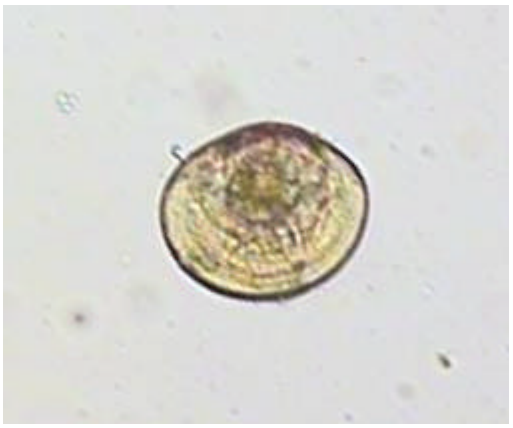
The tellin is observed in the lagoons of the Magdalen Islands (Table 3) and in Malpeque Bay (Sullivan, 1948) starting in mid-July. Sizes range from 75 x 90 µm to 235 x 260 µm. Initially, the larva is pale yellow in colour, becoming darker as it grows. At all stages, vibrant purple coloration can be observed near the umbo and hinge. This coloration is visible only if the microscope's condenser is adjusted so as to allow all the light to pass through the larva. At 120 µm, the umbo extends above the hinge. At that point, the larva is almost circular. The umbo is protuberant in the advanced stages, giving it the appearance of a bubble when observed (Figure 21).

A



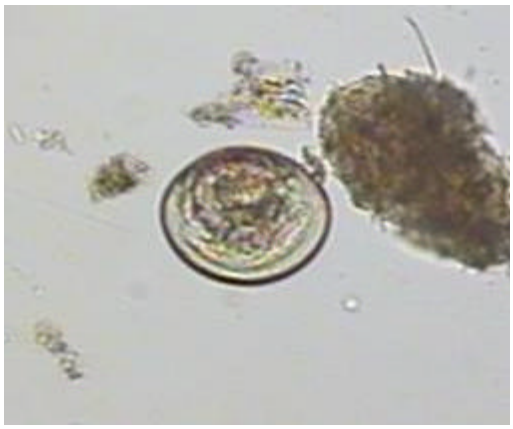
122 x 100 µm

D



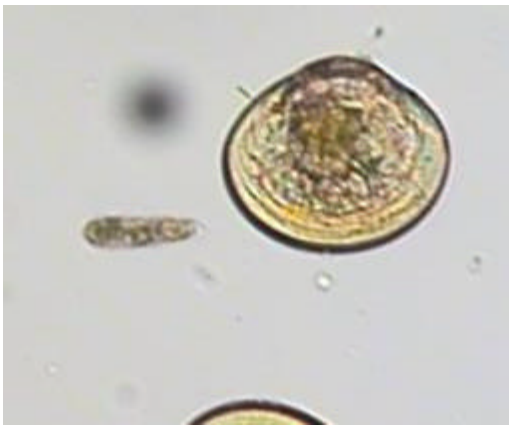
165 x 141 µm

B



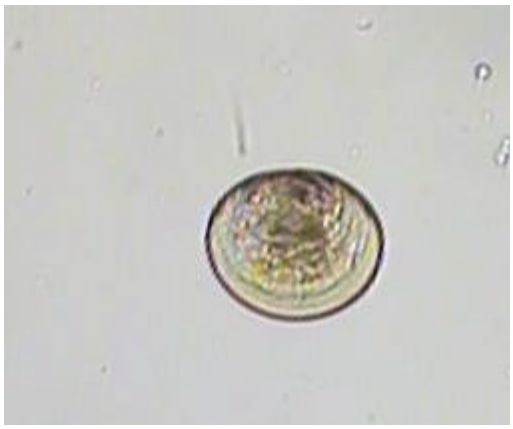
133 x 110 µm

E



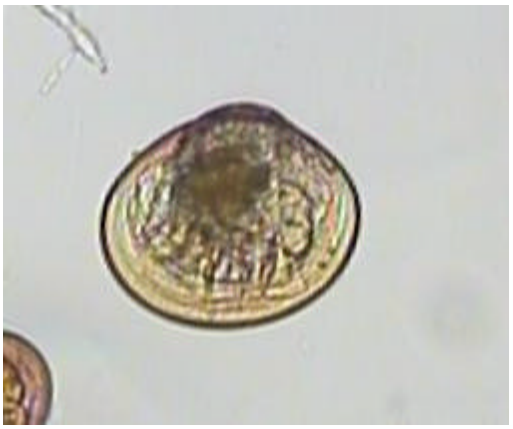
179 x 155 µm

C



142 x 117 µm

F



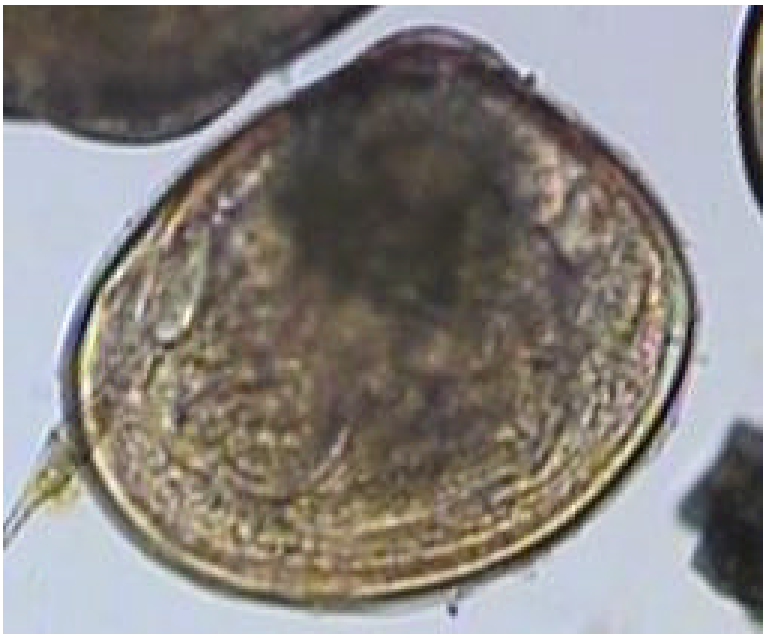
206 x 177 µm

Figure 21. Larvae of the northern dwarf tellin (*Tellina agilis*).

G

315 x 277 µm

H



Photos F. Aucoin

338 x 308 µm

Figure 21 (contd.). Larvae of the northern dwarf tellin (*Tellina agilis*).

5.2.6 Atlantic Flat Lepton (*Mysella planulata*)

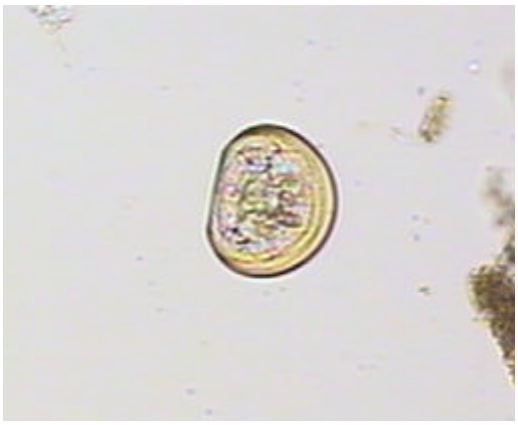
Illustration adapted from Sullivan (1948)

Larvae are observed in the lagoons of the Magdalen Islands starting in mid-June. They have also been seen until late August during samplings at sea (Table 3). Sizes range from 125 x 100 µm to 345 x 290 µm. When the D-shape appears, the larva is a brilliant yellow colour, the hinge is straight, and the umbo appears below it. The larva remains symmetrically shaped until about 240 µm, at which size the umbo extends above the hinge line and becomes more protuberant. Coloration sometimes becomes more orange (Figure 22).

A

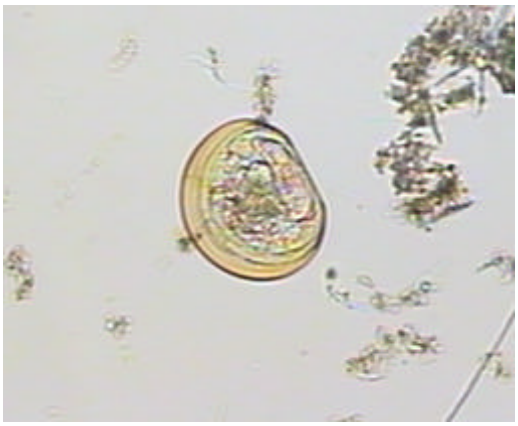
124 x 102 µm

B



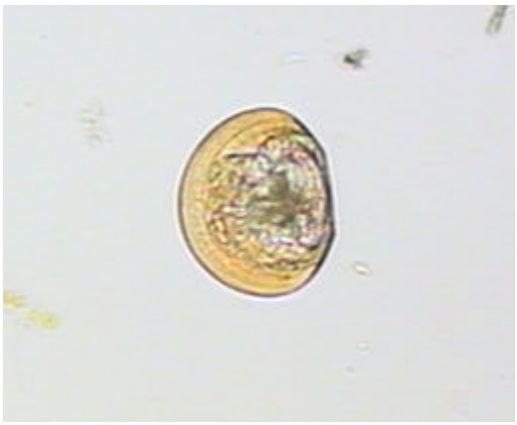
144 x 113 µm

C



157 x 124 µm

D

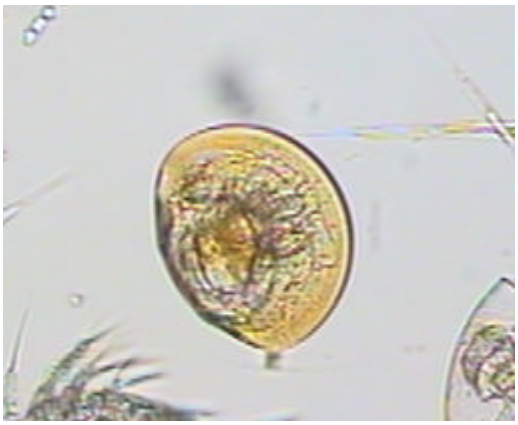


176 x 145 µm

E

191 x 156 µm

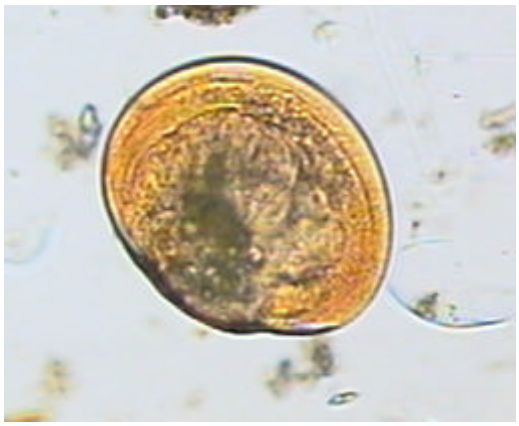
F



220 x 178 µm

Figure 22. Larvae of the Atlantic flat lepton (*Mysella planulata*).

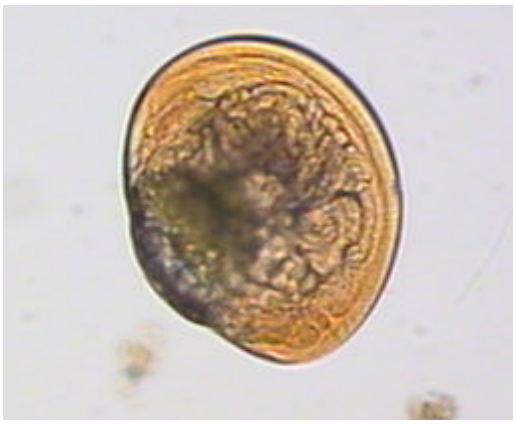
G



Photos F. Aucoin

294 x 248 µm

H



316 x 262 µm

Figure 22 (contd.). Larvae of the Atlantic flat lepton (*Mysella planulata*).

5.2.7 Common Shipworm *(Teredo navalis)*

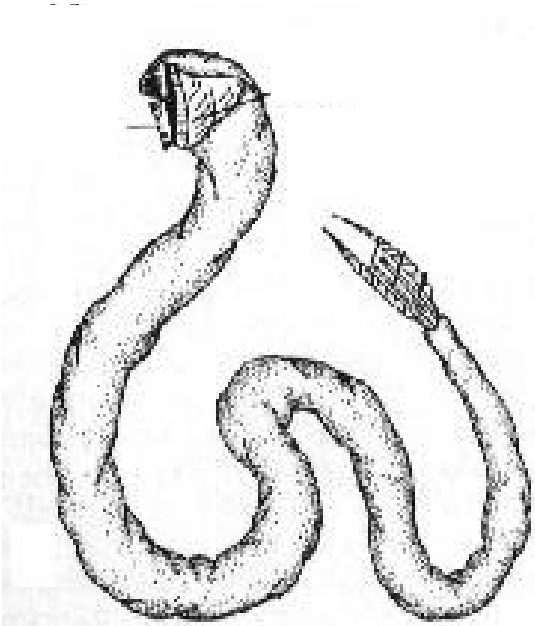
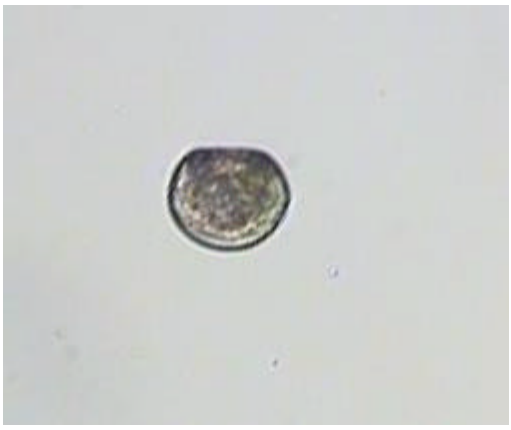


Illustration from Turner (1966)

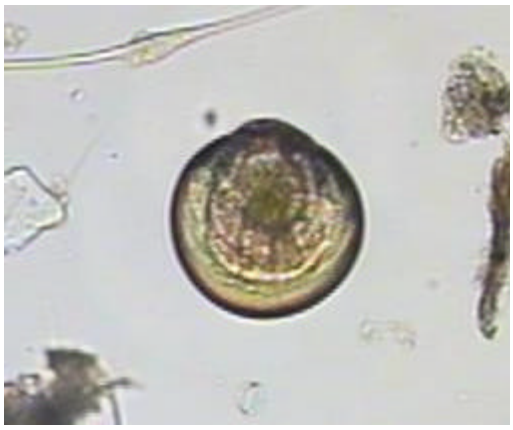
The larvae of this vermiform bivalve are observed in the lagoons of the Magdalen Islands starting in mid-June and at sea until late August (Table 3). Sizes range from 95 x 95 µm to 250 x 220 µm. The D-larvae are small in size (80 µm) with two black dots resembling a pair of eyes, one on either side of the hinge. A clear space is visible between the visceral mass and the outline. During development, the outline becomes thicker and black. The umbo is always central (Figure 23).

A



96 x 80 µm

D



155 x 150 µm

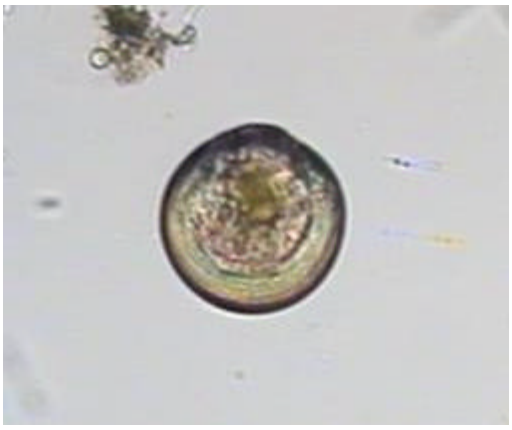
B

120 x 110 µm

E

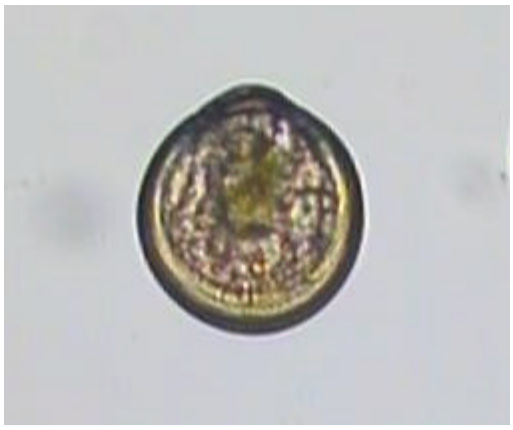
168 x 178 µm

C



149 x 151 µm

F



182 x 198 µm

Figure 23. Larvae of the common shipworm (Teredo navalis).

G

Photos F. Aucoin

209 x 235 µm

Figure 23 (contd.). Larvae of the common shipworm *(Teredo navalis)*.

5.2.8 Echinoderms

Larvae of sea stars, sea urchins, and other echinoderms can be observed from early summer until mid-July. After fertilization, the primitive larvae (Figure 24) of these species develop into blastulae (Figure 25) and then become gastrulae (Figure 26). At that point, they use external cilia to move around or else are carried by the current.

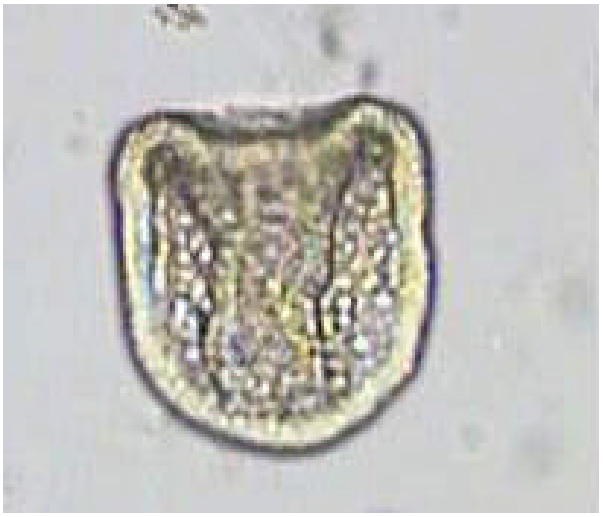


Photo F. Aucoin

Figure 24. Echinoderm primitive larva (181 x 169 µm).

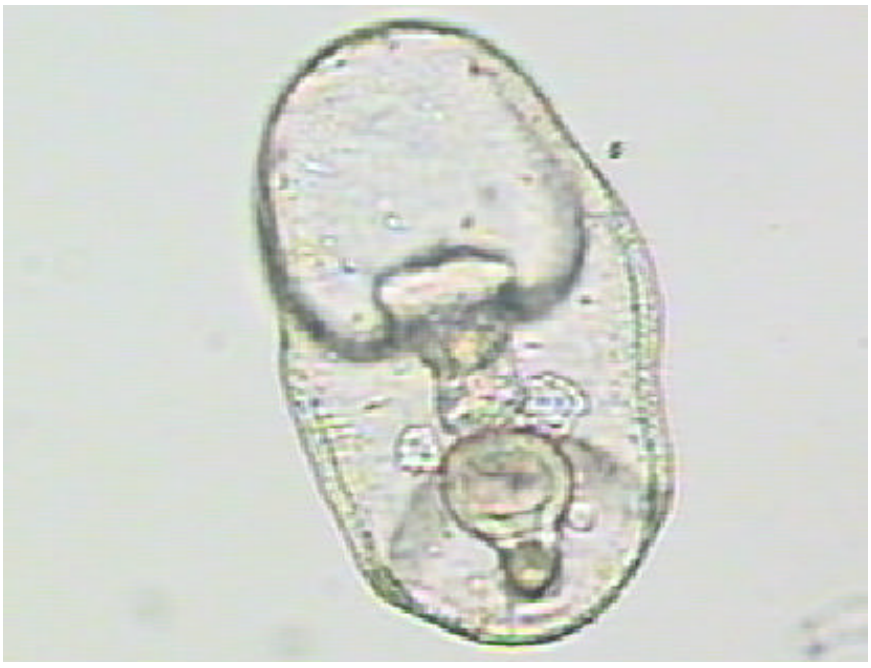


Photo F. Aucoin

Figure 25. Echinoderm blastula larva, 376 m.

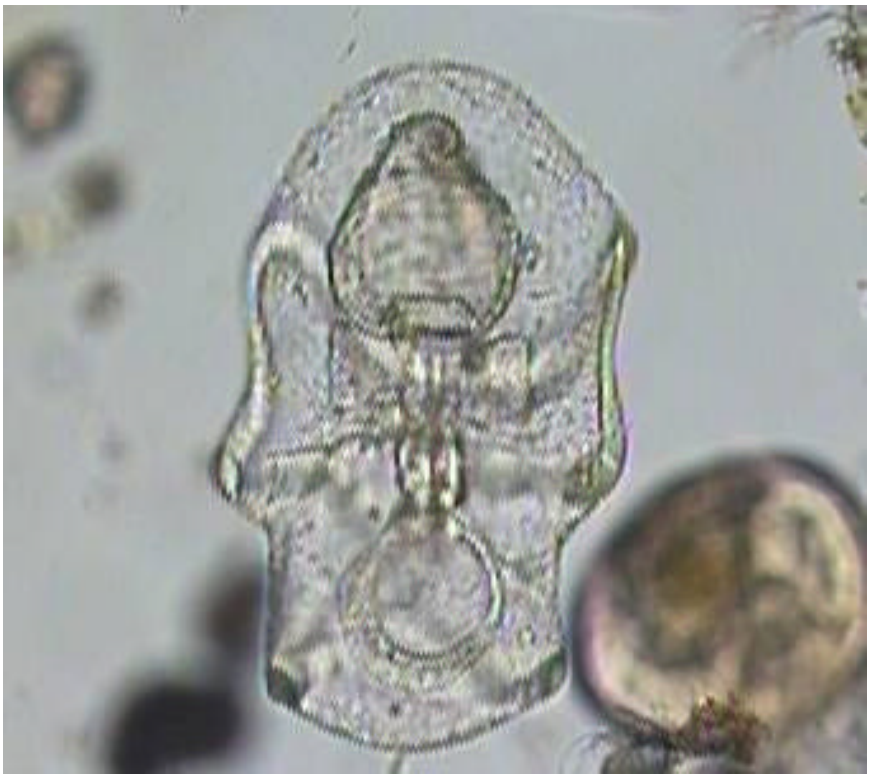


Photo F. Aucoin

Figure 26. Echinoderm gastrula larva, 431 µm.

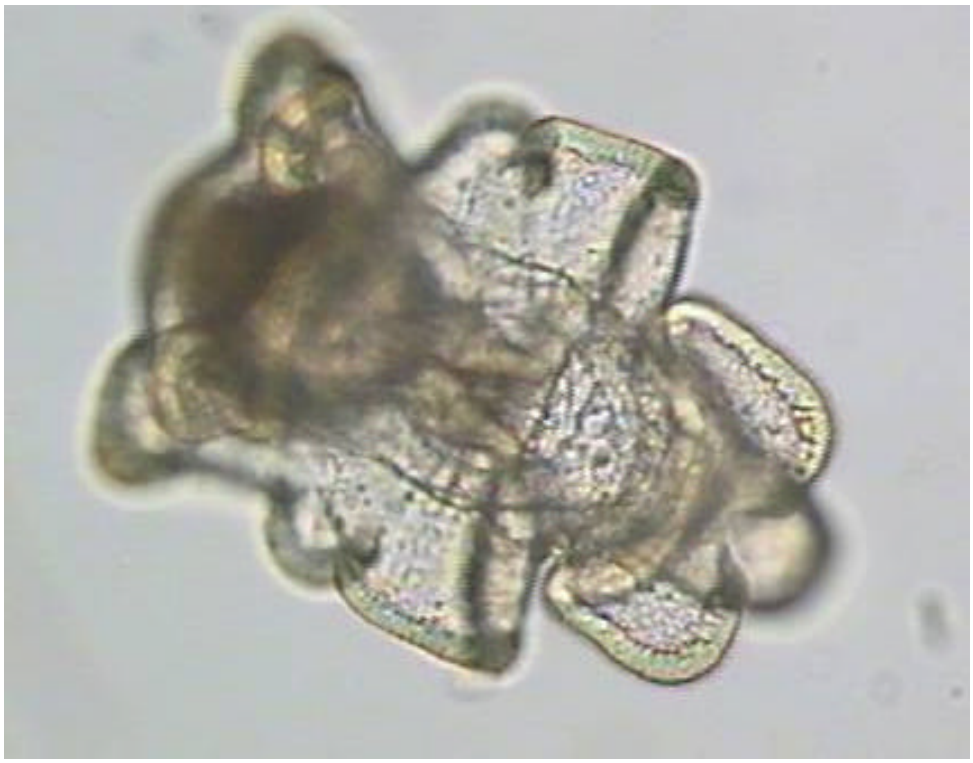
As a rule, the larvae are bilaterally symmetrical, meaning that the left half is the mirror image of the right. In the early stages, the different species of echinoderms are quite similar. However, the larvae of sea stars and sea cucumbers have no skeleton (Figure 27), whereas those of sea urchins and brittle stars have small skeletal spicules (Figure 28). During the course of development, arms of varying length appear in several species of larvae.

5.2.8.1 Northern Sea Star (*Asterias vulgaris)*

Photo M. Nadeau

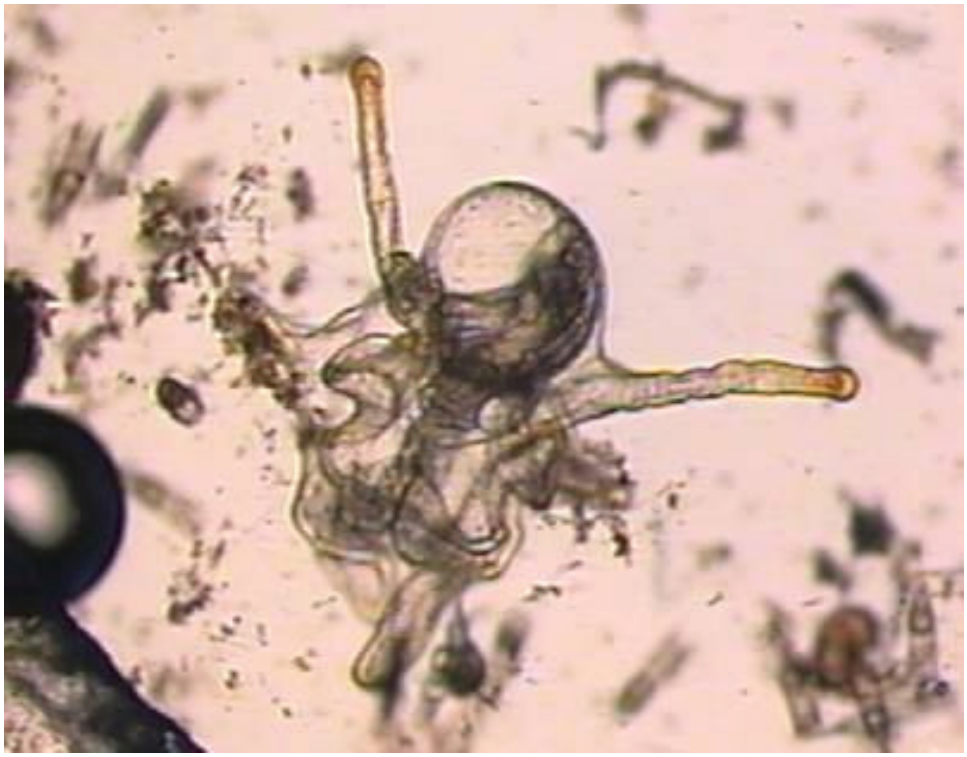
Sea star larvae grow from 250 µm to 1.5 mm during the course of their development. They can be distinguished from other species starting mainly at the bipinnaria stage (Figure 27A). After a few days, the larvae take on a more complex shape that includes arms, becoming brachiolaria larvae (Figure 27B). The ciliated arms, which are longer and more numerous than in other larvae, and the larva's unique adhesion system enable it to attach itself to a substrate. When the larva measures about 800 µm (Figure 27C), the body of the sea star with five embryonic arms appears at one end. At this stage, the larva settles on a substrate, its larval tissues are invaginated, and it metamorphoses into a juvenile sea star (Figure 27D).

A



520 µm

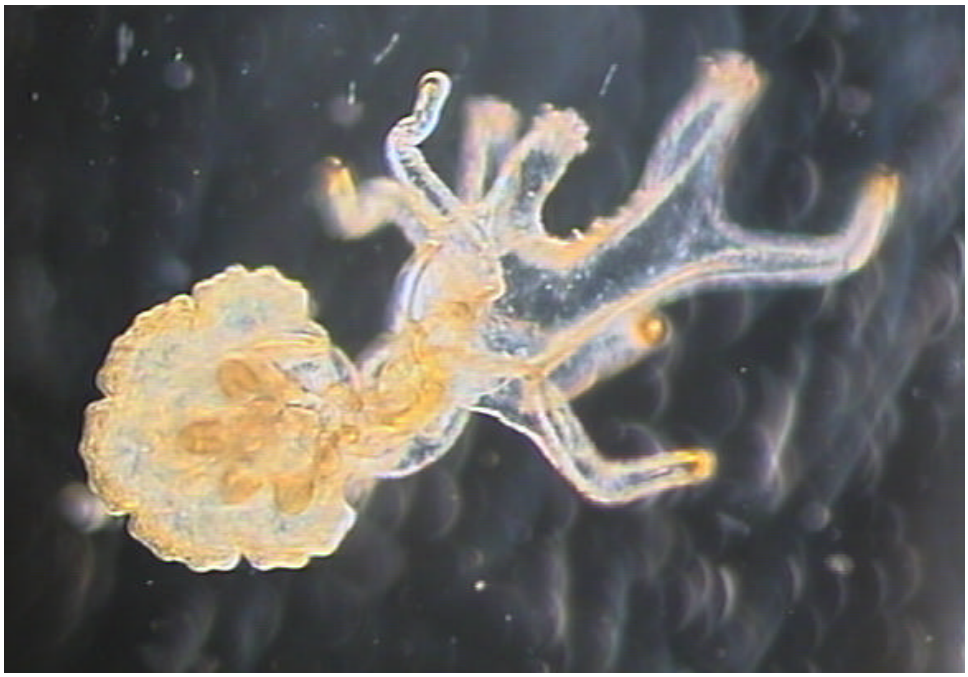
B



600 µm

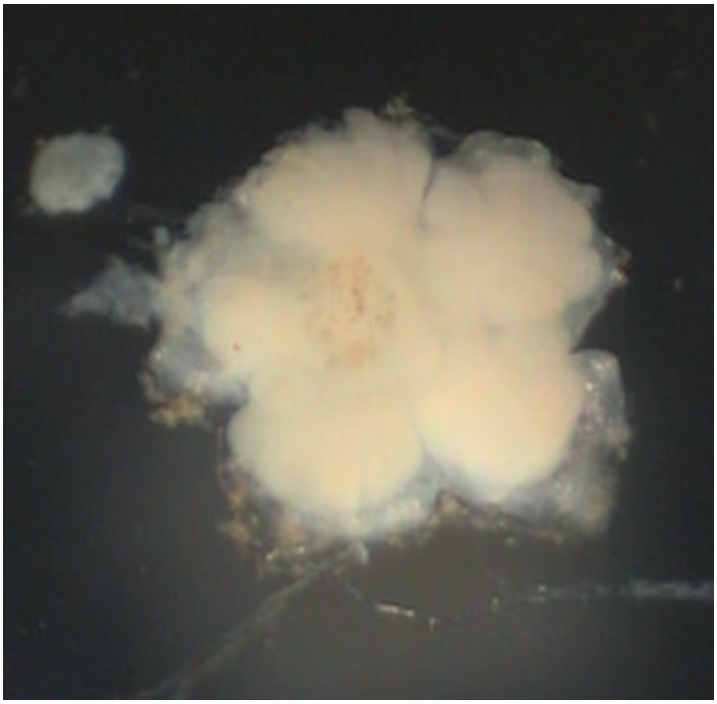
Figure 27. Larva of the northern sea star (Asterias vulgaris).

C



819 µm

D



Photos F. Aucoin

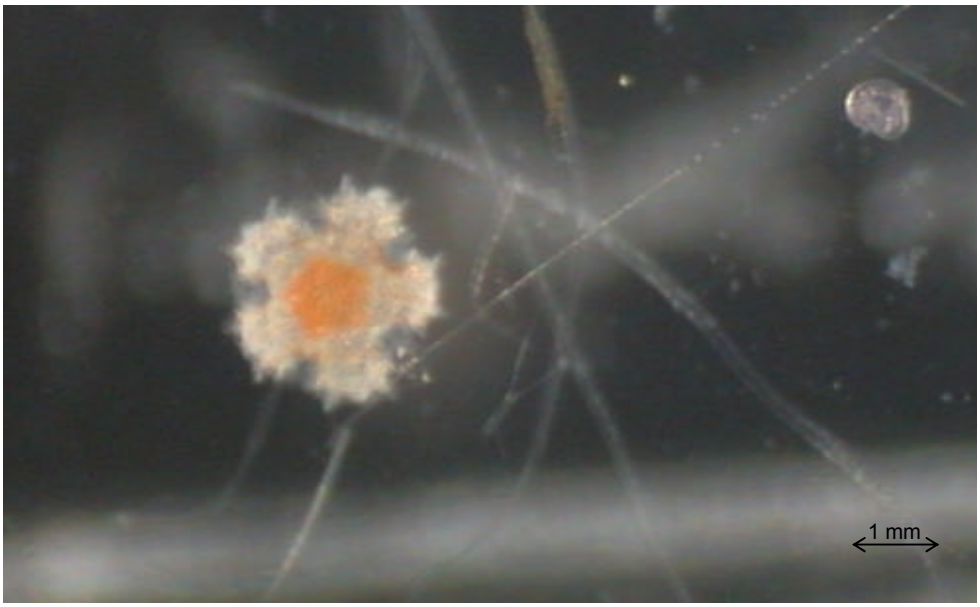
368 µm

Figure 27 (contd.). Larva of the northern sea star (*Asterias vulgaris*).

5.2.8.2 Other Echinoderm Species

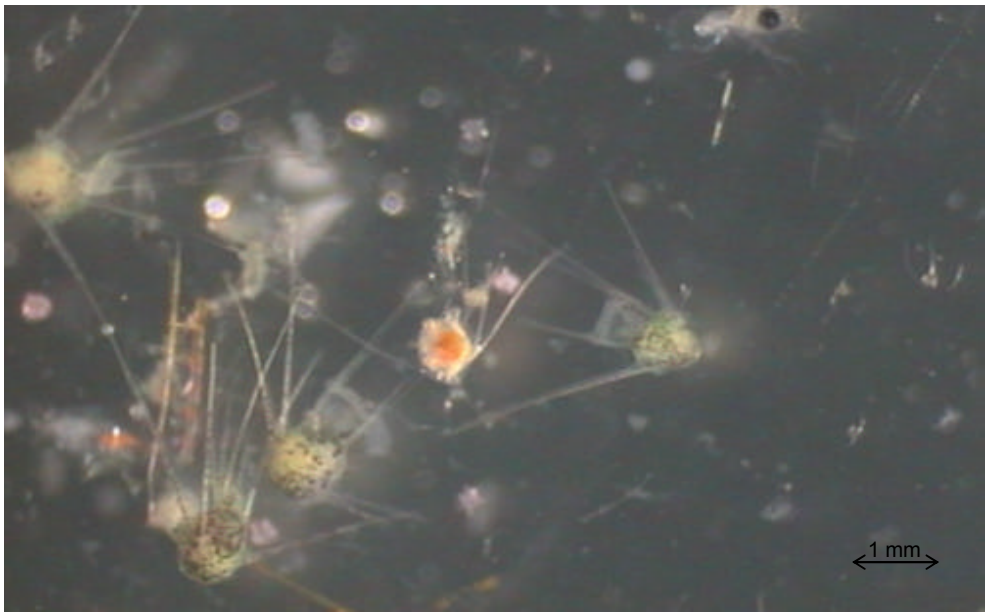
During sample analyses, the larvae of various echinoderm species, such as sea urchins, brittle stars, and sea cucumbers (Figure 28), may be observed.

A



Brittle star

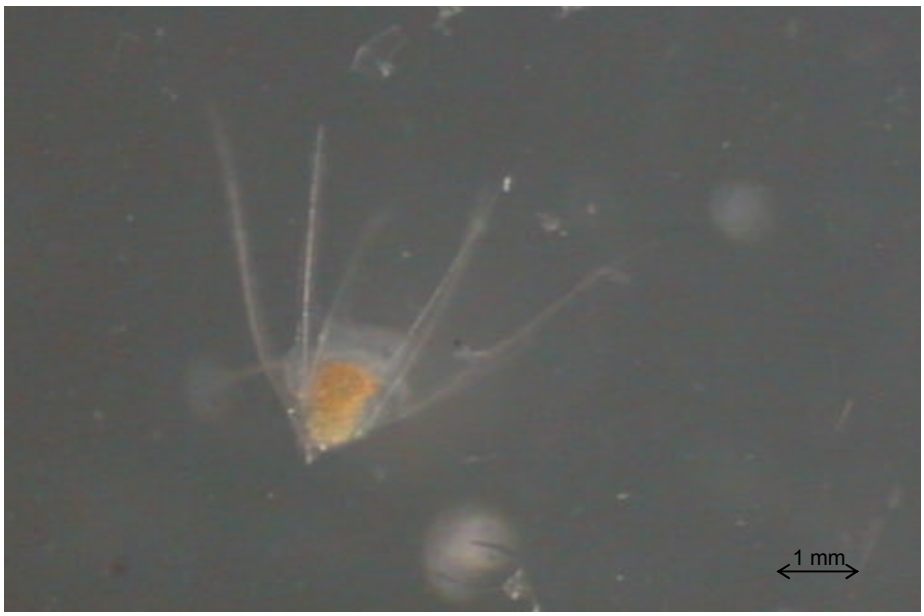
B



Brittle star and sea

Figure 28. Echinoderm larvae.

C



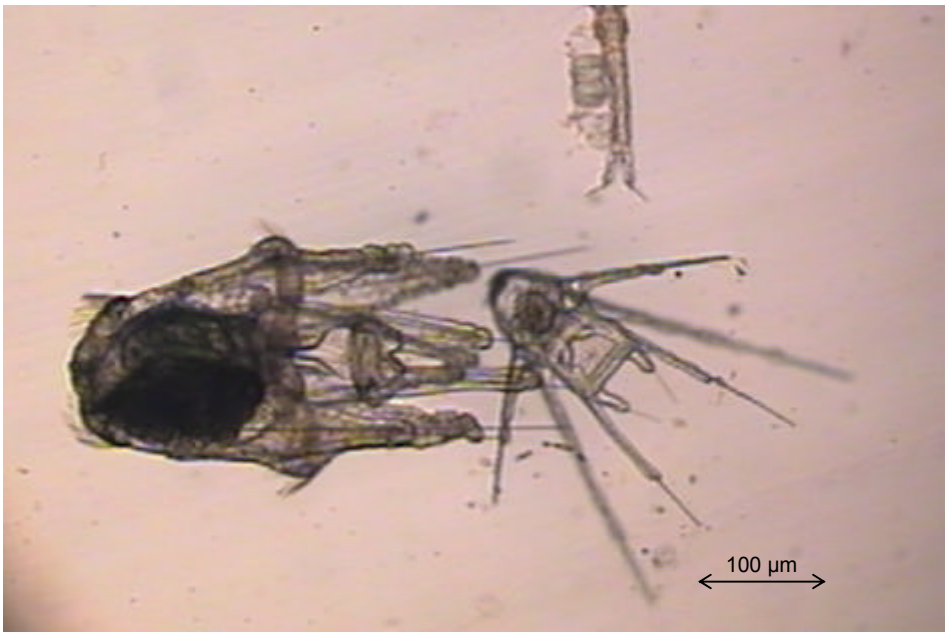
Brittle star

D

Unidentified species

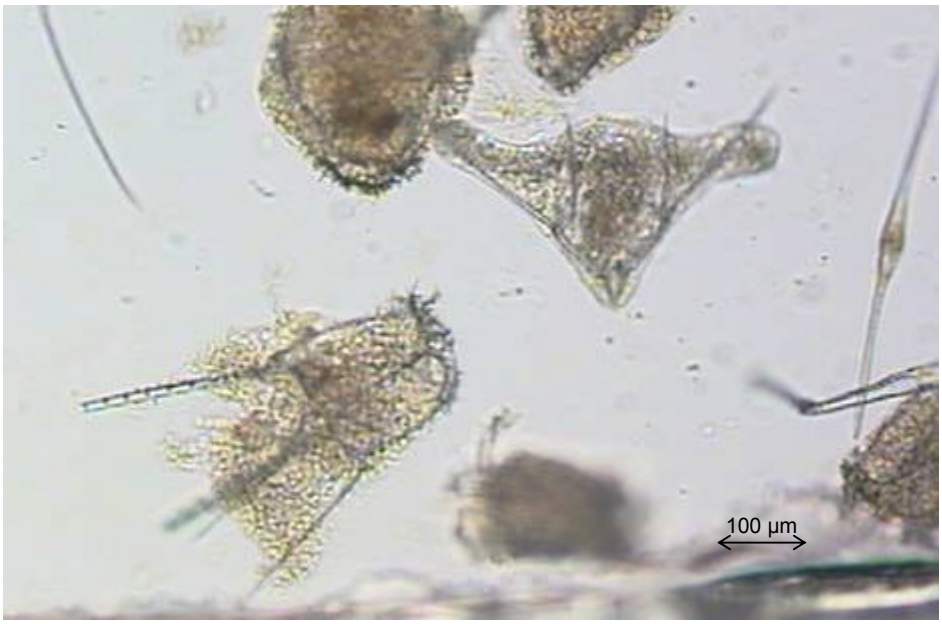
Figure 28 (contd.). Echinoderm larvae.

E



Sea urchin and unidentified species

F



Photos F. Aucoin

Unidentified species

Figure 28 (contd.). Echinoderm larvae.

5.2.9. Barnacle (*Balanus sp.)*

Photo S. Doiron

On New Brunswick's east coast, barnacles are collected during the same period as oysters, which can pose a considerable problem for oyster farmers. Barnacle larvae are therefore found in the water at the same time as oyster larvae, i.e., from early July until late August. The barnacle's first larval stage, called the nauplius stage (Figure 29), is characterized by two horns, one on either side of the head. At the cypris stage, the larva, which is looking for a place to settle, does not feed. It can live for up to 13 days on its energy reserves.

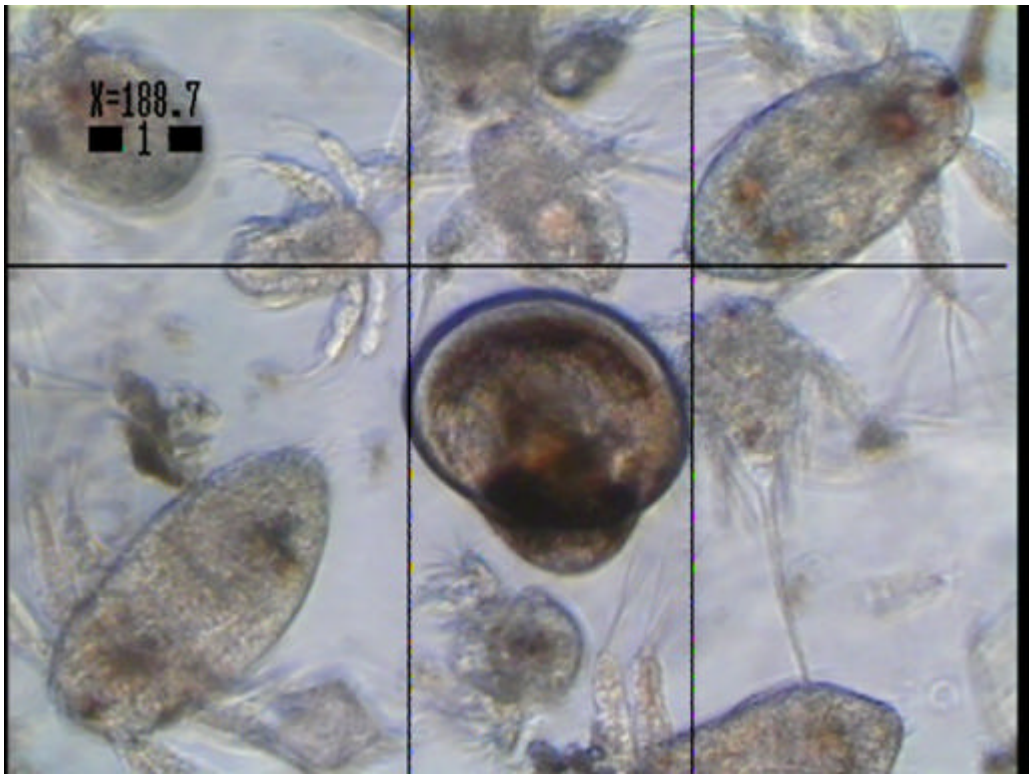


Photo S. Doiron

Figure 29. Barnacle larvae (nauplius) surrounding a 189-µm oyster larva.

5.3 Comparison between Certain Species

This section presents the distinctive features of species whose larvae could be confused when found in the same sample. The data are grouped together according to two periods of the year: May to July and August to October.

5.3.1 May to July

From May to July, larvae of the blue mussel, the soft-shell clam, the common razor clam, the Arctic rock borer, the flat lepton, and the common shipworm can be observed at the same time (Table 3). Of these species, it is possible to confuse blue mussel larvae with those of the soft-shell clam, the common razor clam, and the rock borer. It is also difficult to differentiate between the rock borer and the soft-shell clam and between the lepton and the razor clam.

5.3.1.1 Blue Mussel and Soft-shell Clam

Various features distinguish blue mussel larvae from soft-shell clam larvae. At the beginning of the D-veliger stage, the difference between the length and width of the blue mussel is about 30 µm, while it is 15 µm for the clam. Mussel larvae remain D-shaped until they reach a length of 150 µm, whereas clam larvae change shape when they are about 125 µm long. The clam's umbo becomes quite rounded, and the shoulders are visible. It then becomes angular and keeps this shape until the end of the larval stage. Mussel larvae, however, become rounded during the course of their development; the umbo is indistinct but can become protuberant and centred as the larvae grow (260 µm). The two species are not the same colour: mussel larvae change from yellow to brown, while clam larvae are greenish grey.

5.3.1.2 Blue Mussel and Common Razor Clam

Blue mussel and common razor clam larvae are the same colour. However, unlike mussel larvae, the left and right sides of razor clam larvae are not the same shape. The left side of the razor clam most

resembles that of mussel larvae. The difference lies mainly in the shape of the hinge. When the larva measures about 150 µm, the hinge on the left side of the razor clam is smaller than that of the mussel, and the shoulders are already apparent. At this size, the razor clam's umbo appears; lower than the shoulders, it exposes a hook-shaped black line on either side. The mussel's umbo is not apparent or does not extend above the hinge.

5.3.1.3 Blue Mussel and Arctic Rock Borer

Arctic rock borer larvae present characteristics very similar to those of soft-shell clam larvae, but the two species can be distinguished by the umbo. In mussels, the umbo is indistinct, while the rock borer's is angular in shape.

5.3.1.4 Soft-shell Clam and Arctic Rock Borer

It is possible to mix up the clam and the rock borer, both of which have an angular umbo. However, the clam has shorter shoulders than the rock borer, as well as a thinner outline, a more pronounced slope on the anterior side, and darker brown pigmentation.

5.3.1.5 Flat Lepton and Common Razor Clam

A wide yellow band distinguishes lepton larvae from razor clam larvae. In the advanced stages, the shoulders of the lepton are shorter as well.

5.3.2 From August On

In August, new species, such as sea scallops, horse mussels, and jingle shells (Tableau 3), start to appear. Scallop and horse mussel larvae can be confused with those of the blue mussel, which are still present in the water. Samples may also contain oyster larvae, which resemble jingle shell and shipworm larvae.

5.3.2.1 Blue Mussel and Sea Scallop

Blue mussel larvae can be confused with sea scallop larvae. However, the hinge of the D-veliger larva of the mussel is longer than that of the scallop. The D-shape of the scallop is rounder, and it is more delicate in appearance. Its hinge will always remain short, whereas that of the mussel larva will grow in proportion to its increasing size. The mussel's umbo is more protuberant, and the outline is thicker.

5.3.2.2 Blue Mussel and Horse Mussel

Blue mussel larvae between 250 and 300 µm can be confused with horse mussel larvae of the same size. However, the umbo of the horse mussel is more protuberant than that of the blue mussel. In addition, horse mussel larvae look more robust and have darker coloration throughout their development. Lastly, the outline of horse mussel larvae is black and thicker.

5.3.2.3 Eastern Oyster and Jingle Shell

In the early stages, eastern oyster larvae are more symmetrical than jingle shell larvae. The anteroventral edge of the eastern oyster larva is uniformly rounded, whereas the jingle shell develops a notch during the larval cycle. Jingle shell larvae are more yellow than oyster larvae, which have some purplish hues as well. Also, jingle shell larvae are more delicate (transparent) than oyster larvae.

5.3.3.4 Eastern Oyster and Common Shipworm

Oyster larvae can be confused with shipworm larvae. The shipworm has a very thick, black outline. The umbo of the oyster larvae moves off-centre during growth, whereas the umbo of the shipworm larva always remains centred. In oysters, the sides get longer; in shipworms, it is the ventral part that gets longer.

5.4 Identification Exercises

The photos below (Figure 30) can be used as part of a larval identification exercise. The sampling date is recorded at the bottom of the photos, and the line below the date is approximately 100 µm in length. The answers are found at the end of this section.

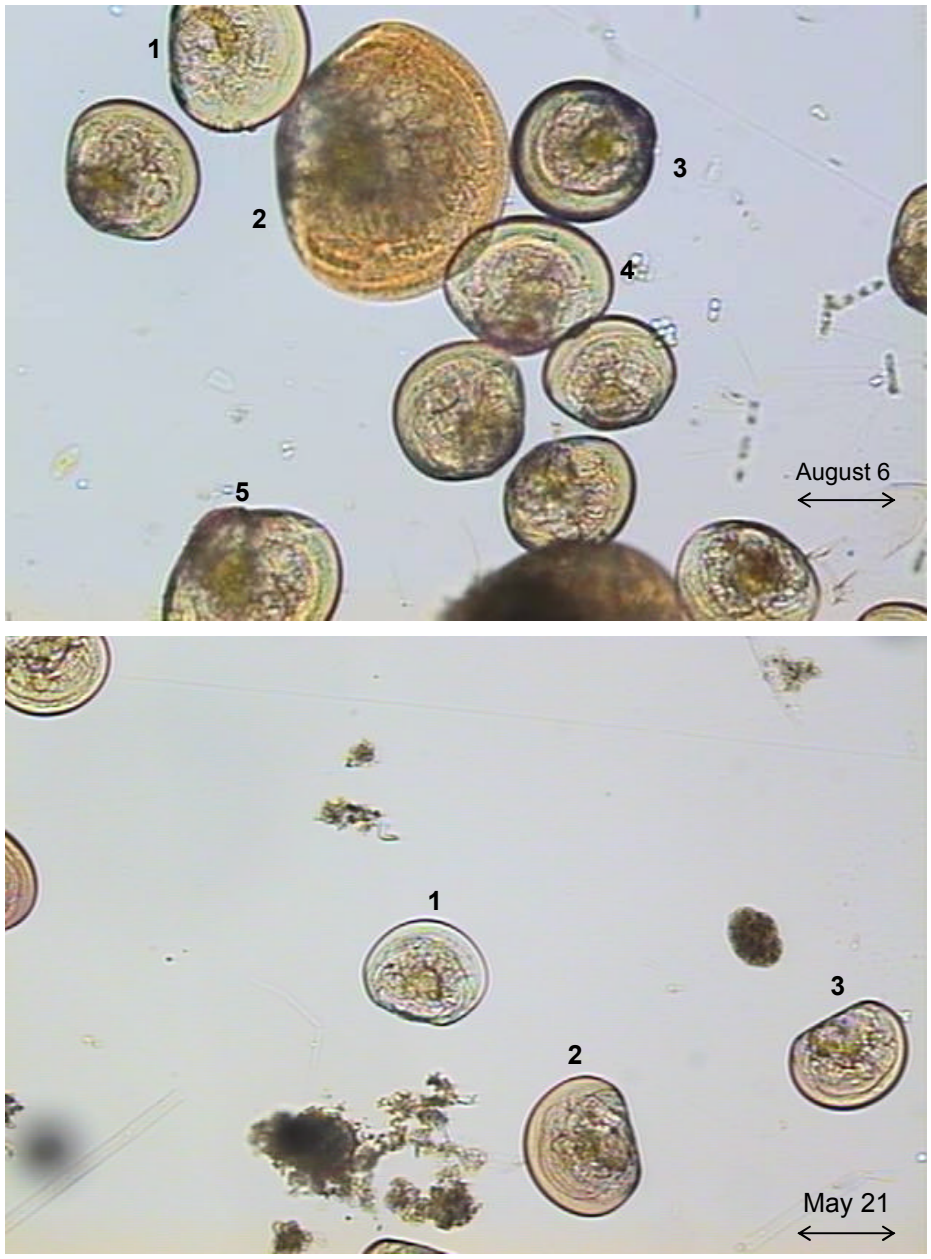
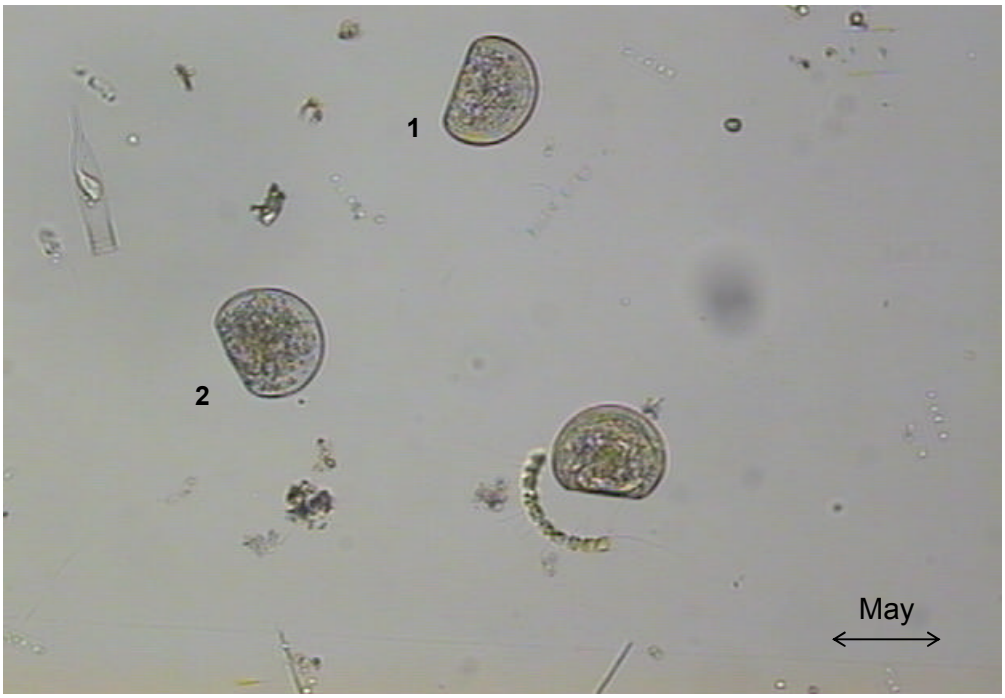


Figure 30. Who am I?

C



D

Figure 30 (contd.). Who am I?

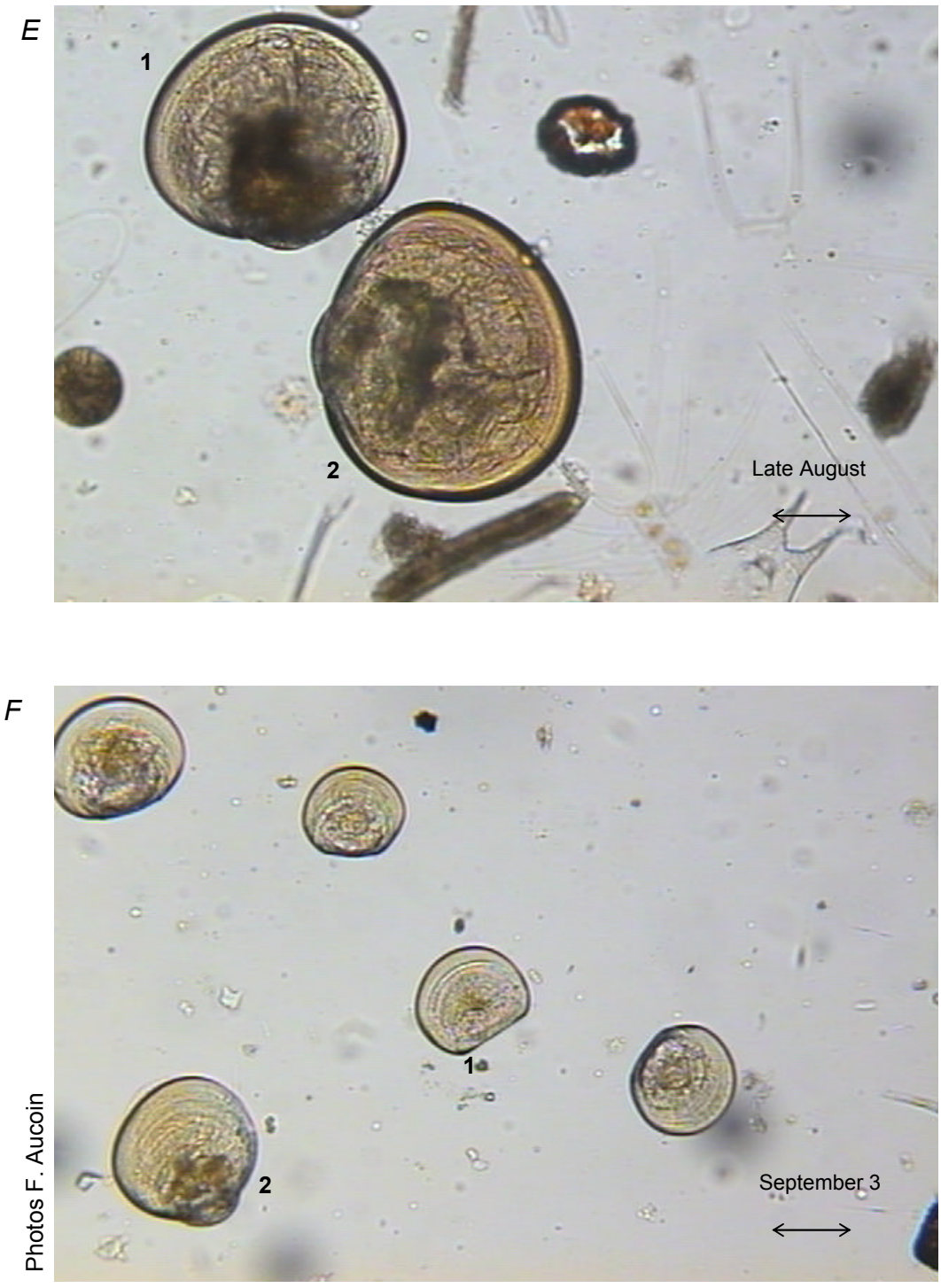


Figure 30 (contd.). Who am I?

Answers – section 5.4

A) 1 - Blue mussel
2 - Flat lepton
3 - Common shipworm
4, 5 - Northern dwarf tellin

B) 1 - Soft-shell clam
2, 3 - Blue mussel

C) 1 - Blue mussel
2 - Soft-shell clam

D) 1 - Northern dwarf tellin
2 - Blue mussel

E) 1 - Horse mussel
2 - Blue mussel

F) 1 - Sea scallop
2 - Jingle shell

6. CONCLUSION

The information contained in this guide should be of assistance to inexperienced observers faced with the task of analyzing a larval sample. Of course, the guide does not cover all the species that may be found in a sample, primarily because much of the data come from activities carried out at sea and in lagoons in the Magdalen Islands area or from projects conducted at the experimental hatchery in Shippagan. Any gaps can be filled in by consulting various references, a few of which are listed in section 8.

7. ACKNOWLEDGMENTS

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