Chapter 3

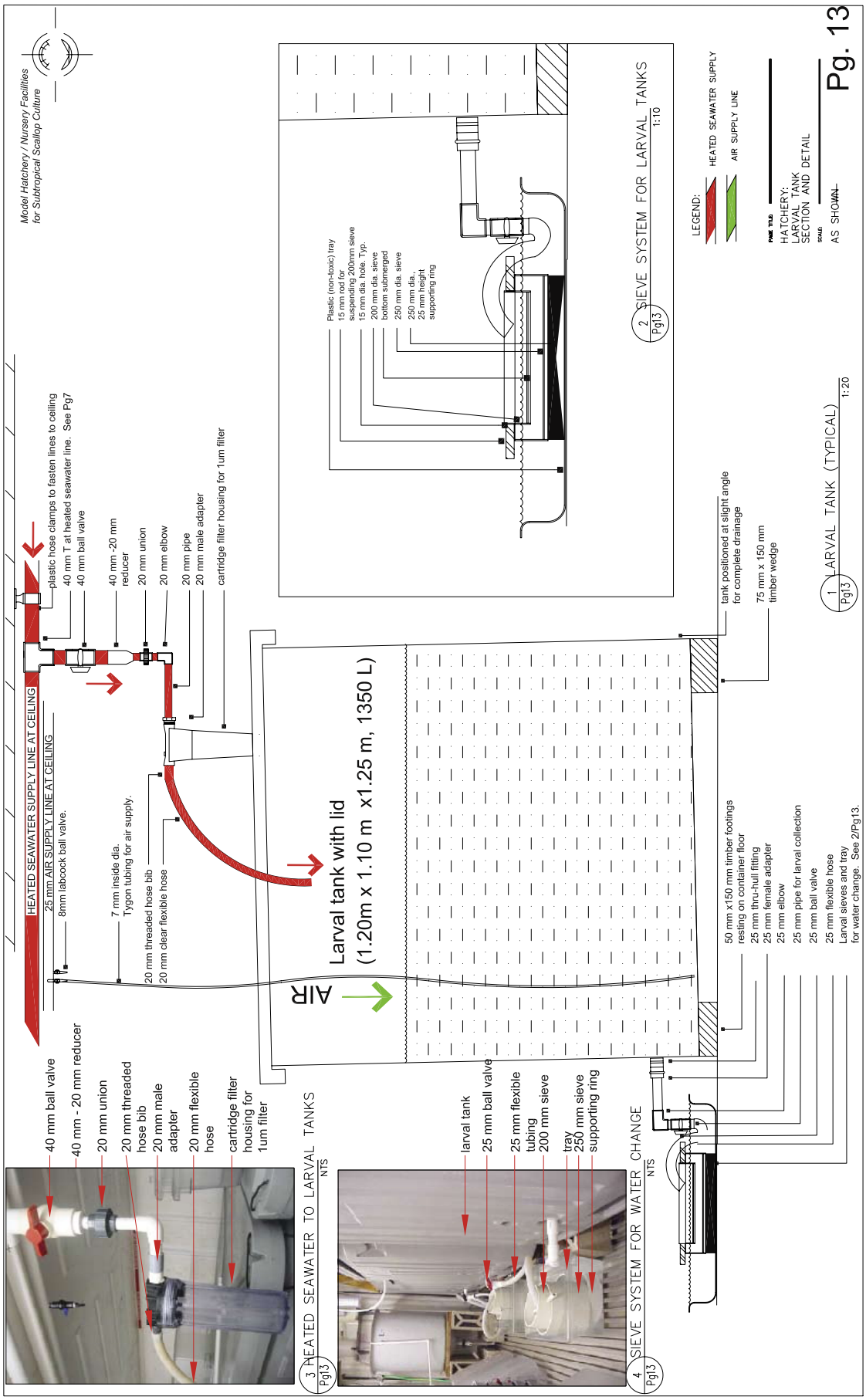
Hatchery: facilities and techniques for larval culture

3.1 HATCHERY FACILITIES

The goal of the hatchery is to produce quantities of juvenile scallops by inducing spawning of adults, and rearing larvae and post-larvae under controlled conditions. For reliable production of larvae and post-larvae, strict adherence to protocols for each stage of culture and routine chores for daily care is necessary. Throughout larval and post-larval rearing, cleanliness in the hatchery is essential to prevent large-scale mortality and loss. For this reason, details of routine cleaning of seawater supplies and tanks followed in Bermuda are provided in this Chapter. These protocols have proved extremely valuable at the Bermuda hatchery, in ensuring regular maintenance.

Technical drawing, Pg. 13

Hatchery: Larval tank section and detail



This Chapter describes the hatchery facilities necessary for the development of eggs to straight-hinge stage (Figure 1.7), and for the rearing of larvae to the time of settlement.

The overall plan of the facility, showing larval tanks and air compressor is seen on technical drawing – page 6A and described in Chapter 1. A total of four square insulated "BONAR" tanks (1 344 litres total capacity) are used for larval rearing. Larval tanks are supplied with compressed air and heated seawater filtered twice to 1 µm. Description of the heating system and heating lines in the hatchery are provided in Chapter 1 (see Section 1.1.3) and drawings are on technical drawings – pages 5A and 5B.

3.1.1 Larval tanks

Refer to Technical Drawing – page 13. In general, the larger the tank the better; it is preferable to minimize the surface area to water volume ratio as surfaces tend to have higher numbers of bacteria. In Bermuda, square tanks with maximum volume of 1 344 litres are used as standard larval rearing vessels. These insulated tanks have double walls filled with foam, and molded legs; they are often used for fish transport, for their insulating capacity, and for this reason, they were selected for scallop larval rearing in Bermuda, as larvae are cultured at a temperature higher than the ambient. Lids are provided with the tanks, and are used for maintaining a constant seawater temperature. For larval rearing, tanks are filled to a maximum of 1 000 litres, and hence referred to throughout this manual as 1 000 litres tanks. Larval tanks are filled with heated seawater, supplied from the heating unit (see Chapter 1). As seen in the technical drawing diagram – 1/Pg13, the seawater line is affixed to the ceiling by plastic hose clamps. A 50 mm T is glued in-line for supply of seawater to two larval tanks. A 50 mm ball valve regulates the flow of seawater to the tank; it is reduced to 20 mm by a 50 mm to 20 mm bushing. A 20 mm union connects the heated seawater supply to a 20 mm elbow fitted to a cartridge containing a 1 µm filter. The 20 mm union above the cartridge allows for dismantling of the cartridge-housing unit for cleaning in-between water changes. This filter system provides additional filtration after heating and prior to delivery to the larval tank. The cartridges used have 20 mm fittings included; on the outflow a 20 mm ID clear flexible hose is used to fill the larval tank. The technical drawing photo – 3/Pg13 illustrates the filter set up in detail.

The air supply to the larval tank is also illustrated in technical drawing – 1/Pg13. Air supply is regulated by labcock ball valves and connected to the airline (see diagram – 2/Pg 7). A 7 mm ID Tygon tube is fitted to a labcock ball valve running down the length of the tank. For larvae, a low volume air flow is provided, equivalent to one bubble at a time, sufficient to oxygenate the tank and prevent algal cells from sinking.

In the hatchery, larval tanks are placed directly on the floor, resting on timber footings at each corner. A gentle slope is provided for complete drainage of the tank, by raising the back side using additional timber wedges. The drain valve is located on the front of the tank. For controlled drainage, necessary for collecting of larvae, a 25 mm thru-hull bulkhead fitting is glued into an existing drain hole. A 25 mm female adapter is threaded into the thru-hull fitting; this is in turn glued to a 25 mm elbow. The flow of water is regulated by a 25 mm one-way ball valve. A 25 mm pipe is dry fitted to the valve and to a 25 mm ID hose for collection of larvae; in this way, a gentle outflow of water is obtained from the larval tank, minimizing any crushing of larvae.

The technical drawing diagram – 2/Pg13, shows, in detail, the equipment utilized for collection of larvae during water transfers. As larvae are culled at every water change, two sieves are placed one within the other; the smaller 20 cm diameter sieve is balanced inside the larger 25 cm diameter sieve, and is supported by a 15 mm transverse pipe, resting on top of the lower sieve. Sieves are kept in plastic non-toxic trays (used in

the restaurant trade), and supported by a 2.5 cm high ring off the bottom; this set up allows collected larvae in both sieves to remain continuously submerged, and for those collected on the bottom sieve to avoid direct contact with the tray and subsequent crushing and damaging of shell. Care is taken that the top of the bottom sieve is above the tray level, so as to not lose any larvae during collection. In this way, water flowing from the larval tank passes through both sieves, where larvae are separated by size and collected; water remaining in the tray, overflows and is discarded without any loss of larvae. Water level in the tray and flow rate from the tank must be monitored through the entire draining process to ensure that the sieve does not become clogged with larvae and overflows; this would cause larval loss. For culling larvae, larger larvae are collected first in the 20 cm sieve (with larger mesh aperture) and smaller larvae are collected in the 25 cm bottom sieve (with smaller mesh aperture). The technical drawing photo – 4/Pg13 illustrates the set-up of trays and sieves for collection of larvae during a water change.

3.2 SCIENTIFIC BACKGROUND – EMBRYONIC AND LARVAL DEVELOPMENT

There are many problems which may arise in the reliable hatchery production of bivalve species. Successful hatchery production of larvae and spat is very dependent on the skill and experience of the staff. As mentioned at the beginning of this Chapter, a keen awareness of the importance of hygiene is essential. In general terms, higher mortalities of larvae are often associated with higher temperatures triggering greater bacterial proliferation and infection. Chapter 1 describes the procedures followed for spawning induction of adults and fertilization of the eggs. In the following section, procedures followed for embryonic development and larval rearing are discussed.

3.2.1 Embryonic development to D-larvae

A generalized life cycle for pectinids is given in Chapter 1 (see Figure 1.7), as well as a brief synopsis of the sequence of events following fertilization, prior to distribution of eggs in culture tanks for both *Euvola ziczac* and *Argopecten gibbus* (see Section 1.3.3). In Figure 3.1, the change in shape of the various stages of development from fertilized egg, through D-larvae to pediveliger for sand scallop larvae is shown. Development of *E. ziczac* and *A. gibbus* larvae is similar to that seen for other pectinids, which has been described in great detail in the literature (Costello *et al.* 1973; Culliney, 1974; Paulet, Lucas and Gerard, 1988; Kasyanov, 1991; Cragg and Crisp, 1991). For the purpose of this manual, a brief generalized description of each stage is given in the appropriate sections as a quick reference. However, as the rate at which larvae develop varies with species, the short section below gives that observed for the sand and calico scallop in Bermuda.

Embryos are left undisturbed for the first 48 hours. During this time period, repeated cleavages lead to formation of the spherical blastula; timing varies among species and was not determined for either the zigzag or calico scallop, but was determined for

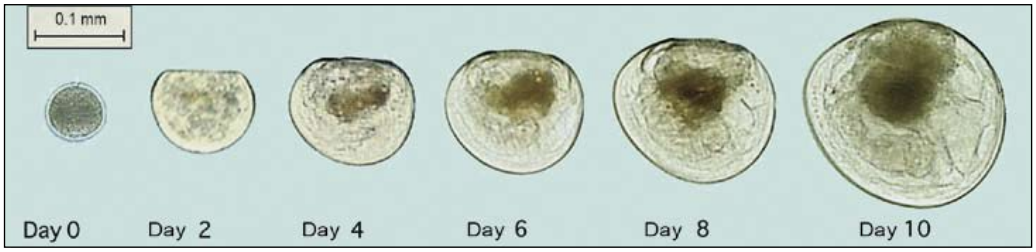


Figure 3.1: Developmental changes of sand scallop larvae to metamorphosis.

a related species, *Aequipecten irradians concentricus*, to be of five hours and fifteen minutes (Sastry, 1965). The rotating ciliated gastrula appears thereafter (nine hours after insemination for *A. concentricus*). Changes from a ciliated gastrula to the top-shaped trochophore are gradual (see Life cycle – section 1.2.3).

First observations at the BBSR hatchery are made 24 hours after distribution of embryos in culture tanks, when samples are taken by skimming the surface of the water with a 20 µm sieve and examined under a compound microscope. At this time, trochophore larvae and often, early veliger larvae are observed for both *E. ziczac* and *A. gibbus* cultures. Pectinid trochophore larvae are characteristic in that the apical end is rounded and surmounted with a tuft of long cilia; the other end of the larva is tapered with indentations on either side of the larva. During this stage, there is little calcification, and shell secretion is initiated at the end of the trochophore stage. Due to this soft-body characteristic they are very fragile; for this reason, 24 hours larval cultures are left undisturbed, as collection on sieves would be damaging.

Figure 3.2 shows veliger larvae of *E. ziczac*, characterised by their velum, a distinct organ emerging during the transition from trochophore to veliger. As these early 24 hours veliger larvae exhibit an active swimming behaviour both for *A. gibbus* and *E. ziczac*, addition of formaldehyde is required for a clear photograph; this also causes the velum to retract, for this reason, only a few of the larvae seen have been caught with the velum extended. Veliger larvae remain uniformly suspended in the water column. To ensure complete development for a maximum number of embryos, a 48-hour time period is allocated prior to the first collection of larvae. This allows time for the developing larvae to take on the D-shaped outline characteristic of the prodissoconch-I shell. Complete development to the D-larval stage (or straight-hinge stage) thus occurs 48 hours after fertilization (Figure 3.3). In contrast to the 24-hour veligers, D-larvae of the sand and calico scallop remain for the most part still and swim occasionally upon being disturbed. Costello *et al.* (1973) report a similar developmental rate for *A. gibbus* D-larvae.

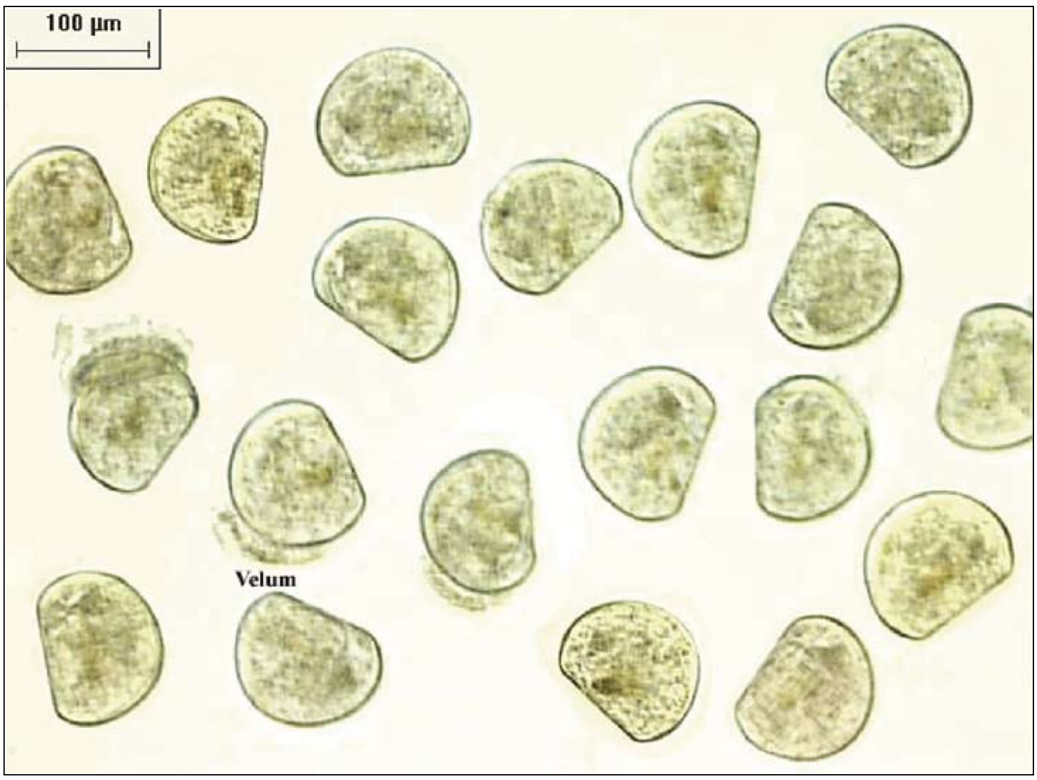


Figure 3.2: One-day old *E. ziczac* veligers showing extended velum.

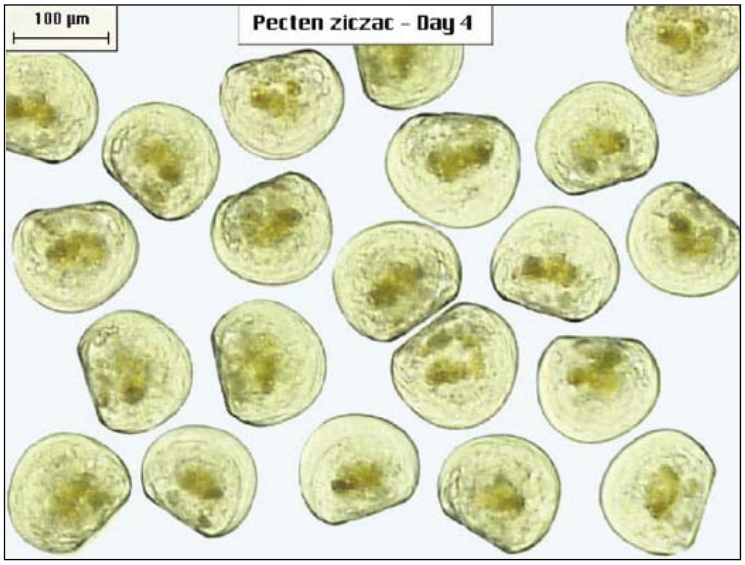


Figure 3.3: Straight-hinge or D-larvae stage of *E. ziczac*.

3.2.1.1 Fertilized eggs: characteristics and developmental requirements

Egg diameter for pectinids generally range from 57–79 µm diameter (Cragg and Crisp, 1991). Within a species, egg size, may vary slightly among batches; according to Kraeuter, Castagna and Dessel (1982), this size difference, although slight, may reflect the amount of reserves stored in the egg and impact subsequent development and growth. These authors suggest eliminating smaller size eggs and concentrate on better care of larger egg sizes that are more likely to survive. Reduced survival of certain batches in hatcheries, especially towards the end of the hatchery season (as seen in Chapter 1), is often related to higher temperatures and bacterial contamination; however, a significant portion of these losses may be due to use of smaller eggs with reduced stored reserves. Mean egg size for *E. ziczac* is found to be 64.8±6.8 µm (n= 50), and for *A. gibbus* 55.9±3.2 µm (n= 50). Size measured for *A. gibbus* in Bermuda is slightly lower than recorded by Costello *et al.* (1973) – 60 µm diameter – for the same species. This difference may be attributed to environmental differences reflected in stored reserves.

Differences among batches of calico scallop eggs are observed at the Bermuda hatchery, namely with respect to buoyancy. Some batches are neutrally buoyant and remain suspended throughout the water column; however, other batches are negatively buoyant and sink to the bottom of the culture tank. This difference in buoyancy among batches has never been associated with a change in developmental success of the embryos or of the larvae thereafter at the Bermuda hatchery. This weight difference may be explained in part by the differences in amount of reserves as reported by Kraeuter, Castagna and Dessel (1982). Negative buoyancy has also been reported for *Patinopecten yessoensis* resulting in a monolayer of eggs at the bottom of the culture tanks (Bourne, Hodgson and Whyte, 1989). For this reason, it is best not to aerate, as embryos will collect in windrows at the edge of the tank because of water circulation. On the other hand, in *E. ziczac*, fertilized eggs are neutrally buoyant remaining suspended throughout the water column.

All fertilized eggs are pooled at the hatchery in Bermuda without any size screening, but with some subjective selection related to shape, development and uniformity. For example, those eggs released as clumps, or potentially self-fertilized, were classified as "bad". An attempt is made at rearing "good" and "bad" in separate culture vessels. It is uncertain whether this arbitrary selection enhances embryonic development, and batches of "bad" eggs lead at times to surprisingly good D-larval cultures. The density at which eggs are distributed varies with species. Calico scallops have been reportedly

reared at as high a density as 25 eggs.ml^{-1} (Costello *et al*. 1973). On the other hand, lower densities (10 eggs.ml^{-1}) are used for the queen scallop (Neima and Kenchington, 1997). In Bermuda, it has been observed that a higher survival to Day 2 larvae is obtained for the sand scallop for batches with an initial egg density less than or equal to 10 eggs.ml^{-1}.

Egg size and rearing density used are thus two factors, which may affect development success to the D-larva stage. Sperm concentration has also been shown to be a major factor in the production of normal bivalve larvae in the laboratory (Loosanoff and Davis, 1963; Gruffydd and Beaumont, 1970). A ratio of 1:6 (egg:sperm) is a standard objective; in the hatchery 1–2 ml of sperm solution per 1 litre of egg solution is found sufficient for successful fertilization. Beaumont and Budd (1983) have shown that there are significant genetic effects during fertilization and the early larval life in *Pecten maximus*. In their study, the origin of both eggs and sperms independently affected the number of eggs, which produced larvae. For this reason, it is routine procedure to fertilize each female with several sperm solutions, in hopes of enhancing developmental success to D-larval stage. An additional consideration needs to be taken for hermaphroditic species, such as zigzag and calico scallops, to minimize abnormal development. In these species, the probability of self-fertilization during laboratory spawning is enhanced, and self-fertilization has been also associated with detrimental effects in development. Beaumont and Budd (1983) found a severe reduction in growth rate of *P. maximus* veligers in all self-fertilized cultures. Sastry (1965) found similar unsatisfactory results in selfed eggs of *A. irradians concentricus*. It has always been a preventive measure during hatchery procedures in Bermuda to avoid self-fertilization in the laboratory and ensure cross-fertilization using several males.

As seen in Table 3.1, variations in percentage D-larvae obtained among and within batches are large. Variations among batches may be a reflection of one or several of the factors mentioned above, namely egg size (or stored reserves) and density. Variations within batches may also be a reflection of the arbitrary selection conducted during distribution of eggs into culture tanks. Other factors, such as variations in salinity (Gruffydd and Beaumont, 1970), and in dissolved organic matter (Crisp, 1982) have also

Table 3.1: Yields of Day-2 larvae obtained for several hatchery seasons following controlled fertilization of *E. ziczac* and *A. gibbus* in the hatchery. Ranges shown indicate yields obtained for all larval tanks in one spawning. Single numbers indicate mean yield for one spawning.

Species	Year	D-larvae (%)	
Sand scallop (*Euvola ziczac*)			
	1998	7.5–57 %	– Spawn 1
		<1–20 %	– Spawn 2
	2000	66–>90 %	– Spawn 1
		1.23–50 %	– Spawn 2
	2001	31.6 %	– Spawn 1
		<1 %	– Spawn 2
		8.2 %	– Spawn 3
	2002	1.6–6.1 %	– Spawn 1
Calico scallop (*Argopecten gibbus*)			
	1997	66–86 %	– Spawn 1
	1998	35–75 %	– Spawn 1
	2000	37.5–>90 %	– Spawn 1
	2001	18.4–54.3 %	– Spawn 1
		22.6–50.3 %	– Spawn 2
		41.6–49.6 %	– Spawn 3
	2002	22.4–39.5 %	– Spawn 1
		19.7–28.4 %	– Spawn 2
	2003	33.4–58.2 %	– Spawn 1
		43.5–66.9 %	– Spawn 2

been associated with variations in yield and normality of *P. maximus* D-larvae. These are most likely not a consideration in Bermuda and have not been investigated.

3.2.2 Larval development

The characteristic "D" shape of early larvae (Figure 3.3) has two valves, a complete digestive system and a velum. The velum is ciliated along its outer margin and enables the larva to swim, enabling it to maintain itself in the water column. As it is swimming, the velum collects unicellular phytoplankton upon which the larva feeds. From hereon, larvae are fed daily a food ration consisting of live algal species, cultured on-site (see Chapter 2).

3.2.2.1 Veliger larvae

As mentioned in the description of embryonic development, the shell appears in the early stages of development (24 hours after fertilization). The veliger shell (prodissoconch-I) is produced by the secretory cells of the shell gland. Kasyanov (1991) gives detailed schematics of the development of the Japanese scallop, *Mizuhopecten yessoensis*. The larval body of the veliger is covered by a semi-transparent shell through which the internal organs are discernible. The digestive system of bivalve larvae consists of the ectodermal fore-gut, the endodermal mid-gut and the ectodermal hind-gut. Food is captured and transported to the mouth opening by ciliary bands on the velum. This function of the velum is as important as the locomotory function. The veliger larva swims actively with the velum extended. The mouth opening situated at the edge of the lower part of the velum leads to the oesophagus, which in turn leads to the stomach. The digestive gland is large and is formed as two stomach pouches. Accumulations of granules of nutritive substances are seen in the cells of the gland. The gland contains digestive enzymes and also opens into the stomach. The stomach leads posteriorly to a short intestine opening to the exterior as anus. The anterior adductor muscle is located dorsal to the anterior attachment of the velum. The respiratory and circulatory systems are absent in the veliger of bivalve mollusc. The influx of oxygen and the excretion of carbonic acid occur by diffusion. The nervous system is considerably developed and is represented by the cerebral ganglion and two pedal ganglia. The main sense organ in the veliger larva is the apical plate of the velum. Straight-hinge larvae vary in length among pectinid species; for example, 78 µm for *A. irradians concentricus*, and 90 µm for *Placopecten magellanicus* (Sastry, 1965; Couturier, Dabinett and Lanteigne, 1996). In Bermuda, shell length for D-larvae of *A. gibbus* ranges from 92.7±4.7 µm to 101.0±4.22 µm, and for *E. ziczac* from 93.1±8.3 µm to 110.4±5.0 µm.

3.2.2.2 Pediveliger larvae

The shape of the D-larvae gradually changes with age. As veligers develop, a slight reduction in the hinge-length with the extension of umbones over the hinge region is seen. Costello *et al.* (1973) report the appearance of the umbo at 140 µm for calico scallops; in Bermuda, this appearance is seen as early as Day-6 old scallops with a mean size of 115 µm shell length (Figure 3.4). The degree of umbo development varies among species, and is for example more conspicuous in the sand scallop than in the calico scallop. Shell growth in the anterior-posterior direction also takes place. The internal organs, such as the stomach, develop. The foot begins to develop as a small projection ventral to the mouth; it is recognizable especially when the larva has retracted into the shell. In 9 Day old veligers, an "eyespot" (darkly colored spot) becomes conspicuous, especially noticeable in *E. ziczac* (Figure 3.5). This appearance of the eye spots with the statocysts is characteristic of pediveligers. The velum reduces gradually and the foot continues to develop; swimming activity also decreases. By the twelfth day the well grown umbones almost mask the hinge line of the early veliger shell. For this reason, the pediveliger shell is triangular, egg-shape.

Bower and Meyer (1990) give very detailed drawings for *Patinopecten yessoensis.* Pediveliger larvae reach the maximum size and develop a functional foot. The foot is a multi-functional organ; it mainly serves to search for a substrate for settlement and attach the larva to the substrate by byssal threads. Swimming larvae can extend the

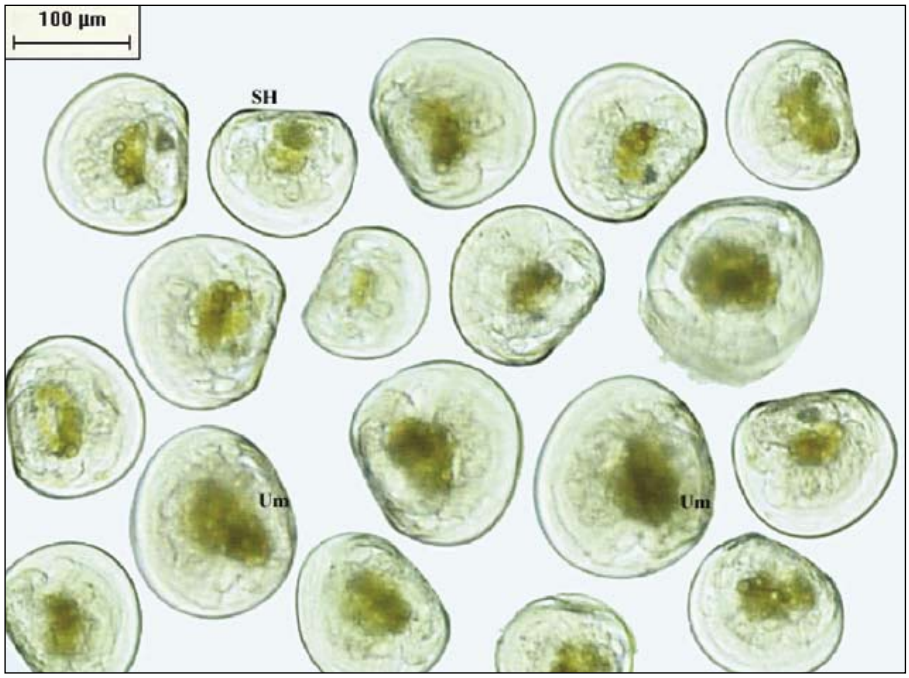


Figure 3.4: Day-6 sand scallop larvae showing initial development of umbones (Um) compared to straight-hinge characteristic (SH) of D-larvae.

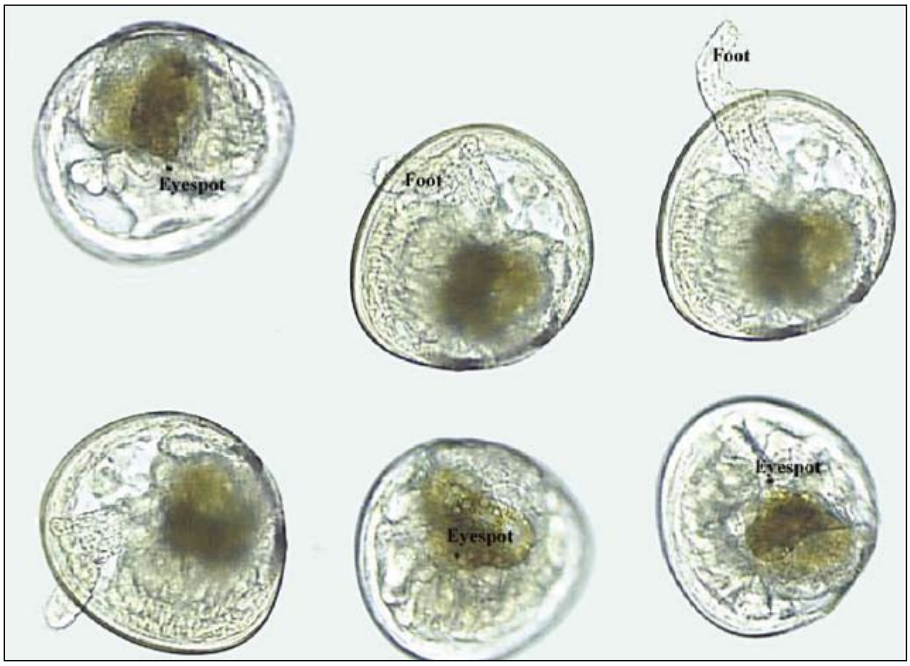


Figure 3.5: Day-11 pediveligers of *E. ziczac* showing eyespot and a well-developed foot in and out of the shell.

foot which, when brought into contact with the substrate, facilitates crawling over the substrate. The result of crawling can be that: 1) the larva attaches itself to the substrate, or 2) the larva retracts the foot, leaves this substrate and resumes swimming in search of a more adequate substrate. At this time, larvae are more often collected from the bottom water, rather than throughout the water column as in the earlier stages. The

most important changes in the larvae take place between the twelfth and fourteenth days of development. The umbones are fully grown, overhang the straight-hinge of the early veliger shell, and are directed posteriorly. The shell has a curved appearance at the hinge attachment. This prodissoconch-II stage is identified as such to distinguish it from the early veliger and pediveliger stages. The anatomy of the prodissoconch shows important differences from the earlier stages. The mouth is located in an anterior and dorsal position, bringing it to the adult position. The mantle is well developed. The foot is enlarged and is a wedge-shaped organ with cilia at the free or distal end. The foot has a cleft in the middle region and the byssal gland is located there. A pair of statocysts is present. The gills are developed as a pair of ciliated folds ventral to the foot. With development of new organs and the loss of some larval organs, metamorphosis is completed. The average size of the prodissoconch-II shell for Day-14 calico scallops is of 200 µm shell length, and for zigzag scallops of 215 µm.

Prodissoconch-II larva attaches to the substratum with the byssal threads; in the hatchery, surfaces are provided during this settlement phase. Thereafter, rapid changes in shell morphology and growth of internal organs take place resulting in the adult scallop form. Post-larval development is discussed in Chapter 4.

3.3 SCIENTIFIC BACKGROUND – FACTORS INFLUENCING LARVAL REARING

Larvae can be grown in conical or flat-bottomed vessels. Both types have been tried for rearing *E. ziczac* and *A. gibbus*. Differences in yield between conical and flat-bottomed were not truly tested, but were not apparent. Considering the restriction in space of the model hatchery, it is found that maximal tank capacity is best achieved with flat-bottomed tanks. Square "BONAR" tanks (1 000 litres capacity) are preferred as the main larval tanks mainly due to their insulation characteristic, allowing for rearing of larvae at a temperature higher than ambient. Temperature, salinity, food ration and rearing density are important factors in development of larvae. They are discussed in the following sections in relation to procedures used in Bermuda.

3.3.1 Temperature

For incubation of fertilized eggs, and larval rearing of both the calico and sand scallop, seawater temperature is increased by 6–8 °C above the ambient; such that rearing temperature is maintained at 24±1 °C for embryonic development to settlement stage. This yields an average larval life of 13 days, when pediveligers are ready for settlement in nursery systems. Costello *et al.* (1973) reared *A. gibbus* under similar conditions (T= 23±2 °C and S= 35 ppt). Velez and Freites (1993) have also reported successful culture of *E. ziczac* larvae at a salinity of 37 ppt and a temperature of 26 °C, yielding pediveligers within 10–12 days after fertilization.

In their review Cragg and Crisp (1991) found that time to metamorphosis in pectinids is related to temperature. Optimum embryonic and larval development varies with temperature and salinity, dependent on the scallop species and on a specific site. For example, Yamamoto (1968) and Bourne, Hodgson and Whyte (1989) report slight variations in the range of optimal temperatures for *P. yessoensis* (T= 10–15 °C and S= 30–40 ppt for the former, and 15–18 °C and 29 ppt for the latter). For the same species, Maru (1985) showed that optimum development of embryos occurred at a higher temperature of 20 °C. However, this does not imply that larval development will be optimal at the same temperature, and consideration to size-specific survival rate must also be given. This was shown for *Pecten fumatus* by Heasman, O'Connor and Frazer (1996). Embryos of this species were seen to develop best at the lowest

temperatures (15–18 °C), whereas larvae initially grew rapidly at 24 °C, but did not survive to metamorphosis; a constant temperature of 21 °C was thus found to yield a maximum number of larvae for settlement. Generally, bivalve larval growth increases with temperature, up to some optimum level, which is species dependent (Bayne, 1983). However, a further increase in temperature causes growth to decline. This was shown for *Chlamys hastata,* where larval growth was faster at 16 °C (5.8 µm/day) than at 12 °C (4.8 µm/day), but was much slower at 19 °C (2.5 µm/day). Similarly, larvae reached a mature stage more quickly when reared at 16 °C, as compared to 12 °C (Hodgson and Bourne, 1988). It is thus of benefit to investigate the highest temperature threshold for larval rearing for the species studies. As a general rule, bivalve larvae reared at temperatures close to their tolerance limits suffer high mortality (Ansell, 1961).

The rearing temperature used for both embryonic development and larvae in Bermuda is similar and was determined through trial and error, rather than a scientific study. The optimal temperature for egg incubation may therefore be worth investigating in these two species, as results in percentage yield of D-larvae varies widely over the years (Table 3.1). However, it has to be noted that bacterial proliferation is associated with high temperatures, and the balance between optimal larval development and low bacterial numbers, needs to be achieved. On the other hand, although scientific investigations may be worthwhile in determining optimal rearing temperatures for larvae, in light of increasing veliger and pediveliger yield, it appears that T= 24 °C yields satisfactory results for both of these scallop species.

3.3.2 Density

Density-dependent mortalities have been described by some workers (Loosanoff and Davis, 1963; Gruffydd and Beaumont, 1972). Initial densities of 5–6 larvae.ml^{-1} have been described as satisfactory for some bivalve larvae (Jespersen and Olsen, 1982, DiSalvo *et al*., 1984). Hodgson and Bourne (1988) report that highest survival for *P. yessoensis* was observed when initial density was 2 larvae.ml^{-1}. Densities used for *A. gibbus* (15 eggs.ml^{-1}) and *E. ziczac* (10 eggs.ml^{-1}) fall into the average range used. Velez, Alifa and Perez (1993) maintained the density at 5–10 larvae.ml^{-1} for *E. ziczac* throughout its larval life; and do report a low tolerance of this species to high density, especially as they approach settlement. On the other hand, Costello *et al.* (1973) incubated *A. gibbus* eggs at 25 eggs.ml^{-1} initially, reducing the concentration to 10 larvae.ml^{-1} at the D-larval stage. It is difficult to evaluate the effect of this initial high density as D-larval yields are not reported.

3.3.3 Salinity

Most rearing of pectinid larvae has been carried out using the local seawater supply, also used for the maintenance of the adults, with salinities within the range of 30–35 ppt. Reduced salinities adversely affect growth of veligers and severely affect embryonic development (Gruffydd and Beaumont, 1972). The degree to which development is affected is species dependent. Embryonic development of the Japanese scallop can take place over the range of about 14-21.5 ppt salinity with a marked reduction in the rate of development at either end of this range (Maru, 1985). Gruffydd (1976) found that survival of *Chlamys islanladica* veligers over a 24-hour exposure period was little affected by salinities as low as about 21ppt, but markedly reduced by salinities of about 14 ppt with salinities of about 7 ppt causing 100 percent mortalities. Culliney (1974) noted that veligers of the queen scallop, *P. magellanicus*, could survive for 48 h at salinities as low as 10 ppt, though there was evidence of tissue swelling and the larvae were incapable of normal swimming. Bourne, Hodgson and Whyte (1989) show little effect of salinity on larval growth of the Japanese scallop; and growth rate averaged 6.9 µm per day at a temperature of 18 °C. Pectinid species showing tolerance

to differences in salinities, such as the bay scallop, are species naturally found in salinity fluctuating environments. The bay scallop, related to the calico scallop studied in Bermuda, is found in bays, sounds and estuaries, where heavy rains will cause salinity reductions at times to as low as 10–12 ppt (Duggan, 1975). It may explain its steady larval growth rate of 10–15 µm.day^{-1} when reared between 25 and 30 ppt. On the other hand, *A. gibbus* is not normally found in the natural environment in low or fluctuating salinity conditions. It can be assumed that its tolerance to lower salinity or to fluctuating salinity will be low, and be reflected in poor larval growth.

Salinity of ambient seawater in Bermuda is constant at 36 ppt throughout the year. There are no adjustments made to the rearing salinity, as both species of scallops used are well adapted to the ambient conditions. Interest was generated in investigating the larval survival and growth of calico scallop larvae with varying salinities for the potential of culturing this species in Gulf of Mexico waters, where salinities can fluctuate daily from 20 to 35 ppt (Norman Blake, *pers. comm.*). The bay scallop, *A. irradians*, appears to tolerate these fluctuations in salinity; the question was the degree of tolerance of *A. gibbus*. Fluctuations could not be simulated at the hatchery in Bermuda; however, three salinities were tested for rearing of larvae, using 3-litre beakers. It was found that, although *A. gibbus* shows some tolerance to salinity reduced by 8 ppt and even 16 ppt, decreased salinity does seem to a have a negative marked effect in both survival rate and growth. The difference in survival to the pediveliger stage for larvae reared at ambient salinity (36 ppt) and at 20 ppt approximates 12 percent. Both shell and tissue growth of calico scallop larvae were also seen to be negatively affected by reduced salinity, especially towards the end of the larval life. This would most probably affect settlement of these larvae and the post-larval yield and growth of surviving scallops.

3.3.4 Food ration

Molluscan veliger larvae feed by means of ciliary currents on the velum. Hence, once the straight hinge larval stage is reached, the larvae are planktotrophic and feed on unicellular algae. Rates of clearance of particles from suspension are dependent on particle size, concentration of particles, larval size, density of larvae and temperature (Bayne, 1983). Hence, adequate diet in the hatchery environment needs to be assessed in terms of algal species used (see Chapter 2) and amount of algal cells provided (food ration). The optimal or critical cell concentration provided can be defined as that density where all food cells are taken in and no pseudofaeces are produced (Schulte, 1975). Cary, Leighton and Phleger (1981) showed on video films of *Hinnites multirugosus* larvae (purple-hinge rock scallop) that at high concentrations, mechanical interference was observed, coupled with heavy pseudofaeces production and severe packing of the gut. These authors contend that a finite larval-algal cell encounter/ingestion ratio exists beyond which increasing cell concentrations promote less growth due to the factors previously described. Furthermore, bacterial contamination at high concentrations and the build-up of ectometabolites may render an acceptable diet toxic to developing bivalves (Loosanoff and Davis, 1963).

The protocol used at the hatchery is derived from several in-house trials, as there was little reference in the literature on optimal food ration for calico and sand scallops. Velez, Alifa and Perez (1993) report rearing *E. ziczac* larvae to the pediveliger stage with a food ration of 30 000 cells.ml^{-1}.day^{-1} to 70 000 cells.ml^{-1}.day^{-1} for a larval density of 5–10 larvae.ml^{-1}. With this regime and a constant temperature of 26 °C, the pediveliger stage was achieved in 10–12 days after fertilization; survival rate is not provided. Rojas, Velez and Azuaje (1988) recommend an initial density of 5 larvae.ml^{-1} for *E. ziczac* fed a ration of 10 cells.ml^{-1} with a diet based on *Isochrysis* aff. *galbana* (clone: T-Iso) and *T. pseudonana* (clone: 3H), and end with a density reduced to 2-3 larvae.ml^{-1} with a ration of 70 cells.ml^{-1}. Preliminary studies in Bermuda (Hohn,

Sarkis and Helm, 2001) on the sand scallop showed that food rations comparable to those of Velez, Alifa and Perez (1993) throughout larval life yielded minimal survival to the pediveliger stage (51 percent of Day-2 larvae), compared to those fed the standard and lower food ration (77 percent of Day-2 larvae). On the other hand, there was no significant difference in shell growth for larvae fed the highest food ration. Costello *et al.* (1973) reported a daily ration of 60 000 cells.ml^{-1} for calico scallop larvae throughout their larval life when reared at 10 larvae.ml^{-1} and a temperature of 23±2 °C; although growth and development to the pediveliger stage was achieved, survival rate is not given. In a hatchery where maximal production is aimed for, it is worthwhile to investigate more closely the larval requirements with size.

3.3.4.1 Effect of food ration on calico scallop larvae

Preliminary studies in 2-litre culture beakers showed that the standard ration initially used at the hatchery was inadequate at certain times throughout the 13 day larval life of calico scallops, in terms of shell growth (Hohn, Sarkis and Helm, 2001). Based on this preliminary study, where three food rations were tested, a second ration schedule was tested using large scale larval cultures (1 000 l), throughout a complete larval cycle during a hatchery run. This second ration schedule provides double the standard amount of algal cells on a daily basis. Hence, control rations ranged from 10 cells.µl^{-1} to a maximum of 21 cells.µl^{-1} and tested ration ranged from 20 cells.µl^{-1} to a maximum of 42 cells.µl^{-1}. Both rations proved adequate in providing nutritional requirements to larvae for growth to the pediveliger stage. The higher food ration seemed to benefit larvae in the middle of the cycle (Day-5 and Day-9), reflected in survival rate only. Nonetheless, a higher yield of pediveligers was obtained from larvae fed the standard (and lower) food ration. Although, differences in food ration were not seen for shell growth, accumulation of reserves reflected in tissue growth was noticeably greater at the end of the larval life in scallops fed the higher food ration. It has been reported that higher reserves in larval life may have an effect on settlement rate, and post-larval growth, influencing the storage of energy reserves and survival of spat (Whyte, 1987). It is therefore advantageous to manipulate food ration to achieve not only high survival rate, but also to ensure enhanced settlement and post-larval growth.

As required food rations are species-specific, consideration must be given to environmental parameters to which the species is acclimated. For example, Bourne, Hodgson and Whyte (1989) reported feeding schedule for *P. yessoensis*, following numerous feeding trials as being best as follows: Initially, *C. calcitrans* is fed to veliger larvae and the algal density in the larval tank is 5 000 cells.ml^{-1}. As the larvae grow, additional algal species are fed and the algal density is increased. Such that, by "Day of Setting" (21 days for this species), an algal density of 20 000 cells.ml^{-1} is maintained in the larval tank, composed of a mixture of *C. calcitrans*, *Isochrysis* and *T. pseudonana*. These authors had the use of a coulter counter, which provided a daily measure of the amount of algae consumed by larvae. In this way, a supplement of algae is added to equate the desired density. Being able to measure consumption of algae also enables monitoring of the health of a larval culture, since healthy larvae will actively swim and graze the algae. Unfortunately, the hatchery in Bermuda did not have access to a Coulter Counter, and it was simply assumed that larvae consumed all of the algae provided on a daily basis. In general, clearance rates increase with the size of the larva at any one particle concentration (Wilson, 1980); however, if particle concentration (or algal density) is above a certain threshold, larvae may reject particles, interfering with feeding and ultimately growth and survival (Bayne, 1983). For this reason, feeding rations are maintained relatively low in Bermuda, to avoid excess uneaten algae leading to increased rejection of algal cells, bacterial proliferation and contamination of larval culture. Food rations provided are within the range investigated by Lu and Blake (1996) for a related species, *A. irradians concentricus;* these authors determined

an optimal concentration of 20 cells.µl^{-1} for larvae. Finally, care should also be taken to protect larval tanks from bright sunlight, so that algal blooms do not occur and thus create an overfeeding situation.

3.3.5 Culture systems: flow-through vs. static

Traditionally, bivalve larvae are reared in an aerated static system where treated water is changed regularly throughout the larval cycle. Larvae are provided with a daily food supply and are collected prior to every water change. Routine hatchery protocols for the calico and zigzag scallops in Bermuda involve a static system. Although this protocol provides satisfactory results, this method is labour intensive, and requires at times the use of antibiotics to control bacterial contamination within the larval cultures. These two factors play a limiting role in optimizing hatchery rearing of bivalves in general (Southgate and Ito, 1998; Andersen, Burnell and Bergh, 2000). Flow-through systems have been recently attempted with larvae of *Pinctada margaritifera* L. (Southgate and Beer, 1997; Southgate and Ito, 1998), and with *P. maximus* scallop larvae (Andersen, Burnell and Bergh, 2000). Design of the flow-through system appears to be one of the major factors in success of larval rearing. It is worthy of investigation as an adequate flow-through system for rearing of scallop larvae would offer a number of advantages over the conventional static culture systems, including reduced physical handling of larvae, reduced labour demand, and a reduced dependence on the use of antibiotics due to an improved water quality. Additionally, in a space-limited environment such as Bermuda, a flow-through system might provide a greater tank capacity to space ratio.

In-house studies in Bermuda investigated various designs for a flow-through system, using the available 200 litre conical tanks. Comparisons in the larval and post-larval yield of calico scallops reared in a flow-through system are made with scallops reared in static systems for the same larval batches. The flow-through system used is illustrated in Figure 3.6. The differences with the more conventional static system lie in the nature of the seawater flow and the procedure for maintenance. In flow-through systems, seawater flows at a steady rate continually, reducing the necessity for frequent and complete water exchange. For this reason, procedures for maintenance of this system revolve around the daily cleaning of the banjos on the outflow, and ensuring a constant water flow. Sarkis, Helm and Hohn (2006) explain in greater detail these differences and the procedure followed in flow-through rearing. This study also provides results of comparative larval and post-larval growth using a flow-through system and a static system.

The flow-through system described here and tested was developed over the course of three years; many preliminary studies were conducted to ensure an optimal water circulation within the tank, a constant water flow, and a steady supply of algal food. The difficulty in designing a successful flow-through system lies in the minimal handling of the system, and lack of assessment of the culture throughout the larval development, rendering difficult the identification of time periods where the culture performs well or poorly. Preliminary studies focusing on the latter, indicated that Day-8/Day-9 after fertilization, when larvae are pre-metamorphic, was a critical period; in that, high growth and survival were obtained in the flow-through system until this point, but a collapse in culture ensued thereafter, related in part to excessive food ration leading to the clogging of the outflow, restriction in water flow, and accumulation of detritus within the tank. For this reason, one water change was performed at this time to allow for fractionation and culling of larvae, in order to optimize survival rates to metamorphosis; and food ration was decreased in the latter part of the larval life; at this time, extreme care was taken in maintaining a constant water flow by repeated cleaning of the outflow banjo. Southgate and Beer (1997) in their trials of flow-through for oyster larvae utilized a larger surface area allowing for outflow of water. Despite

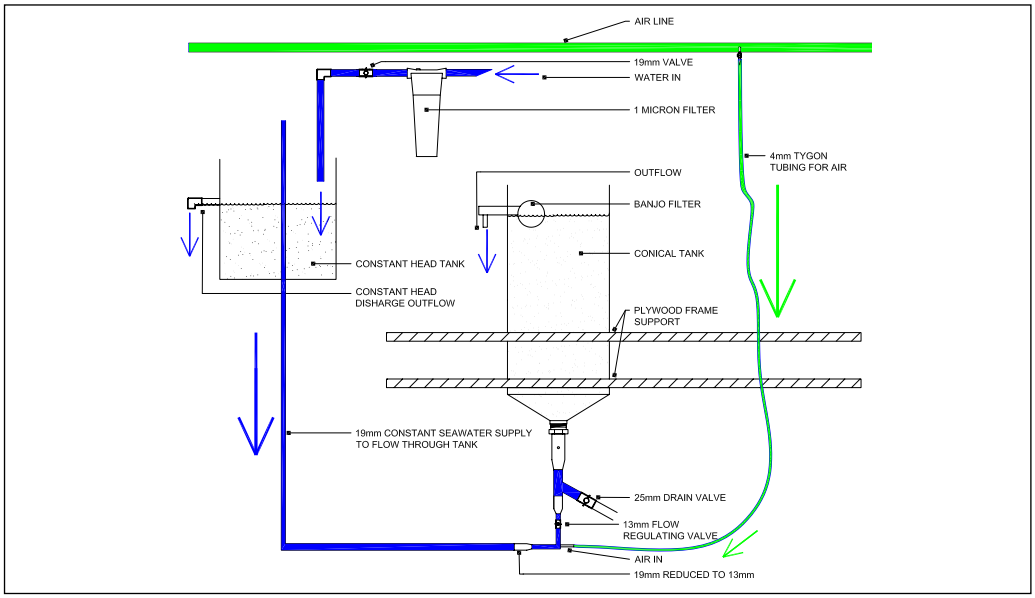


Figure 3.6: Conical tank modified to a flow-through system for larval rearing.

this larger surface area, their results showed a similar trend where larval survival was high during the early part of larval life, and decreased to 7.2 percent survival to the pediveliger stage (Southgate and Ito, 1998). Andersen, Burnell and Bergh (2000) also explained poor larval survival for *P. maximus* reared in a flow-through system by poor water quality caused by overfeeding. Fractionation and culling of larvae during the water change, is likely to enhance growth and survival of larvae. It has been implied that growth of smaller larvae may be inhibited by larger larvae (Bourne, Hodgson and Whyte, 1989); conversely, the presence of dying larvae may have a negative overall effect on the larval culture, as seen in preliminary experiments towards the end of the larval cycle in the flow-through systems. By incorporating a water change towards the latter part of the larval life, and optimizing the daily maintenance procedure, results of this study demonstrate a comparable performance of the flow-through system in terms of pediveliger yield to that of the static system throughout the larval life regardless of density (Sarkis, Helm and Hohn, 2006).

Pediveliger yields are evidently an important assessment of larval rearing conditions, yet, in an aquaculture operation, the true goal is the yield of fixed spat, ensuring an adequate juvenile production. For this reason, the evaluation of the percentage of spat fixed when reared under different larval conditions was determined in the present study. One of the most critical factors is the accumulation of reserves throughout the larval life, related to food ration in both a qualitative and quantitative sense (Farias, Uriarte and Castilla, 1998). It has been shown that rearing conditions affect storage and utilization of biochemical components, and hence metamorphosis and settlement (Gallager and Mann, 1981). Only one diet was provided in the present experiment for all treatments such that nutritional value was not evaluated; the diet chosen was the standard one utilized at BBSR that has proven adequate over the years for ensuring good spat settlement. Quantitatively, food ration was calculated on the basis of water volume within the tank; such that, in the flow-through system, algal ration was calculated based on water flow, equating to approximately 600 litres of water, as opposed to the 200 litres volume of the tank itself. This food ration provided comparable results when larval density was initially at 8 larvae.ml^{-1} or 1.6 million larvae in a 200 litre tank; the number of spat fixed approximated 30 percent of the pediveligers for all treatments, in accordance with results obtained for other pectinid species, as for example a 40 percent yield to 1 mm size for *P. magellanicus* (Couturier, Dabinett and Lanteigne, 1996). On the other hand, it appears that food ration may not have been sufficient, once larval

density was increased, for the accumulation of reserves necessary for settlement. The spat yield for flow-through reared pediveligers at a higher density, was much lower than that for those reared in the comparable static system and the standard hatchery system. This density effect was also reflected in the lower shell growth of the fixed spat, as seen when compared to the static system and when compared to the flow-through reared larvae at lower density (Sarkis, Helm and Hohn, 2006).

In conclusion, the results obtained in flow through larvae culture with calico scallops suggest that in resource-limited regions this concept is worth investigating. The benefits lie in the reduced labour involved, the absence of antibiotic use, and the optimization of space availability. These three factors are of economical importance, possibly rendering a hatchery operation more cost efficient.

3.4 TECHNIQUES – STANDARD PROTOCOL FOR REARING CALICO AND ZIGZAG SCALLOP LARVAE

The rearing of calico scallops was developed and well tested at the Bermuda hatchery between 1999 and 2003. The procedures developed give satisfactory results with respect to both larval yields and shell growth rate. In 4 years of hatchery operation yields of D-larvae for calico scallops determined per spawn, ranged from 29.3–58.1 percent, and yields of pediveligers ready for settlement were in the range of 17.8–55.4 percent; pediveliger yields are calculated as percentage of Day-2 D-larvae. (*Note: Throughout the following sections, Day-2 larvae refer to straight-hinge D-larvae developed two days after fertilization*). These yields are in accordance with results obtained by other authors for pectinid species; Rupp (1997) reported a 12.5 percent pediveliger yield for *Nodipecten nodosus*, Uriarte *et al.* (1996) report a range of 17.6–27.8 percent for *Argopecten purpuratus*, and Couturier, Dabinett and Lanteigne (1996), report 50 percent for *P. magellanicus.* On the other hand, zigzag scallop yields are generally lower, and are indicative of the greater sensitivity of this species to handling and bacterial contamination. The range of D-larvae obtained from the number of fertilized eggs was of 0.96 to 49.2 percent per spawn over four years of operation, and for pediveligers of 2.1 to 11.7 percent.

Shell growth rates, vary among pectinid species, ranging from 4.8 µm.day^{-1} for a cold-water species such as the rock scallop (Bourne, Hodgson and Whyte, 1989) to 14.8 µm. day^{-1} for the tropical scallop *N. nodosus* (DeLa Roche *et al.,* 2002). A mean growth rate of 10 µm.day^{-1} is strived for, when rearing both calico and zigzag larvae in Bermuda; this is a relatively fast growth rate when compared to other pectinid species. Throughout the larval life, shell length and height are measured routinely at every water change to assess the state of the larval culture. Figures 3.7 and 3.8 provide shell growth data for calico and zigzag scallops reared in routine hatchery operation. Data shown is a summary of results from the past four years of operation, illustrating the maximal and minimal shell growth curves for batches with average yields. Shell length of Day-2 zigzag larvae range from 93.1±83.1 µm to 113.3±4.3 µm. Pediveligers for this species reach shell length of 161.3±22.5 µm to 226.6±45.9 µm. For the calico scallop, Day-2 larvae have a shell length of 92.7±4.7 µm to 102.9±4.5 µm. Pediveligers for this species reach shell length of 184.2±14.0 µm to 203.5±16 µm. Pediveliger shell length is lower than that reported in the literature for the same species (Costello *et al*. 1973). This may also be attributed to the fact that settlement was usually initiated as early as possible to avoid sticking of pediveligers on the sides of the culture tanks. For this reason, shell length provided here, may underestimate maximal pediveliger size.

Daily growth rate for the same larval cycles as in Figures 3.7 and 3.8 are shown in Table 3.2. For both the zigzag and calico scallop, the differences in overall shell growth

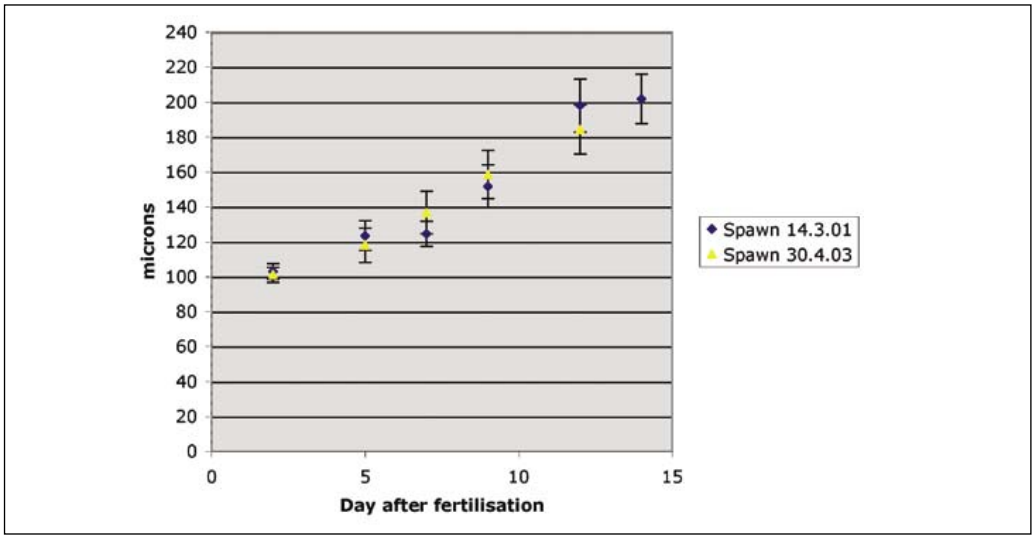


Figure 3.7: Results of shell growth (length) for calico scallop larvae reared in Bermuda. Two curves show maximal and minimal range obtained over 4 years of operation.

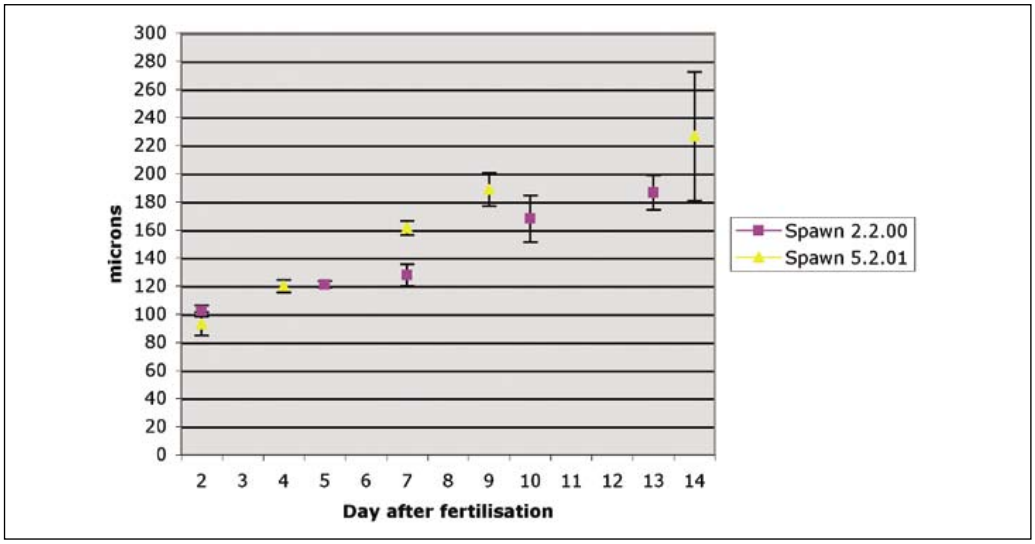


Figure 3.8: Shell growth (length) for zigzag scallop larvae reared in Bermuda. Two growth curves show maximal and minimal length obtained over 4 years of operation.

between a "good" run and a "bad run" can be attributed to the slow growth rate at the beginning of larval life between Day-5 and Day-7. Both the shell length curves and the daily growth rate can be used as a standard range for future hatchery rearing of these species.

Table 3.2: Daily growth rate for both zigzag and calico scallop larvae reared in Bermuda. Results are shown for two larval batches, one illustrating minimal shell growth and the other illustrating maximal shell growth.

Days after fertilization	Zigzag scallop growth rate (µm per day)		Calico scallop growth rate (µm per day)	
	Minimum	Maximum	Minimum	Maximum
D2–D5	9.5	13.5	6.9	5.7
D5–D7	3.3	13.8	0.6	9.3
D7–D10	13.3	13.7	13.6	10.9
D10–D13	6.2	7.5	15.4	8.6

3.4.1 Larval rearing procedure

In light of the in-house studies described above, a standard procedure was developed and adhered to for the rearing of calico and zigzag scallop larvae in Bermuda. The more conventional static rearing system is routinely followed, where square 1 000 litres capacity tanks with lids, are preferred mainly due to their insulation characteristic. This allows for rearing of larvae at a temperature higher than ambient. Temperature fluctuation within larval tanks does not exceed ± 0.5°C between water changes. (*Note: The changeover to flow-through system is considered, but the purchase of new tanks is necessary for this*). Rearing temperature is 24±1 °C and salinity is ambient (36 ppt) from the egg stage to settlement stage. Water change is conducted three times a week; at this time, larvae are collected on two sieves of differing mesh size, such that fastest growing larvae are separated from the slower growing or dying larvae. Collected larvae are transferred to temporary containers (10 litres buckets), while tanks are cleaned and re-filled with treated seawater. Any assessment of larval culture is done during this transfer period. Once tanks are ready, larvae are re-distributed, often pooling larvae of similar size into one tank. Beginning Day-2 after fertilization, a small supply of air is given to the larvae, via a small diameter air tube, reaching the bottom of the larval tank (see technical drawing – page 13). The air supply, controlled by a labcock ball valve, is turned on low to only allow one air bubble at a time. Feeding of larvae is provided in a single batch at the same time each day, and after re-distribution of larvae during water change days.

3.4.1.1 Water change

The schedule followed pertains to a working week from Monday to Friday; although feeding and routine checks are conducted daily (including Saturday and Sunday), water change days are avoided over the weekend. Spawns are conducted as outlined in Chapter 1 (Protocol–4), preferably on a Wednesday; the reason for this is related to the length of the larval life (12–14 days). Depending on batches, larvae are ready to be set either on Day-12 or Day-14 after fertilization. Spawning on a Wednesday, results in Day-2 larvae to be distributed on a Friday, and "Setting Day" to be either on a Monday (Day-12), or a Wednesday (Day-14). In this way, any mortality due to delayed set is avoided. Water change days are therefore, Monday, Wednesday and Friday.

Initially Day-2 D-larvae are collected on 40 and 60 µm sieves. Depending on batches, a certain percentage of larvae are large enough to be collected on a 60 µm sieve; however, for the most part, healthy D-larvae are collected on a 40 µm sieve. Some variations are seen among tanks at times. Any abnormal larvae or undeveloped eggs either pass through the 40 µm sieves to drain, or are thereafter discarded should they prove to be the majority of the collected larval culture. Pull-down, or take-down, of tanks is conducted slowly, to avoid crushing of larvae onto the sieves or damage to the shells. For a 1 000 litres tank, 45–60 minutes is allowed for take-down. The model hatchery has four 1 000 litres larval tanks, take-down is initiated at 30-minute intervals to minimize transfer period for each larval batch. Each larval tank is attributed a number (LI to LIV), facilitating the tracking of larval cultures.

Protocol–11 outlines the details for taking-down of larvae; this applies at every water change; the only difference being the increasing mesh size for larvae as they grow and develop. Table 3.3 is a summary of mesh sizes used throughout larval development for calico and zigzag scallops, as well as the relationship between mesh size and larval size. Throughout tank pull-down, larvae are maintained in water, to avoid dehydration; for this reason, sieves are suspended in a tray, and submerged in seawater. Once a tank is completely drained, a thorough rinsing with filtered seawater is done with a gentle flow to ensure that any larvae collected in corners and drain is washed onto the sieves. Once all larvae are collected from the larval tank, immediate transfer into pre-cleaned 10-litre

Table 3.3: Relationship between mesh size and larval size retained on it as well as mesh sizes used specifically for the sand scallop and the calico scallop throughout larval development.

Mesh size (µm)	Size of larvae retained	Days after fertilization	Mesh size used for zigzag and calico scallops
44	D-stage to 110 µm	2	40 & 60
120	>113 µm	5	40 & 60
150	≥170 µm	7	60 & 80
160	≥212 µm	9	80 & 120
180	≥255 µm	11	80 & 120
200	≥280 µm	13	150 & 120

buckets allows larvae to remain suspended; larvae collected on different mesh size sieves are transferred into separate containers. Buckets are labeled with the larval tank number and size of mesh upon which larvae are retained. Holding of larvae in buckets should not exceed one hour; this is a stressful period for larvae and diseases that may be present could spread rapidly. Samples are taken at this time for counting of larvae. For re-distribution, larvae of similar "health" and size fractions are pooled; if a culture contains a greater percentage of dead or abnormal larvae than healthy larvae, it is discarded. [*Note: Other hatcheries perform bacteriological tests to assess the health of larval cultures; as for example, is reported by Neima and Kenchington (1997), who take samples and plate them on CBS and Marine Agar using standard bacteriological procedures. From these plates, they perform a microbiological screening for a yes/no response. Results of this screening are used to make decisions regarding combining groups of larvae on subsequent change days or discarding larvae*). Larvae are passed through a large mesh sieve (300 µm) for elimination of debris as they are distributed into larval tanks. To avoid damaging larvae, the previously cleaned sieve is held in the larval tank so that the mesh is submerged, and contents of the transfer bucket are gently poured through it. To ensure that all larvae are transferred, the holding bucket is rinsed with filtered seawater and its contents poured through the sieve; this rinsing process is repeated twice.

At the time of distribution, the number of larvae per tank is recorded along with density and volume of water in tank. On a daily basis, routine checks are made at the very beginning of the day and records are made on the hatchery check sheets provided for each tank. Appendix 15 provides a sample check sheet. During these checks, air supply is verified, ensuring that flow is not too high or stopped; temperature of the tanks is recorded and care is taken to clean the thermometer between each tank to avoid contamination; any observations related to the state of the culture, for example detritus on the bottom, is written on the check sheet and algal food ration and composition provided are recorded for each tank.

Sieves required throughout larval and post-larval cultures can be made using available materials. Large diameter pipes can be cut and transformed into sieves by gluing fine mesh on one end. Details of construction are given in Appendix 17.

PROTOCOL–11

TAKE-DOWN OF LARVAL TANKS: LARVAL COLLECTION AND REDISTRIBUTION

1. Backwash as per Appendix 6.
2. Do routine checks of broodstock, larvae, post-larvae and algae.
3. Set-up Heating Unit as per Appendix 4.
4. Rinse 1 µm filter, filter housing and 20 mm inner diameter hose with fresh water. Set up second 1 µm filter onto appropriate fittings above larval tanks. Adjust 20 mm ID hose to filter via hose barb (see technical drawing – page 13).

5. Clean sieves and support rings to be used by scrubbing with a cloth soaked in chlorinated fresh water and rinsing well with fresh water.
6. Clean 4 trays similarly (same trays as used for spawning).
7. Remove 4 hose fittings from chlorinated bin and rinse thoroughly with fresh water.
8. Adjust each labeled hose fitting to respective larval tank. Set up one tray per tank.
9. In each tray, place larger sieve on top of ring so that mesh of sieve is not in direct contact with bottom of tray, and that larvae do not get damaged against tray surface.
10. Suspend smaller diameter sieve into large diameter sieve using a 15 mm pipe. Be careful, smaller diameter sieve, has larger mesh size. Large larvae are collected first; small larvae pass through and collected on second large diameter sieve.
11. Fill tray with filtered seawater. Make sure collecting hose is inside smaller diameter sieve.
12. Open valve of larval tank slowly and ensure that a gentle flow of water occurs. There should not be any air bubbles coming out due to vigorousness of flow.
13. Once first tank is 1/3 down, start second tank.
14. Repeat procedure with all larval tanks.
15. Once all water is collected from the larval tank, rinse sides and bottom of tank with filtered seawater. In this way you are ensuring to collect all larvae.
16. Finish off by washing down drain with filtered seawater.
17. Carefully remove the hose from the sieves.
18. From smaller diameter sieve, wash larvae carefully into a previously cleaned bucket with a gentle flow of filtered seawater from a 20 mm ID hose. Preferably use heated seawater (same temperature as larval rearing temperature).
19. Label bucket with larval tank number and mesh size collected.
20. Place in secure place for counting and shell growth determination.
21. Clean larval tank by rinsing with a vigorous jet of fresh water.
22. Close drain valve, fill bottom of tank with approximately 50 mm of fresh water so that drain area and all corners are submerged. Add one capful of commercial bleach and leave for 10 minutes.
23. Use scrub brush and with chlorinated water scrub sides of tanks and bottom thoroughly.
24. Drain chlorinated water and rinse completely with fresh water including rinsing of lid.
25. Do one final rinse with filtered seawater.
26. Start filling tank with heated seawater to desired volume.
27. Adjust airflow so that air is supplied one bubble at a time.
28. Count collected larvae in buckets and determine survival and shell growth of larvae as described below (see Section 3.4.1.4).
29. Once tank is filled pool larvae according to size and health of culture.
30. Re-suspend in larval tanks maintaining an adequate density (see Table 3.4 below).
31. For re-suspension, pass larval culture through a pre-washed 300 µm sieve held at surface of larval tank so as to remove any debris in culture.
32. Feed larvae as required (see Table 3.5 below and Protocol–12). Replace lid.
33. Once all larvae are re-suspended, clean hatchery as outlined in Appendix 7.

3.4.1.2 Standard rearing density

Larval density within one tank, does not exceed 8 larvae.ml^{-1} initially, and 5 larvae.ml^{-1} towards the end of larval life. Densities gradually decrease with length of larval period as larval mortality naturally occurs. Tank volume is adjusted at times in order to maintain a density of larvae appropriate for growth. Typical densities throughout

larval life are given in Table 3.4. As larvae approach metamorphosis, lower densities (1–2 larva.ml^{-1}) are found more favourable to growth and development.

Table 3.4: Larval densities in rearing tanks throughout larval life during a typical hatchery cycle.

Days after fertilization	Density (larvae.ml^{-1})	Larval stage
0	12.4	Fertilized eggs
1	12.4	"
2	6.2	D-larvae
3	6.2	"
4	6.2	"
5	4.8	Umbone development
6	4.8	"
7	2.9	"
8	2.9	"
9	1.8	Eyespot appearance
10	1.8	"
11	1.8	"
12	1.8	Set

3.4.1.3 Standard food ration

Food ration and composition given during standard hatchery procedure is given in Table 3.5. The first ration of food is provided at the first appearance of veliger larvae (24–48 hours after fertilization). Consequently to food ration studies conducted in-house (see section 3.3.4.1), algal food ration is increased on Day-9 and Day-10 of larval life.

Table 3.5: Food ration and composition used in rearing of calico and zigzag scallop larvae.

Days after fertilization	Algal Species and Ratio	Cells.µl^{-1}
0	0	0
1	T-Iso	7
2	T-Iso:Chaeto (1:1)	10
3	T-Iso:Chaeto (1:1)	10
4	T-Iso:Chaeto (1:1)	10
5	T-Iso:Chaeto (1:1)	12
6	T-Iso:Chaeto (1:1)	14
7	T-Iso:Chaeto (1:1)	18
8	T-Iso:Chaeto:Tetra (1:1:1)	18
9	T-Iso:Chaeto:Tetra (1:1:1)	21
10	T-Iso:Chaeto:Tetra (1:1:1)	21
11	T-Iso:Chaeto:Tetra (1:1:1)	18
12	T-Iso:Chaeto:Tetra (1:1:1)	18
13	T-Iso:Chaeto:Tetra (1:1:1)	18
14	T-Iso:Chaeto:Tetra (1:1:1)	18

3.4.1.4 Counting larvae and determining survival rate and shell growth

To determine survival rate, counting of larvae in a sub-sample is done following transfer to the holding buckets. Larvae are gently mixed using a homemade plunger (see Appendix 8). Thorough mixing is obtained by a continuous up and down motion with the plunger, taking care not to touch the bottom of the bucket, to avoid crushing larvae, and staying below the surface of the water, to prevent any splashing or bubbles, which may be damaging to the larvae. During mixing, aliquots of larvae are sampled using an Eppendorf pipette. Aliquots of 1 ml are placed onto a Sedgewick Rafter Cell, and fixed with two or three drops of 10 percent formalin. Counts are made systematically by moving from one end of the grid, scanning the slide up and down to the other end. For larvae located on lines of the grid, care must be taken not to count

them twice. Triplicate aliquots are taken for each larval fraction. To determine survival, the average number of larvae counted in three aliquots is calculated, and used in the following equation:

Total number of larvae collected =
Average (larvae per ml) x Volume of seawater in bucket (ml)

During routine hatchery procedure, the same aliquot of larvae used for assessing survival is used for assessing shell growth; length is defined as the maximum distance across the shell, parallel to the hinge; height is perpendicular to length, being the maximum distance from the hinge to the edge growth. Measuring 20 larvae, usually gives a good indication of growth rate between water changes. Comparisons are made with growth curves given in Figures 3.7 and 3.8. For any scientific studies however, a minimum number of 50 larvae should be measured. In the hatchery, a compound microscope using an ocular micrometer is sufficient for assessing length; some hatcheries have access to more sophisticated equipment, such as an image analysis program connected to a camera. Although these are extremely useful in detailed scientific studies, they are not necessary for routine assessment of growth.

3.4.1.5 Setting of larvae

The detailed procedures for setting larvae and post-larval rearing are given in Chapter 4. Determining maturity of larvae and readiness to set is discussed in this section as the conclusion to larval life. As larvae approach metamorphosis, selecting the right day for initiating settlement is crucial. The reason being that if larvae are ready to settle, but maintained in larval tanks, high loss of pediveligers will most probably occur. This loss may be due to two factors. The first is linked to the substrate search behaviour of pediveligers, which will lead them to settle on the sides of the larval tanks; they then, become difficult to dislodge, and become damaged in the process. The second factor is the occurrence of high mortality in metamorphic larvae observed when left too long in larval tanks. In order to avoid this loss of pediveligers, certain criteria are followed at the hatchery in Bermuda to decide whether or not larval batches are ready for settlement.

This set of criteria includes size, morphology and behaviour, and is similar to that used by other aquaculturists for the determination of mature larvae. Bourne, Hodgson and Whyte (1989) found that mature Japanese scallop larvae are 260–280 µm in shell length, and collected on a 180–200 µm screen; this is similar to size criteria for *P. magellanicus* (Neima and Kenchington, 1997). The presence of a well-developed foot, developing gill bars and eyespots approximately 10 µm in diameter, aid in the identification of mature larvae. Neima and Kenchington (1997) calculate percent eyespots and foot activity in samples for a more accurate determination. Eyespots are not as conspicuous in all pectinid species, as is the case for calico scallops, and may be difficult to use as a criteria for these species. A change in larval behaviour, from continuous swimming to periods of swimming interspersed with periods of crawling with the foot on a substrate, is most important. At this time, larvae are often collected from the bottom of larval tanks, rather than from the surface layers, unlike during the earlier stages of larval life. In holding containers, mature larvae will often clump together to form mucous strands in the water. This behaviour is called "rafting" and is a sign of healthy and vigorous larvae that are ready to metamorphose.

Criteria used for initiating settlement at BBSR are:

1) Active substrate-search behaviour of larvae, with foot extension and crawling observed when larvae are placed under the microscope.

2) Clinging of larvae to each other when they are in the transfer bucket; this "rafting" behaviour creates a very distinct line formation.
3) All larvae are collected on 120 µm and 150 µm mesh sieves, with the greatest majority on the latter. Mean shell length ranges from 180–200 µm for calico scallops, and 180–225 µm for zigzag scallops. Because not all larvae develop at the same rate, the smaller size fraction may be kept in a larval tank for a further two days prior to setting. This has occurred several times in Bermuda, with comparable settlement and post-larval development of the smaller size fraction (and delayed set) to the larger size fraction.

If these observations are made, it is best to initiate settlement in larvae and end larval rearing. If not, an additional two days in larval tanks is preferable for a better settlement yield. Chapter 4 describes various methods for setting of mature larvae and their subsequent post-larval requirements.