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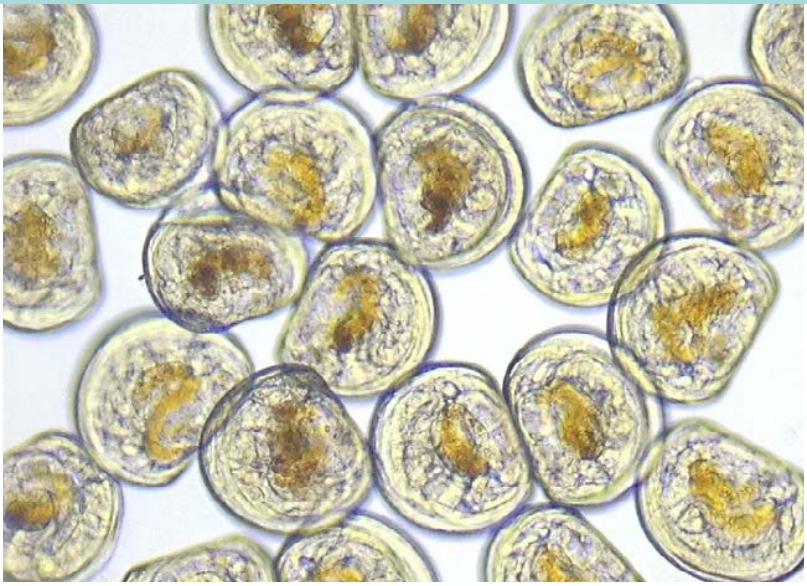
Hatchery-based seed production of the Japanese scallop, *Mizuhopecten yessoensis*


Cover photographs:
Semi-continuous batch culture vessels of microalgae (top left); Yesso scallop D-larvae (top centre); conical tanks used for flow-through rearing of scallop larvae (top right); adult Yesso scallop specimens ready to spawn (bottom left); and Yesso scallop seed (bottom right) (Courtesy of Samia Sarkis).

Hatchery-based seed production of the Japanese scallop, *Mizuhopecten yessoensis*

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Preparation of this document

This manual focuses on hatchery seed production of the Japanese or Yesso scallop, *Mizuhopecten yessoensis*, and is intended as a practical compilation of techniques for all stages of seed culture. Scallops are a high value lower trophic marine bivalve species with minimal impacts to the natural environment when cultured. Producing this species in a consistent and sustainable manner is best achieved through hatchery seed production.

The information presented and the techniques described are a result of scientifically-based experimental studies on the culture of several scallop species tested at pilot-scale and implemented at commercial-scale for the Japanese scallop in various parts of the world. The manual also discusses the transfer of seed out of the nursery and methodologies for transport to the farm sites.

This manual differs from others in that it pertains specifically to the Japanese scallop, a species with a proven potential for commercial culture, and provides a range of hatchery methodologies including low and high technologies applicable to small and large-scale operations.

The overarching goal for the publication of this manual is to contribute to the growth of aquaculture worldwide and improve its performance in the production of nutritional food as well as export products to generate foreign exchange. The larger sized Japanese scallop yields one of the most sought-after shellfish food products; by lending itself to hatchery seed production and suspended farm culture of market-sized animals, it is most likely one of the best suited species of scallops to commercial aquaculture and one of the lower trophic species with an impactful contribution to global aquaculture growth.

This document is one of three technical guides on shellfish culture produced as part of a project funded and implemented by the Food and Agriculture Organization of the United Nations (TCP/DRK/3803) in enhancing coastal livelihoods and food security in the Democratic People's Republic of Korea.

Abstract

This guide is intended as a standalone practical manual for the culture of the Japanese or Yesso scallop, *Mizuhopecten yessoensis*. It is written for hatchery staff as a reference for daily operating procedures and for developing a site-specific and resource-specific seed production strategy. To that end, the whole production cycle is addressed, from broodstock conditioning to transport of seed to the farm. It is the aquaculturist's decision as to whether all stages are required to achieve the target production in a given site and hatchery facility. Standard and more recent emerging techniques are included where possible, for the equal benefit of low and high technological operations.

The manual starts with a brief overview of the anatomy and morphology of the scallop and describes the main organs of the adult specimen and the stages of its life cycle; the anticipated development time between each stage throughout its culture is added for the aquaculturist's benefit. This is followed by a chapter on the culture of live microalgae for food; different approaches to culturing large-scale microalgae are given, including traditional batch culture to the more recent newly designed photobioreactors. Protocols are given from stock to intermediate microalgal cultures for the inoculation of large-scale vessels. The integration of probiotic bacteria as an alternative to standard antimicrobial drugs is described in a separate chapter; this is a critical component of this manual as it is a current and important shift in sustaining optimal larval and spat performance. The need for biosecurity in a full cycle hatchery operation is emphasized and conceptually illustrated in Chapter 4; as much has been written on the design of bivalve hatcheries, the reader is directed to further readings for details.

Culture protocols for scallops start in Chapter 5 with the holding and conditioning of broodstock; assessments of the gametogenic stage, the manipulation of holding temperature to maintain and/or enhance gametogenesis and food requirements are all discussed to ensure the supply of broodstock for spawning when needed. Larval culture is one of the longest chapter of this manual and describes rearing in both static and flow-through systems with the expected growth and survival rate for the Yesso scallop spat. Post-larval culture in the nursery chapter is divided into early post-set up to Day-14, rearing of 1 mm spat and raising seed up to 5 mm or more in a land-based environment; as for larvae, expected performance for the Japanese scallop is given based on real-life experience. The final chapter discusses different strategies affecting the time and size at which seed are transferred out of the nursery facility to either intermediate outdoor nurseries (such as ponds) or to the farm sites. Considerations to be taken into account when selecting a strategy are given. The alternative "remote setting" approach, which bypasses the land-based nursery stage, is also discussed; this is especially useful to large-scale aquaculture operations.

The manual ends with a list of suggested readings providing further details on procedures at various stages, and appendices of working templates for recording data by hatchery staff.

Keywords: Japanese scallop, hatchery, seed production, broodstock conditioning, remote setting, nursery, seed transport

Contents

Figures

Tables

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A practical manual such as this one could not be written without the hard work of many researchers, hatchery managers and technicians; they cannot all be named here, but their dedication, long hours and culturing skills are the backbone of this manual. Thank you to those who provided their photos of larval and early spat stages.

Huge thanks to the many hatcheries which enabled research for the advancement of scallop aquaculture and for the preparation of this manual; special thanks to the Canada Blossom Team who shared their knowledge on Japanese scallop broodstock temperature requirements, to the staff at Coastal Shellfish Corporation for their assistance in applying known techniques to the large-scale culture of this species, and to Nova Harvest for their development in probiotic integration to shellfish culture.

A special thanks to Jaramar Balmori (Mowi Canada West) for her microbiological expertise and assistance for the "probiotic" chapter. Guilherme Rupp (Center for Development of Aquaculture, Santa Catarina, Brazil) is gratefully acknowledged for generously taking the time to scientifically review the entire manual. Massimiliano Lipperi (@studiowildart) applied his artistic talent to producing beautiful illustrations of various scallop culture stages. Graphic design and layout was carried out by Jose Luis Castilla Civit. Last but not least, appreciation is also due to the staff at the FAO Country Office in the Democratic People's Republic of Korea for their support and project coordination.

This manual is dedicated to Neil Bourne, former researcher at the Pacific Biological Station in Nanaimo, British Columbia, Canada, whose work on the larval rearing of the Japanese scallop continues to be relied upon by many to this day and which is the scientific basis for the techniques related in this document.

Acronyms and abbreviations

CFU	colony-forming units
CO_2	carbon dioxide
DI	de-ionized water
GI	Gonadic Index
HCl	hydrogen chloride
LED	light-emitting diode
MI	Muscle Index
PVC	polyvinyl chloride
RPM	revolutions per minute
SI	Système International
SW	seawater
TCBS	thiosulfate citrate bile salts sucrose
TSA	tryptic-soy agar
UV	ultraviolet

<	less than
>	greater than
µm	micrometer (micron)
mm	millimetre
cm	centimetre
µl	microlitre
ml or **mL**	millilitre
L	litre
g	gram

Conversions

1 µm	0.001 mm = 0.000001 m
1 mm	0.001 m = 1 000 µm = 0.0394 inch
1 cm	0.01 m = 10 mm = 0.394 inch
1 µg	0.001 mg = 0.000001 g
1 mg	0.001 g = 1 000 µg
1 g	1 000 000 µg = 1 000 mg = 0.001 kg
1 µl	0.001 ml = 0.000001 l
1 ml	0.001 l = 1 000 µl = 1 cm^3
1 L	1 000 000 µl = 1 000 ml = 0.264 US gallons
1 m^3	1 000 l = 264.16 US gallons
1 cm^3	0.001 l = 1 ml

Scientific units

Scientists have a different way of writing some of the units described in this glossary. They use what is called the Système International (SI). The units are referred to as SI units. For example: 1 ppt, which can be written as 1 g/l is written as 1 $g.l^{-1}$ in scientific journals, 1 g/kg as 1 $g.kg^{-1}$, 12 mg/kg as 12 $mg.kg^{-1}$ and 95 µg/kg as 95 $µg.kg^{-1}$. A stocking density of 11 kg/m^3 would be written as 11 $kg.m^{-3}$. This SI system is not normally used in daily hatchery records, however for the purpose of standardization, it is used throughout this publication.

1. The Japanese scallop

IN THIS CHAPTER – General characteristics of the Japanese scallop, its distribution and life cycle.

1.1 BEHAVIOUR AND DISTRIBUTION

The Japanese scallop, *Mizuhopecten yessoensis* (Jay, 1857), is a cold water marine bivalve species, in the family of Pectinidae. It is synonym with *Patinopecten yessoensis* (Jay, 1857), and also referred to as Yesso or Ezo scallop. Scallops begin their lives as bysally attached juveniles, but rapidly grow into free-living adults; they are one of the few groups of bivalves capable of rapidly swimming short distances, and in some cases even migrate across the ocean floor. The Yesso scallop does not recess into the substrate but lies on top of the seabed; if faced with a predator, it may attempt to escape by swimming swiftly but erratically through the water by clapping its shells together.

Yesso scallops naturally occur in sheltered, shallow bays and inlets adjacent to rocky shores (4–10 m) and up to depth of 40 m. Inshore distribution is limited by ice depth during the winter. Seawater salinity ranges from 32–34 ppt, optimal growth temperature is 4–8 °C with a wide tolerance range between 2–26 °C.

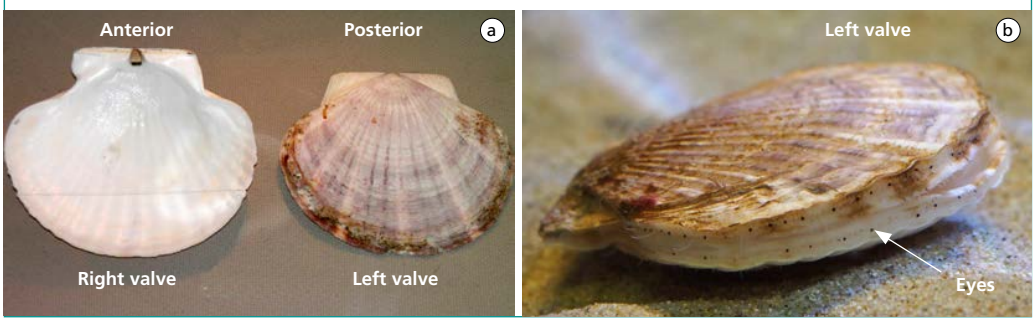
The species is common along the coast of northern Japan. Its native distribution ranges from the Sea of Japan (Democratic People's Republic of Korea, Republic of Korea, and Russian Federation) to the Pacific coast of Japan. The species was introduced for culture purposes in the Northeast Atlantic, the Mediterranean and the Pacific coast of Canada.

1.2 MORPHOLOGY AND ANATOMY

A scallop shell consists of two valves, left and right, and the Yesso scallop rests on its right valve. The left valve is flat or slightly concave, and the right or bottom valve is slightly more convex (Figure 1.1). Both valves are similar in size and are ridged in the Yesso scallop. These "ribs" result in a relatively strong and heavy shell. The shell is streamlined to facilitate ease of movement during swimming. The shells radiate from a winged or eared umbo, seen on either side of the shell's midpoint. Each valve is regarded as having a dorsal, ventral, anterior and posterior margin. The hinge serves as a reference point and is considered the "dorsal" region; the underside is the "ventral"

FIGURE 1.1

Specimens of *M. yessoensis* – (a) left and right valves showing anterior and posterior regions of the right valve; and (b) live scallop sitting on its right valve and showing eyes



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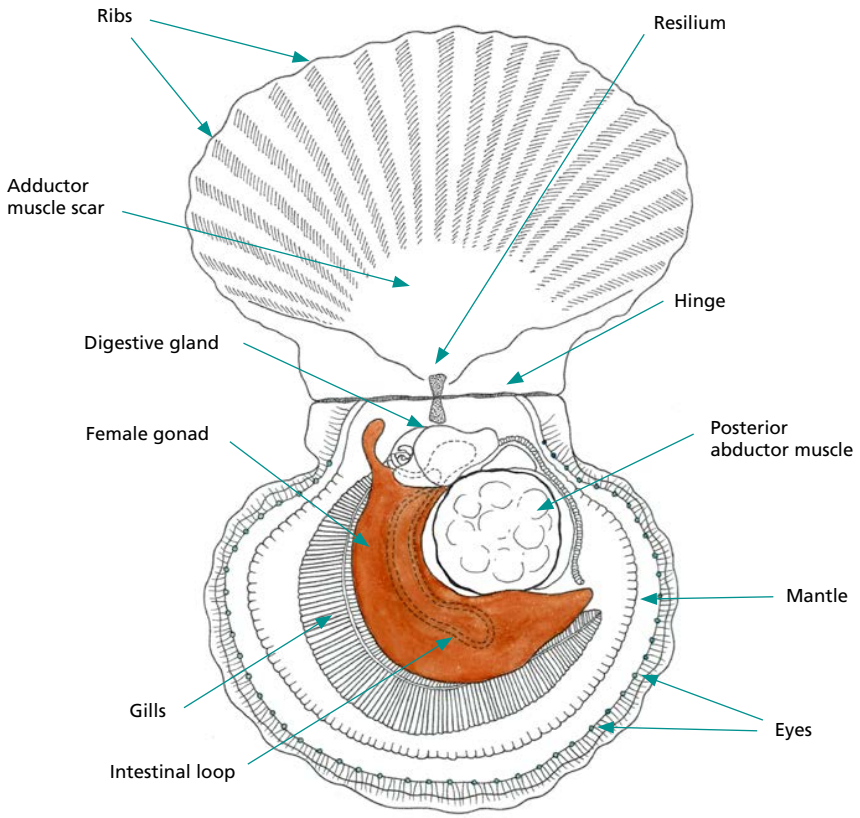
region; the "anterior" region is that closest to the extreme point of the shell margin and the "posterior" is opposite.

As the scallop grows, it lays down distinct growth lines as concentric circles around the perimeter of the shell; these originate at the centre of the hinge (or beak), surrounded by the umbo. Growth rings increase in size downwards until they reach the curved ventral edge of the shell. Slower growth during the winter months concentrates the lines into an "annulus" and can be used as an indicator of age; although, any disruption in feeding, or rough handling, high stocking density in culture operations, might also result in the formation of non-annual concentrations of lines. The main organs of an adult Yesso scallop are illustrated in Figure 1.2.

FIGURE 1.2
Anatomy of the Japanese scallop, *M. yessoensis*



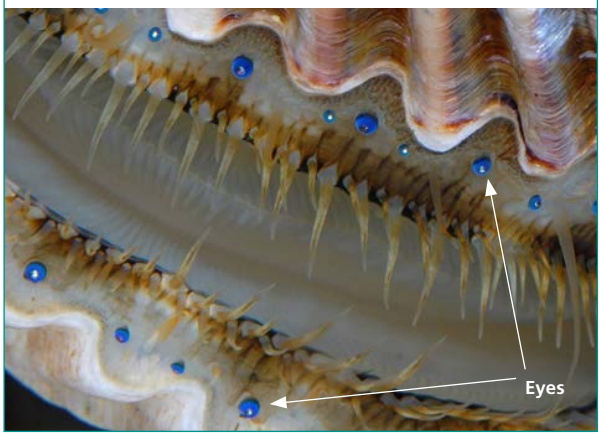
The mantle – It consists of three folds, each serving a separate function. The first fold lies immediately interior to the shell and is responsible for the synthesis of the shell along the thickened outside margin. Attached next is the sensory fold with its tactile organs (tentacles) and eyes (ocelli). The third fold is the velar fold, a muscular flap that plays an important role in swimming. Acting as a flapper valve, it extends to seal the valve margins and is used while swimming. The scallop's movements are achieved with rapid flushing of the water from its mantle cavity; its configuration controls to some degree the direction and distribution of the expelled water. These behaviours are termed coughing, jumping and swimming.

Adductor muscle – Scallops have a single central well-developed adductor muscle, and inside of each shell there is a characteristic central scar, which is the point of attachment for the muscle (Figure 1.2). Scallops have a well-developed nervous system and have a

ring of simple eyes (about 1 mm) situated around the edge of their mantles (Figures 1.2 and 1.3). The nervous system of scallops is controlled by three yellowish paired ganglia, located at various parts throughout their anatomy; the cerebral or cerebropleural ganglia, the pedal ganglia, and the largest visceral or parietovisceral ganglia. The visceral ganglia connects to the circumpallial nerve ring, which loops around the mantle and connects to all of the scallop's tentacles and eyes. The cerebral ganglia controls the scallop's mouth and the statocyst (sensing its position). The pedal ganglia controls movement and sensation in its muscular foot. The foot is most prominent during the early life stage (pediveliger) at the time of metamorphosis and settlement; it is used for crawling and manipulating or attaching threads secreted by the byssal gland located at it base. In adults, the foot becomes vestigial and useless.

Eyes – The characteristic scallop eyes focus and retro-reflect light (Figure 1.3). They rely on concave, parabolic mirror of guanine crystals and possess a double-layered retina, with the outer retina responding most strongly to light and the inner to abrupt darkness. This grants scallops contrast definition and the ability to detect changing patterns of light and motion. Scallops primarily rely on their eyes as an "early-warning" threat detection system, scanning around them for movement and shadows, which could potentially indicate predators. In some cases, they may alter their swimming or feeding behaviour based on the turbidity or clarity of the water, as they detect the movement of particulate matter in the water column.

FIGURE 1.3
Series of blue-green "eyes", distinct in the Yesso scallop, are clearly captured here in a specimen of the Atlantic bay scallop, *Argopecten irradians*



Gills – Gills are large leaf-like organs located on each side of the visceral mass and used for respiration and feeding (Figure 1.2). Water flows through the mantle cavity and across the gill plates, driven by the synchronous beating of ciliated regions on both the ventral mantle surface and the gill filaments. There is no siphon region; the inhalant current flows in across the circumference of the shell margin and the exhalant current flows out of a narrow post-dorsal region of the shell.

Digestive gland – Scallops are filter feeders. The cilia-driven water delivers food particles to the gill surface; particles become trapped in mucus and then digested in the digestive gland (Figure 1.2). This organ includes the oesophagus, stomach, intestine, rectum and anus. Other particles are rejected as pseudofaeces and flushed into the excurrent flow.

Gonad – Yesso scallops are dioecious and sexes are separate. The gonad is distinct crescent-shaped and lies antero-ventral to the adductor muscle and over the visceral mass. Female gonads become bright orange as they mature, and male gonads are white. Yesso scallops are protandrous hermaphrodites initially maturing as males and changing sex to female as they age.

1.3 LIFE CYCLE

The Japanese scallop releases eggs and sperm in the water column, and fertilisation of oocytes follows. Development of fertilised embryos into subsequent larval, post-larval and juvenile stages is illustrated in Figure 1.4. Development rate is dependent in great

part on rearing temperature and the expected time period between stages is given in Table 1.1.

FIGURE 1.4
Life cycle of *M. yessoensis* showing all stages from spawning to larval and post-larval development and juvenile

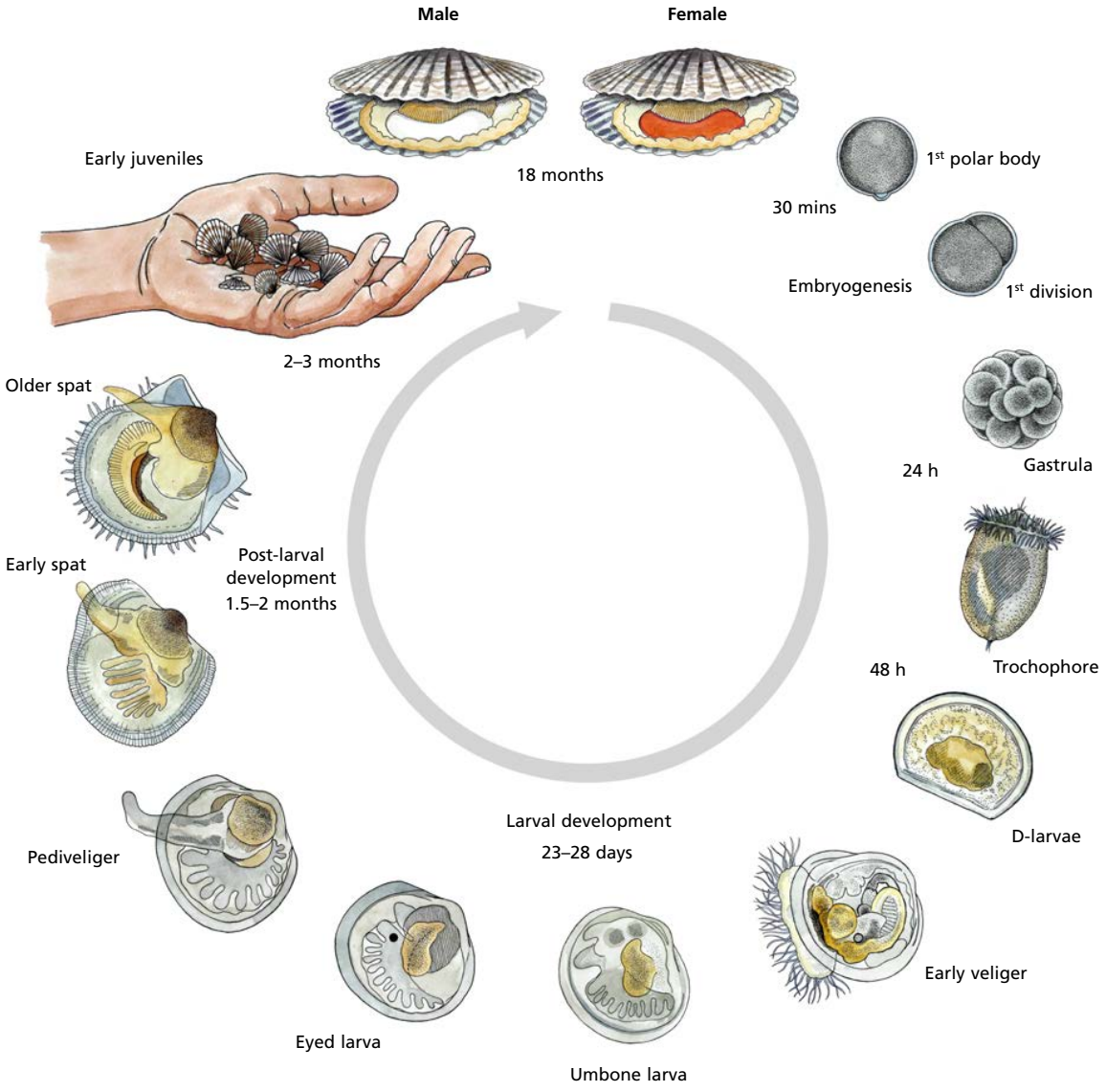


TABLE 1.1
Description and timeline for development of early life stages of *M. yessoensis*, reared at 13±0.5 °C

Description	Expected time after addition of sperm
First polar body	30 mins
First mitotic division (2-celled "snowman")	>1 h
Trochophore	24 h
First D-larvae	48 h
D-larvae	72 h
Umboned veliger larvae	Day 12
Eyed veliger larvae	Day-20–22
Pediveliger (foot development)	Day-23–28

Embryonic development: fertilised egg to D-larva – The first polar body appears within 15–30 minutes of fertilisation followed by the second polar body (Figure 1.4). Expulsion of both first and second polar bodies occurs, and the first division or blastomere (pear-shaped) can be observed within 45 minutes of fertilisation. Multi-cellular division occurs during the next 2 hours, and the gastrula stage develops within the first 12 h following fertilisation. Gastrulae develop into active trochophores in 24–36 h. Trochophores are characterised by the apical tuft – a cilia banding around the zygote (Figure 1.4).

Early larvae – At a rearing temperature of 13±0.5 °C, first D-larvae of the Yesso scallop can be seen as early as 48 h, but more frequently at 72–96 h after fertilisation, averaging 115±5 µm in shell length, and larvae with prodissoconch II develop shortly after (Figures 1.4 and 1.5). This veliger stage is characterised by the swimming and feeding ciliated organ, called the velum; it consists of two large ciliated lobes developed from the prototroch (Figure 1.5). When the velum is retracted, larvae appear under the microscope as transparent semi-circles or D-shaped, hence referred to as "D-larvae" (Figure 1.4). Tissues can be seen, and consumed phytoplankton can be seen in the digestive gland appearing as a prominent dark brown area. Larvae have a shell made of aragonite (calcium-based mineral) as do the adults. As the larva grows, the umbo becomes more prominent and triangular, and the resemblance to a capital D decreases (Figure 1.4).

Mature larvae – Umbonate larvae occur around Day-11 after fertilisation. Eyespots, indicating the approach to metamorphosis and settlement, are frequently observed around Day-21. The pediveliger stage, characterised by the development of a ciliate foot, is first seen in Yesso scallops around Day-22, with a shell length averaging 234±15 µm. Length/height relationship changes towards the end of the larval life, and pediveligers and post-set stages exhibit a higher rate of height growth than earlier D-larval stage. Metamorphosis occurs between Day-24 and up to Day-28 (dependent on rearing conditions) for pediveligers ranging in size from 243±15 µm to 280±14 µm; a thickening of the shell at the end of metamorphosis indicates an early fixed dissoconch.

Early post-set spat (<1 month) – Following settlement, early spat are found attached within the first day; spat grow quickly and are well formed following ten days of settlement. At this time, the dissoconch appears as a colourless band with growth lines

FIGURE 1.5
Larval stages of scallops – (a) early veliger larva; (b) D-larvae; and (c) umbone larvae

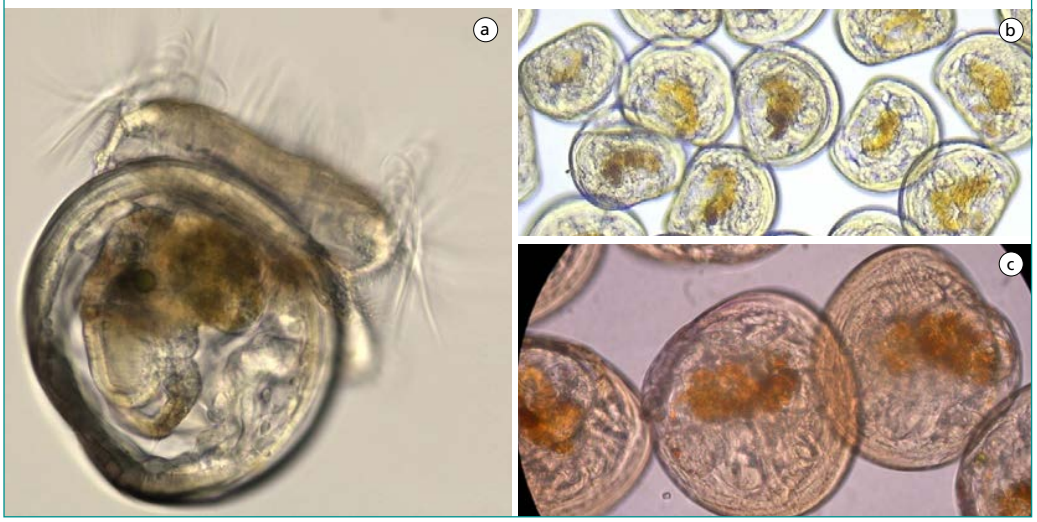


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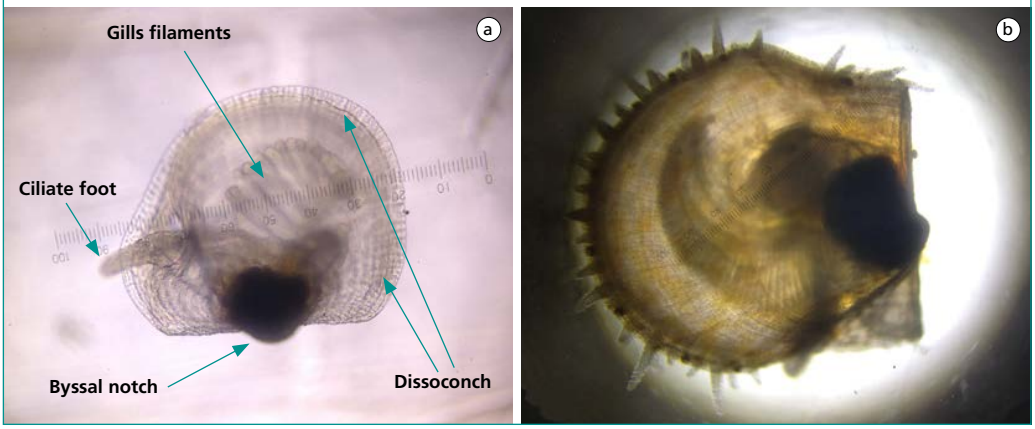
FIGURE 1.6
Post-set stages of *M. yessoensis* – (a) early spat with dissoconch; and (b) older spat >1 month post-set translucent



(Figure 1.6a). Post-larvae (2–3 weeks) show almost complete internal development with a ciliate foot, an outline of the byssal notch, and a group of rudimentary gill filaments.

Older post-set spat (>1 month) – Older spat (1.5–2 months) begin to resemble adult morphology and are no longer attached to the substrate (Figure 1.6b). *M. yessoensis* attains 4–8 mm shell height (growth determined by height rather than length), 4–5 months following settlement (Figure 1.7a).

Juveniles – Once transferred at sea, juveniles growth rate averages 8 mm.month^{-1}, reaching 40 mm within 6 months of transfer at sea (Figure 1.7b). Market size adults (120±10 mm shell height) are obtained 18 months following transfer at sea.

FIGURE 1.7
Juveniles of *M. yessoensis* – (a) 8 to 15 mm seed with semi-translucent shell; and (b) robust 30 mm juveniles with opaque shell

2. Microalgal culture

IN THIS CHAPTER – How to produce a live high quality microalgae food source.

Scallops filter feed on phytoplankton throughout their lives as larvae, juveniles and adults. The microalgal culture hatchery unit is vital to successful seed production; without high quality live microalgae, survival and growth of larvae and post-larvae will be poor.

Goal of microalgal facility – Produce large volumes of healthy monocultures of selected algal species, grown from a reservoir of starter stock cultures; this is referred to as the "Progressive batch culture" process described in detail in Section 2.1.

Microalgal species suited for the Yesso scallop:

Flagellate species - *Isochrysis galbana, Pavlova lutheri, Tetraselmis* sp.
Diatom species - *Chaetoceros muelleri or calcitrans, Thalassiossira pseudonana*

Feeding larvae, seed and broodstock – The variety of algal species grown serves different needs throughout the production cycle with respect to size and digestibility, culture characteristics and nutritional value. Live diets for scallops are composed of at least three algal species, which will provide all of the essential constituents suited to the requirements of the life stage. For the algal culture facility, this means the large-scale culture of 4–6 different algal species in total.

Note: The use of commercially available diets as substitutes to live microalgae for broodstock conditioning and older spat can alleviate the demands on the algal culture unit. Details are given in Chapters 5 and 7.

Microbiological techniques – Extreme care is needed when culturing microalgae, as a failure in algal culture production has repercussions on the entire hatchery. Contamination of algal cultures may occur via the seawater supply, air supply, and cross-contamination from nearby algal cultures. Cleanliness and careful microbiological transfer techniques are to be applied daily and to all culture steps without fault to maintain algal cultures free of contaminants and excessive bacteria.

Algal culture facility components – Capacity for sterilisation and filtration (1 µm) of seawater for all vessels, from stock to large harvestable cultures.

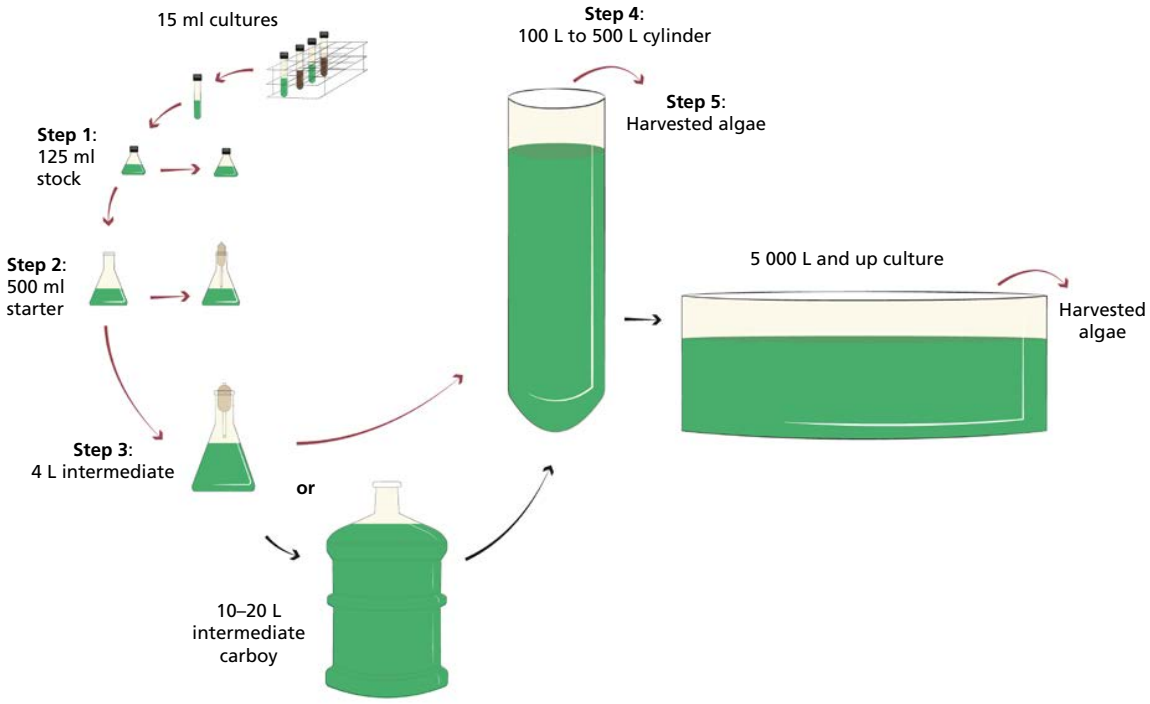
- Seawater temperature control (T = 24 °C).
- Light banks.
- Filtered aeration.
- Carbon dioxide (CO_2) input for pH control.
- Freshwater for cleaning.

The design, installation and operation of an algal culture facility for bivalves is described in detail in other technical publications (see Further Readings section).

2.1 MICROALGAL CULTURE OVERVIEW

Progressive batch culture process – is the growing of algae to large harvestable volumes through a series of culture vessels increasing in volume at every stage; small-volume cultures of concentrated algae inoculum are transferred to larger volumes of treated enriched seawater (Figure 2.1). All algal vessels are maintained at constant temperature (typically T = 23±1 °C), lighting regime (24 h illumination; or 12 h illumination) and pH (7.5–8.5); nutrients are added to sterile seawater for algal growth. Smaller volumes (stock starter and intermediate cultures) are usually reared as batch or static cultures; larger volumes can be cultured as semi-continuous or continuous cultures.

FIGURE 2.1
Progressive batch culture for live microalgal species



STEP 1

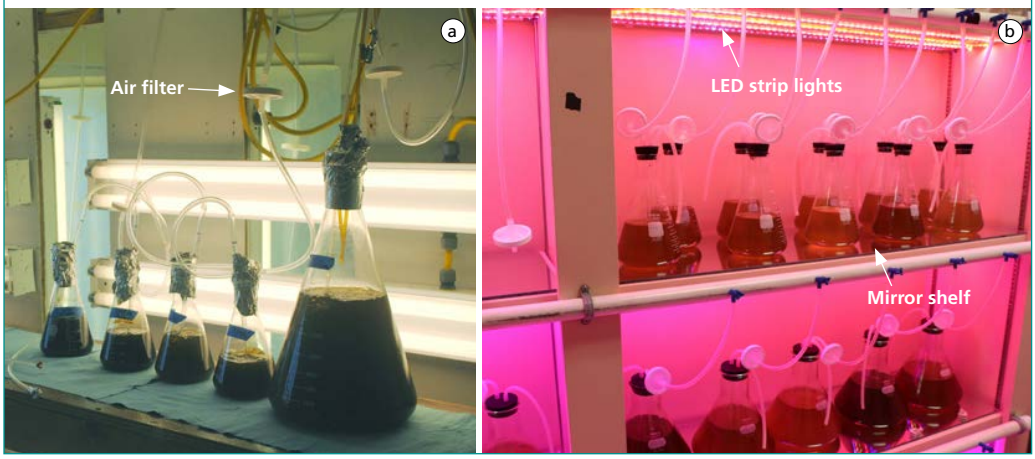
Stock cultures – Algal master cultures can be isolated from natural seawater or purchased by the aquaculturist. These are typically received in 15 ml test tubes, when purchased (Figure 2.1). They can be maintained in similar 15 ml test tubes or transferred to 125–250 ml flasks (Figure 2.2), loosely closed with a screw cap; this allows some oxygen in culture, and there is no need for aeration. The use of flasks is often preferred over test tubes, as it ensures the stocking of a larger volume of inoculum. Stock culture flasks are kept in an isolated section of the dry laboratory area, in order to prevent contamination from other flasks, or in an incubator. They are maintained through monthly subsampling and re-inoculations as a backup, in case of failure in the starter, intermediate or larger algal culture system.

FIGURE 2.2
Stock cultures maintained in 250 ml Ernlemeyer flasks

STEP 2

Starter cultures – Stock cultures are used to inoculate larger starter cultures (500 ml flasks); these can be aerated or not. Aeration maintains cells in suspension, especially useful for some species such as *Tetraselmis* sp. (Figure 2.3). Aeration is also the means by which carbon dioxide (CO_2) is added to the microalgal cultures; air supply is supplemented with CO_2 (2–5 %) to stabilise the pH of the culture, which ensures that sufficient carbon is available for photosynthesis as cells divide and the density of the culture increases. Standard lighting is by cool white fluorescent lamps (40 watts) (Figure 2.3a); Gro-Lux® LED strip lights are also used by some aquaculturists (Figure 2.3b). To increase illumination, algal flasks can be placed on a mirror shelf as illustrated in Figure 2.3b.

FIGURE 2.3
Starter algal cultures in 500 ml flasks, aerated – (a) illumination with standard cool white fluorescent lamps; starter cultures top shelf, intermediate cultures (4 L) bottom shelf; and (b) illumination with Gro-Lux® LED strip lights and mirror shelves



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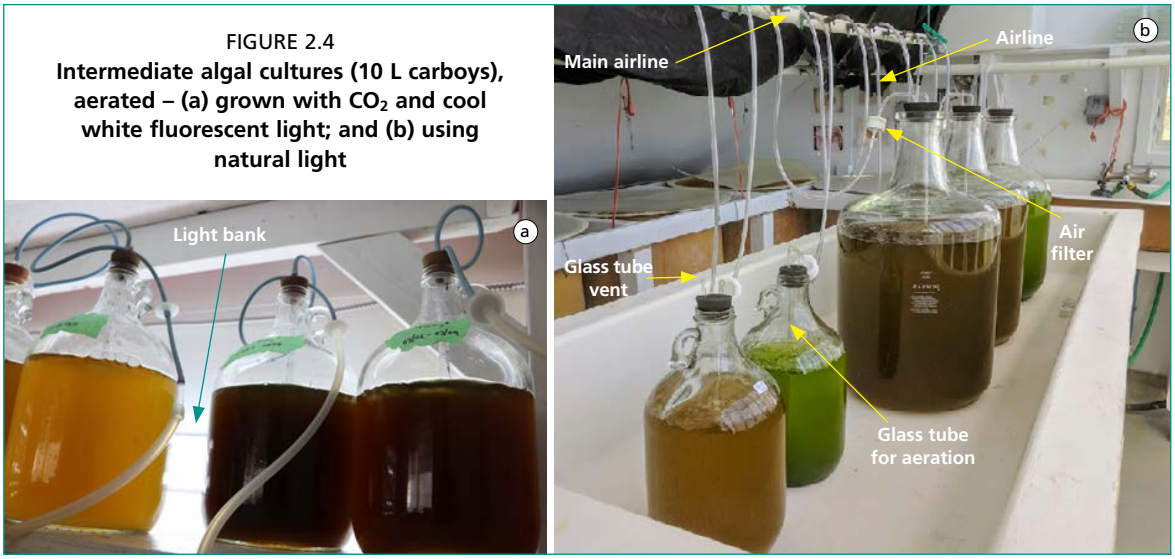
STEP 3

Intermediate cultures – In turn, starter cultures inoculate intermediate cultures (4 L flasks to 20 L carboys); these are typically aerated, and densities are operator–dependent, but average 15 000 cells.µl^{-1} and can reach up to 30 000 cells.µl^{-1} (Figure 2.4).

FIGURE 2.4
Intermediate algal cultures (10 L carboys), aerated – (a) grown with CO_2 and cool white fluorescent light; and (b) using natural light



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STEP 4

Large cultures – Intermediate cultures are used to inoculate large harvestable size cultures (Figure 2.1); typically 100–500 L translucent fiberglass cylinders or polyethylene bags. Round fiberglass tanks (5 000 L and up) can also be inoculated and grown as batch cultures. Algae can be harvested 4–5 days after inoculation of a large culture vessel. Large-scale cultures can be grown as semi-continuous batch cultures, or as continuous cultures (Figure 2.5). Semi-continuous batch cultures follow the same inoculation protocol as intermediate cultures; the difference is that when the culture is harvested down to ⅓ of the vessel, new sterile seawater and nutrients is added to the vessel and the culture re-grows to full volume. Semi-continuous cultures can be maintained in the same vessel for up to 30 days (Figure 2.5a). Continuous cultures can be grown in polyethylene bags supported by a wire frame (Figure 2.5b); these receive a continuous input of seawater and nutrient and can be harvested on a continuous daily basis once suitable densities are reached. Details on protocols for large-scale cultures of both batch and continuous systems are available in other technical guides (see Further Readings section).

FIGURE 2.5
Large-scale algal cultures – (a) 100 L semi-continuous batch cultures; and (b) 500 L continuous cultures, showing nutrient line and harvest line

Alternative large cultures – Other systems for growing algae in large volumes include the more recent technology of photobioreactors (Figure 2.6). Photobioreactor systems provide higher algae densities, more efficient space usage (a smaller footprint), and lower labour requirements. The disadvantages are that they are costly and rely on computer software for good functioning; technical support should be easily accessible in case of breakdown. Training by the manufacturer is needed.

2.2 MICROALGAL PROTOCOLS

Daily monitoring – Constant monitoring of algal cultures is critical and must become part of an automatic check at the beginning and end of the day. Protocol 2.2.1 is an example of daily tasks to ensure the availability of healthy and "clean" algal cultures free of ciliates or other undesirable microorganisms for high quality algae production.

Inoculations – The protocols used for preparing algal vessels and inoculating them is similar for all steps in the progressive batch culture (see Protocol 2.2.2). An increasing larger volume of inoculum is used at every step of the process, in order to obtain sufficient harvestable algal volumes to supply the hatchery needs. Rigorous use of microbiological techniques is required throughout. Differences in large algal vessel types are at the discretion of the aquaculturist and are dictated by the scale of algal production required.

FIGURE 2.6
(a) Photobioreactors for growing live microalgal cultures; and (b) close up computer monitor for data entry and nutrient bottles

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2.2.1 PROTOCOL: Daily algal checks

Note: Keep the algae laboratory as clean as possible. Wash hands with antibacterial soap before working with algae cultures. Wear laboratory gloves to prevent contaminating surfaces and cultures.

Beginning of day

- Swirl stock cultures to resuspend algae and record room/incubator temperature.
- Check small and intermediate cultures and note if any are growing poorly or crashed; check that all are correctly aerated (if using air); if not, swirl gently without moistening the plug. Record air temperature and check lighting.
- Check all large vessels similarly. If any crashed cultures, drain tank immediately, and freshwater rinse before preparation for new inoculate. Do not leave crashed cultures in tank, and do not leave dirty algal tanks to avoid any cross contamination to healthy cultures.
- Check air supply line for condensation build-up and purge, if required.
- Correct any aeration or temperature problems.
- Check air filter and replace, if needed.
- Record CO_2 tank pressure; always have two tanks for back up. Order new tank as soon as one is empty.
- Replace any non-working light bulbs or LED strip lights.
- Add bleach in chlorine bath at entrance of algal room to clean boots, if needed.
- Remove items from autoclave and place on cooling rack or storage shelves.
- Reload and start autoclave, if needed.

Rest of day

- Collect algal samples for checks and counts for feeding.
- Check pH if there is concern of fluctuations.
- Calculate amount of algae required for feeding and harvest.
- Distribute algae for feeding immediately after harvest. If algae are held in harvest containers for any length of time, make sure they are well aerated.
- Do new inoculations.

End of day

- Walk through facility, re-check aeration, lighting and temperature level.

2.2.2 PROTOCOL: Inoculating and maintaining starter algal cultures

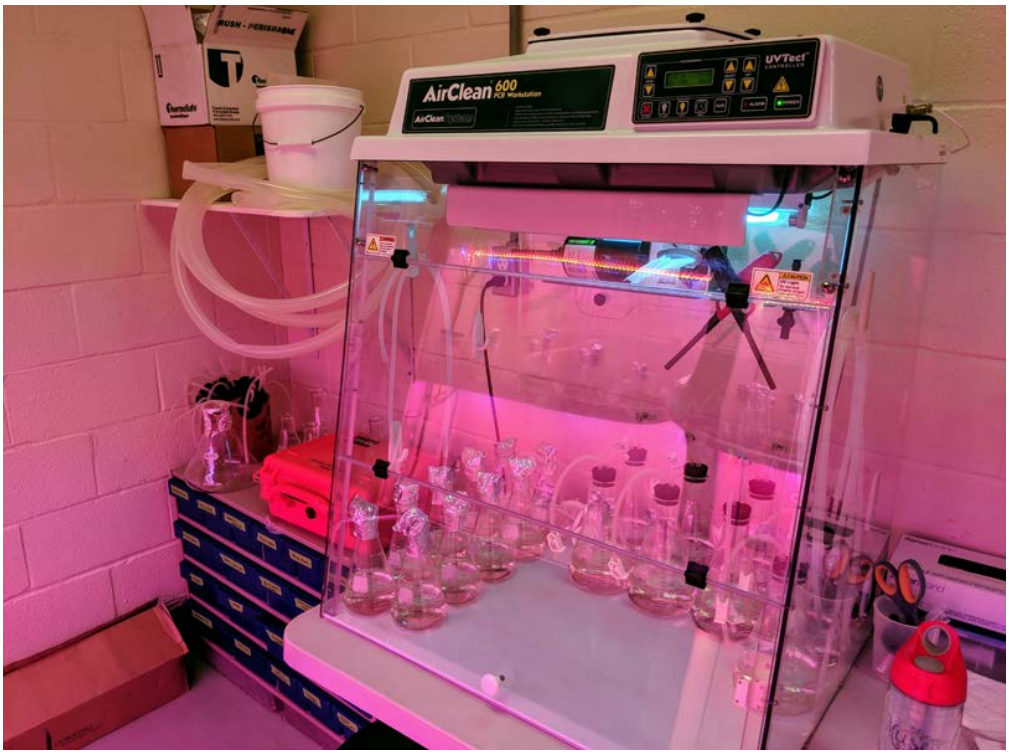
MATERIALS

- 10 % hydrogen chloride (HCl) bath
- De-ionized water
- Flasks: 125 ml and 500 ml
- Sterile graduated pipettes: 5 ml and 10 ml
- Bunsen burner (or small propane torch) and lighter
- Pipette bulb (3-way) for dispensing nutrients
- Algal nutrient media: F/2 commercially purchased or other, vitamin solution, sodium metasilicate solution (3 % W/V)
- Fume hood
- Cotton plugs or steri-stoppers
- Algal stocks

METHOD

Preparing flasks

1. Clean 2 flasks for each algal species in a 10 % HCl bath; rinse twice with freshwater and one final rinse with distilled water.
2. Fill 500 ml flask with 350 ml of 1 µm filtered seawater.
3. Close flask loosely with plug (e.g. cotton plug and foil).
4. Sterilise in autoclave.



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New sterile flasks under fume hood

Inoculating 500 ml starter cultures with 125 ml stock cultures

5. Work under the fume hood.
6. Wear gloves and spray with ethanol to sterilise.
7. Use microbiological sterile techniques for all transfers.
8. Prepare work area, with a Bunsen burner or small propane torch nearby, nutrient solution (F/2 [(1)], vitamin and sodium metasilicate).
9. Use a 10 ml pipette, transfer 30–35 ml of stock culture to 500 ml flask, staying close to flame at all times. Do not touch mouth of either flask with pipette. Plug flask quickly after transfer.
10. Label flask with species and date of transfer.

2.2.2 PROTOCOL (continued)

11. Working close to flame, remove cap of nutrient solution and place on clean surface area, maintain mouth of nutrient media container close to flame.
12. Using a sterile pipette, collect nutrient media. Dosage is 1 $ml.l^{-1}$ of F/2 nutrients to algal inoculum; for e.g. for a 500 ml algal flask, 0.5 ml nutrient solution will be required.
13. Close nutrient container immediately after pipetting by holding close to flame, flaming mouth and cap before closing.
14. Always keeping pipette tip with nutrients close to flame.
15. Hold algal flask in one hand, and pipette in the other close to flame.
16. Remove flask plug and keep in palm of hand; add nutrients to algal inoculum.
17. Close flask immediately with cotton plug in hand. Discard pipette. Swirl flask to mix nutrients and algae.
18. Using a second sterile pipette, add 2 $ml.l^{-1}$ of sodium metasilicate for diatom species. For e.g. for 500 ml flaks, add 1 ml in total.
19. Flame aerating tube and insert into 500 ml flask, holding close to flame.
20. Move completed flask to light bank, and connect to airline; bubbling ensures homogeneous mixing.

Maintenance of 500 ml starter cultures

21. 500 ml cultures will be ready for transfer within 3–4 days of inoculation.
22. 500 ml culture is used as inoculum for new 500 ml flask, and for new intermediate flask.
23. For 500 ml flask, transfer 100–200 ml of inoculum (from old 500 ml flask) to new autoclaved 500 ml flask containing seawater. Add nutrients using same dosage as above.
24. For intermediate culture, transfer remaining of old 500 ml flask. Take care not to pour the bottom of the culture as it may contain some detritus.
25. Add nutrients and aeration line. Plug and move to light bank for growth.

(1) There are several nutrient solutions suitable for microalgal culture and dosage may differ with nutrient composition. F/2 is a common, commercially available solution.

Determining algal culture density – Subsamples of algal cultures ready for harvest are checked under the microscope to make sure that they are free of ciliates and other foreign and undesirable organisms. Clean cultures are counted on a daily basis for feeding of larvae and spat. Algae can be counted using sophisticated equipment such as a Coulter Counter, or less costly tools such as an XpertSea digitized bucket (XperCount), and more typically a haemocytometer cell under a compound microscope. Assessing density of microalgal culture is needed to calculate the food ration for scallops; this is dependent on age (for larval/post-larval stage), stocking density (for broodstock/older spat) and volume of culture tank.

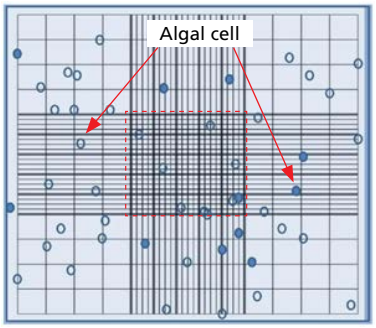
2.2.3 PROTOCOL: Counting algae and estimating algal culture density

MATERIALS

- Haemocytometer cell
- Small vial or test tube or well plate
- 10 % formaldehyde
- Pasteur pipette and bulb
- Clicker

METHOD

1. Collect a subsample of algal culture and transfer to vial.
2. Add 2–3 drops of 10 % formalin if using a flagellate species to halt swimming and movement. Mix.
3. Mount cover slip on haemocytometer cell.
4. Collect 1 ml of algal sample using a Pasteur pipette and introduce a drop into chamber at edge of cover slip. Do not force sample in, allow it to run by capillary action. Make sure not to have any air bubbles in cell.
5. Fill grooves of cell completely with algal sample.
6. Using a clicker (counter), count number of algal cells in at least three of the 25-square grid in the centre of the cell (highlighted in red in photo).
7. If algal culture is not very dense (as in photo), count all of the cells in 25-square grid.
8. Calculate average number of cells per square.
9. To obtain cell density: Average number of cells × 250. This gives the number of algal cells per µl.
10. Multiply by 1 000 for number of cells per ml.



Haemocytometer cell grid

Explanation

Each cell is 0.004 mm^3
By multiplying by 250, you obtain number of cells per mm^3
1 mm^3 = 1 µl

Note: To avoid counting the same algal cell twice, select top border and right border of each small square (within 25-square grid); count only algal cells sitting on top and right border.

Example

1. Count algal cells in 3 squares within 25-square grid.
   Count in square 1 = 56 algal cells
   Count in square 2 = 48 algal cells
   Count in square 3 = 51 algal cells
2. Calculate average = 51.6 algal cells per square.
3. Calculate culture cell density = 51.6 × 250 = 12 916 cells.μl^{-1} or 12 916 000 cells.ml^{-1}.

3. Probiotic bacteria culture

IN THIS CHAPTER – Steps for culturing and introducing probiotic bacteria in larval and spat culture systems.

Probiotics should be considered in any new hatchery operation as a biocontrol agent. Shellfish aquaculturists worldwide are increasingly turning to the use of probiotic bacteria for controlling pathogens as an alternative to that of antimicrobial drugs. Bacterial balance has been shown to be important in maintaining healthy larval cultures. Reports are available for probiotic use in oyster larval culture; however, little is reported for scallop culture. Preliminary trials have been successfully conducted on spat rearing of the Japanese scallop; more trials are needed to optimise procedures for administering probiotics to larval and spat cultures. This section provides a brief overview on probiotics in general, but focuses on procedures specific to probiotic bacteria. The culture and monitoring of probiotic bacteria, co-cultivation of probiotic bacteria with microalgae used as feed, and a general approach on their integration to larval and spat cultures are described.

Types of probiotics – Probiotics generally refer to organisms which beneficially affect the host; benefits are often achieved by improving microbial balance of the host or of the ambient environment.

Some microalgae species have probiotic properties; for example, *Skeletonema costatum*, a commonly used microalgae, produces an extract inhibiting growth in several *Vibrio* species and can potentially act in a manner beneficial to shellfish larviculture. It is reported that when *S. costatum* is added to the developing *M. yessoensis* trochophores at a ratio of 4 000 cells.ml^{-1}, it reduces bacterial contamination during this early vulnerable stage. Other microalgae species, *Phaedactylum tricornutum* and *Tetraselmis suecica* also inhibit *Vibrio* sp. and are used as probiotics.

A diverse range of Gram-positive bacteria is used worldwide as probiotics, known to have beneficial effects on the digestive system of bivalve larvae. *Pseudoalteromonas* sp. is one species, which is commercially available and successfully used for commercial oyster culture for its antibacterial and antivirulent compound production.

Selecting a probiotic bacteria species – Selecting a probiotic bacteria species for scallop culture is at the trial and error stage, and a screening process by the aquaculturist will most likely be necessary to find a species specific probiotic. Potential probiotics may be obtained from various sources such as: a) isolates from local seawater, b) commercial suppliers, and c) intestinal tract of the aquatic species itself. The probiotic selected needs to fulfil the greatest number of the following properties:

a) harmless to the host;
b) accepted by the host through ingestion, colonisation and proliferation within the host;
c) reach target organs where it can perform; and
d) contain no virulent resistance or antibacterial genes.

The first property (a) is key and the aquaculturist must ensure that the probiotic species does not cause any toxic side effects to the host. As this is species specific, it

is an essential part of the screening process and should be tested at small scale before introducing to the entire operation. For example, *Pseudoalteromonas,* used for oyster culture, is reported to be toxic to other invertebrate species.

Probiotics can be used singly or in combination; those based on mixed strains are found to be more effective. For example, a co-culture of *Roseobacter* BS107 and *Vibrio anguillarum* enhances the survival of larval scallop (*Pecten maximus*). The aquaculturist will need some time to conduct trials to determine the most suited species and best protocols; the ultimate goal is to ensure a continuous presence of probiotic bacteria in the scallop cultures at a density sufficient to provide larvae with antimicrobial defence mechanism.

Administering a probiotic – A proper administration method creates favourable conditions in which the probiotic performs well. Probiotic bacteria have shown compatibility with several microalgae species used as standard scallop food; probiotic bacteria can be co-cultivated with live microalgal food and introduced to larvae with the food ration. This method improves the ingestion of probiotics by scallop larvae and spat.

Alternatively, probiotics can be introduced directly to the culture, by adding them as a bacterial suspension into larval and post-larval tanks.

3.1 PROBIOTIC BACTERIA CULTURE EQUIPMENT

Dedicated space – Probiotic bacteria can be cultured in the algal dry laboratory area of a hatchery complex. Ideally, stock and starter cultures should be kept separate from those of microalgae; but practically, these can be maintained in the same climate-controlled room on separate shelves; adherence to strict protocols to eliminate the risk of cross-contamination is a must throughout all steps of culture.

Equipment and materials – A fume hood is essential for inoculation and transfers of probiotic bacteria. Additional small equipment is required for the culture of probiotics; a list is given in Table 3.1. The agar media used depends on the probiotic bacteria species cultured and on the need for differentiation of *Vibrio* species, Gram-positive and Gram-negative bacteria.

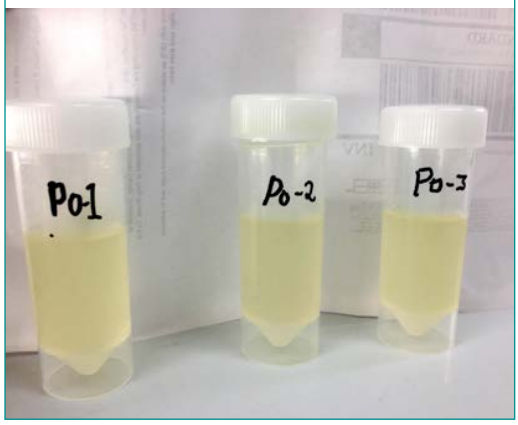
TABLE 3.1
List of small equipment specific to probiotic bacteria culture, additional to that used for microalgal cultures

Description	Minimum quantity
BD Difco™ dehydrated culture media: Marine Agar 2216 or Difco™ Marine Broth 2216 powder	1 (500 g)
Thermo scientific Remel Simmons Citrate Agar dehydrated[(1)] or Thiosulfate Citrate Bile Salts Sucrose (TCBS) Agar	1 (500 g) Package of 10 (15 × 100 mm plate)
Tryptic-Soy Agar (TSA) plates – purchased ready to use[(2)] (Merck)	
Dispenser for Marine Broth to stocks: Scilogex SCI-Spense Bottletop Chemical Dispenser, 1–10 ml volume	1
Premiere Benchtop centrifuge 1 000–4 000 revolutions per minute (RPM) holding 50 ml test tubes	1
Sterile centrifuge tubes with flat caps (50 ml) RCF (relative centrifugal force) 12 000/g	Packs of 25
Magnetic stirrer hot plate (to hold 250 ml^{-1} L flasks)	1
Shaker table (able to hold up to 1 L Erlenmeyer flask and set 25 RPM)	1
Fume hood	1
Eppendorf pipette and tips (up to 1 ml)	1
Glass spreader	2
Petri dishes	As required

[(1)] TCBS agar is selective and used to assess presence of *Vibrio* species; plates can be made or purchased.
[(2)] TSA is a universal medium, which supports the growth of both Gram-positive and Gram-negative bacteria.

3.2 PROBIOTIC BACTERIA CULTURE PROTOCOLS

FIGURE 3.1
Seed culture received from commercial supplier in 30 ml vial



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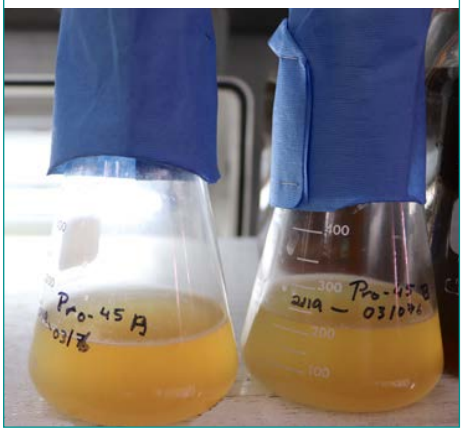
Seed cultures – Probiotic bacteria are received in small containers, such as 30 ml vials (Figure 3.1). Cultures are removed from the shipped box as soon as they are received and placed under the fume hood until they are ready for use. These seed cultures are used to inoculate the aquaculturist's own stock cultures as a backup for the probiotic bacteria cultured on site. The first step is to check the cleanliness of seed cultures received by checking for *Vibrio* (see Protocol 3.2.4). A subsample of seed culture is plated on TCBS agar; if *Vibrio* are present, they will grow on the plates. A clean culture will not show any growth on TCBS.

Batch culture – Once seed cultures are verified as free of undesirable organisms, they are grown using bacteria specific nutrient media. All materials and media are autoclaved prior to use, similar to the procedure for algal cultures. The progressive batch approach described for microalgae is also used for the culture of probiotic bacteria; seed cultures received are sub-sampled to ensure a stock culture as back- up and to inoculate starter cultures (Figure 3.2); from these, probiotic bacteria are harvested for co-cultivation with algae in larger volumes or introduced directly into larval and post-larval tanks. Note that the total volume of probiotic bacteria produced is orders of magnitude smaller than that of food microalgae and requires substantially less space in the algal facility.

FIGURE 3.2
Stock cultures of probiotic bacteria used to inoculate 500 ml starter cultures



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Harvestable target – As a guide, culturing a total of approximately 2 L of probiotic bacteria every 3.5 days should suffice a hatchery/nursery of 150 000 L continuous algal capacity. It is recommended that new stock cultures be received from the supplier every 4–6 months.

Protocols – Six protocols follow, outlining procedures from the time the probiotic bacteria shipment is received to the production of starter cultures. Two types of agar plates are prepared: a) TCBS agar is highly selective for *Vibrio*, undesirable in an isolated probiotic bacteria seed culture (see Protocol 3.2.1). The streak method is applied to the seed cultures received (see Protocol 3.2.4); b) Marine agar or TSA are used to monitor probiotic bacterial growth (see Protocol 3.2.5). One common nutrient medium used for growing bacteria is Marine Broth or Marine Agar, and is prepared by the algologist (see Protocol 3.2.2). The density of bacteria is estimated based on the number of colony-forming units (CFU), determined through a series of dilutions (see Protocol 3.2.6). Probiotic bacteria are cultured independently of microalgae initially as starter cultures (see Protocol 3.2.3); these are grown to densities high enough for harvest used for co-cultivation with microalgae or for direct addition to tanks (see Protocol 3.3.1).

3.2.1 PROTOCOL: Preparing thiosulfate citrate bile salts sucrose (TCBS) agar plates

MATERIALS

- Scale
- TCBS
- 1 L flask
- De-ionized water (DI)
- Seawater (SW)
- Magnetic stirrer
- Magnetic stirrer hot plate
- Steri stoppers
- Aluminium foil
- Graduated cylinder
- Parafilm

METHOD

Note: TCBS plates are used to check for *Vibrio* sp. in cultures. Manufacturer's instructions should be on TCBS container.

1. Weigh the amount of TCBS needed for the required volume (88 $g.l^{-1}$ DI water).
2. Pour TCBS Agar into a 1 L flask.
3. Measure required volume as per manufacturer's instruction, using 50 % SW and 50 % DI water.
4. Pour DI water into the flask sides, making sure all the powder falls to the bottom of the flask.
5. Put one magnetic stirrer into the flask.
6. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder (take care not let it boil over the flask).
7. Cover with a steri stopper and aluminium foil.
8. Do NOT autoclave.
9. Leave to cool to 40–45 °C.
10. Pour into plates, filling around half the plate.
11. Leave for a day, making sure there is no water condensation on the top of the plate.
12. Next day, check for condensation and contamination. If there is none, make packages of 5 using parafilm.
13. Keep the plates upside down and in the fridge until use.

3.2.2 PROTOCOL: Preparing marine broth for probiotic bacteria cultures

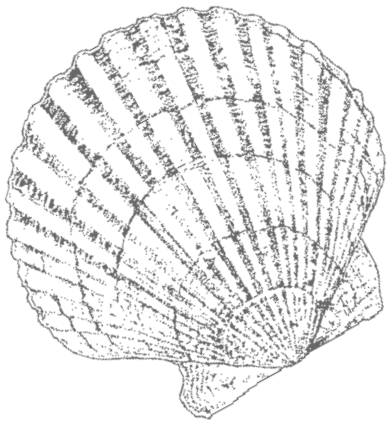
MATERIALS

- Difco™ Marine Broth 2216 powder
- Scale ±0.01 g
- Magnetic stirrer hot plate
- Graduated cylinder (500 ml or 1 000 ml)
- De-ionized water (DI)
- Six 1 L Ernlemeyer flasks
- Magnetic stirrer
- Steri stoppers or cotton plugs (made with cheesecloth)
- Aluminium foil
- Autoclave

METHOD

Note: Manufacturer's instructions should be on the side of the container.

1. Prepare a 1 L flask with 500 ml purified (or DI water if you have it); add one magnetic stirrer to flask.
2. Weigh 37.4 g Difco™ Marine Broth powder.
3. Pour into a 1 L flask filled with 500 ml DI water.
4. Pour an additional 500 ml of purified water onto the flask sides, making sure all powder falls to the bottom of the flask.
5. Transfer flask to a magnetic heated stir plate.
6. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder (take care not to let it boil over the flask).
7. Transfer to a new 1 L Ernlemeyer flask, leaving the magnetic stirrer in the old flask.
8. Plug flask with a steri stopper (or cotton plug and aluminium foil similar to that used for algal culture growth flasks).
9. Repeat Steps 1–8 two additional times to have 3 × 1 L flasks of Marine Broth ready for autoclave.
10. Autoclave at 121 °C for 15 minutes.
11. Remove from autoclave and leave to rest for one day before use.



3.2.3 PROTOCOL: Culturing probiotic bacteria – seed culture to starter culture

MATERIALS

- Fume hood
- Shaker table
- Autoclaved Marine Broth
- Autoclaved flasks (250 ml and 500 ml)
- Steri stopper or cotton plugs
- Aluminium foil
- Sterile pipettes
- Bunsen burner or propane torch
- Three 30 ml seed cultures

METHOD

Example: Receiving 3 × 30 ml seed cultures

1. As soon as cultures are received, open box, loosely open cap to allow some oxygen in but avoid contamination.
2. Clear fume hood of any materials, keeping only Bunsen burner and clean surface.
3. Seed cultures received should be cloudy. Note state of cultures once removed from shipping box.
4. As a further check collect one drop of culture from each vial, place on slide, and check motility under the microscope. Plate 1 µl from each vial to check that cultures are viable (see Protocol 3.2.1 for TCBS plates and Protocol 3.2.4 for rapid streak plating procedure).
5. Place seed cultures under fume hood until ready to transfer into Marine Broth.
6. You will first inoculate 250 ml flasks as stock cultures; these will be kept as back-up and used for inoculating 500 ml starter cultures.
7. Working under the fume hood, and using sterile microbiological techniques, transfer 100 ml autoclaved Marine Broth into 3 × 250 ml flasks.
8. Using 1 of 3 seed cultures received – Transfer seed culture into one 250 ml flask with Marine Broth. Close with steri stopper. Label starter culture with probiotic reference number and date.
9. Repeat Steps 1–8 for second and third seed culture.
10. Place 250 ml flasks on a shaker table (25 RPM) and maintain overnight at 25 °C for cultures to grow. The agitation keeps the solution aerobic. Cultures on shaker can be kept in a dark incubator or lighted room.
11. Once agitated for one night, 250 ml cultures of probiotic bacteria can be kept in a small incubator in the algae room at 25 °C.

Shaker and probiotic bacteria stock cultures

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12. Monitor probiotic bacteria growth by subsampling once a week for CFU (see Protocol 3.2.6 for CFU assessment).
13. Once CFU in stock cultures reach 10^6, prepare 3 × 500 ml flasks for stock cultures.
14. Transfer 250 ml of (autoclaved) Marine Broth into each 500 ml flask.
15. Using sterile microbiological techniques, working by the flame, transfer 100 ml stock culture to 500 ml flask containing Marine Broth. These are now your starter solutions for growing probiotic bacteria for inoculum to larger flasks or harvest.
16. Label new 500 ml starter cultures accordingly with date of inoculation.
17. Transfer remaining stock culture into new 250 ml flask to maintain stocks.
18. Initially, CFU is monitored weekly to assess growth; afterwards, monitoring every 2 weeks should be sufficient. A minimum density of 10^6 is required for harvest.
19. Establish a sub-culture schedule to maintain stocks every 2 weeks or when CFU reaches 10^6.

3.2.4 PROTOCOL: Streak plate method to identify *Vibrio* or bacteria in a culture

MATERIALS

- Marine agar or TCBS plates (as required)
- Samples
- Inoculating loop

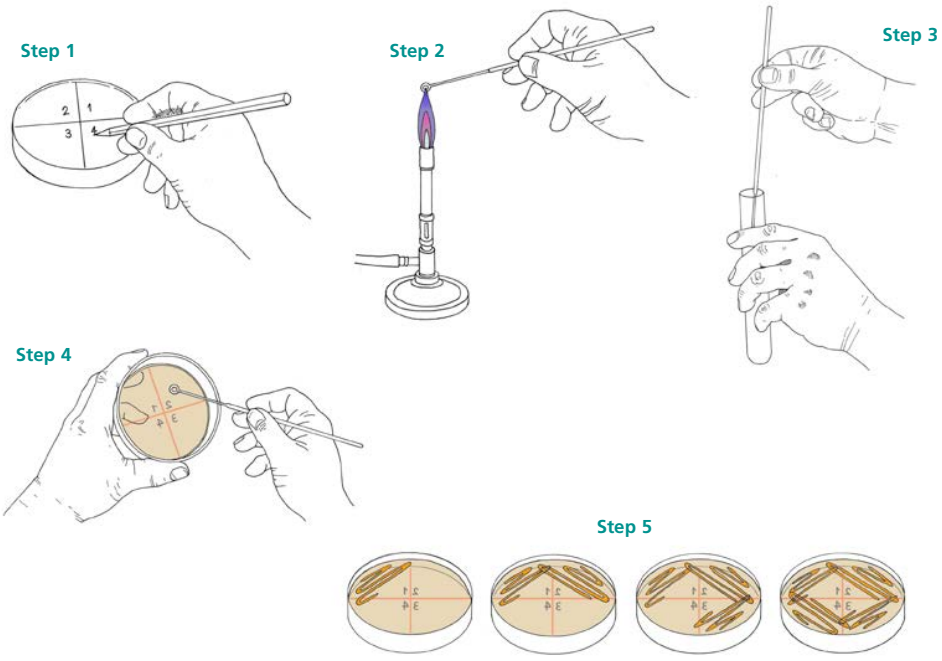
METHOD

Streaking is a rapid method used to identify presence of *Vibrio* or bacteria in a culture.

Routine checks using TCBS plates are commonly done in algal cultures and larval/nursery tanks to assess presence of *Vibrio*.

1. Take the number of plates required from the fridge and place into the fume hood until they are at room temperature.
2. Divide and mark plates, as needed. Label each plate with the sample that will be plated and the date (Step 1 in Figure 3.3).
3. Sterilise the inoculating loop using a Bunsen burner (or propane torch) by keeping the loop into the flame until it is red hot. Allow it to cool (Step 2 in Figure 3.3).
4. Insert sterilised loop into tube containing sample and collect a drop of culture (Step 3 in Figure 3.3).
5. Immediately streak the inoculating loop very gently over the space of the plate designated for the sample with a single zig-zag streak (Step 4 in Figure 3.3).
6. Flame the loop again and allow to cool before taking the next sample.
7. Repeat until all your samples have been plated (Step 5 in Figure 3.3).
8. Flame your loop once more for a final sterilisation.
9. Close plate with lid, invert plate and place at 26 °C for 48–72 h in a small countertop incubator.

FIGURE 3.3
Stepwise procedure for streak plate method



3.2.5 PROTOCOL: Preparing marine agar plates for assessing bacterial counts

MATERIALS

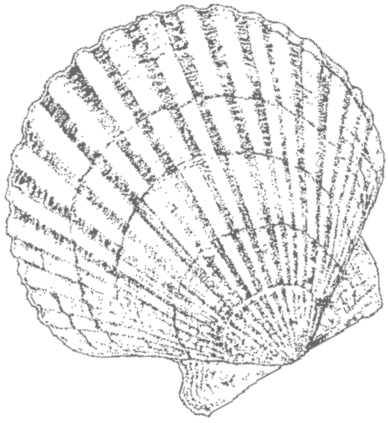
- Scale
- Marine Agar or TSA
- 1 L flask
- De-ionized water (DI)
- Magnetic stirrer
- Magnetic stirrer hot plate
- Steri stoppers or cotton plug
- Aluminium foil
- Graduated cylinder
- Sterile Marine Agar/TSA plates
- Parafilm

METHOD

Marine Agar plates supports bacteria growth and is used to assess CFU in probiotic bacteria cultures. Tryptic (trypticase) soy agar (TSA) can also be used.

Manufacturer's instructions should be on Marine Agar container.

1. Weigh the amount of Marine Agar needed for your required volume (55.1 $g.l^{-1}$ DI water).
2. Pour Marine Agar into a 1 L flask.
3. Measure volume of DI water needed.
4. Pour DI water into the flask sides, making sure all the powder falls to the bottom of the flask.
5. Place one magnetic stirrer into the flask.
6. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder (do NOT let it boil over the flask).
7. Plug with steri stopper or cotton and cover with aluminium foil. Label with autoclavable tape.
8. Autoclave at 121 °C for 15 minutes.
9. Leave to cool to 40–45 °C.
10. Pour into plates, filling around half the plate.
11. Leave for a day, making sure there is no water condensation on the top of the plate.
12. Next day, check for condensation and contamination. If there is none, make packages of 5 using parafilm.
13. Keep the plates upside down and in the fridge.



3.2.6 PROTOCOL: Determining colony-forming units (CFU) in bacteria cultures

MATERIALS

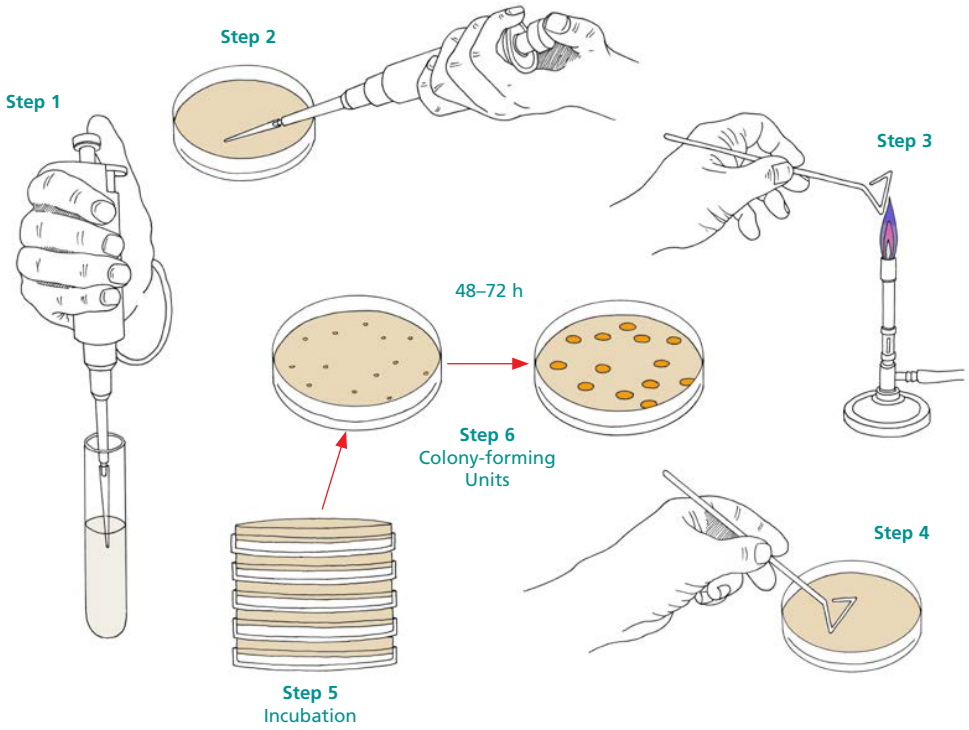
- Fume hood
- Bunsen burner or propane torch
- Four 15 ml test tubes with caps
- 60 ml of Marine Broth
- 10 ml sterile pipettes, 1 pipette bulb
- Marine Agar or TSA plates
- Glass spreader
- Eppendorf pipette and tips
- Autoclaved seawater

METHOD

This protocol uses the serial dilution technique where the sample is diluted enough so that bacterial colonies grown on the marine agar plates are differentiated and can be counted.

1. Prepare fume hood and materials. All materials, Marine Broth and seawater is autoclaved prior to subsampling for CFU.
2. Label test tubes: 1:10, 1:100, 1:1 000, 1:10 000.
3. Label TSA plates: 1:1 000, 1:10 000 with date of plating.
4. Add 9 ml of Marine Broth to each test tube with a 10 ml sterile pipette. Always work close to flame, using microbiological techniques. Discard pipette.
5. For 1:10 dilution – add 1 ml of probiotic solution to 15 ml test tube with new sterile 10 ml pipette and mix.
6. For 1:100 dilution – add 1 ml of 1:10 dilution to 15 ml test tube. Screw cap on and mix by inverting test tube gently a few times.
7. For 1:1 000 dilution – add 1 ml of 1:100 dilution to 15 ml test tube. Screw cap on and mix.
8. For 1:10 000 dilution – add 1 ml of 1:1 000 dilution to 15 ml test tube. Screw cap on and mix.
9. Remove probiotic culture and any other material from fume hood for next steps; keep only dilutions (Steps 6–9) and TSA plates.
10. Mix 1:1 000 dilution by inverting test tube a few times and collect 100 µl aliquot using Eppendorf pipette (Step 1 in Figure 3.4).
11. Remove lid from TSA plate, and dispense aliquot in the middle of the TSA plate with matching label (Step 2 in Figure 3.4).
12. Discard Eppendorf tip.
13. Sterilise the glass spreader by dipping it into 70 % ethanol, and then flame it using Bunsen burner (Step 3 in Figure 3.4).
14. Let spreader cool by placing gently on TSA (not directly on probiotic aliquot).
15. Spread the 100 µl aliquot on the plate by moving the spreader around the surface of the plate making a circular motion until all the sample has been absorbed in the plate (Step 4 in Figure 3.4).
16. Close plate with lid. Do not invert plate right away. Let dry 5 minutes to make sure all probiotic sample is absorbed in agar.
17. Once sample is absorbed, place TSA plate upside down in incubator (26 °C) for 48–72 h (Step 5 in Figure 3.4). Temperature for incubation can vary dependent on probiotic bacteria species used.
18. Sterilise glass spreader and repeat Steps 11–18 with 1:10 000 dilution.
19. After incubation, there should be no more than 30–300 colonies on plate (Step 6 in Figure 3.4). Identify colonies of probiotic bacteria and make sure to differentiate with naturally occurring colonies.
20. Count probiotic colonies with a clicker and record.
21. Calculate CFU.ml^{-1} as follows: Number of colonies counted per plated volume × dilution factor. Dilution factor for 1:1 000 is 1 000.
    Dilution factor for 1:10 000 is 10 000.

FIGURE 3.4
Stepwise dilution procedure for determining CFU in probiotic bacteria culture



3.3 CO-CULTIVATION OF PROBIOTIC BACTERIA AND MICROALGAE

The compatibility of probiotic bacteria and microalgae species used for food must be assessed prior to introduction of probiotic to entire algal culture system.

STEP 1

Assess the compatibility of probiotic bacteria with the microalgal species used in the hatchery, if unknown.

Table 3.2 illustrates the importance of this assessment before integrating probiotic bacteria in algal lines. Information for two strains of *Pseudoalteromonas* is given, and incompatibility with some microalgal species and the difference between strains is seen.

TABLE 3.2
Compatibility of standard hatchery algal species with probiotic bacteria *Pseudoalteromonas* sp. "C" is compatible; "IC" is incompatible, "NT" is not tested

Algal species	*Pseudoalteromonas* sp. strain compatibility	
	P02-1	P02-45
Tahitian isochrysis	C	C
Chaetoceros calcitrans	C	C
Chaetoceros gracilis	IC	C
Rhodomonas	NT	C
Tetraselmis sp. (Plat P)	NT	C
Tetraselmis sp. (429)	C	C
Thalassiosira pseudonana (3H)	C	IC
Skeletonema costatum	NT	C
Pavlova lutheri	NT	C

Source: Aquatechnics supplier, 2018.

STEP 2

Harvest the probiotic bacteria by removing the Marine Broth in which it is grown. This is done by centrifuging a known concentration of bacteria culture; the bacteria is concentrated, the supernatant is discarded and the remaining bacterial pellet used for inoculating. Bacterial pellets are dissolved in filtered, autoclaved seawater.

For co-cultivation, pellets are used to inoculate intermediate algal cultures using microbiological techniques or are introduced via the algal nutrient pumping system for large-scale culture systems (see 3.3.1 for Protocol on harvest and co-cultivation)

For direct addition to larval and spat culture tanks, pellets are resuspended in seawater prior to addition to tanks to ensure adequate mixing in the tanks.

How much probiotic bacteria is needed? – The bacterial isolate will co-cultivate with algae when transferred at concentrations of 10^6–10^8 CFU; optimal concentration is dependent on the species of algae. Resulting co-cultures are monitored daily, algal cell density is assessed (cells.µl^{-1}) (see Protocol 3.3.2 for bacteria counts in co-cultures).

During the initial research and development phase, co-cultivated algal culture growth is compared to a standard algal culture without probiotic addition. At least three species of microalgae used in food ration should be tested simultaneously with the same bacteria culture, in order to avoid any uncertainties as to results obtained. Ultimately, all microalgal species used in the hatchery and/or nursery should be assessed for compatibility.

How often is probiotic bacteria added? – The goal is to maintain a CFU of 10^6 on a daily basis in the co-culture; and this will most likely be achieved through routine injections of probiotic bacteria in the co-culture at least twice a week. Typically, in the 3 days following injection, *Pseuodalteromonas* CFU remains steady around 250×10^6, and in some cases increases up to 10^9 CFU; if there is no additional injection, a rapid decrease in CFU is seen, with no probiotic bacteria detected 5 days post-injection. This has been reported in the co-culture of three algal species (*Tetraselmis* sp., *Chaetoceros muelleri* and *Pavlova lutheri*) during preliminary trials.

To achieve success, the emphasis is on the cleanliness of the algal culture; for algal cultures where naturally occurring bacteria level is high, co-cultivation of probiotic bacteria will most likely be unsuccessful, and natural bacteria will prevail.

Differentiating bacteria species – The aquaculturist will establish a database with photos to facilitate the identification of probiotic bacteria. Identification is based on shape and colour; photos are useful to differentiate with any naturally occurring bacteria species in microalgal cultures and/or seawater (see Section 3.3.3).

3.3.1 PROTOCOL: Scaling up probiotic bacteria culture, harvesting and co-culturing with microalgae

MATERIALS

- Fume hood
- Centrifuge
- 1 L flasks (autoclaved)
- 500 ml flasks (autoclaved)
- Marine Broth (autoclaved)
- 12 centrifuge tubes and lids (50 ml)
- Clean and healthy 4 L and 20 L algal culture
- Steri stoppers or cotton plugs
- Aluminium foil
- Sterile pipettes (25 ml)
- Bunsen burner or propane torch
- 1 L sterile 1 µm filtered seawater
- 1 L sterile harvest flask (dry)
- 500 ml probiotic stock cultures

METHOD

IMPORTANT
Marine Broth in probiotic culture has to be discarded when harvesting bacteria and before transfer to algal culture for co-cultivation

Note: Probiotic cultures can be used to inoculate individual algal vessels (4 to 20 L) or a continuous culture system by injection into nutrient supply. As is recommended for algal cultures, the greater the volume of the inoculum, the faster the growth of the culture. A 1-week old 500 ml culture can reach 1.9×10^7 CFU in one week, compared to 5.2×10^6 CFU in two weeks with a lower volume inoculum.

Scaling up probiotic bacteria cultures from 500 ml to 1 L

1. Prepare previously autoclaved 1 L flasks with 600 ml Marine Broth.
2. Working under the fume hood near the flame, and using sterile microbiological techniques, transfer 200 ml of starter culture into 1 L flask containing Marine Broth. Close and label. Grow for 2 weeks or until CFU reaches 10^6
3. Divide remaining starter culture into two. Transfer half into a new 500 ml flask containing Marine Broth. Close and label accordingly. Repeat with second half; these are your new duplicate starter cultures; they are used for re-inoculating 1 L harvest cultures if these become contaminated.
4. Harvest cultures are sub-cultured for co-cultivation with algae.

Harvesting probiotic bacteria (from 1 L flask)

5. To maintain 1 L cultures for continuous harvest, re-inoculate new 1 L flasks with 600 ml Marine Broth at every harvest.
6. Place 1 L new flasks with 600 ml Marine Broth under the fume hood.
7. **Re-inoculate new 1 L flask:**
   Working near the flame, collect 50–100 ml of probiotic culture (10^6 density at 10–14 days old) from old 1 L starter cultures.
8. Transfer inoculum to new 1 L flask with Marine Broth. Grow for 10–14 days or until CFU reach 10^6. This will be used for the next harvest.
9. Remaining probiotic culture from old 1 L flask will be used for co-cultivation with algae.
10. **Harvest bacteria:**
    Collect 45 ml of probiotic bacteria culture from remaining 750 ml harvest culture using a graduated 50 ml pipette.
11. Dispense 45 ml into centrifuge tube and close tube with screw cap. Repeat to centrifuge entire harvest culture. The number of centrifuge tubes to be filled depends on available spaces in the centrifuge.
12. It is important to have equal measures in each centrifuge tube in order to keep centrifuge balanced.

3.3.1 PROTOCOL (continued)

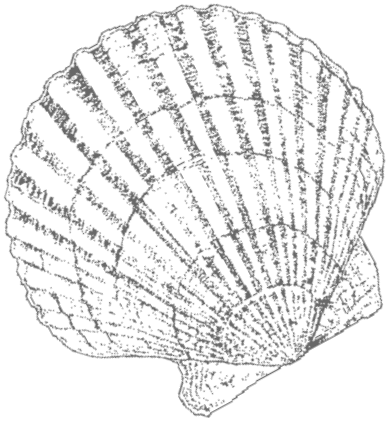
13. Alternatively, if probiotic culture is very concentrated, collect 17.5 ml of probiotic culture and dilute each test tube with 17.5 ml Marine Broth. This will allow for better precipitation and pellet formation during centrifuging.
14. Place tubes at opposite ends of centrifuge to keep it balanced.
15. Set centrifuge to 4 000 RPM for 10 minutes.
16. Once done, remove tubes from centrifuge.
17. Decant the supernatant, making sure not to disturb the probiotic bacteria pellet or precipitate.
18. Re-suspend the pellet in the same tube using sterile seawater (if <95 % of supernatant has been discarded, an additional rinse with sterile seawater will eliminate any remaining Marine Broth).

Inoculate single vessel microalgal culture

19. Working close to the flame, transfer bacteria/seawater solution into selected algal culture vessel.
20. Grow co-cultivated bacteria and algae under same conditions as algal cultures.
21. Monitor daily, collect a subsample to determine CFU and assess algal density. This will be an indicator of growth for both probiotic bacteria and microalgae.
22. Compare to algal growth in vessels with no probiotic addition.
23. Steps 7–23 can be followed as part of the screening process to assess the performance of the probiotic bacteria species, and the quantity of bacteria needed in co-cultivation. As a starting point, for a single 500 L bag, approximately 250 ml of probiotic bacteria culture is centrifuged.

Introduce probiotic bacteria to continuous large-scale microalgal system

24. Wash bacteria/seawater suspension (Step 19) into a 2 L sterile harvest flask. This is sufficient for a 150 000 L algal production system.
25. Pour harvested probiotic bacteria in nutrient reservoir supplying all algae.
26. Once both bacteria and algae reach optimal densities, feed to larvae or spat as per standard procedures.
27. Monitor CFU in larval and spat tanks three times a week.
28. Harvest probiotic bacteria when required and add to algal system to maintain CFU in co-cultivated algae. For a 150 000 L continuous algal system, this is done every 3.5 days.
29. Compare scallop growth and survival when fed co-cultivated probiotic bacteria/algae to standard techniques and assess effectiveness of probiotic bacteria.



3.3.2 PROTOCOL: Determining bacteria counts in co-cultures

MATERIALS

- 15 ml test tubes
- TSA plates
- Eppendorf pipette and tips
- Sterile seawater

METHOD

Note: Several TSA plates are prepared, comparing full concentration of the co-culture and diluted co-culture samples. If concentration of probiotic bacteria is high, it may be too difficult to count the colonies in a fully concentrated sample. The aliquot volume subsampled can also be adjusted dependent on the expected probiotic concentration.

1. Collect a sample of algal culture in a sterile 15 ml test tube, labelled with algal culture/Date plated/Day after injection of probiotic/Full ("Full" indicates full concentration of co-cultivated sample).
2. Label one more test tube similarly for a 1:10 dilution of co-cultivated sample.
3. Label three plates adjusting the aliquot volume plated, resulting in labels. For example:
   - Algal culture/Date plated/Day after injection of probiotic/0.1 ml/Full (for 0.1 ml aliquot of fully concentrated co-cultivated algae).
   - Algal culture/Date plated/Day after injection of probiotic/0.1 ml/1:10 (for 0.1 ml aliquot of diluted co-cultivated algal sample).
4. Add 9 ml of sterile seawater to test tube labelled "1:10".
5. Add 1 ml of co-cultivated algal culture into 1:10, 15 ml test tube and mix.
6. Collect 100 µl from co-cultivated algal test tube labelled "Full".
7. Remove lid from TSA plate and dispense aliquot on TSA plate labelled accordingly.
8. See Protocol 3.2.6 for details on plating.
9. Repeat collecting 100 µl from co-cultivated algal test tube labelled "1:10".
10. Once all plates are completed and probiotic bacteria samples are absorbed by agar, place TSA plate upside down in incubator (26 °C) for 48–72 h. Incubation temperature depends on probiotic species and can be as high as 32 °C.

3.3.3 Notes on identifying probiotic bacteria colonies

Bacteria colonies differ in appearance, size and colour, and the aquaculturist will have to learn to differentiate probiotic bacteria from naturally occurring bacteria. Here is an example on the approach used for the identification of bacteria colonies using a *Pseudoalteromonas* probiotic seed culture.

1. This seed culture has two distinct bacteria: Large orange and large creamy.
2. Algal culture bags have naturally occurring bacteria, which can be whitish pinhead size colonies.
3. To determine CFUs in probiotic cultures: A series of dilutions in Marine Broth is needed.
4. To determine CFUs in algal culture, a Fully concentrated sample and 1:10 dilution is required until the standard operating procedure is established.
5. A description of the probiotic bacteria colonies based on size, shape, colour, texture assists the technicians in the identification. In our example: probiotic bacteria colonies can be described as large, round, slightly raised, opaque, and a buttery surface.

6. The time of incubation varies among bacteria species. It takes 48 h of incubation for *Pseudoalteromonas* colonies to fully grow (at 26 °C), but 24 h for naturally occurring pinhead size bacteria.
7. Photos can be used as a reference to identify CFU in algal cultures and larval tanks (Figure 3.5).

FIGURE 3.5
Colony-forming units of probiotic bacteria on TSA plates – (a) too many colonies to count; top 1:10, bottom 1:1 000; (b) co-cultivation in continuous culture (500 L) showing probiotic bacteria colonies and naturally occurring bacteria in algae; post-inoculation (left), Day-1 after inoculation (right); and (c) top: pure probiotic culture 1:10 000 dilution; bottom: co-cultivated in 20 L and 3 L batch culture full concentrations (left and right)

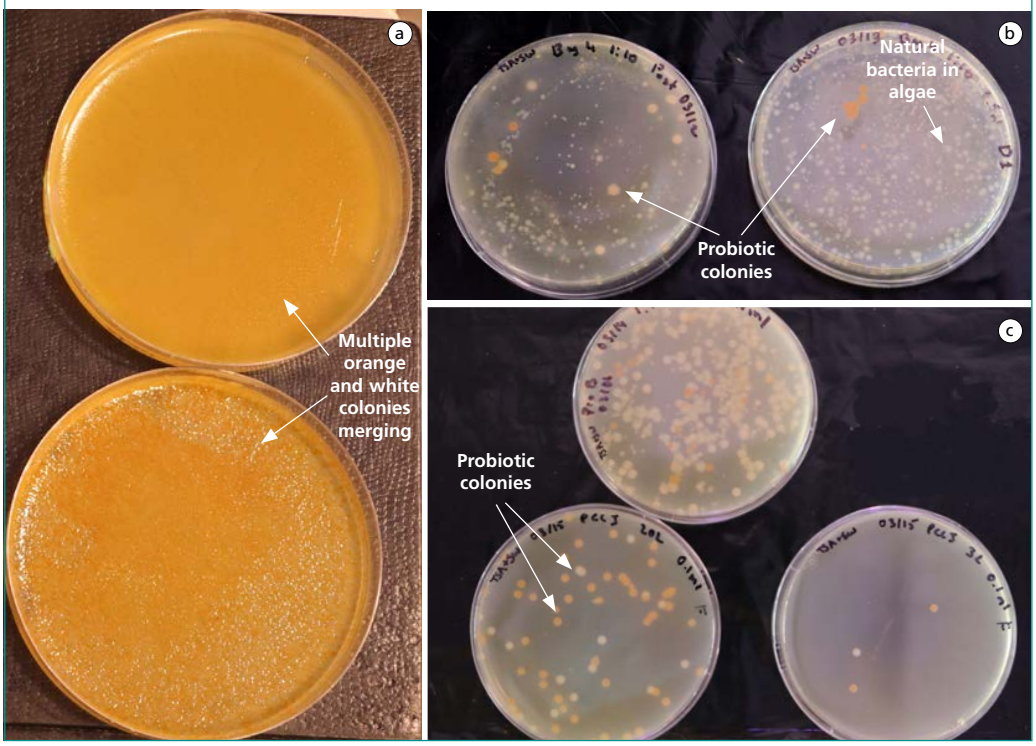


Figure 3.5 illustrates the challenge in assessing the dilution factor which enables differentiation of colonies and assessment of CFU; and in distinguishing between probiotic bacteria and naturally occurring bacteria found in algal cultures. Figure 3.5a shows plates with complete coverage of probiotic bacteria colonies illustrated by a uniform orange plate; even a dilution of 1:1 000 (bottom plate) results in too many colonies, which have merged and make it impossible to count. Figure 3.5b illustrates probiotic bacteria co-cultivated in continuous 500 L algal vessel plated post-inoculation of bacteria (see left plate) and Day-1 after inoculation (see right plate) both at 1:10 dilution. Probiotic bacteria colonies are distinct in post-inoculation plate (Figure 3.5b left) with some pinhead-size natural bacteria seen; this indicates the presence of naturally occurring bacteria in algal culture. In Day-1 plate, naturally occurring bacteria are seen in increasing numbers whereas probiotic bacteria colonies numbers decreased. This potentially indicates an unfavourable environment for probiotic bacteria overtaken by the naturally occurring bacteria. On the other hand, probiotic bacteria co-cultivated in small batch cultures illustrate a cleaner culture with undetected naturally occurring bacteria (Figure 3.5c). Top plate in Figure 3.5c is a pure probiotic bacteria culture illustrating a high CFU but possible to count at a dilution of 1:10 000.

3.4 INTEGRATING PROBIOTIC BACTERIA TO LARVAL AND POST-LARVAL CULTURE SYSTEMS

Initiate trials – Dedicate replicate larval and post-larval tanks for first trials. Add co-cultivated microalgae on daily basis as per standard feeding, monitor growth and survival of scallops, and compare with culture tanks fed standard microalgae. The goal is to achieve comparable, if not better, growth and survival without the use of antimicrobial drugs.

Probiotic bacteria species will differ in their effect on *M. yessoensis* early stages. Switching to a different probiotic bacteria species is recommended if co-cultivation and/or larval and spat responses are poor.

As a starting point, real life preliminary trials starting with Day-6 *M. yessoensis* larvae using *Pseudoalteromonas* indicated comparable growth and survival rate between probiotic and control batches with a survival rate >95 percent for larvae fed probiotic-enriched algae.

4. Hatchery facility layout

IN THIS CHAPTER – A conceptual design for a hatchery complex including all sectors of seed production is illustrated.

A brief overview of the hatchery complex is illustrated in Figure 4.1. Coarsely filtered seawater is pumped to the hatchery complex and treated according to the needs of the various scallop life stages. Any large equipment dedicated to seawater treatment, such as heating or cooling, protein skimmers and/or UV sterilisation are maintained separate from scallop and algal culture vessels. Smaller filtration and/or sterilisation units are frequently installed inline in relevant hatchery sectors. There are four main sections to the hatchery facility: broodstock, hatchery (larval culture), nursery (post-larvae and spat culture), and the algal culture areas. All are isolated from one another in order to avoid cross-contamination; biosecurity in a hatchery complex is a major component of successful seed production. Within the algal sector, additional precautions are taken to isolate stock, starter and intermediate cultures from large algal cultures. A dry laboratory area is needed in both the larval and algae sector. Design and construction for scallop or bivalve hatcheries are given in great detail in other manuals (see Further reading list).

4.1 SEAWATER TREATMENT PER SECTOR

Broodstock – Seawater is coarsely filtered. Temperature is ambient or according to conditioning cycle selected. This requires chilling and heating the incoming seawater at different stages of the conditioning.

Hatchery – Seawater is at minimum filtered to 1 µm and in some cases sterilised using UV or equivalent. Rearing temperature for *M. yessoensis* ranges from 12–14 °C and requires heating of the incoming seawater.

Nursery – Seawater is initially filtered as for the hatchery; filtration is reduced to 10 µm within one month of settlement. Seawater temperature is as for larvae, and requires heating of the incoming seawater; the capacity to reduce temperature by 1 °C in tanks with a continuous flow of seawater to ambient is needed for acclimatation at the end of the nursery period.

Algal cultures – Seawater is sterilised for all stages (stock to large-scale); UV is used for all; chemical sterilisation can be used for larger round batch tanks. Optimal temperature range for culturing algae is 22–24 °C.

Intermediate nursery – This is not shown in Figure 4.1, but seawater for intermediate outdoor nursery tanks or ponds is coarsely filtered using a sand filter or equivalent. Temperature is at ambient.

All sectors require a continuous air supply; this is further filtered by inline filters to individual vessels for the algal sector and for the hatchery. If air quality is poor, inline air filters are also added to the nursery sector.

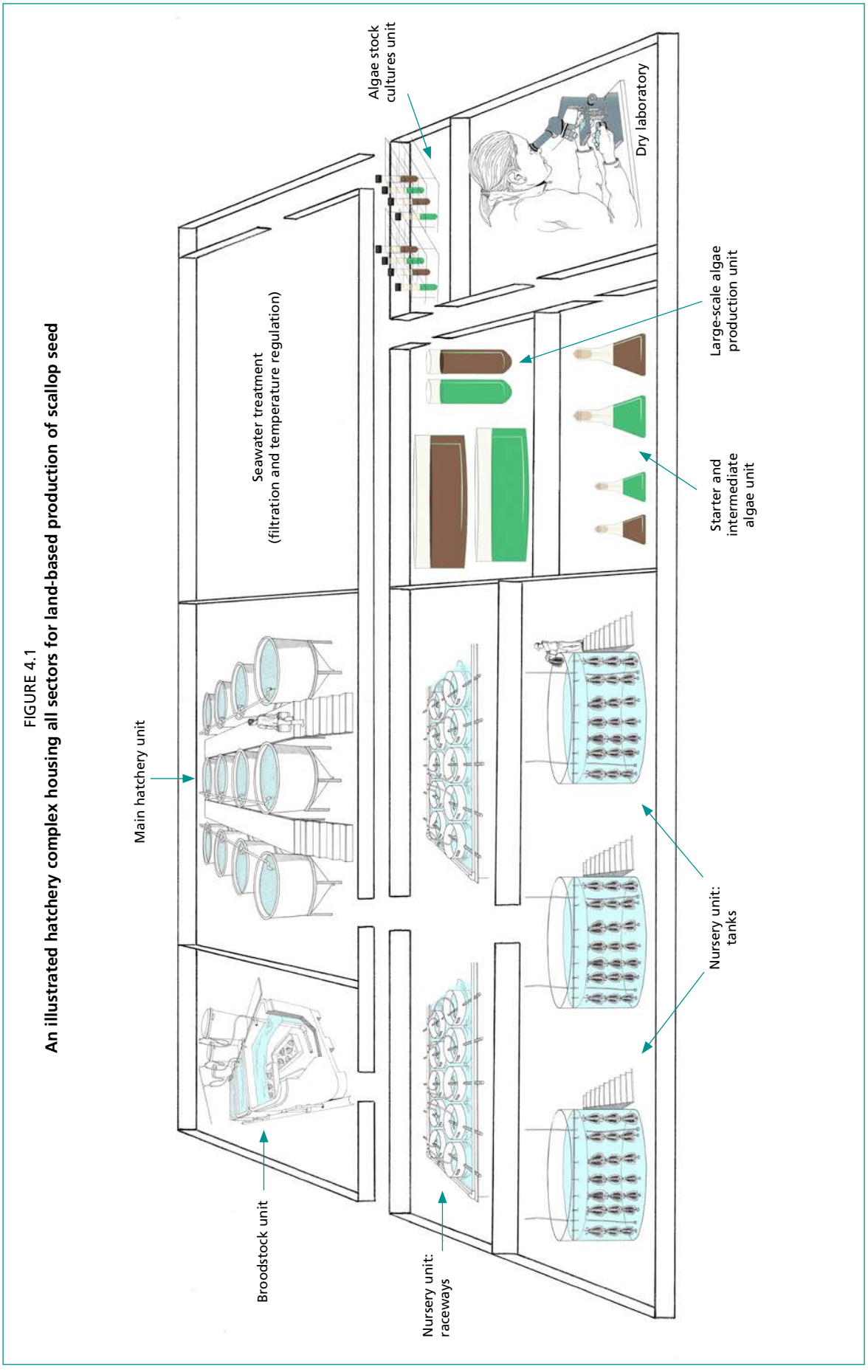


FIGURE 4.1
An illustrated hatchery complex housing all sectors for land-based production of scallop seed

5. Broodstock holding and conditioning

IN THIS CHAPTER – How to maintain or condition broodstock to ensure reliable spawning in the hatchery and how to implement a controlled spawn.

Mature scallops, both male and female, are required for spawning under controlled conditions, producing gametes and D-larvae for the commencement of the larval rearing cycle.

Yesso scallop gametogenic cycle – The seasonality of the gametogenic cycle is site and temperature dependent. Generally, Japanese scallops show active gametogenesis at seawater temperatures of 4 °C, increasing with temperatures of 6 °C.

Obtaining mature broodstock – There are two main approaches to obtaining mature broodstock ready for spawn.

1. *The aquaculturist relies on the natural gametogenic cycle*. This is supported by favourable environmental factors and is the simplest and less costly approach. It is used for species, which exhibit a distinct reproductive cycle and spawn naturally several times a year. This is achieved by collecting naturally ripened broodstock from the farm one week prior to natural spawn.

   To facilitate collection by farm personnel, the hatchery manager identifies a healthy stock of reproductively mature scallops; these are maintained on a dedicated longline with labelled nets. Ripe broodstock can be held at T = 6 °C in the hatchery for up to 14 days before spawning if fed daily. A few days before spawning, seawater temperature in the broodstock tank is gradually increased by 1 °C every two days until it reaches 8 °C; scallops can be maintained at this temperature for up to one week before spawning, but careful monitoring is required at this stage to detect and/or prevent spontaneous spawning. Although raising the temperature to 8 °C does not result in a marked increase in gonadic weight, it seems to enhance egg quality and larval viability.

   Feeding is stopped 24 h prior to spawning; this minimises the amount of faecal material excreted during spawning and yields a cleaner solution of eggs; in turn, this reduces the risk of bacterial contamination during embryogenesis.

2. *The aquaculturist controls the reproductive cycle*. This is most useful for species, which only spawn naturally once a year, have unpredictable spawning timing, and/or inhabit inconsistent natural environmental conditions. Conditioning broodstock enables the timing of spawning and allows to extend the natural spawning season; the disadvantages of conditioning are that it requires additional labour, space and feed volumes. Conditioning broodstock is achieved by collecting broodstock individuals which have started gametogenesis from the farm.

   In the hatchery, the aquaculturist manipulates temperature and food ration to accelerate the gametogenic process. Details are given in Section 5.3.

A mix of the two approaches is usually the strategy adopted by most commercial hatcheries.

5.1 REPRODUCTIVE CYCLE OVERVIEW

Gonad development – Gonad weight in adult scallops increases to a maximal value pre-spawn; this requires a substantial amount of energy, especially for female gametes (or eggs). Energy is initially obtained directly from the external food source through the digestive gland; as gametes mature – or when food supply is scarce – energy for gonad development is obtained from the reserves stored in the adductor muscle. If the natural reproductive cycle is not known, it is strongly advised to assess the gonadic and muscle indices on a monthly basis for the first 12 months. Indices are based on gonad and tissue weight; their relative fluctuations on a yearly basis provides a trend as to the changes in reserves for both tissues, and an indication of gamete ripeness. A high gonadic index is indicative of mature gonads, and a lower index reflects the onset of gametogenesis or spent gonads. A simultaneous monthly assessment of gonadosomatic indices and visual stages over a 1-year period will validate the latter; this will allow the aquaculturist to select ripe broodstock based on rapid visual assessments in subsequent years. For a more in-depth scientific analysis, cytological changes can be examined in gametes using histology to assess gamete development in a definitive manner. This is not usually conducted in a commercial hatchery environment.

5.1.1 Determining gonadosomatic indices

Gonadic index (GI) and muscle index (MI) – Using GI and MI to assess the gametogenic cycle is a simple, fast and inexpensive procedure which provides reliable quantitative information on gonad stage and mobilisation of reserves. Expressing indices in terms of shell weight is recognized to be most accurate; such that gonadic and muscle indices can be expressed as:

$$\text{Tissue wet weight} \div \text{dry shell weight} \times 100$$

or

$$\text{Tissue dry weight} \div \text{dry shell weight} \times 100$$

Dry weight is more accurate; however, a comparable trend can be obtained using wet weight, if a drying oven is not available. Other methods can be used where indices are determined based on "soft tissue weight" rather than shell weight; however it is recognized that using shell weight is more accurate. See Protocol 5.1.3 for determining GI and MI.

Seawater quality at site of broodstock sampling is determined during collection; this information assists in understanding seawater conditions favourable to natural gametogenesis, namely temperature and serves to implement and adapt a conditioning programme, if needed. A professional multiparameter meter (e.g. YSI™ Pro Plus meter) is sufficient for obtaining basic data such as temperature, salinity, pH and oxygen in the field.

5.1.2 Visual assessment

Gonad visual assessment – Visual assessment of gonads is common practice in hatcheries, because it is a non-destructive, rapid method and can provide a rough estimate of the gonadal status when conducted by an experienced technician. However, this is a subjective method and is not as accurate as tissue indices. Ideally, for the selection of broodstock for a scheduled spawn or for a conditioning regime, tissue indices are conducted on a subsample for an overview of the gametogenic stage of the whole stock and visual assessment is used to separate the broodstock according to level of ripeness. Table 5.1 provides the description of stages in a visual scale; the number

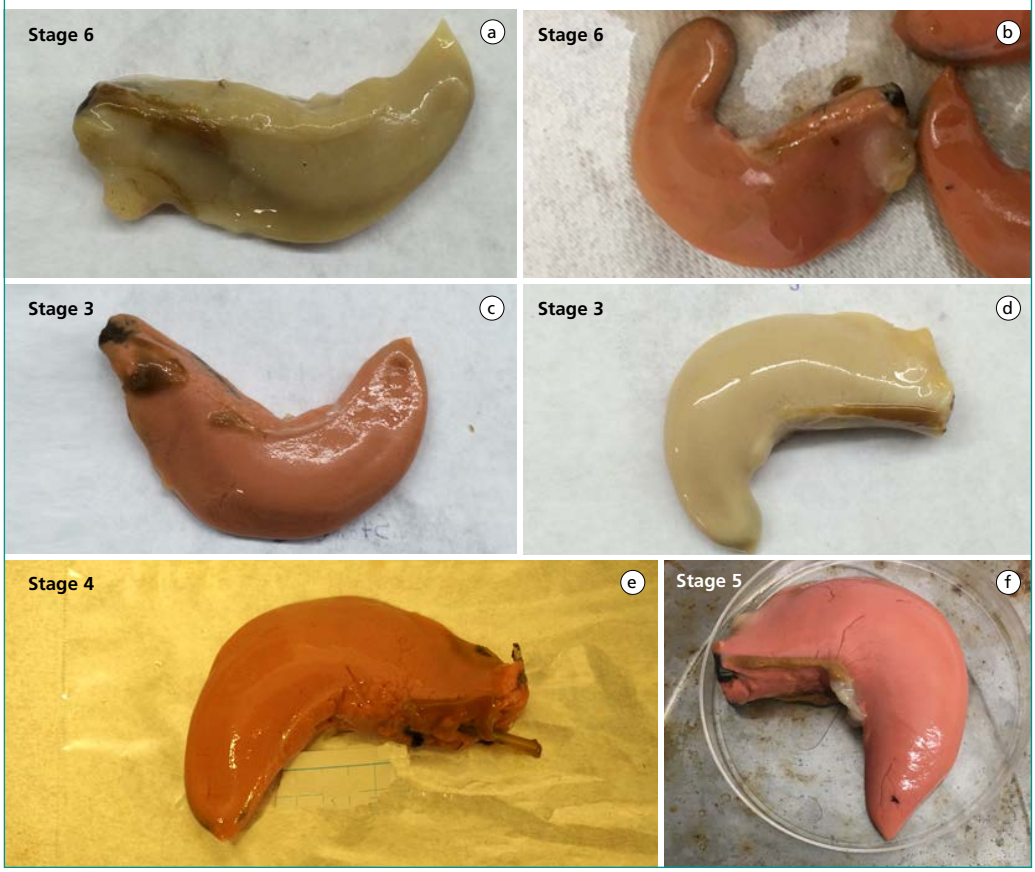
of stages within a scale and consequently its defining characteristics may vary slightly according to the preference of the aquaculturist. Photos of the Yesso female gonads are given in Figure 5.1 with visual stages subjectively attributed by an aquaculturist. See Protocol 5.1.4 for steps to assess gonad stages visually.

TABLE 5.1
Description of arbitrary visual index assessing reproductive status of adult scallops

Visual Index	Description	Gonad condition
0	Gonad is completely empty. Gametes are absent or largely so. Not possible to differentiate sex. Gonad is small, thin, flaccid and translucent. Intestinal loop is clearly visible.	Immature or spent
1	Gonad is translucid, white to grey. Slightly possible to differentiate sex.	"
2	Gonad is small, but pale. Sex is clearly differentiated: male (white) and female (orange). Intestinal loop is visible.	Active
3	Gonad is larger than visual grade 2 and increasing in turgor. Gonad is less granular in appearance. Intestinal loop is partially obscured.	"
4	Gonad is large and turgid. Ovarian tissue appears brighter, uniform in colour and texture. Very little of intestinal loop is visible (usually only a small portion at the distal extremity of the gonad).	"
5	Gonad is very large and thick as if ready to burst. Ovarian tissue is bright, uniform in colour, glossy and highly turgid. Gonoducts are usually large and conspicuous. Intestinal loop is not visible.	Ripe
6	Gonad is reduced in size compared to visual grade 4 and 5 and has lost turgor. Ovary appears mottled or lattice-like (as if partially emptied). Intestinal loop is usually visible but not always.	Partially spent

Source: Adapted from Williams, 2005.

FIGURE 5.1
Stages of Japanese scallop gonad – (a) male partially spent Stage 6; (b) female partially spent Stage 6; (c) female Stage 3 with intestinal loop; (d) male Stage 3 with intestinal loop; (e) female Stage 4, bright orange and turgid, some flaccidity along tip edge; and (f) Stage 5 female gonad with gonoducts



5.1.3 PROTOCOL: Determining gonadic and muscle indices

MATERIALS

- Dissecting kit
- Scale (±0.1 g)
- Absorbent paper
- Petri dish
- Vernier caliper

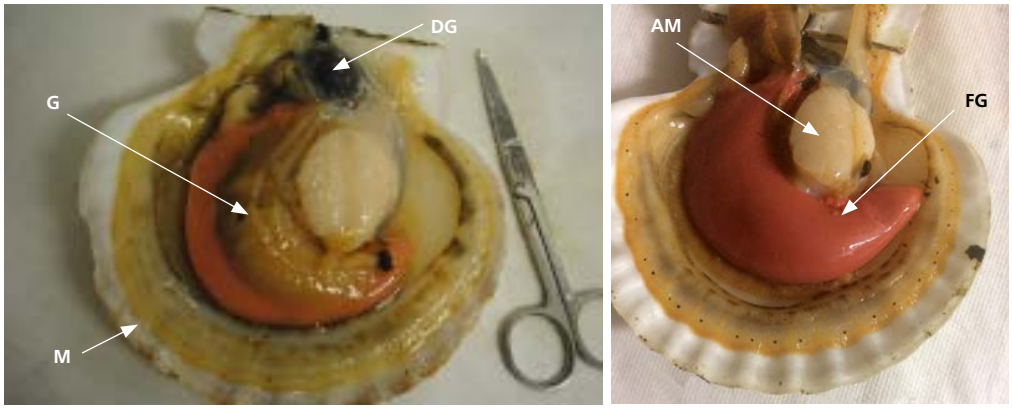
METHOD

Preparation of dissection

1. Set up balance, dissection kit with scissors, forceps and scalpel, and Vernier caliper for measuring scallops. Use a waterproof notebook to record all information.
2. A minimum of 10 females from each site or conditioning tank should be dissected. Males are only dissected, if there are any uncertainties or for a more scientific and comprehensive analysis.
3. Keep track of scallop source; attribute each scallop a number and label according to site or tank from which it is collected.
4. Record date of collection and dissection in laboratory book (or "lab book").
5. In the lab book, organize columns for scallop number, collection site or tank number, total shell weight (g), wet gonad weight (g), wet muscle weight (g), rest (rest of tissue wet weight in g), empty shell weight (g), and shell height (mm).

Dissection procedure (wet weight)

1. Work with 2 or 3 scallops at a time.
2. Place scallops convex bottom valve on a counter to allow valves to open.
3. Tare balance to read zero.
4. Blot dry each scallop with absorbent paper, wipe off any detritus accumulated on shell, and place on balance for total wet weight. Record data in lab book next to appropriate scallop number.
5. When a scallop is seen to gape, slide the scalpel in the opening close to hinge of the scallop, and staying as close to upper flat shell as possible, cut the adductor muscle from upper shell.
6. Separate the two valves and lay the convex bottom valve on counter for dissection.



G = gills; **DG** = digestive gland; **M** = mantle; **FG** = female gonad; **AM** = adductor muscle

5.1.3 PROTOCOL (continued)

7. Cut the adductor muscle from the bottom shell, scraping as closely to shell as possible for a clean cut (Figure 5.2 – Step 1).
8. Drain excess water before weighing and blot dry.
9. Lift the gills and using forceps hold the gonad.
10. Separate the gonad from the muscle by cutting around the muscle using scissors (Figure 5.2 – Step 2).
11. Cut gonad from digestive gland. Cut vestigial foot from gonad. Cut the gill from gonad.
12. Record if male or female (male-white, female-orange). If gonad is not developed and sex cannot be assessed, record as "undifferentiated".
13. Tare clean petri dish on balance. Balance reads 0.00 g.
14. Blot gonad dry with paper towel. Place gonad on petri dish and record weight.
15. Wipe petri dish clean, tare again.
16. Cut the mantle around the muscle to remove the muscle. Make sure you have all muscle tissue from ventral (bottom) and dorsal (upper) shell (Figure 5.2 – Step 3).
17. Blot muscle dry with absorbent paper.
18. Place muscle on petri dish and record weight.
19. Wipe petri dish clean, tare again.
20. Scrape all other tissue from both shells. Blot as dry as possible.
21. Place on petri dish and record weight under the "Rest" column to obtain total soft tissue weight.
22. Take both ventral and dorsal shells, dry with paper towel.
23. Tare balance to zero and weigh empty shells.
24. Reconstruct scallop (flat shell on bottom), and measure height (hinge to opposite side) with a Vernier caliper to ±0.1 mm. Record.
25. Repeat procedure for remaining scallops in sample.
26. **For conditioned scallops:** The number of scallops sub-sampled must be large enough to give the aquaculturist an indication of the conditioned stock, but does not have to be statistically representative, as this would deplete the broodstock available for spawning.
27. Subsample a few scallops from each conditioned tank and repeat Steps 1–24.
28. Clean all equipment thoroughly, especially balance, dissecting tools and Vernier caliper to prevent any salt corrosion.
29. Enter data as shown in the worksheet template (Appendix I).
30. Dissected gonads and muscles can be placed in a labelled ziplock bag and stored in a freezer to weight at a later date, if needed.

Recording gonad wet weight

Recording dry weight of empty shell (both valves)

5.1.3 PROTOCOL (continued)

Calculating indices

1. Data is entered in Excel sheet
2. Wet Gonadic Indices (GI) are calculated as:

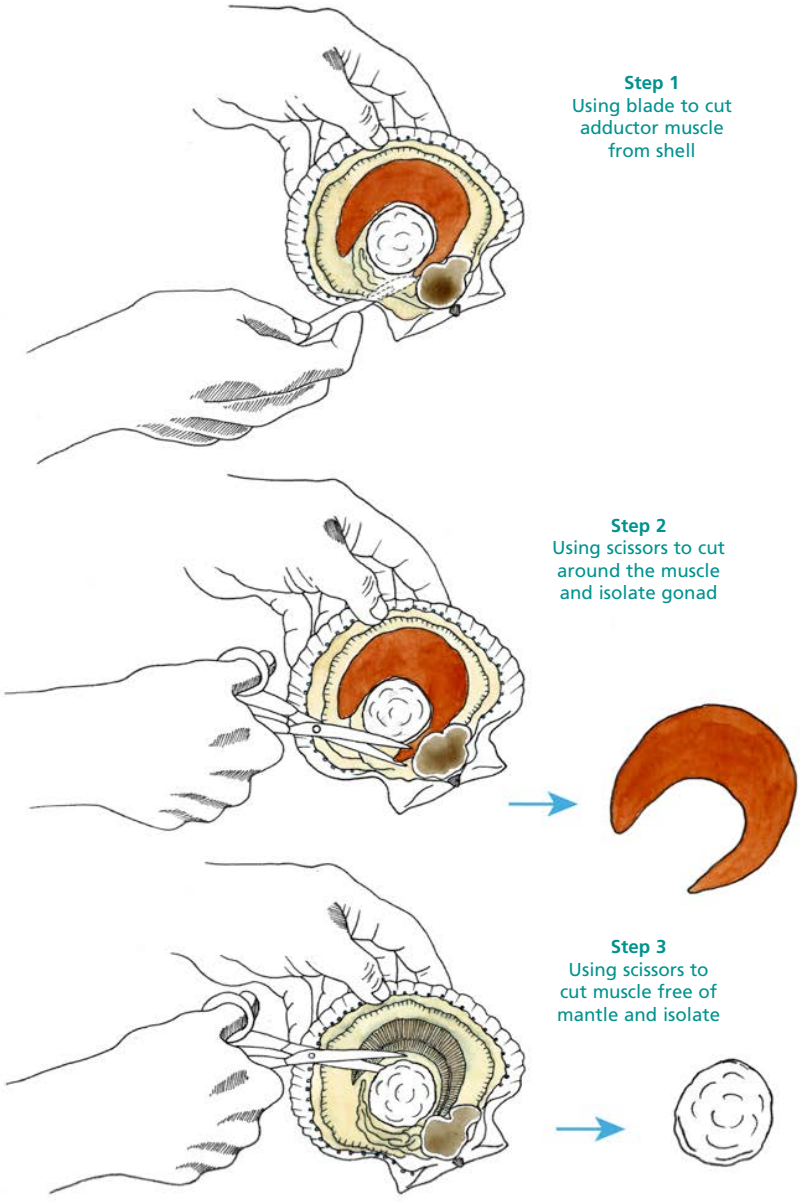
   gonad weight (g) ÷ empty shell weight (g) × 100

3. Wet Muscle Indices (MI) are calculated as:

   muscle wet weight (g) ÷ empty shell weight (g) × 100

Note for dry weight indices: The same procedure is followed, but organs are dried in a drying oven at temperature of 60–80 °C for 48 h or until constant weight, before weighing.

FIGURE 5.2
Dissecting gonad and muscle from mature scallop to determine gonadic and muscle indices



5.1.4 PROTOCOL: Assessing reproductive status visually

MATERIALS

- Light torch
- Zip tie to maintain valves opened
- Sub-sample of scallops

METHOD

1. Collect a subsample of the broodstock (females >85 mm shell height).
2. Expose to air.
3. When valves open, place a round smooth object between the two valves (a zip tie) to maintain valves opened long enough for a visual inspection of the gonad.
4. Use a light torch to shine onto the gonad.
5. Check the gonad against photos in Figure 5.1 and inspect the gonad for:
   - Intestinal loop – progressively less clear as gonads ripen.
   - Uniformity in colour – if gonad appears mottled or lattice-like or intersped with isolated specs of translucent sections (or acini), it has likely partially spawned or atrophied due to poor conditions. If it is uniform in colour but pale, it is in early development stage.
   - Colour, brightness of gonad – the deeper red in colour and brighter, the closer is the female gonad to ripeness.
   - Size of gonad – as it ripens, gonad will appear increasingly rounder and more swollen; a flaccid, flat or thin gonad is not close to ripeness stage.
   - Gonoducts – these are very obvious in ripe gonads.
6. Give a visual stage number to gonad as per the visual scale in Table 5.1.

Male Yesso scallop

Female Yesso scallop

Note: A visual stage of 3 represents a GI of 18 (based on wet tissue weight/dry shell weight). Spawning is induced with a minimum visual stage >3.5 (GI > 20) to obtain viable larvae.

5.2 HOLDING BROODSTOCK

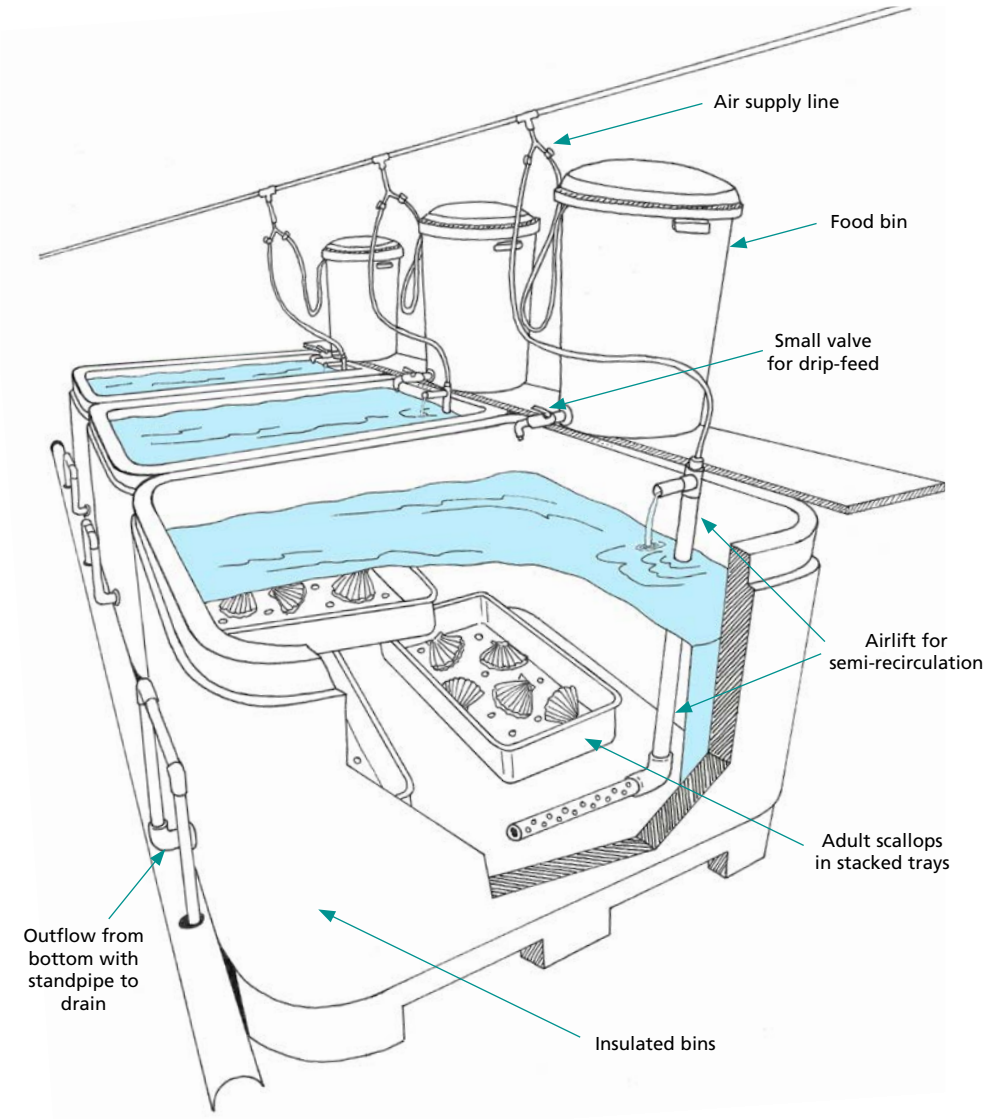
Water flow and temperature – Broodstock is kept in coarsely filtered seawater, typically in an open flow-through or semi-recirculated tank system. The key to an efficient broodstock tank system is to ensure a) a good seawater flow providing a 100 percent exchange of seawater every 2 h, b) the ability to control the seawater temperature to ±1 °C, and c) the ease of maintaining bottom of tank clean through removal of faecal material produced by broodstock feeding.

Tank systems – Dependent on the level of conditioning and broodstock selection, broodstock tank systems can be simple flow-through tanks, or more complex compartmentalised systems. As a guide, broodstock tanks approximating 600 L, with a 100 percent exchange of water every 90 minutes, are suitable to hold 40–50 scallop broodstock (90–120 mm shell height). Water exchange can be optimised throughout the tank with an airlift system, which partially recirculates the seawater and enhances food distribution. The difference between other bivalves and scallops is their tendency

to move and swim away from disturbance. This causes "clamping"– as they move or swim, they tend to clamp down on one another's shells and damage both shells and mantle. Figure 5.3 illustrates the use of trays, stacked to increase available surface area and increase capacity to hold broodstock. This is not ideal as faecal material from scallops in top trays will fall through lower trays, but it is space efficient; monitoring of scallops and changing tray placement is required in this system to ensure comparable water quality for all.

Broodstock held for longer than four days need to be fed on a daily basis. Feed can be distributed by batch and drip-fed over a period of 24 h. A rigorous cleaning protocol must be implemented to avoid a high deposition of faecal matter by fed scallops and consequently a decline in water quality.

FIGURE 5.3
Broodstock tank with airlift pipe, stacked trays and a dedicated batch-feeding tub



5.3 CONDITIONING BROODSTOCK

Goal – Generally, several spawns a year are required to attain targeted seed production. A continuous conditioning programme can provide readily available broodstock to accommodate the spawning demands on a when needed basis or is implemented when natural seawater parameters do not support consistent gonad development.

Conditioning – Conditioning a broodstock is labour intensive, it requires the capacity to control seawater temperature in large volumes and demands a high volume of feed. For these reasons, a strategic approach to conditioning and obtaining ripe broodstock is necessary to be as cost-effective as possible.

Role of temperature in conditioning – Conditioning of broodstock relates mainly to the manipulation of the seawater temperature; the aquaculturist simulates the seawater temperature cycle to which scallops are exposed in their natural environment, in an accelerated fashion. Dependent on the gametogenic stage of scallops when collected, the temperature cycle used for conditioning will differ.

Gametogenic stage at start of conditioning – The time at which the broodstock is brought into the hatchery depends on whether the objective is to trigger or enhance gametogenesis activity or ripen the oocytes in the final stages. However, the gametogenic stage at which broodstock are collected will affect the duration of the conditioning period. A broodstock collected at the beginning of the gametogenic cycle (visual stage <2.5) will require a longer conditioning period (minimum three months), starting at a lower temperature (2 °C). On the other hand, broodstock collected at a more advanced stage of gametogenesis is conditioned more quickly; for example, animals with a visual stage of 3, can be conditioned within one month starting at a temperature of 4 °C. Table 5.2 provides some guidance on the expected duration of conditioning for scallops of different levels of maturity and shows the equivalence between gonadic index (GI) and visual stage in each case.

TABLE 5.2
Level of condition required for Yesso scallops collected at different gametogenic stages

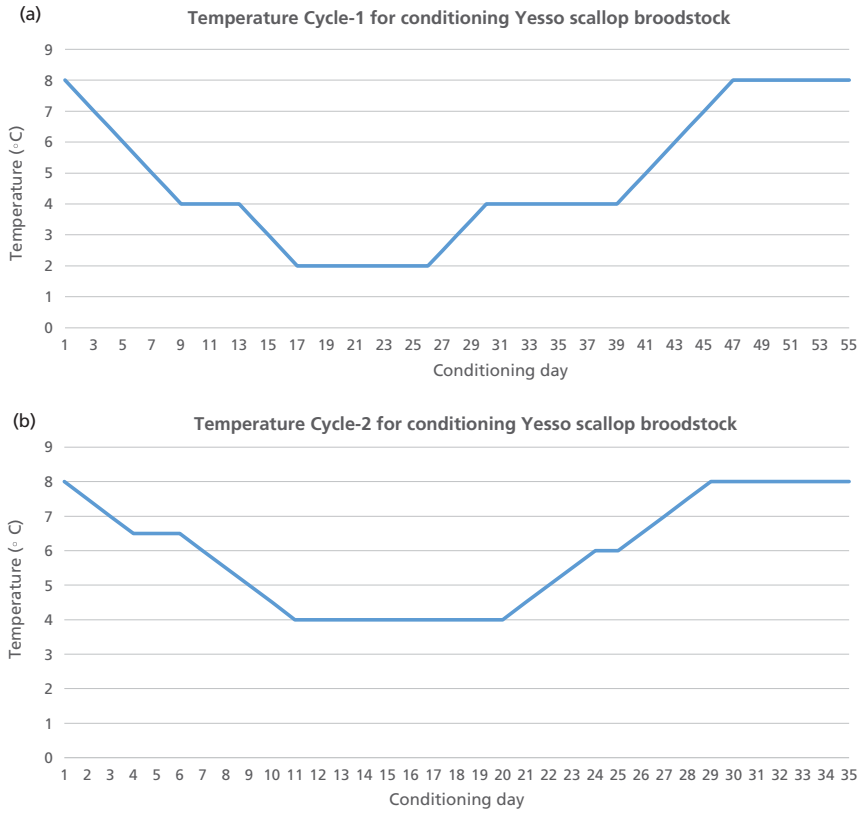
Duration of conditioning	Visual stage (1–6)	Gonadic Index (GI) (wet weight ÷ shell weight × 100)	Initial conditioning temperature (°C)
4–5 months	1.5	13.5	2±0.1
80 days	2.5	16	2±0.1
40 days	3	>16	4±0.1
7 days	6	>19.5	4±0.1

5.3.1 Overview of temperature cycles for broodstock conditioning

Temperature cycles – A full conditioning regime consists of a 2-part conditioning programme, preceded by a gradual stepwise acclimation from the ambient seawater (e.g. 8 °C) to the start of the conditioning temperature cycle (2 °C or 4 °C) (Figure 5.4). The first part of the temperature regime, when temperature is at a minimum, stimulates broodstock to accumulate reserves necessary to oocyte production; this occurs at 2±0.2 °C for scallops at the beginning of gametogenesis (Figure 5.4a), or at 4±0.2 °C for scallops which are at a more advanced gametogenic stage (Figure 5.4b). The second part of the cycle subjects scallops to a gradual increase in temperature for oocyte maturation. The longer conditioning period at colder water temperatures (2 °C) is referred to as Cycle-1 in the rest of this document; the shorter temperature regime initiated at 4 °C is referred to as Cycle-2.

FIGURE 5.4

Two temperature cycles for conditioning Yesso scallops adapted by S. Sarkis – (a) 55-day temperature Cycle-1 for scallops at the start of gametogenesis; and (b) 35-day temperature Cycle-2 for scallops which have partially spawned or with a starting gonadic index close to 16



Source: adapted by S. Sarkis (unpublished).

Duration of temperature cycle – The more advanced the scallops are in the gametogenic cycle, the shorter the duration of conditioning and the easier the process. Broodstock selected for conditioning should at minimum have differentiated gonads with a visual stage of 1.5–2.

Visual stage 0: Conditioning scallops with undifferentiated gonads (visual stage 0) is difficult; it is a long and costly process and may result in sub-optimal egg quality. Cycle-1 will have to be repeated several times before reaching a broodstock ready for spawning. Hence, the recommendation above to start with a visual stage of 1.5–2.

Visual stage <2.5: Scallops exhibiting visual stage 2 or thereabouts will require a prolonged exposure to the 4 °C phase in Cycle-1 before increasing temperatures to 6 °C and completing the cycle. Evaluation of the gonadic and muscle indices mid 4 °C phase will assist the aquaculturist in assessing the need for a prolonged cold phase.

Visual stage >3: Scallops exhibiting close to visual stage 3 (or GI approaching 18) will be ready for spawning within a 40-day conditioning cycle, following Cycle-2 (Figure 5.3b), and should reach a GI of 27 at this time.

Relationship between GI, MI and conditioning – Yesso scallops release viable eggs developing into viable larvae when mean gonadic index is >20.

1. Exposure to 2 °C during conditioning, results in increased muscle reserves, reflected as a high muscle index (MI).

2. Exposure to 4 °C results in a gradual increase in gonad weight and yields a higher gonadic index (GI) with input from muscle reserves. This reduces muscle weight and gives a lower MI.
3. Subsequent exposure to 6 °C results in continued increase in gonad weight with reliance on external food.
4. Maintaining scallops at 8 °C for one week prior to spawning, does not cause any change in GI or MI; however, this further ripens the oocytes, and possibly improves egg quality and larval viability.
5. Ripe scallops can be maintained at optimal gametogenic stage until spawning at a sub-optimal T = 5–6 °C, to prevent spontaneous release of eggs and sperm.
6. Partially spawned broodstock can be re-conditioned rapidly for a subsequent spawn within a 7-day period using Cycle-2.

Broodstock induced to spawn and spent should be routinely replaced by backup broodstock from the farm, such that broodstock are collected from longlines and/or natural beds on a regular basis throughout the spawning season. The aquaculturist can also save time by re-conditioning partially spent broodstock.

For successful conditioning, seawater quality must remain constant, with the seawater temperature cycle strictly adhered to and a high food ration given daily.

5.4 FEEDING BROODSTOCK

Food ration – Daily feeding is a must for adequate conditioning and for maintaining scallops at optimal gametogenic stage in the hatchery. During conditioning the diet given aims to provide the essential nutrients specific to egg development; standard practice is to calculate food ration as four percent per day based on dry weight of broodstock. Using 100 percent live microalgae as food supply places a high labour demand on the algal sector. Commercially available diets can supplement part of the daily feed requirements; rations are calculated according to the manufacturer's instructions. A detailed protocol for calculating live microalgae food ration is given in Protocol 5.4.1.

Food composition – A diverse diet will enhance scallop gonadic development and scallop health. The following has been used successfully for the Yesso scallop:

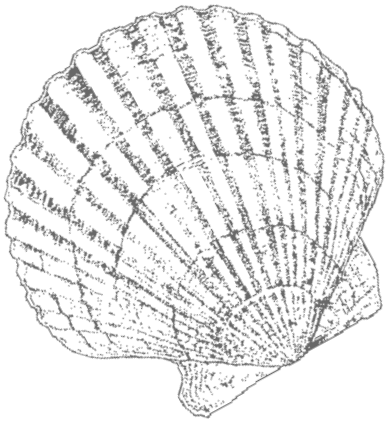
1. Live microalgae – includes standard diatom species (*Chaetoceros muelleri*, *Chaetoceros calcitrans*, *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*), and flagellates (*Isochrysis galbana*, *Pavlova lutheri* and *Tetraselmis* sp.).
2. Shellfish Diet 1800® – a commercially available product, consisting of 6 microalgal species (*Isochrysis*, *Pavlova*, *Tetraselmis*, *Chaetoceros calcitrans*, *Thalassiosira weissflogii*, and *Thalassiosira pseudonana*). This algal diet is often used as a supplement to the live microalgae for all life stages of bivalves.
3. AlgaMac-Enhance – a spray dried formulation that is high in DHA (docosahexaenoic acid – required by maturing scallops) and astaxanthin (A carotenoid). It contains intact cells of two algae, *Schizochytrium* and *Haematococcus*, non-genetically modified organisms (GMOs), known to improve the growth of juvenile bivalves. The product also contains inactivated yeast, which serves as an additional source of amino acids, nucleic acids, vitamins and beta-glucans. It is sold as a supplement for maturation and conditioning in aquaculture.

Mixing live and commercial foods – Live microalgae undoubtedly provides the best nutrition, but costs in terms of space and labour need to be assessed by the aquaculturist; this has to be balanced with the purchase costs of commercial diets. Commercial feeds used as partial substitutes for live microalgae, result in successful gonad development,

comparable egg fertilisation rate and high larval viability. The relative contribution of live to commercial can vary with live microalgae quality and quantity; the following proportions are suitable for conditioning of the Yesso scallop:

- Shellfish Diet 1800® can be used for up to 50 percent of total broodstock diet.
- AlgaMac Pro Plus or Enhance can be used for up to 35 percent of total broodstock diet.

A daily feeding record is kept by the aquaculturist to ensure appropriate food ration throughout the conditioning period; a template is provided in Appendix II.



5.4.1 PROTOCOL: Calculating algal food ration for broodstock

METHOD

Food ration for broodstock is calculated on a percentage tissue weight basis. Total tissue weight in a broodstock tank can be estimated as follows:

1. Count the number of scallops per broodstock tank.
2. Estimate total wet weight per tank (approx. 80 g per scallop for the Japanese scallop).
3. Estimate total dry weight per tank (approx. 8 g per scallop).
4. Create an Excel spreadsheet.

To calculate live algae ration based on dry weight food ration

Standard food ration is given at 4 % dry weight of animal tissue.
Average weight for microalgae 0.02 mg dry weight per cell.

- except for *Tetraselmis* sp. (0.2 mg dry weight per cell) and
- *Chaetoceros calcitrans* (0.007 mg dry weight per cell).

For ease of calculation: Use 0.02 mg for all flagellates and diatoms and 0.2 mg for *Tetraselmis* per cell.

5. Give a mixed 1:1:1 ratio of flagellate:diatom: *Tetraselmis*.

Diet equivalent to:

- ⅔ mix of flagellates and diatoms = 2.7% of total diet and
- ⅓ *Tetraselmis* = 1.3 % of total diet

6. Calculate flagellate/diatom mix:
ml of algal culture to be given = (2.7 × mg dry weight biomass) ÷ 100 ÷ (cell density × dry weight microalgae)

Example:
For an algal cell density of 10 million cells.ml^{-1},
dry weight of microalgae = 10 × 0.02 = 0.2 mg
Scallop dry biomass in broodstock tank = 60 g = 60 000 mg
ml of microalgae to give= (2.7 × 60 000) ÷ 100 ÷ (10 × 0.02) = 1620 ÷ 0.2 = **8 100 ml** or **8.1 L** (for 24 h period)

7. Calculate *Tetraselmis* 1.3%:
ml of algal culture to be given= (1.3 × mg dry weight biomass) ÷ 100 ÷ (cell density × dry weight microalgae)

Example:
For a cell density of 5 million cells.ml^{-1}
dry weight of microalgae = 5 × 0.2 = 1 mg
Scallop dry biomass in broodstock tank = 60 g = 60 000 mg
ml to be given = (1.3 × 60 000) ÷ 100 ÷ (5 × 0.2) = **780 ml** or **0.78 L** (for a 24 h period)

Total food ration to give (sum of 6 and 7): 8.1 L (flagellate/diatom mix) + 0.78 L (Tetra) = 8.88 L total microalgae administered over 24 h period.

5.5 SPAWNING INDUCTION

Broodstock is spawned under controlled conditions, eggs and sperm are collected, eggs are fertilised and embryos are distributed into larval tanks for development.

Inducing spawning – Techniques for spawning scallops are well known. In general, spawning of scallops can be induced using thermal shock, air exposure, increased supply of food, and chemical exposure. Yesso scallops respond to an air exposure of 2–4 h and release oocytes within one hour following transfer to a warm water bath.

Overview spawning protocol – In brief, a GI taken the week of spawning dictates the spawning date for the hatchery staff. Scallops are collected and the ripest are selected by doing a rapid visual assessment of the gonads (see Table 5.1). The aquaculturist aims for a spawning stock with a ratio of 1:4 male:female, in order to have an adequate pool of sperm to egg ratio. Scallops are scrubbed clean, any epiphytes are removed and they are exposed to air in a clean area of the hatchery for 2 hours; there should be minimal disturbance of scallops during this time. Following air exposure, scallops are transferred to a warm water bath (T = 13±1 °C), approximately 4–5 °C higher than that held prior to induction. Females and males are kept separate; this enables controlled addition of sperm to egg solution. Females can be placed in a saltwater table (Table spawn) or in lantern nets (5 scallops per layer) suspended in a tank (Tank spawn). Using a saltwater table will allow for a more careful monitoring and fertilisation procedure; however, the tank approach allows for a larger scale induction and yields comparable results in terms of larval production and percentage of pediveligers.

Protocol 5.5.1 describes a mixed approach of saltwater table and of the larger scale method using round larval tanks (5 000 L and up). The saltwater table method facilitates the monitoring of the spawn, allows for an accurate count of eggs released and ensures that the correct ratio of sperm:egg is added; this optimises the fertilisation rate and maximises the D-larval yield. A Table spawn should at the very least be carried out during the first spawns of the hatchery to obtain a better understanding of scallop fecundity and fertilisation rate. The larger scale spawning method (Tank spawn) using round tanks makes it difficult to accurately assess the number of eggs released and suitable volumes of sperm solution for fertilization. A high sperm:egg ratio increases the risk of abnormal development and/or mortality, as does a high egg density. When carefully executed with previous knowledge obtained during a Table spawn, spawning directly in tanks does provide a simpler large-scale approach to spawning.

Spawning timeline – First response is usually seen by males within 45 minutes following transfer to warm water bath; females usually first respond within 1–1½ hour after transfer to warm water.

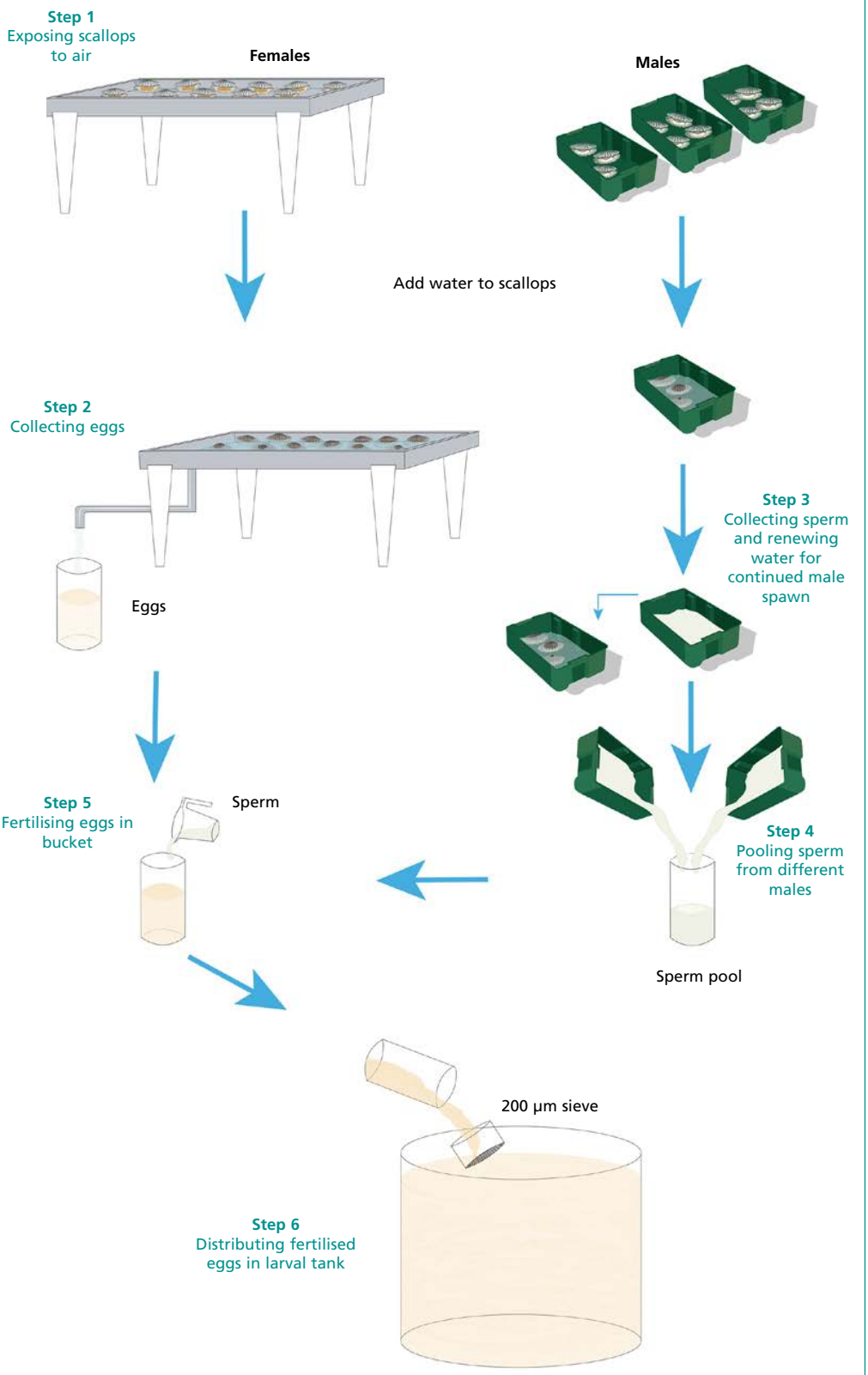
Sperm collection – As males spawn and water in tray becomes milky, sperm from various males is collected. It is important to collect sperm at frequent short intervals and keep track of the time each pool is created. As sperm lose viability with time, the newest pools are used to fertilise the eggs.

For the table spawn – Eggs are collected at regular intervals as they pass through the saltwater table drain and are fertilised within 30 minutes of collection. Evidence of fertilisation is checked before distribution into larval tanks (Figure 5.5a).

For the tank spawn – Sperm is added into the tank itself as eggs are released; it is important to keep track of the volume of sperm to avoid a high ratio of sperm:egg leading to abnormal embryo development. The total sperm volume is added over a

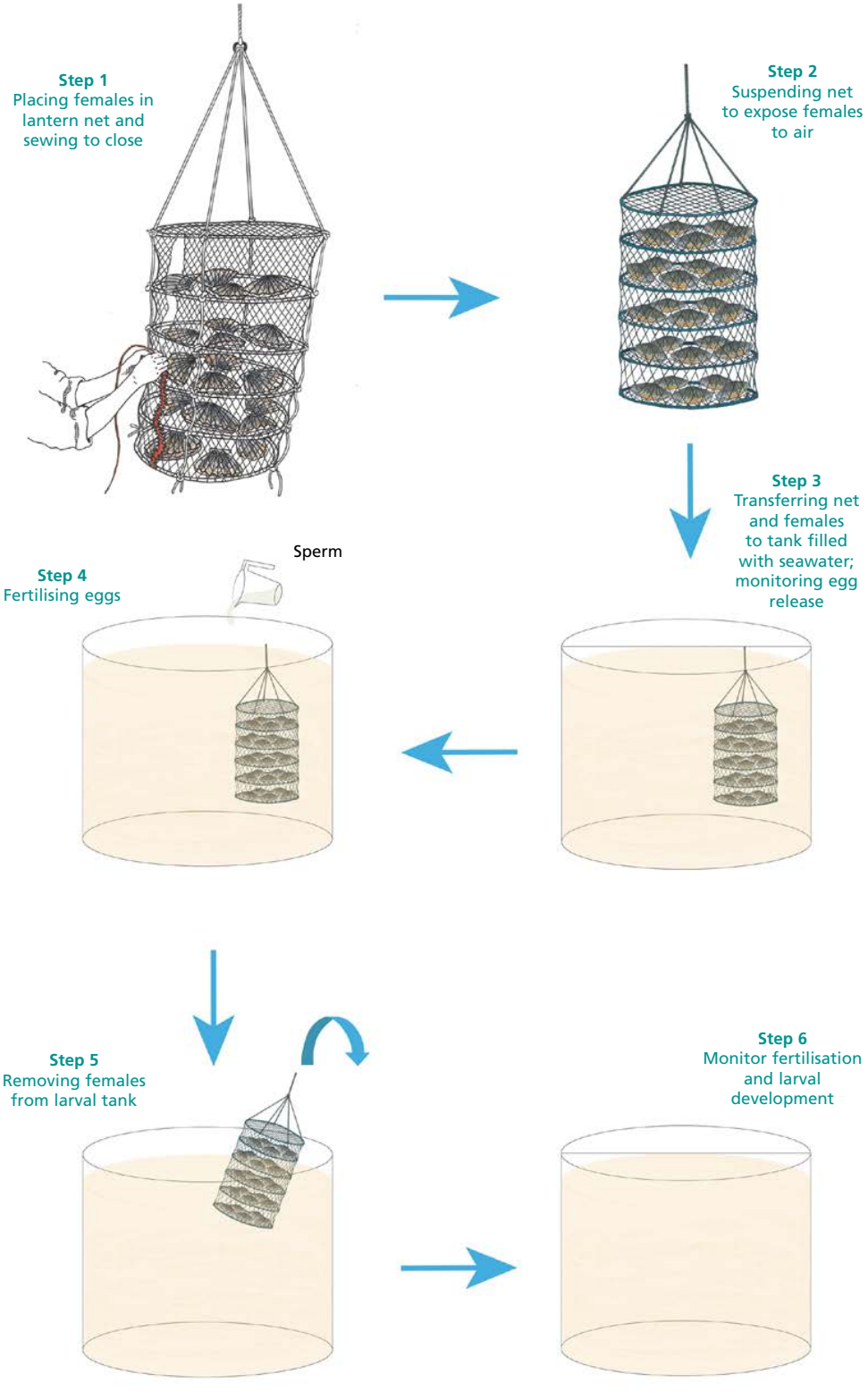
FIGURE 5.5a

Step-by-step illustration of spawning set-up for scallops, collecting gametes, and distributing fertilised eggs in larval tank – saltwater table spawn



2-hour interval, with the last addition made once females are removed from the tank (Figure 5.5b).

FIGURE 5.5b
Step-by-step illustration of spawning set-up for scallops, collecting gametes, and distributing fertilised eggs in larval tank – tank spawn



Approximately 2–3 h after warm water immersion, the rate and intensity of egg release is seen to decrease; at this time, female scallops are removed from the spawning table, and final egg collection is done. The first three hours of spawning yield the highest number of eggs; however, scallops can continue to release eggs and sperm for 12 h or more. To halt spawning, broodstock is transferred to a cold water tank. Average number of eggs released per female is expected to approximate 30 million eggs; this can most likely be improved through conditioning and spawning techniques.

Scallop eggs are distributed in relatively low densities compared to other bivalves; standard densities for scallops range from 5–20 eggs.ml^{-1}. For the Yesso scallop, densities ranging from 5–8.5 eggs.ml^{-1} result in a 42–55 percent D-larval development; higher densities should be tested as they may prove suitable.

Flat bottom tanks with a very slight aeration are used for the development of embryos to prevent the aggregation of the early immobile embryos at the bottom of the tank.

5.5.1 PROTOCOL: Spawning Japanese scallop in saltwater table and in large round larval tanks

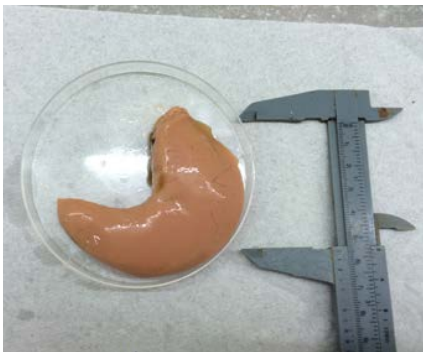
MATERIALS

- Hatchery logbook
- Saltwater table with open-flow capacity; outflow connected to clean hose
- 5-layer lantern nets or equivalent
- Buckets (10–20 L)
- Plungers for tanks and buckets
- Cleaning agent: Bleach, Virkon™ or equivalent
- 150 µm and 500 µm sieves
- Shallow trays or equivalent to isolate males
- Sedgewick-Rafter cell
- Eppendorf pipette
- Compound microscope with ocular micrometre or with a digital camera for measuring

METHOD

CHECK gonadic state of the scallops 3–4 days prior to spawn and isolate scallops selected for spawning. Visual index has to be >3.5; Gonadic index has to be ≥20 (GI based on shell dry weight).

SEAWATER used at all stages of spawning and egg fertilisation is preferably sterilised (UV or other); if sterilisation is not possible, double filter seawater to 1 µm.



Stage 5 female Yesso scallop ready for spawn

©FAO/S. Sarkis

Day before spawn: preparation

1. Do not feed selected broodstock the day before inducing spawn. This will minimise the amount of pseudofaeces produced during spawning and result in a cleaner egg solution.
2. Clean all tanks identified for D-larval development with a bleaching agent, scrub and rinse three times with freshwater. If used immediately, rinse one more time with filtered/sterilised saltwater.
3. Check that all airlines to tank are clean; each with an inline air filter. For large tanks, more than one airline may be needed to ensure a homogeneous and gentle flow in tank.

5.5.1 PROTOCOL (continued)

Option to use tank spawning for large-scale, add Step 4 to preparation:

4. Clean number of lantern nets required (5 scallops per layer), by immersing in a bin filled with a solution of freshwater and bleach (1 $ml.l^{-1}$ of bleach per litre of water) for up to 24 h before rinsing with freshwater. Suspend to dry until spawning the next day. If used immediately, rinse one more time with filtered/sterilised saltwater.

Spawning day

5. Start preparing spawn early in the day.
6. Clean saltwater table using freshwater and bleach (or equivalent); make sure to scrub corners and clean outflow drain and hose.
7. Select scallops keeping males isolated from females.
8. Remove any epiphytes from scallops using a metal scraper; scrub scallops vigorously using a brush and rinse well in 1 µm filtered seawater.
9. Distribute females on dry saltwater table for exposure to air; distribute males in smaller trays (4 per tray) or in a second saltwater table, if available (Figure 5.5a). If scallops originate from different conditioning regimes, keep batches separate throughout spawning and label each batch.

Stage 5 female Yesso scallop ready for spawn

10. Record initial time of air exposure; leave for 2–4 h. After 2 h, test one scallop by pushing down gently on its valve; if scallop responds slowly, terminate air exposure. If reaction is quick, leave exposed to air and test every 45 minutes.
11. During air exposure prepare all equipment/materials and continue filling tanks for fertilised eggs. Final temperature in tanks should be at 13±1 °C.
    a. Set up clean filters for filling larval tanks.
    b. Fill tanks with double filtered 1 µm seawater.
    c. Monitor water temperature as tanks fill; record final temperature and salinity once filled.
    d. Set up 1 µm filter for saltwater table.
    e. Clean 10–20 L buckets for sperm and egg collection.

Following air exposure

Table spawn

12. Fill saltwater table with 13±1 °C (UV or double 1 µm filtered seawater) and distribute females in table.
13. Fill trays (or separate saltwater table) with saltwater as for females (Step 12) and distribute males.

Tank spawn

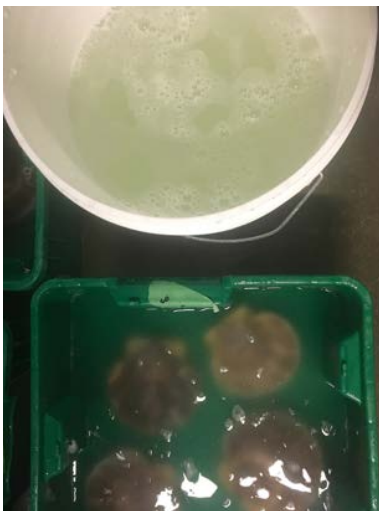
14. Place females in lantern net (5 per layer) and suspend in hatchery tank (Figure 5.5b). Record female origin per tank. Aerate tank gently. Record time of immersion.

Note: For Tank spawn, scallops can be distributed in nets and exposed to air; or left on a table for this period.

5.5.1 PROTOCOL (continued)

Egg and sperm release and collection

15. Leave scallops undisturbed following immersion, minimising movement around saltwater table and males and dimming lights in spawning area.
16. Males usually release first; it is expected that they start to spawn within 30–45 minutes after immersion in warm water; earliest response known is 1 minute after immersion. If males do not release sperm as planned, change water (they shouldn't stay in the same water for too long). If after 1 hour, males are still not seen to spawn, dissect one male from broodstock, collect sperm from gonad using a Pasteur pipette, dilute in a 50 ml beaker with filtered seawater and distribute into spawning trays.
17. Record time males first seen to spawn. Once males start to spawn, leave in tray until water becomes quite milky.
    a. Collect sperm from tray into a bucket when water gets milky.
    b. Add new UV or 1 µm heated seawater (leaving some sperm in tray to keep triggering release).
    c. Label the bucket with the time of collection.
    d. Pool sperm from various trays into one bucket.
18. Females should release eggs within 1–1.5 h following immersion in warm water; earliest response known is 25 minutes after immersion. Record time of first response and allow eggs to be released for approximately 30 minutes before first collection into bucket.



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Bucket with pool of sperm collected from all males (top); spawning males showing milky water (bottom)

19. Collecting eggs from saltwater table: Place drain hose into 150 µm sieve over a bucket. Crack one-way valve to allow for a gentle flow of egg solution in sieve. Care must be taken not to break the eggs. Fill bucket up to ⅔ of total capacity.
20. Collect eggs every 30 minutes or when you see too high a density in table. After each collection, re-fill spawning table with new 13±1 °C 1 µm filtered and/or UV disinfected seawater.

Fertilisation

Table spawn

21. Count and fertilise eggs in bucket as follows:
    a. Count number of eggs per bucket by sub-sampling 1 ml aliquots with an Eppendorf pipette as described in Protocol 6.2.1.
    b. Measure a sub-sample by recording the diameter under the microscope.
    c. Add 1 ml of sperm per 1 L of egg solution. Use newest pool of sperm released; try not to use sperm older than one hour.
    d. Mix sperm by plunging with gentle and long up and down motions. Plunger should not hit bottom of bucket (otherwise crushing the eggs) and should not come out of the surface water (also damaging eggs).
    e. Leave eggs to fertilise in bucket for 20 minutes or until first polar body is seen.
22. To distribute eggs in larval tanks; pass eggs through a 500 µm sieve to remove any detritus or faeces (Figure 6.4).
23. Distribute fertilised eggs at a minimum density of 10 eggs.ml^{-1}.

5.5.1 PROTOCOL (continued)

Tank spawn

24. Check for eggs using flashlight. Females should spawn approximately 1–1.5 h after immersion in warm water. If egg release is not seen, add some sperm as a trigger.
25. Record time when eggs first observed.
26. After approximately 20 minutes of spawn, add a first batch of sperm into tank; total volume of sperm should be equivalent to 1 ml of sperm per 1 L of water in tank.
    a. Collect a first sample of sperm into a graduated beaker and pour into tank.
    b. Mix sperm gently in tank using long and slow up and down motions with tank plunger.
    c. Make sure to mix gently and thoroughly entire volume, going around the tank.
27. Label tank; record number of litres of sperm added and time of addition in order to keep track of total volume added throughout spawn.
28. Allow females to spawn in tank for approximately 1.5 h after the first egg release is noticed; a good proportion, if not all, eggs should be released by that time.
29. Gradually add sperm solution as eggs are released in tank.
30. Plunge slowly after each addition, so that eggs are not disturbed.
31. Record volume of sperm added after each addition.
32. Estimate egg density in tank; if it exceeds 8 eggs.ml^{-1}, remove females.
33. Once females are removed from tank, record total amount of sperm added. Total amount of sperm in a 5 000 L tank should be between 5–8 L of sperm (or 1–1.5 L of sperm per 1 000 L of seawater).
34. To continue spawn, transfer nets with spawning females to new larval tanks filled with seawater. Record time of second immersion.
35. Make sure total amount of sperm is added to first set of tanks, as these will most likely be the largest volume and best eggs of the spawn.
36. Fertilise the second set of tanks as described in Steps 25 to 31.
37. Once egg release seems to slow down or stop (or 2 hours after second immersion) remove nets with scallops from tanks.
38. Remove females from nets and transfer to a holding broodstock tank filled with flowing coarsely filtered seawater at 6 °C. To completely stop egg release, decrease the temperature to 4 °C.
39. Repeat Step 38 for males, placing them in a separate tank to avoid continued induction and fertilisation.
40. Record time that spawning is terminated.
41. Gently aerate all hatchery tanks with fertilised eggs – a very slow stream of rising bubbles (one bubble at a time) coming to the surface should be visible. Too much aeration will trap the fertilised eggs on edge of the tank. Many aquaculturists do not aerate at this stage; however, this helps to keep embryos in suspension and prevent accumulation at the bottom of the tank.
42. Leave eggs undisturbed with airline until D-larvae development.
43. Sub-sample for the following two or three days to monitor division and stages. Record in hatchery logbook.
44. Add the first food ration at the late trochophore/early D-larvae stage. See Table 6.2.
45. A D-larvae culture should be ready for take down on Day-3 after fertilisation; it can extend to Day-4 dependent on time of day when eggs initially fertilised and rearing temperature.

Note: Scallops often only partially spawn and can be re-conditioned subsequently.

6. Larval rearing

IN THIS CHAPTER – How to culture larvae for successful metamorphosis into spat by managing tank seawater flow systems, seawater requirements, food composition and ration.

Larval cycle – Larval rearing refers to the culture of D-larvae to the metamorphosis stage, where larvae change from a planktonic to a benthic stage. The larval stage for the Japanese scallop ranges on average between 23 and 28 days (see Table 1.1) when reared at T = 13–14 °C. The first sign of metamorphosis is the presence of an "eye" on D-larvae; this is followed by the appearance of a foot. Substrate search behaviour can be seen under the microscope as larvae extend the foot out of the shell; this leads to "rafting" of competent larvae (or larvae ready for settlement) and can be observed during water exchanges in holding containers. "Rafting" appears as filaments of larvae in the water column. Techniques for larval culture of scallops are well known and a 40 percent survival from D-larvae of pediveligers is standard.

Larval tanks – Larvae can be reared in static or flow-through systems, or a combination of both. Larval tanks are typically flat-bottom round or semi-round tanks for static systems, ranging from 200–20 000 L and up. Flow-through larval tanks are typically cone-shaped and smaller up to 1 000 L in volume (Figures 6.1 and 6.2). Static systems require 100 percent water changes 2–3 times a week to maintain "clean" seawater culture conditions. Flow-through systems rely on a continuous low flow of incoming seawater; this improves water quality and requires minimal water change throughout the duration of the larval life. Consequently, labour requirement in the hatchery is reduced and larval cultures are less prone to bacterial contamination.

FIGURE 6.1
Larval tanks – (a) conical tanks used for flow-through rearing; and (b) illustrated series of larval tanks in hatchery

A general flow-through larval tank system is illustrated in Figure 6.2. Incoming seawater is filtered twice (1 µm) and often collected in a small header tank prior to the larval tank; this ensures a constant flow rate of seawater into the larval tank, minimising

FIGURE 6.2
Schematic of a larval flow-through tank suitable for scallop rearing, showing direction of incoming seawater flow

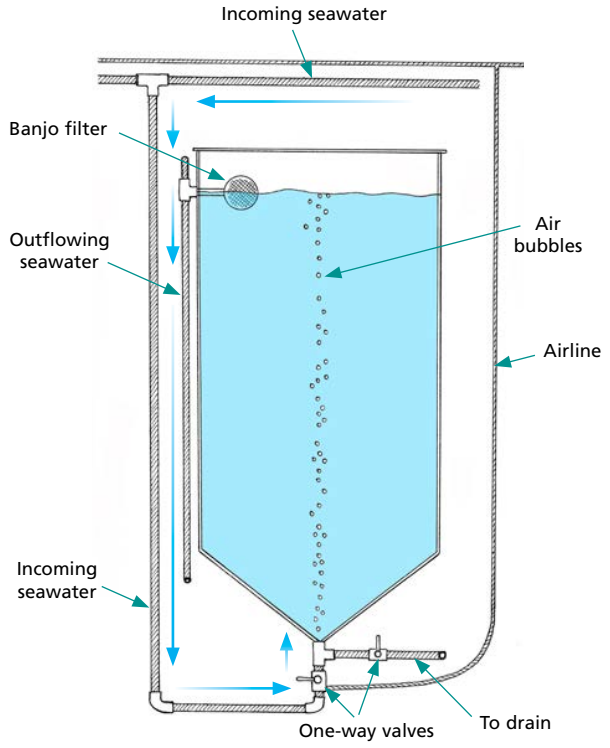
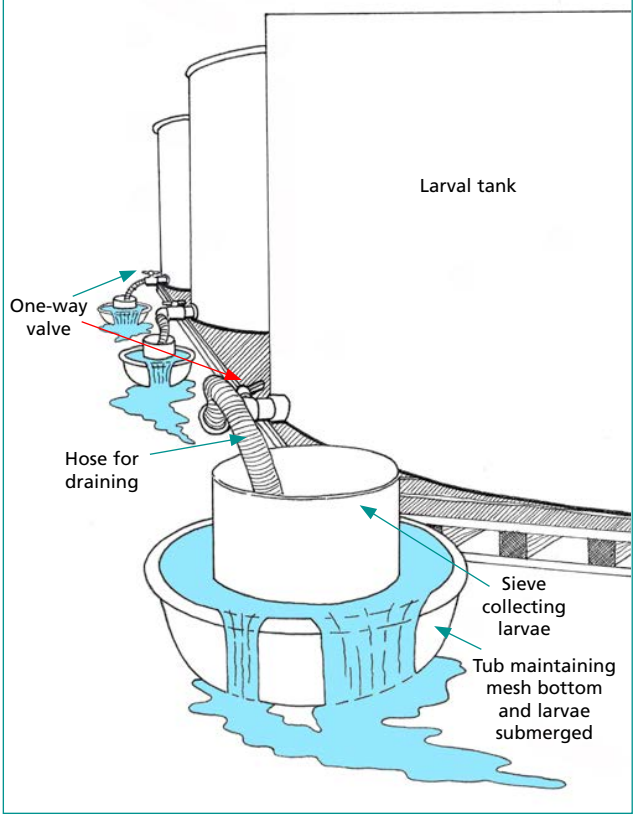


FIGURE 6.3
Take down larval tank for water exchange with larvae collected on meshed sieve



surges occurring in the main seawater line. In turn, the level of seawater in the header tank is maintained constant. Incoming seawater flows down the tank supply line to the bottom of cone, through gravity. Influx of seawater into the bottom of the tank is regulated by a valve. Air supply is mixed with incoming seawater, resulting in a slow stream of air bubbles rising to the surface of the larval tank. Outflow of water occurs at the top of the tank, through a banjo filter which prevents larvae from being discharged with the outgoing seawater. Suction pressure at the outflow must be considered and is minimised by fitting an appropriate diameter banjo. Outflow discharge rate of 0.8 $ml.min^{-1}$ for every cm^2 of banjo mesh surface area prevents trapping of larvae on mesh. Further details on setting up a flow-through system are available (see Further Readings).

6.1 GENERAL LARVAL REARING PROCEDURES

Use of sieves – The entire larval culture is collected by draining a tank through an appropriate mesh sieve (Figure 6.3). Larvae are collected to monitor growth, survival of the culture, and for transfer to new high quality seawater especially needed for static systems. Mesh size of sieve increases as larvae grow, to ensure that larvae are retained when draining of the tank, and range from 40–200 µm. Following monitoring, larvae are re-distributed in clean tanks filled with filtered seawater. Sieves are made in-house in PVC (polyvinyl chloride), fiberglass, or with hard plastic baskets (see Protocol 6.1.1); mesh used must be uniform to ensure that larvae are not lost. Sieves should have some support on the bottom so that mesh is not in direct contact with surfaces; if there is no bottom ring, always store sieve upside down so that mesh does not touch surface. Any small hole in mesh will result in a loss of larvae or spat. Storage must be in a dedicated area to avoid any spillage or tearing of mesh. If well taken care of, sieves can last several years. Care must also be taken when cleaning sieves to avoid tear. At every use, check sieve for any holes. At the beginning of every hatchery season, check all sieves and re-mesh, if needed.

6.1.1 PROTOCOL: Making sieves and banjos for larval and spat collection

MATERIALS

Glue material
- PVC frames – PVC cement glue and cleaner
- Fiberglass frames – Epoxy West System resin or cyanoacrylate superglue and accelerator

Frames
- PVC, fiberglass or plastic
- Permanent marker for labelling sieve
- Nitex® mesh 40–200 µm
- Hose clamps
- Razor blade or cutter knife
- Disposable brushes for epoxy resin

METHOD

PVC frames

To make sieve for larval and spat collection:

1. Cut PVC ring to appropriate height.
2. Sand surface of PVC frame on both sides to make it as smooth as possible.
3. If sieve is to be used as an inset, drill two 15 mm holes in opposite sides approximately 40 mm from the top. This will be used for suspending the sieve into a larger one.
4. Cut a square piece of mesh, so that entire surface of sieve is covered, with 3–4 cm of extra mesh hanging on side.
5. Lay mesh on top of frame.
6. Adjust hose clamp so that it can be placed on top of mesh and frame; some mesh will extend below clamp.
7. Tighten hose clamp along top of frame with a screwdriver, while pulling on hanging mesh.
8. Try to eliminate wrinkles in mesh by pulling uniformly around perimeter of sieve.
9. Mesh should be tight on surface of frame.
10. Label size of mesh on side of PVC frame with a permanent marker.
11. Once mesh is tight and hose clamp secure (approximately 1 cm from top of sieve) use PVC cleaner to prepare surface of frame for gluing.
12. Glue mesh to PVC using PVC cement along surface and side down to hose clamp.
13. Let dry for 24 h.
14. Once dry, make sure mesh is uniformly glued so that larvae cannot accumulate in small unglued areas (pay special attention to the rim on the inside of the sieve; larvae can become trapped between mesh and frame if not glued properly).
15. Remove hose clamp and cut unglued mesh with a razor blade.

Pulling mesh tight with hose clamp

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PVC sieves stored upside down

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6.1.1 PROTOCOL (continued)

To make banjo filters for flow-through tanks:

16. Cut PVC ring to appropriate height.
17. Drill a 15–25 mm hole on side to fit PVC connectors. This will secure banjo filter to larval tank.
18. Follow same steps as for PVC frame sieves (4–12) to mesh one side
19. Repeat on other side of the frame.
20. Let dry for 24 hours and follow Steps 14–15.

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Banjo filters

Fiberglass frames

21. Smooth surface of frame.
22. Follow Steps 3–10 above.
23. Make up a small volume (60 ml) of Epoxy West System (mixing hardener with resin using manufacturer's instructions).
24. Using a disposable paint brush, fix mesh to fiberglass dabbing top of mesh so that resin fills any uneven surface, gaps or holes.
25. Continue with paint brush on sides, taking care not to have the epoxy resin dripping onto clamp as this will also glue the clamp to the frame.
26. Once the first coat is completed, apply a second coat paying special attention to uneven surfaces.
27. Leave sieve to dry overnight in a dry and heated area.
28. Once dry, remove the clamp.
29. Cut excess mesh with a razor blade or cutter knife.

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Fiberglass sieve with independent support

6.1.1 PROTOCOL (continued)

Plastic sieves

Plastic sieves can be made with cheap plastic tubs purchased in department stores

30. Cut out bottom of container.
31. Cut a piece of mesh large enough to cover the bottom of container.
32. Clean plastic edge upon which mesh will be glued.
33. Using cyanoacrylate glue, secure mesh on plastic edge by gluing along edge.
34. Spray with the cure accelerator for instant gluing.
35. Continue gluing mesh by going around whole perimeter of container.

Plastic sieve

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Cleaning sieves

Before use for larval or spat collection, sieves have to be cleaned to avoid contamination of culture. Sieves are also cleaned after use to prevent any build-up of detrital material.

36. Use a damp soft cloth or sponge dipped into a cleaning agent diluted with freshwater.
37. Wipe around sieve frame inside and outside, and on mesh inside and outside with damp cloth.
38. Hose down thoroughly three times with freshwater.
39. Store in a dedicated area for drying until next use.

Water quality, temperature and density

Water quality – Water quality is the key factor in successful larval rearing. It is maintained through frequent water changes, when larvae are provided with new seawater. A complete water exchange involves the draining of the whole culture tank and the collection of all larvae for transfer into a new culture tank. This enables a comprehensive assessment of the growth and survival rate of the larval batch, and the aquaculturist can make any adjustments to culture parameters to improve larval performance at this time.

Flow-through systems – Continuous treated seawater flows into the larval tank at a rate equivalent to 2–3 times a full water exchange over a 24 h period. This maintains optimal water quality; in this case, manual water exchanges are not needed as frequently as for static systems. The cone shape in a flow-through tank results in the "trapping" of dead larvae or detritus when the incoming flow is stopped; occasionally, the drain can be opened briefly to remove accumulated matter. The flow-through tank will need to be thoroughly flushed and cleaned some time during the larval life to eliminate all detrital matter and prevent subsequent bacteria or *Vibrio* contamination. A manual water exchange can be scheduled every 7–10 days or more frequently dependent on incoming seawater quality and the health of the larval culture. The most critical period is as larvae approach the vulnerable pediveliger stage, and water exchange is a must at this time to successfully complete larval development.

Maintaining water quality on a daily basis in a flow through tank involves mostly the daily cleaning of the banjo filters. Unconsumed algae and/or pseudofaeces accumulate on banjo filters, and if not cleaned, the filter will clog and affect water flow. Banjos of increasing mesh size (40–150 µm) are used on the outflow as larvae grow to reduce clogging by the increasing amount of detrital matter associated with increasing food ration.

Daily checks for a flow-through tank system include:
- monitoring flow rate;
- cleaning banjo on outflow; and
- monitoring temperature – this is especially important as rearing temperature will be affected if incoming flow is inconsistent.

Static systems – Seawater is oxygenated via a gentle aeration. daily checks for static tanks include:
- visual check of tank and larval culture – bottom of tank should be clear of detritus or any trace of bacteria or *Vibrio* (e.g. *Pseudomonas* leaves red streaks on bottom of tank);
- monitor temperature, oxygen and other key parameters, if needed; and
- check aeration produces a slow stream of bubbles rising to the surface.

The hatchery manager sets a water exchange schedule to ensure that there is a complete (100 %) water exchange of larval culture at least three times a week. A detailed protocol for water exchange is given in Protocol 6.1.2.

The schedule utilised depends on the hatchery capacity, namely the:
- size of the larval tanks;
- available supply of seawater at the appropriate temperature and filtration; and
- number of hatchery staff available at any one day.

Table 6.1 below gives a few examples of water exchange schedules; these can be adapted dependent mainly on the seawater quality and on the health of the larval batch. For static tanks, 30 percent of the water can be exchanged between full 100 percent exchanges to optimise seawater quality if considered poor. For flow-through systems, a 100 percent exchange every 7–10 days is often sufficient; if there are concerns with respect to water quality or to larval batch, the number of water exchanges per week can be increased. A complete water exchange is a must if there is any evidence of contamination in the larval tank.

TABLE 6.1
Water exchange schedule scenarios for static and flow-through tanks

Water exchange scenarios	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
	%						
	Static						
Schedule A	100	30	30	30	100	-	30
Schedule B	100	-	100	-	100	-	-
	Flow-through						
Schedule A	100	-	-	-	-	-	-
Schedule B	100	-	-	100	-	-	-

Temperature – Larvae of Yesso scallops are successfully reared in 1–5 µm filtered seawater, at a temperature of 13–14 °C; this will result in a 23 to 25-day larval life.

Density – Larval density in the culture tank is reduced as larvae grow; initial density is 5–5.5 larvae.ml^{-1} and is usually decreased to an optimal density of 1–2 larvae.ml^{-1} at the pediveliger stage. However, comparable metamorphosis and settlement occurs for Yesso scallop pediveligers reared at densities of 3 larvae.ml^{-1} prior to settling.

In a flow-through system, larval densities are calculated based on the incoming flow rate; this results in a high-density system. For example: For a flow-through tank

with a 3-fold water exchange per 24 h, the initial stocking density is also 3-fold or 15 larvae.ml^{-1} (3 × 5 larvae.ml^{-1} as for static tanks), and final stocking density is 6 larvae.ml^{-1} (or 3 × 2 larvae.ml^{-1} as for static tanks).

Care must be taken to adjust larval densities in flow-through tanks as they grow; this is monitored during manual water changes.

6.1.2 PROTOCOL: Takedown of larval tanks for water change

MATERIALS

- Log book
- Cleaning agent (clorox bleach or Virkon™)
- Soft cloth for sieves and buckets
- Scrubbing brushes for tanks
- Holding buckets (10–20 L)
- UV and 1 µm filtered seawater supply (to wash sieves and fill holding buckets)
- Plungers for buckets
- Appropriate sieves
- Sedgewick-Rafter cell
- Eppendorf pipette
- Compound microscope with ocular micrometer, or with digital camera and computer set up for measuring

METHOD

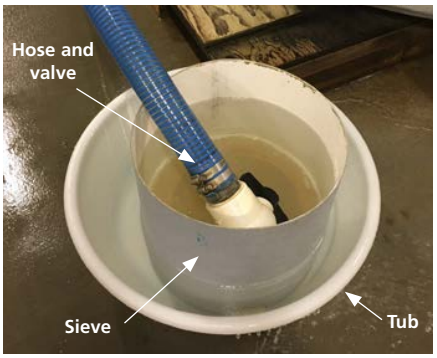
Day before 100 % take-down preparation

1. For any empty larval tanks, clean tank as per standard protocol.
2. Make sure airlines are clean with bacteria filters inline.
3. Make sure any cartridge filters are clean and air dried before use.

Note: If larval tanks are >10 000 L, draining the tank and collecting larvae with one hose only will take a few hours. PVC siphons with a one-way valve can be placed inside the tank, 30 cm off the bottom, to collect additional water and larvae; this accelerates collection, monitoring and transfer of the larval culture to a new tank, and prevents any damage to the shells by maintaining a suitable outflow rate while draining.

Day of take-down

4. Start as soon as possible in the morning.
5. Backwash and set up filtration system for double filtered 1 µm seawater supply and UV treated, if possible, to larval tanks.
6. Do routine checks of larval tanks before takedown (temperature; ~but also salinity, pH, oxygen if there are concerns with seawater quality).
7. Clean hoses, sieves, tubs, buckets using bleach, a freshwater rinse (3×) and one saltwater rinse (use same heated and filtered seawater as for larval culture).
8. Set up sieves in tubs, with hose connected to drain valve of tank.
9. Fill tub with treated seawater (filtered/sterilised and heated.



Collecting larvae in sieve

10. Place sieve in tub filled with seawater; this ensures that larvae collected always remain immersed in seawater. Make sure the sieve has a supporting base to maintain screen off the bottom of the tub; this can be a 5 cm high PVC ring of the same diameter. This maintains larvae in suspension off the hard bottom of the tub and avoids damaging larval shells during take down.

6.1.2 PROTOCOL (continued)

11. Place hose at an angle alongside sieve wall, to ensure that larvae flow out gently and are not damaged against sieve walls or pressed into the screen.
12. Open valve of larval tank slowly and ensure a gentle flow. There should not be white foam or bubbling during take-down due to vigorous flow (see photo).
13. Once first tank is ⅓–½ empty, start the second tank. Timing takedown for a series of hatchery tanks depends on availability of sieves, seawater supply, rate of filling of new clean tanks and the numbers of tanks to take down on the day.
14. Once larval tank level is above drain, stop flow from hose. Carefully remove hose and siphon from sieve.

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Vigorous flow during tank take down damages larvae

15. Keep the bottom of the larval culture separate from the rest, as it will contain detrital material from pseudofaeces, faeces and any dead larvae and/or unconsumed algae.
16. **To separate remaining bottom fraction of culture** – place a new sieve in tub and collect remainder.
17. Sides and bottom of tank should be washed with filtered and heated seawater to make sure that all larvae are collected (if the culture is healthy).
18. Gently wash larvae collected on sieves using heated filtered seawater supply into a clean 10–20 L bucket (make sure water used for washing larvae is the same filtration and temperature as larval rearing water).
19. Label bucket with tank number, sieve mesh size and record time when transferred in bucket. Larvae should not stay in the bucket for more than 1 h.
20. Set up an airline in the bucket with a low airflow, such that bubbles are seen rising in a steady stream to the surface of the bucket.
21. Examine both fractions of the culture (suspended and bottom). If the bottom fraction does not look healthy, with much detritus or moribund larvae sitting on the bottom, discard.
22. If the bottom fraction is healthy and consists of live swimming larvae, it can be added to the initial larval collection. If in doubt, it is better to discard, or pool all bottom cultures and re-distribute in a separate larval tank.

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Larvae held in bucket with gentle aeration

23. Clean larval tanks once empty.
24. Refill empty tanks as soon as they are clean, and adjust airflow in larval tanks, such that bubbles are seen to rise to the surface of the tank and spread throughout entire surface. Bubbling is not vigorous.
25. Count collected larvae in bucket and determine survival and shell growth of larvae (see Protocol 6.2.3).
26. Re-distribute larvae into filled tanks; dependent on numbers of larvae available, pool larvae according to size and health of culture into one tank.
27. Day-3 to Day-8 larvae are re-distributed at densities of 4–5.5 larvae.ml^{-1}; larvae older than Day-9 are re-distributed at densities ≤3 larvae.ml^{-1}.

6.1.2 PROTOCOL (continued)

28. **To re-distribute** – pass larval culture through a clean 500 µm sieve. Lower sieve below the surface so that half of the mesh is submerged. Pour the larval culture from holding bucket gently into the sieve so that any large debris or foreign larvae in culture are removed (Figure 6.4).
29. Feed larvae, as required (see Table 6.2).

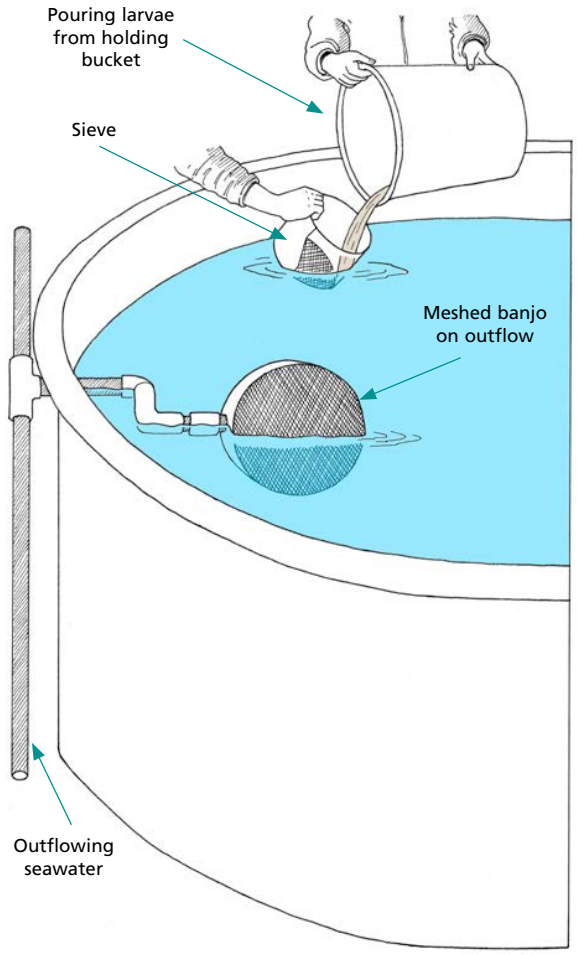
Once all larvae are re-suspended:

- Clean all materials and filters used.
- Drain all hatchery lines so that no stagnant water remains overnight.
- Hose down hatchery floor with freshwater and cleaning agent.

6.1.3 Larval tank aeration

Aeration is provided throughout the larval life as this maintains algal food cells and larvae in suspension. Embryo development into D-larvae is conducted in a static system. During the first 48–72 h of embryo development, minimal aeration is provided (one bubble at a time) to avoid disturbance and damage. Throughout larval life for static systems, aeration of the tank ensures a homogeneous distribution of algal food and suitable oxygenation to larvae; one to several airlines is used per tank dependent on the size of the tank. Air is often filtered using a 0.45 µm bacteria filter. Aeration throughout the larval life is gentle, and air is regulated by stopcock valves such that air bubbles can be seen rising to the surface from each line and joining at the surface. Aeration lines are weighed down but NOT fitted with air stones, as small air bubbles generated by air stones trap larvae.

FIGURE 6.4
Re-distributing collected larvae into a new clean flow-through tank by passing through a large mesh sieve to remove detritus



6.1.4 Larval food ration and distribution

Live microalgae cultured at the hatchery typically supplies 100 percent of the food administered to scallop larvae and <1.5 mm spat.

Distributing algal food – Dependent on the scale of the hatchery, algae can be drip-fed over 24 h into larval tanks from a 20–100 L bin. Alternatively, harvested algae are pumped to a food reservoir and diluted with filtered seawater; algal ratio is administered continuously over 24h and regulated via a dosage pump.

Food ration and composition – Both ration and composition change throughout the duration of larval life, to suit the metabolic requirements of larvae. Egg viability, embryogenesis, development rate and survival rate of early larvae depend in great part on egg quality. This in turn, depends on the condition of the broodstock and its gametogenic state. During the first few days of larval life, bivalves rely partly on the lipid reserves in the eggs; food ration is maintained low. Throughout larval life, the larvae's lipid-based metabolism slowly changes into a carbohydrate-based metabolism, which the aquaculturist satisfies by altering the algal composition. In general, scallops do not require as high an algal ration as other bivalves.

Daily larval food ration guide – The food ration for the Yesso scallop is well-tested and given in Table 6.2. A first ration of 5 000 cells.ml^{-1}, composed of a smaller algal-celled species (*Chaetoceros calcitrans/muelleri*; *Isochrysis* sp.) is given to larvae as soon as early D-larvae are observed, usually Day 2 after fertilisation. Food ration is slowly increased as larvae grow, to a maximum of 21 000 cells.ml^{-1} as they approach competency. There is a transition stage during metamorphosis when larvae lose their velum and do not feed. It is recommended to reduce feeding of the larval culture as larvae develop into pediveligers to prevent an excess of unconsumed algae and accumulation of detrital matter in the larval tank. Once pediveligers are observed in the culture, food ratio is slightly reduced to 18 000 cells.ml^{-1}, especially if setting of the whole larval batch spans over a few days.

Calculating algal food ration for larvae is based on larval tank volume. Algal density of algal vessels to be harvested is determined as described in Protocol 2.2.3. The amount of algae to feed each larval tank is calculated as shown in the example below:

EXAMPLE – How to determine volume of algae to feed larvae

Tank volume = 5 000 L
Algal density = 10 000 cells.ml^{-1}
Food ration = 10 000 cells.ml^{-1}

Volume of algal culture to give (ml):

Food ration (cells.ml^{-1}) ÷ **algal density** (cells.ml^{-1}) × **tank volume** (L)

$$\frac{10\,000}{10\,000} \times 5\,000 = 5\,000 \text{ ml}$$

or **5 L** of algae to distribute over 24 h to larval culture in 5 000 L larval tank

Calculating volume required for *Tetraselmis* sp. *Tetraselmis* is 10 times bigger than the other microalgal species used.

For a 5 000 L tank, an algal density of 10 000 cells.ml^{-1} and a food ration of 3 000 cells.ml^{-1}

Volume of Tetra culture to give (ml):

Food ration (cells.ml^{-1}) ÷ **algal density** (cells.ml^{-1}) × **tank volume** (L)

$$\frac{3\,000}{10\,000} \times 5\,000 = 1\,500 \text{ ml}$$

or **1.5 L** of algae

To compensate for the larger algal cell size for *Tetraselmis*:

1 500 ml ÷ 10 = 150 ml to distribute over 24 h to larval culture in 5 000 L tank

Table 6.2 is intended to be used as a datasheet to record actual food ration and composition given, dependent on availability. It should be posted in the algal laboratory next to the algal counting station; this will help the algal culturist to plan the algal production based on the daily harvest volume required. Columns for the actual food ration given are useful in the case of a change of ration or composition due to insufficient algae.

The algal composition assumed for Table 6.2 is initially an equal mix of 2–3 algal species consisting of flagellates and diatoms. Ration is usually composed of a 1:1 ratio of flagellates to diatoms. Flagellate species often used are *Isochrysis galbana* (clone T-Iso) and *Pavlova lutheri*; standard diatom species are initially *Chaetoceros muelleri* or *Chaetoceros calcitrans*. *Thalassiosira pseudonana* (clone 3H) is added to the diatom mix around Day-9. As larvae grow and approach the umbone stage, the larger flagellate *Tetraselmis suecica* or *Tetraselmis chuii* is added as a third part of the ratio for its high lipid content.

As mentioned above, food ration is decreased during metamorphosis when larvae lose their velum. This is reflected in Table 6.2, starting Day-23 or when pediveligers are first observed.

TABLE 6.2
Datasheet for food ration for Yesso scallops cultured at 13±1 °C

Larval stage and size (µm)	Days after fertilisation	Planned ration (cells.ml^{-1})	Planned algal ratio	Actual food ration given (cells.ml^{-1})	Actual algal composition
Early D-larvae 110 µm	Day-3	5 000	Chaet or Iso		
D-larvae	Day-4	6 000	1:1 Chaet:Iso		
	Day-5	7 000			
	Day-6	8 000			
	Day-7	8 000			
	Day-8	10 000			
	Day-9	10 000	1:1:1 Chaet:Iso:3H		
	Day-10	12 000			
	Day-11	12 000			
Umbone 170 µm	Day-12	14 000	Add Tetra 1:1:1 Flagellates:Diatoms:Tetra		
	Day-13	14 000			
	Day-14	15 000			
	Day-15	15 000			
	Day-16	15 000			
	Day-17	18000			
	Day-18	20 000			
	Day-19	20 000			
220 µm	Day-20	21 000			
Eyed	Day-21	21 000			
	Day-22	21 000			
Pediveligers 235–250 µm	Day-23	18 000–20 000			
	Day-24	18 000–20 000			
Day of set- based on behaviour	Day-25	18 000–20 000			

Note: Abbreviations in Table 6.2 are as follows: *Chaetoceros muelleri* or *calcitrans* (Chaet), *Isochrysis galbana* or Tahitian clone (Iso), *Thalassiosira pseudonana* (clone 3H) and *Tetraselmis* sp. (Tetra). *Pavlova lutheri* (Pav) can be substituted for Iso.

6.1.5 Use of antibiotics and probiotics

Antibiotics – Minimising bacterial contamination is inherent to the hatchery biosecurity operational mode. Lids on static larval tanks, cleanliness of all materials used, dedicated equipment to the larval section are such examples. For static systems, antibiotics can be used during water exchanges to reduce the risk of contamination; a small antibiotic dose can be added to the holding buckets post-drain and prior to re-distribution. Florfenicol (an alternative to chloramphenicol) is often used in large-scale operations (also in veterinary medicine and the salmon industry); a dose of 2 $mg.l^{-1}$ mixed into the holding buckets for a period of 1 h enhances the larvae's immunity to harmful bacteria.

Probiotics – Probiotics is the more ethically acceptable and sustainable alternative to the use of antimicrobial drugs for the control of bacterial contamination in hatchery cultures; their use is expanding in the shellfish industry and are routinely used in some commercial oyster; however, little is reported for scallop culture. Preliminary trials on Yesso larvae and spat are promising. There are two methods of integrating probiotics in larval culture. Chapter 3 provides further details on this including a description of probiotic culture techniques.

6.2 LARVAL GROWTH AND SURVIVAL

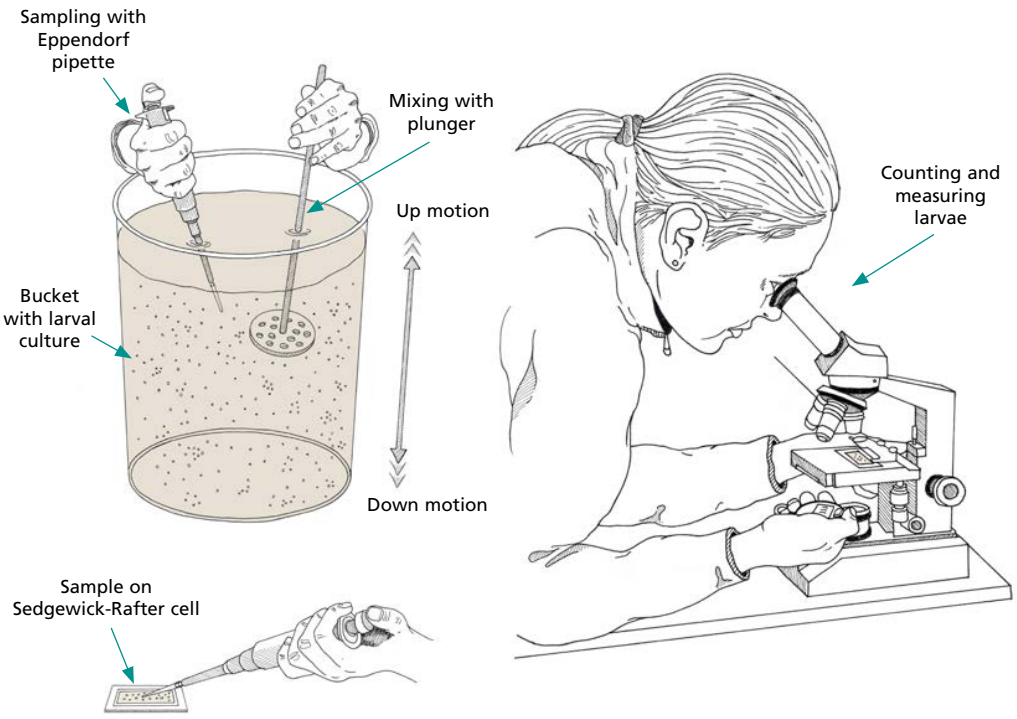
Larval cultures are routinely monitored for growth and survival. This provides the aquaculturist with a good understanding of the performance of the larval batch and an early warning of potential issues due to handling, poor seawater quality, poor algal food quality and bacterial/*Vibrio* contaminations.

Measuring growth – Assessing growth throughout larval life is based on shell length. An accurate assessment is obtained following a 100 percent water change, when the entire larval culture of a given tank is collected on a sieve prior to transfer to a new clean tank. For flow through systems, where 100 percent water change is less frequent, a subsample for shell length can be obtained by "scooping" a sample from the surface using a small sieve. This is a quick and basic assessment of growth but will not accurately reflect survival of the whole culture, as only healthy larvae suspended in the water column will be scooped.

Length can be measured on a subsample of 30–50 larvae using an ocular micrometre on a compound scope. The microscope is calibrated when first used; generally, with an objective of ×10, 1 unit equates 9.6 µm (often rounded off to 10 µm for ease of calculation), and with an objective of ×4, 1 unit equates 25 µm. For round eggs, the diameter is recorded as measurement of size; for D-larvae, length is equivalent to the straight hinge of larvae; as larvae grow and develop an umbone, the largest distance parallel to the hinge is taken as "length".

Estimating survival – Assessing survival is based on larval counts. Counts of live and dead larvae per ml are made on subsamples of the whole larval culture collected from one tank following water exchange. At this time, all larvae are collected from a rearing tank and washed into a holding bucket. Larvae are gently mixed for subsampling; triplicate samples for each larval fraction are taken and the average number of larvae calculated (Figure 6.5). This provides a "production yield" value and is used to assess the performance of the larval batch and its contribution to the annual production target. Standard procedures used for counting larvae are given in Protocol 6.2.1.

FIGURE 6.5
Collecting and measuring larvae



6.2.1 PROTOCOL: Counting and measuring larvae

MATERIALS

- 10 to 20 L bucket
- Homemade plunger
- Eppendorf pipette
- Sedgewick-Rafter cell
- 10 % formalin
- Compound microscope with ocular micrometre or with digital camera

METHOD

1. Once larvae are collected from a rearing tank and washed into a bucket, mix using a homemade plunger: mix thoroughly using 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 damage larvae.
2. During mixing take an aliquot (100 µl) of larvae using an Eppendorf pipette.
3. Place sample onto a Sedgewick-Rafter cell and fix with two or three drops of 10 % formalin.
4. Count larvae in a systematic fashion 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 the same larvae twice.
5. Measure larvae using an ocular micrometre along the straight edge of the shell; this will give you shell length.
6. Record the number of units on the micrometre and convert to µm according to your initial calibration.
7. Triplicate aliquots are taken for each larval fraction.
8. To determine survival, the average number of larvae counted in three aliquots is calculated, and used in the following equation:

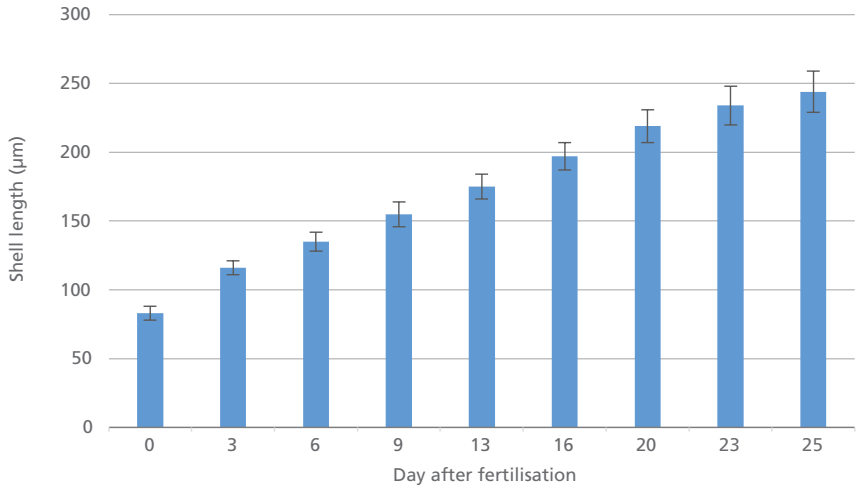
6.2.1 PROTOCOL (continued)

Total number of larvae collected = Average (larvae.ml^{-1}) × Volume of seawater in bucket (ml)

9. Survival is calculated from previous water change throughout larval life. A good culture will have a survival >90 % between water changes.
10. Overall performance of the larval batch is expressed as survival rate of pediveligers; this is calculated from Day-3 D-larvae (or Day-4 if development is slow).

Yesso larval growth – Yesso scallop D-larvae average 110 µm in shell length, growing to 244–280 µm prior to settlement. A larval growth rate of 5–10 µm.day^{-1} can be expected for a healthy larval batch. Competent larvae ready for settlement are recorded between Day-25 and Day-31 after fertilisation. The expected average shell growth for *M. yessoensis* larvae is given in Figure 6.6. Rearing temperature, water quality, algal supply and quality, air supply and circulation in tank are all key factors affecting the duration of larval life and survival rates. Inconsistency in the implementation of rearing techniques results in poor survival, and strict adherence to protocols is a must to achieve production targets.

FIGURE 6.6
Expected trend for shell growth for larvae reared in a static system at standard densities (n = 50)



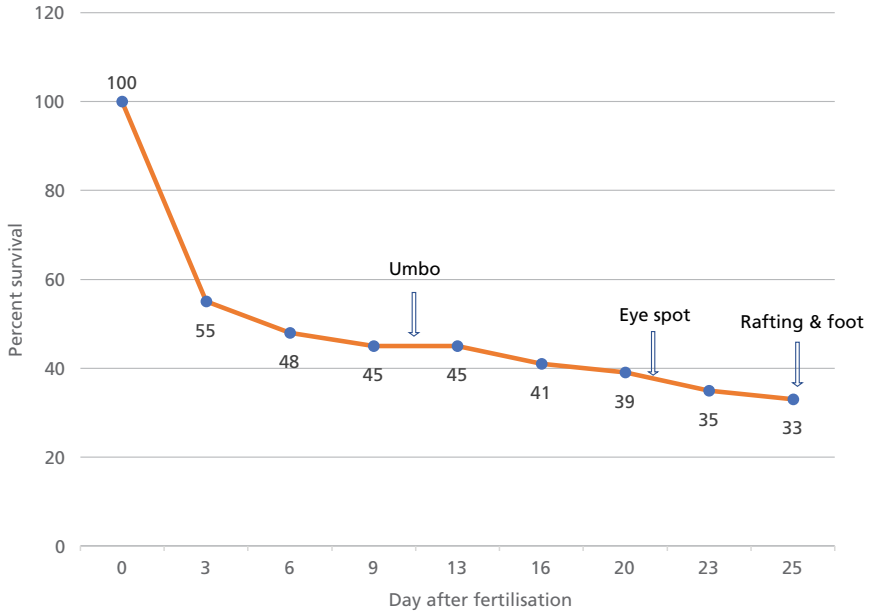
As larvae approach Day-23 of culture, signs of readiness for set or competence of larvae for metamorphosis can be observed; this is an indication to the aquaculturist to prepare settlement conditions for the next rearing stage.

Expected Yesso survival rate – Survival rate from D-larvae to pediveliger stage is a standard measure of the performance of a larval batch. A 40 percent survival of pediveligers from D-larvae can be expected for a successful larval batch.

Survival rate between 100 percent water changes provides an indication of the larval health on a weekly basis. A healthy larval batch which remains clean of bacterial or other contamination has an expected survival rate >90 percent between 100 percent water changes. Survival rate decreases with larval life, with the highest mortality rate generally seen shortly before metamorphosis.

Figure 6.7 illustrates a typical survival trend when rearing Yesso scallop larvae at a T = 13.5 °C. There are three critical points in larval rearing to which the aquaculturist must pay extra attention. The first is during embryogenenis where highest mortalities are encountered during development to the D-larval stage (Day-3 or Day-4). Thereafter, if protocols are strictly adhered to, high survival between water changes can be expected between Day-3 and Day-21. As eyed larvae approach metamorphosis, they become more vulnerable; this is the second critical point during the larval hatchery phase, as an increased mortality rate between water changes is likely. If optimal conditions are maintained, the aquaculturist can expect a 40–60 percent survival of pediveligers from D-larvae. Collecting and setting pediveligers in a timely fashion is the third critical point. As rafting and substrate search behaviour with foot extension is observed, setting must be initiated by the hatchery staff; if pediveligers are kept in larval tanks too long, the aquaculturist can lose more than 50 percent of the larval batch, as pediveligers will set on the sides and bottom of the larvae tank. At this point, it is very difficult to retrieve set larvae without damage to the shell. Note that in a large-scale operation, portions of the larval culture can be set at different times. This is done by grading larvae, setting those that are ready, and re-distributing the slower growing ones in the larval system for a few more days. The next section discusses grading larvae.

FIGURE 6.7
Average percent survival of *M. yessoensis* larvae determined during water changes in a 5 000 L static tank (T = 13.5 °C)



6.3 GRADING LARVAE

Larvae are graded according to size throughout the duration of the larval life. This has a threefold purpose: 1) It allows for selection of faster growing larvae; 2) it allows the slower growing larvae to catch up in shell growth by removing the larger-sized larval fraction; and 3) it helps in controlling bacterial contamination by separating the larval size fractions and removing smaller deformed or dead larvae.

Mesh size for grading – Meshed sieves are used for separating different size fractions of the larval culture. Day-3 D-larvae with an average shell height of 115 µm are collected from the tank and passed through a 60 µm and 40 µm mesh; the 40 µm portion often consists of undeveloped embryos or detritus and is generally discarded. As larvae

grow, they are collected on 80 µm, 120 µm, 150 µm mesh and 180 µm sieves. While grading, care must be taken not to damage the larvae as they are poured through the sieve; best practice is to have the sieve partially submerged in a tub of water, so that larvae accumulating on the mesh remain in water at all times (see Protocol 6.1.2).

Table 6.3 provides a guide on mesh size used for grading as larvae grow. The grading guide given is compiled from several sources; ranges may differ slightly, dependent on mesh type used and sieve characteristics. Shell length will vary with Day after fertilization from batch to batch. Setting is from Day-23 on depending on shell growth rate.

TABLE 6.3
Percentage of larvae retained on sieves of increasing mesh size (µm) according to their respective shell length, throughout the larval life

Day after fertilisation	Larvae retained on mesh size in µm (%)						
	40	60	80	120	150	180	Shell length range (µm)
0	100						78–88
3	0	100					108–118
7		70	30				126–144
9		7	93				131–155
13			60	40			160–170
16			20	80			175–190
21				45	55		185–200
25				12	88		190–220
28				15	82	3	200–260
31					35	65	240–275

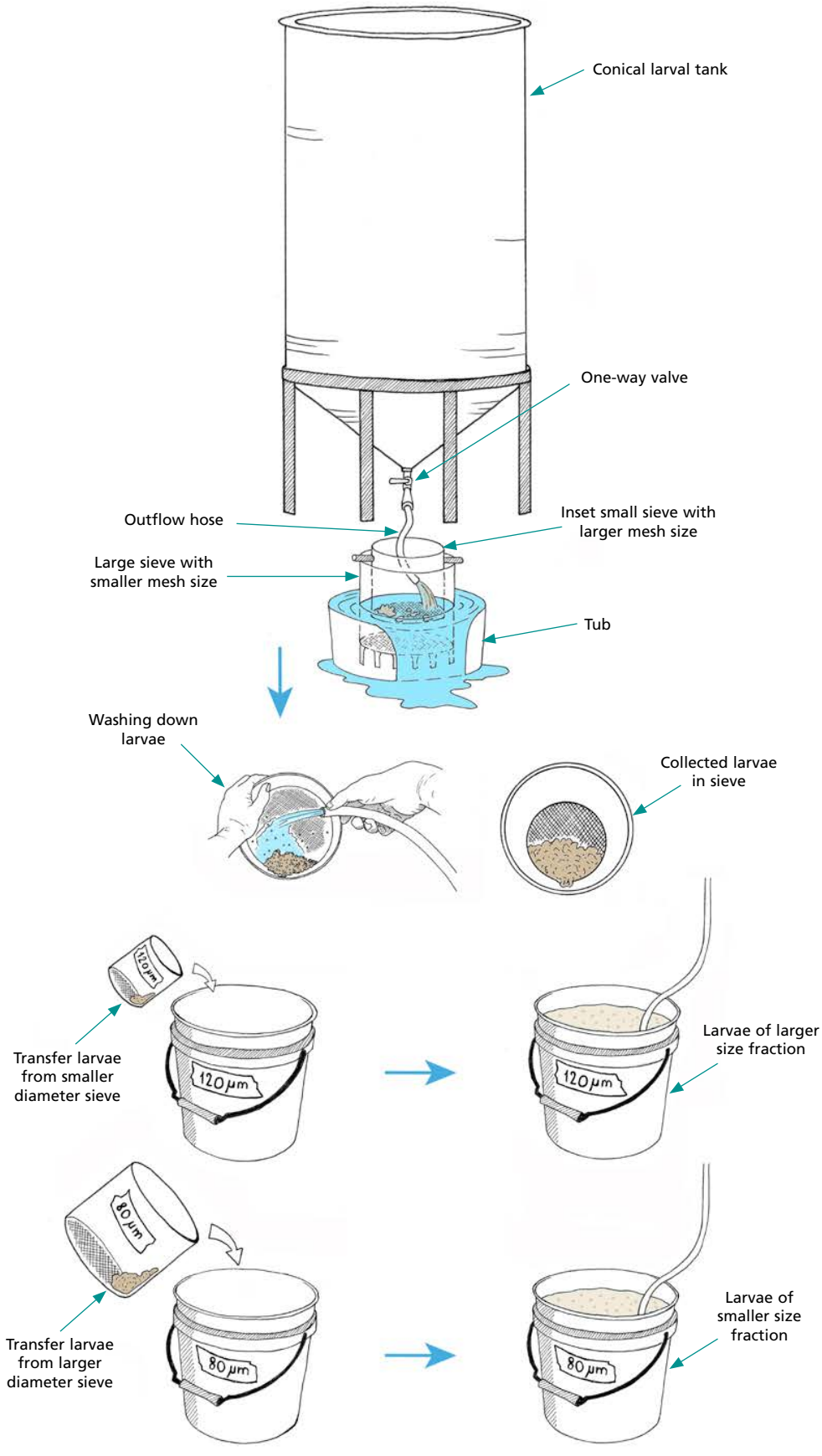
Grading methods – Grading can be done either during takedown of larval tanks or after collection into a bucket through a grading sieve.

A. Grading during takedown involves the use of an inset sieve of larger mesh size placed inside a larger diameter sieve with smaller mesh size. Larvae are automatically graded as they are collected, with larger larvae retained in the inset sieve and small larvae retained by the bottom sieve. Larvae are thereafter washed into two separate buckets (Figure 6.8).

B. Alternatively, grading can be done once larvae are collected in the holding bucket; they are passed through a grading sieve placed over a bucket; larger larvae are retained in the grading sieve and smaller larvae are passed through. The disadvantages to this method are that it requires an additional step in handling, and in most cases, when collected on the grading sieve, there is a risk of damaging larvae retained on the mesh out of water.

Re-distributing larvae – Standard practice is as per Protocol 6.1.2 and Figure 6.4. Larvae are passed through a large mesh sieve held partially submerged in the new tank. Larvae are poured gently from the bucket to avoid crushing of larvae against the mesh while passing through.

FIGURE 6.8
Grading and collecting larvae during a full water exchange



6.4 SETTING PEDIVELIGERS

The settlement period is one of the most critical stages in a hatchery operation. The setting system and process are designed to obtain a maximum survival of pediveligers to 1 mm spat. Dependent on the system used, the timing of when to introduce competent larvae for set will differ slightly.

Setting systems – Scallop pediveligers can be set using two different systems, shallow raceways or round nursery tanks.

In raceways, competent larvae are set on meshed sieves installed as a downwelling system; such systems yield high settlement rates but require intensive labour and a large surface area within the infrastructure of the hatchery complex. If space or labour are insufficient to accommodate a raceway setting system for the targeted production, a small-scale raceway system is useful to monitor settlement and survival rate to post-set during the first year of operation; this enables the aquaculturist to adapt the nursery tank system for maximal survival and growth.

In tanks, individual mesh substrates are used to fill the tank vertically. Round tanks enable the setting of a higher number of pediveligers within a given area, but yield a typical lower settlement rate. This system is less labour intensive during post-set rearing but becomes tasking when it is time for transfer of seed to the sea-based farm.

Knowing when to set – Larvae change as they become competent to set. Larvae will develop at different rates, and grading of larvae into larger and smaller size fractions is necessary to differentiate competent and non-competent larvae. The assessment is similar regardless of the setting system used. Criteria for set are based on the distinct changes in morphology and behaviour (described in Table 6.4), and involve an increasing number of "eyed" larvae, the development of a foot, "rafting" in holding buckets, substrate search behaviour with extension of the foot, and a thickening of the shell. The latter indicates the disappearance of the prodissoconch larval shell and formation of spat dissoconch shell. Table 6.4 guides the aquaculturist as to the readiness of larvae to settle. It also describes the morphological changes observed during and post settlement.

TABLE 6.4
Criteria for setting Yesso scallop larvae

Larval stage	Description	Behaviour	Observations in holding bucket
"Eyed" larvae	An eyespot appears at the back of the digestive gland; this spot is inconspicuous and irregular at first but becomes in 2–3 days more regular in shape (round) and conspicuous with a dark brown colour.	Larvae are still swimming, but eyespot indicates end of swimming stage and approach of metamorphosis. Not always easy to see the eyespot, and lack of observation does not necessarily mean its absence.	Swimming larvae suspended in water column.
Early pediveliger	Development of a foot. Use of both velum and foot alternatively.	Swimming, but can be occasionally seen under the microscope to extend foot out of shell, in "substrate-search" mode.	Rafting of larvae and suspended in water column, with some "crawlers" exhibiting substrate search behaviour on bottom of bucket.
Thickening of shell margin	In preparation for development of dissoconch shell.		
Metamorphosis	Disappearance of velum. Retention of functional foot. Beginning of dissoconch shell.	Spat can attach and detach dependent on favourable conditions.	
Settled larvae	Foot, velum and eyespot degenerate. Gills and adductor muscle develop. Appearance of dissoconch shell.	Spat are fixed to substrate.	

Source: Adapted from various sources by S. Sarkis.

The most reliable and easily assessed criterium for setting is the rafting behaviour for larvae retained on a 150–180 µm sieve. This is easily identified by untrained eyes and is a sure sign of larvae approaching metamorphosis.

Setting density – Setting density is calculated differently for a tank and a raceway system.

In a tank system, densities are calculated as per larvae, on a volume basis. A density range of 1–8 larvae.ml^{-1} for setting of Japanese scallop larvae has been tried in tank systems. Scientific recommendations for an optimal settlement rate are in the range of 1–1.5 larvae.ml^{-1}. On a large-scale, setting at densities of 2–2.2 larvae.ml^{-1} are known to yield up to 14 percent survival to 1 mm spat (1 month post-set) if protocols are strictly implemented. Higher setting densities may result in a high settlement, but subsequent survival and shell growth of post-set will most likely be poor, due to overcrowding. This author recommends a setting density for *M. yessoensis* larvae of 1–2.5 larvae.ml^{-1} when using a tank system.

For raceways, setting density is based per surface area of the meshed sieve. Competent larvae are distributed at an initial density of 30 larvae.cm^{-2} of sieve; surface area is the main limiting factor in scaling up the raceway methodology. A minimum settlement rate of 16 percent is expected for Yesso scallops set in raceways (Day-14 after set).

Duration of set – Generally, competent larvae are fixed following 10–14 days after setting. Numbers of swimming larvae are monitored and recorded during this period and decline to zero, as settlement phase is completed. Settlement rate refers to the number of fixed larvae counted on Day-10 or until "swimmers" are no longer observed.

6.4.1 Raceway setting

Setting in a raceway system is used for settlement of pediveligers by some commercial scallop hatcheries and for rearing seed up to 5 mm. A brief overview of the procedures used is given in this manual; details are available in other technical guides (see Further Readings).

A known number of larvae is poured gently directly into the sieve used for setting (Figure 6.9). Sieves with scallop pediveligers are suspended in a shallow raceway (a minimum of 15 cm deep) and receive continuous filtered seawater and algae; the latter is pumped from an external sump tank to the raceway. Seawater characteristics are similar to those used for larvae (double filtered to 1 µm and T = 13–14 °C).

Downwelling system – A downwelling system is used to enhance settlement. Incoming seawater and algae from the sump tank are distributed into the raceway through a PVC line drilled at regular intervals and fit with adapters; these connect to Tygon® tubes which supply incoming seawater and algae directly into each sieve (Figures 6.9 and 6.10). Incoming water and algae pass from the top of the sieve through the mesh to flow out of the raceway through a drain valve. For the first 48 h, the system operates as a recirculated system. Thereafter, new water flows into the sump tank, and the system is changed to semi-recirculated; part of the water flows back into the sump tank; the other part drains out. This increases the residence time for the algae in the raceway, as a portion of unconsumed algae are recirculated to raceway and maintains the temperature constant. Spat are drip-fed continuously over a 24 h period. Settlement yield and growth rate is often higher in a raceway system, mainly because monitoring stock density is relatively easy.

The downwelling system is continued until spat reach approximately 2 mm shell height; after that, spat growth benefits from an upwelling system (see Section 7.1.2).

FIGURE 6.9
Setting pediveligers in sieves suspended off the bottom in a downwelling raceway system. Algal food reservoir and incoming seawater lines are also shown

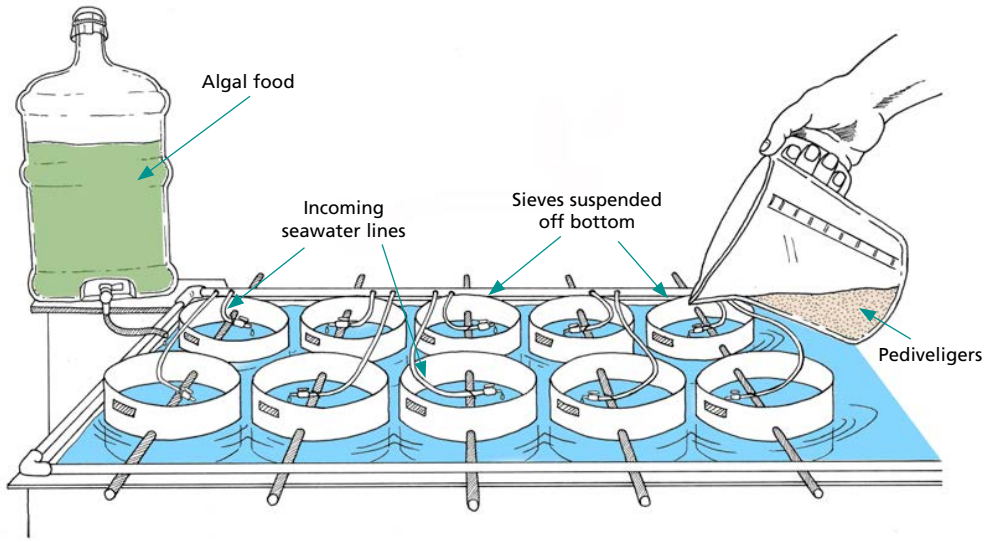
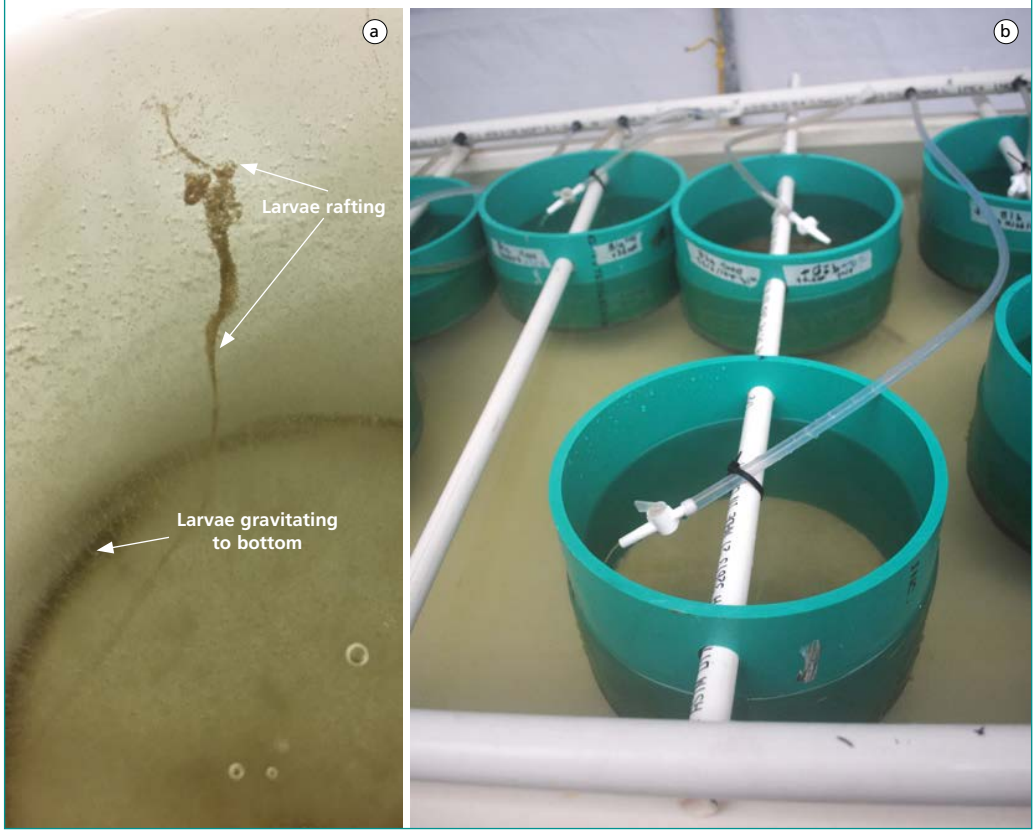


FIGURE 6.10
Settling larvae – (a) rafting behaviour of larvae in holding bucket; (b) close up of valve regulating incoming flow in downwelling sieve



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6.4.2 PROTOCOL: Setting and rearing mature Yesso scallops in a semi-recirculated raceway system from Day-0 to Day-14 after set

MATERIALS

- Sieves 120–150 µm
- 10–20 L buckets
- Saltwater table or extra raceways

METHOD

Setting day = Day-0 of set

1. Clean sieves on sides and mesh, inside and outside.
2. Clean raceway.
3. Fill raceways and sump tank with double filtered 1 µm seawater at T = 12±1 °C.
4. Once filled, turn off incoming flow and place raceway on re-circulating system only.
5. Pool larvae and pass through 150 µm and 120 µm sieves.
6. Resuspend each fraction in a 10–20 L bucket and count (see Protocol 6.2.1).
7. Calculate volume to set per sieve as per example below:
   Larval density: 400 larvae per ml
   Sieve diameter = 25 cm
   Target setting density = 30 larvae per cm^2
   Total number of larvae to set = 70 000 larvae
   Volume of larval pool to distribute per sieve = 70 000 ÷ 400 = 175 ml
8. Distribute larvae into sieve.
9. Partially open incoming seawater flow to obtain a total flow of 3 $L.min^{-1}$ in the raceway.
10. Restrict flow to individual sieves on setting day, to leave larvae settle without any surface agitation.
11. Calculate food ration (see Table 7.1). Distribute 50 % in the sump tank. Distribute remainder in 20 L carboy and top up with 1 µm filtered seawater.
12. Adjust flow of carboy to drip-feed to distribute total algal solution over 24 h.
13. Record number of larvae set per sieve and label sieve with date of set.
14. Do a daily check of flow, temperature and algal ration.
15. On Day-2 – increase flow slightly to increase drip.
16. On Day-8 – clean raceways. Transfer sieves to clean raceway or saltwater table with spat in the sieve. Do not clean sieves. Do not disturb larvae.
17. On Day-10 – Assess the number of swimmers. Wash spat off the mesh with a gentle stream of filtered seawater into a holding bucket.
18. From Day-10 on, begin routine cleaning of raceway system once a week.
19. Thinning of sieves is started when crowding is observed and spat are seen climbing up the sides of the sieves.
20. For thinning, collect the spat as in Step 17. Divide the culture into two and re-distribute into two sieves.

6.4.3 Tank setting

This system is useful for round tanks of 1 000 L and up.

Substrate type – Substrate type can affect settlement rate and it is recommended to use a substrate providing optimal surface area for settlement as well as allowing for good water flow in the setting tanks. Various substrates specific to scallop settlement have been tested, such as Kinran and Netron™. Less costly versions of a similar mesh are a 3 mm black polyethylene mesh often used in the mussel industry, which can be "fluffed" to increase volume and water flow.

6.4.4 Preparing a tank for setting

Once larvae are metamorphosed and are fixed on the substrate, they are reared in the same tank until they are large enough to be transferred to a pond or farm. For this reason, the settling tank has to be prepared to allow a change from static to continuous flow system, with a semi-recirculating airlift system; this will ensure homogeneous water circulation and distribution of food for fixed spat. Such a system can be used for spat up to 5 mm shell height.

Preparing the substrate – The mesh selected as substrate is usually cut into lengths of approximating 50 to 70 cm in length, or a suitable length for the tank depth. Substrate is cleaned. Standard practice is to soak cleaned substrate in continuous filtered seawater for a period of one week prior to setting; this produces an organic film on the substrate and is believed to favour settlement.

Airlift and setting installation – For setting, tanks and airlines are cleaned as for larvae. The airlift system for recirculation is cleaned and installed (see Protocol 6.4.5; Figure 6.11). Substrate is suspended in the setting tank, filling the entire tank volume, once the airlift is in place. The aquaculturist must consider the best design for suspending the substrate in the setting tank to facilitate retrieval of the substrate and spat when they are ready for transfer out of the nursery to ponds or to the farm. One method is to make sets of 20–25 substrate pieces tied in line on a rope; the rope is in turn attached to opposite sides of the tank (essentially creating a longline within the tank). It may be necessary to weigh the substrate to maintain it vertical in the water column.

Once the airlift and the substrate are in place, the setting tank is filled with filtered and heated seawater (as for larvae); any gaps in tank are filled with single pieces of mesh.

6.4.5 PROTOCOL: Assembling an airlift system for nursery tanks

MATERIALS

Dimensions below are for an airlift system to fit a round tank with diameter approximating 300 cm and height of 200 cm (see Figure 6.11).

For ONE airlift system:

- 15 m of 25 mm PVC pipe (schedule 40)
- Drill and 8 mm bit
- Hacksaw or PVC pipe cutters
- Four 25 mm "90° elbows"
- 25 mm "T" connector
- Sand paper

METHOD

Note: DO NOT GLUE ANY PART – there is no need and system can be easily disassembled and cleaned between spawning seasons.

Bottom pieces

1. Cut four 76 cm pieces of 25 mm pipe.
2. Using magic marker, mark every 8 cm.
3. Flip pipe, and mark every 8 cm on opposite side.
4. Drill marks using 8 mm drill bit.
5. Use sand paper to smooth and clean edges.

6.4.5 PROTOCOL (continued)

Transverse top pieces

1. Cut two 134 cm pieces of 25 mm pipe.
2. Cut two 152 cm pieces of 25 mm pipe.
3. Mark holes every 8 mm on pipe.
4. Mark another set of holes at a slight angle – water will recirculate back at an angle, rather than parallel to surface of water.
5. Drill holes at marks with 8 mm bit.
6. Using sand paper, smooth edges and remove bits of PVC.
7. Cut a 5 cm long piece of 25 mm PVC pipe as a connector.
8. Sand and connect two Ts using the 5 cm piece (with each T facing in opposite direction).

Vertical pieces

1. Cut four 152 cm pieces of 25 mm pipe.

Before assembly

1. Clean all pipes and PVC parts.

Assembly

1. Put aside, four elbows and six Ts for each airlift system.
2. Fit one elbow to each of the 76 cm pipes.
3. Connect one 152 cm vertical to elbow.
4. Fit a T at end of 152 cm pipe.
5. Repeat for each of 76 cm bottom pipes.
6. Inside tank – place assembled vertical and bottom pipes at opposite sides (as for a clock at 12, 3, 6, and 9 o'clock positions), with bottom 76 cm pipes facing towards the centre.
7. Outside of tank – connect one top 133 cm pipe to connector T-section, and one top 152 cm pipe to other side.
8. Take the whole top section and fit to Ts of vertical pipes already in tank, connecting vertical pipes on opposite sides (e.g. 3 and 9 o'clock).
9. Repeat Steps 7 and 8 using remaining two other transverse pipes (connecting 12 and 6 o'clock vertical pipes).

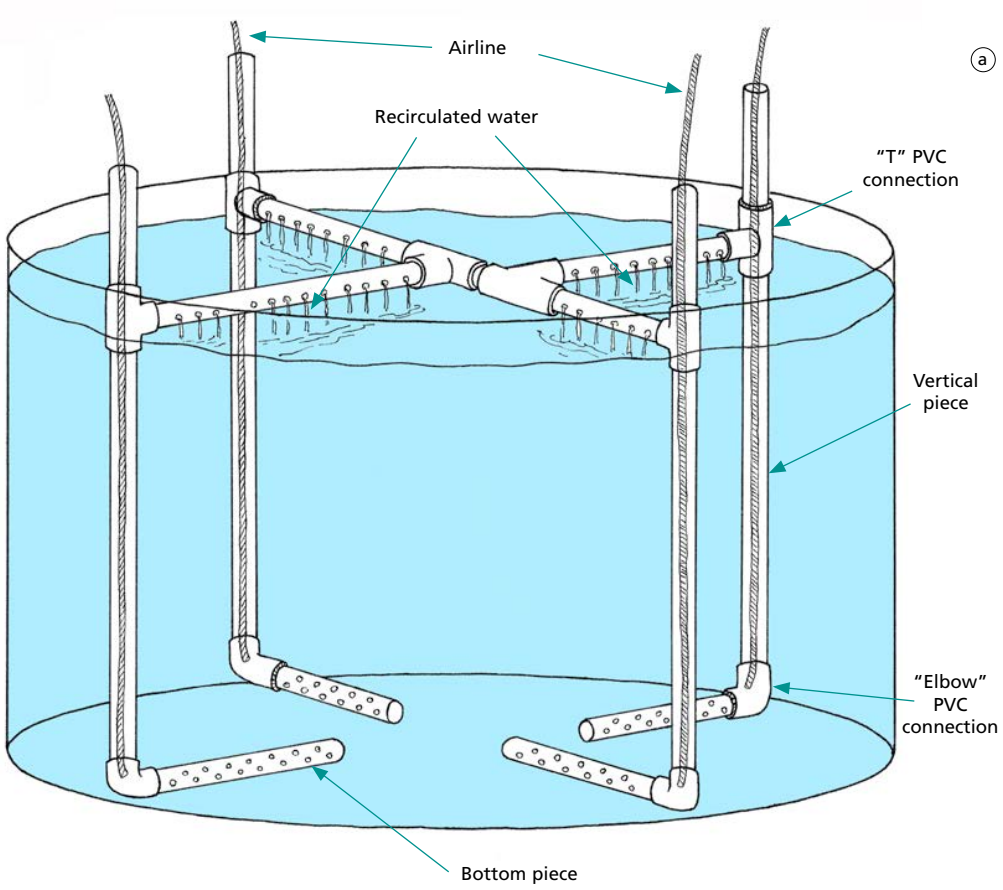
Airline

1. Cut four 7 mm inner diameter tubing to fit from manifold to bottom of each vertical pipe.
2. Cut tubing at top of vertical T and fit with a one stopcock valve in order to adjust airflow when tank is full.

ASSEMBLE AIRLIFT IN TANK BEFORE SETTING LARVAE

Note: For first 10–14 days of setting (until no more swimmers are seen in drain samples) – weigh down airlines and use as regular airlines outside of airlift system.

FIGURE 6.11
Setting tank (a) airlift system assembled; and (b) tank filled with substrate



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6.4.6 PROTOCOL: Grading and setting larvae for round nursery tank system

MATERIALS

- Sieves: 120 µm, 150 µm, 180 µm, 500 µm
- Buckets
- Plunger for buckets
- Sedgewick-Rafter cell
- Eppendorf pipette and tips

METHOD

Day of set ranges from Day-25 to Day-28 after fertilisation
Average size of larvae set ranges from 224±15.6 µm to 247±20.9 µm
Range of settlement rate 4–16 % dependent on larval batches and setting procedures

1. Prepare setting tanks with airlift systems and substrate prior to collecting competent larvae.
2. Takedown larval tanks as in Protocol 6.1.2.
3. Larvae are separated into three fractions (120 µm, 150 µm, 180 µm). Larvae observed to raft in buckets or extend a foot with or without substrate search behaviour under the microscope are considered ready for set.
4. Count and measure larval fractions as per Protocol 6.2.1.
5. Assess the "health" of each larval culture. Larvae of similar health and size fractions are pooled.
6. Healthy smaller size fraction not seen to raft are often pooled with smaller fraction of other tanks and placed back in larval tank for further rearing. Care must be taken to monitor this larval batch carefully, as readiness to set will occur quickly (within 2 days).
7. For setting: pool larvae of same size fractions if they are healthy.
8. Count larvae in each pool. Set at a density of 1.5–2.5 larvae.ml^{-1}
9. Distribute larvae from holding bucket to tank:
   - using a 500 µm sieve, lower sieve halfway into surface of tank water; and
   - gently pour larvae through mesh into tank.
10. Leave larvae undisturbed until Day-2 with no exchange of seawater.
11. A small food ration is given to setting larvae (see Table 7.1). Algae is distributed as for larvae as a batch in a drip-feed system over 24 h.

7. Nursery

IN THIS CHAPTER – How to rear post-set scallops until transfer out of nursery – changing seawater system from static to continuous, managing food demand and stocking density.

Terms for post-set larvae – The terms "spat" and "seed" are commonly used in the nursery phase. The word "spat" relates to bivalve larvae that have set and undergone metamorphosis. The word "seed" is to describe juvenile products supplied by hatcheries to shellfish farmers. The broader term "post-set" is applied to spat and seed. Post-set maturity is referred to as Day after set, with Day of set being 0.

Nursery goal – The goal of the nursery is to produce scallops of size suitable to farm conditions, enabling them to grow and survive at optimal rates post-transfer. The nursery phase involves a) setting larvae until they are fixed spat (10–14 days), b) rearing post-set spat (300 µm) up to >2 mm shell height, or until ready for transfer to intermediate nurseries, such as ponds or sea-based farm sites.

Nursery phases – Nursery culture is typically divided into two phases: an early post-set period to <2 mm shell height, and a subsequent juvenile phase to >5 mm. The size to which an aquaculturist will grow seed is dictated by several factors related to: a) infrastructure of the operation – mainly the scale of the land-based nursery facility; b) the cost-effectiveness of the nursery; and c) the natural conditions prevailing at the farm site.

In other words, size of spat at transfer and time of year at transfer are key to subsequent grow-out performance and to achieving market production target. A good understanding of the physical, chemical and biological characteristics of the farm environment are needed to assess the most cost-effective "Transfer to sea" schedule.

7.1 SEAWATER SYSTEM FOR EARLY POST-SET NURSERY PHASE

This phase is generally an extension of the hatchery larval/setting system where temperature and filtration requirements are similar to that of larvae and food is composed of live microalgae. For the Yesso scallops, seawater is double filtered to 1 µm and T = 12±1 °C.

7.1.1 Raceway set: keeping sieves clean from Day-0 to Day-14

Water flow – In the initial 10–14 days post-set, spat are not disturbed from the sieve. During the first 24 h, supply minimal flow to each sieve to allow the larvae to settle. By Day-2 after set, flow is gradually increased to each sieve but remains gentle to allow spat to fix.

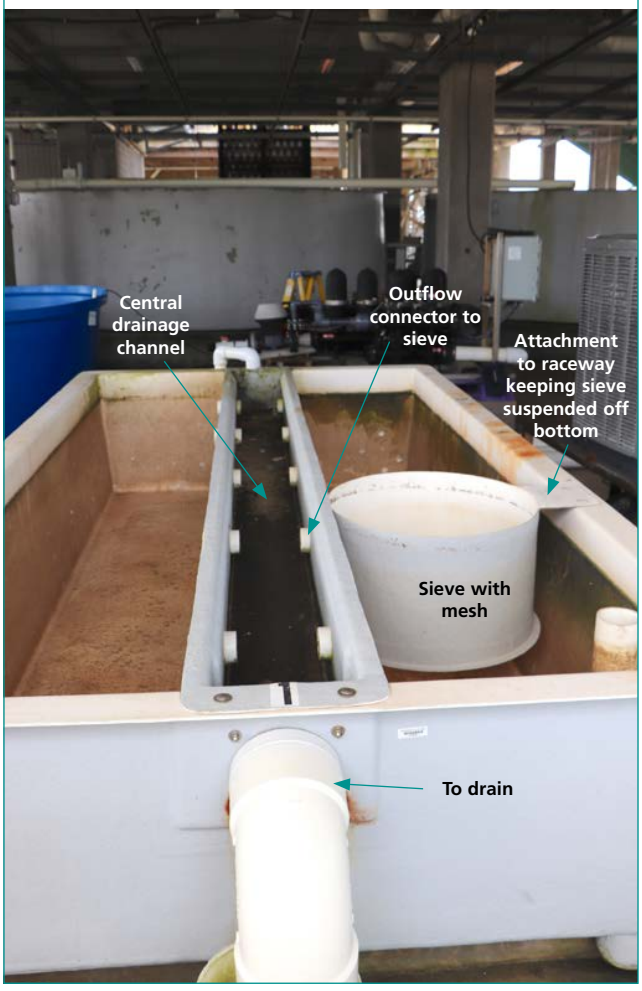
Keeping raceway clean – Extra care is taken during the first clean. As larvae have not fully settled by then, sieves containing spat are transferred to a saltwater table filled with filtered and heated seawater (as per culture requirements). Sump tank and raceways are drained, cleaned and re-filled with seawater. Sieves with settling larvae are placed back into the raceway undisturbed. As of Day-14, spat in sieves are well fixed and gently rinsed with seawater to remove accumulated detrital matter.

7.1.2 Raceway set: growing spat Day-14 to 2+ mm seed

Flow and food ration change – Starting Day-14 after set, seawater flow and food ration are increased, and the protocol for post-larval rearing in terms of cleaning, monitoring and thinning to maintain an optimal biomass per sieve is initiated. Food ration is given in Table 7.1. Flow of raceway is set according to total biomass in the system; as a rule flow is set between 25 $ml.min^{-1}.g^{-1}$ of spat (wet weight) to 50 $ml.min^{-1}.g^{-1}$ of spat. Keeping a record of biomass and maintaining adequate biomass per sieve become critical around the third week after set, as spat begin an exponential increase in growth. If biomass per sieve is not controlled, high mortalities and slow shell growth will ensue.

Changing to upwelling system – Seed measuring 2+ mm continue to grow exponentially requiring an increasing volume of food and space. At this time, they benefit from additional nutrients naturally occurring in seawater. Filtration is reduced to 10 µm for incoming seawater. Increasing faecal production per sieve can lead to clogging and an accumulation of detrital matter within the sieve. At this time, the downwelling system is changed to an upwelling system to improve water quality per sieve. Water flow is reversed; incoming water flows directly into the raceway, regulated by a ball valve; water flows up from the bottom of the raceway through the mesh of the sieve and flows out at the top of the sieve through an outflow pipe drilled at the top of the sieve. The outflow of the sieve is collected in a centre drainage channel (Figure 7.1). Reversing the flow counteracts the increased faecal deposition by spat.

FIGURE 7.1
Downwelling sieve system showing centre channel receiving outflowing water from each sieve going to drain



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Details of all protocols for rearing scallop spat in raceway are available in other technical guides referred to in Further Reading section.

Expected Yesso set in raceway – Setting on sieves is a more controlled approach and high percentage of sets can be obtained. The 16 percent settlement rate reported at Day-14 for Yesso scallops can most likely be improved through additional adjustments in temperature and flow.

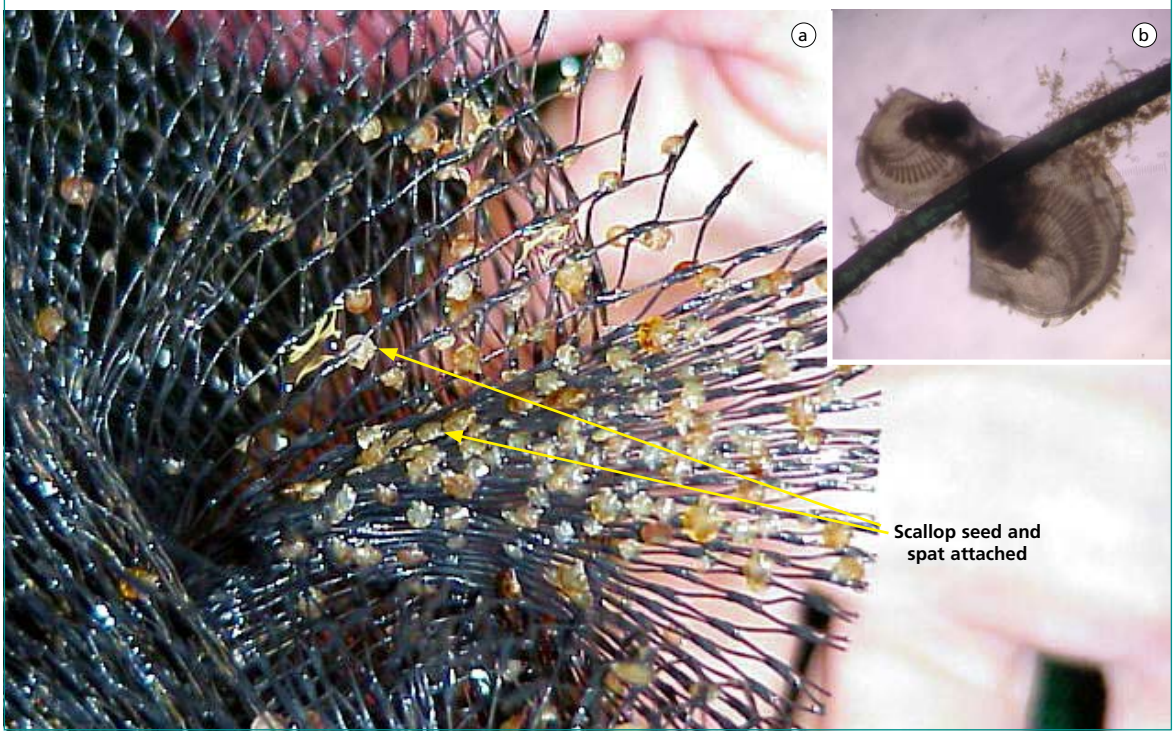
The disadvantage in using raceways is the high demand for space in the nursery to accommodate scallop biomass as they grow. Scallop spat do not perform well if they are crowded or sit on the mesh in more than one layer, unlike oysters or mussels.

7.1.3 Tank set: water exchange and feed from setting day to Day-14

Water exchange – Spat are reared as for larvae in a static system from Day-2 to Day-4 after set, where water is exchanged

several times a week. The difference between a larval and spat water exchange is that spat exchange is done without exposing the substrate (and set scallops) to air. Figure 7.2 shows a close up of spat attached to the substrate; in order to ensure their survival, a monitored volume of new water flows into the top of the tank while an equal volume is drained from the bottom. The water level in the tank remains the same at all time and the substrate and spat remain always submerged. The duration of the water exchange depends on the water flow and size of the tank. The objective is to exchange 100 percent of the water in the tank at a minimum of three times a week. A water exchange schedule is established as for larvae. Outflowing water is drained through a sieve and any non-set larvae or "swimmers" and dead larvae or spat, are collected; this gives an initial estimate of settlement rate and of the state of the culture (see Protocol 7.1.4).

FIGURE 7.2
Settled scallops - (a) seed attached to settling substrate (3 mm mesh); (b) close up of fixed spats



7.1.5 Tank set: continuous flow for Day-14 spat to 2+ mm seed

Changing flow system – Following Day-14 after set, any "swimmers" collected are not worth keeping, and are discarded through flushing of tank. The static closed system is changed to a low continuous flow of 1 µm filtered seawater with new incoming seawater filtered and heated similarly to the first 14 days (Protocol 7.1.7). Aeration in the tank is changed from a gentle aeration via airlines to a pre-assembled airlift system. This semi-recirculating system is a combination of continuous flow and airlift and provides a good water circulation among the suspended substrate pieces. This ensures homogeneous water quality and algal distribution throughout the tank. A rearing temperature for Yesso scallop of 12–13±1 °C provides rapid growth and high survival of spat and should be maintained for as long as possible until 1 week prior to the time for transfer at sea.

7.1.4 PROTOCOL: Spat water exchange using a tank setting system

MATERIALS

- Hose and/or siphons
- Sieves of various mesh size (>120 µm)
- Graduated beaker (3–4 L)
- Clicker
- Compound and dissecting microscope
- Slides
- Ocular micrometre

METHOD

1. Check tanks every morning as for larvae, adjusting air or temperature when required. Record on daily check sheet.
2. For Day-2 to Day-14 after set, nursery tanks follow a similar water exchange schedule as static larval tanks; seawater filtration and temperature are also similar (UV/double filtered 1 µm; T = 13–14 °C).
3. Adjust valves so that outflow rate is equivalent to incoming flow rate.
4. Setting tanks often have drains at the top and bottom. For Day-2 to Day-14, outflow is from the top as a precaution; it avoids lowering the water level inadvertently should incoming water flow stop. It also leaves metamorphosing larvae gravitating to the bottom of the tank undisturbed.
5. Make sure water level in tank does not change; all substrate and spat are continually immersed in seawater.
6. During 100 % water exchange, clean sieve (mesh size >120 µm), hose and tube, and place under bottom drain.
7. Once 100 % water exchange is complete, wash larvae collected into a 3 L beaker (there should not be enough for a 20 L bucket); any live swimming larvae which has not set is referred to as "swimmers".
8. Count and measure larvae; record any mortality.
9. Calculate average shell length and record all data.
10. If there are live larvae in sample and culture is in good health, return larvae to tank, passing through a 500 µm mesh.
11. If there are few larvae or a mortality >10 %, discard.
12. Feed tank AFTER water exchange is complete.
13. Day-0 to Day-14 after set: Live microalgal food is distributed by batch as for larvae until a continuous seawater system is started.
14. Food ration for spat is given in Table 7.1.
15. Early spat are fed by batch; transfer daily algal ration in a food reservoir for each setting tank:
    a. distribute volume of algal species as calculated and dilute with 1 µm filtered seawater (same filtration and temperature as spat tank) to fill food bin;
    b. Drip-feed over 24 h (this should be fast enough to avoid clogging of spigot by overnight and stop algal distribution).

Keeping culture clean – As spat grow, the food ration requirement increases exponentially and generates an increasing amount of detrital matter; this along with dead larvae or spat accumulate on the bottom of the culture tank. Spat tanks are flushed weekly using the bottom drain or a siphon; outgoing water is checked for spat mortality and/or input of other invertebrates from natural seawater.

7.1.6 PROTOCOL: Monitoring settlement rate and growth for spat <1 mm shell height

MATERIALS

- Sieve with 120 µm mesh
- Dissecting microscope
- Petri dish with 1 cm^2 grid drawn on bottom of dish
- Lab wash bottle
- Scale (±0.01 g)
- Laminated grid (20 × 28 cm) of 1 cm^2
- Ocular micrometer or digital camera fitted to microscope

METHOD

Note: Scallop spat <1 mm are fragile and difficult to handle.

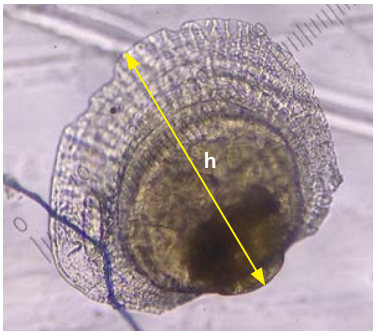
Monitoring shell growth and spat numbers is conducted weekly; alternative tanks can be sampled daily to provide an estimate for the whole batch and minimise handling for any one setting tank.

During 100 % water exchange

1. Collect any dead or live spat washed into sieve during water exchange.
2. Record number of live and dead spat under the microscope.
3. Record any dead larvae seen.
4. Make observations on dissoconch development, presence of detritus, evidence of feeding by appearance of digestive gland (full and brown in colour).

Measure newly settled spat (<1 mm)

5. Measurements are made once a week on live spat.
6. Spat are measured along their shell height (h), not shell length (unlike larvae). See photo.
7. Cut an Eppendorf tip at the end, so that aperture is larger to collect spat.
8. Collect a 1 ml sample using an Eppendorf pipette with newly cut "spat" tip, and place on Sedgewick-Rafter cell. To spread sample evenly, place cover glass over chamber top.
9. Measure a minimum of 30 spat under compound or dissecting scope using an ocular micrometer or digital camera. Note objective size or scale and record number of units.



Spat height (h)

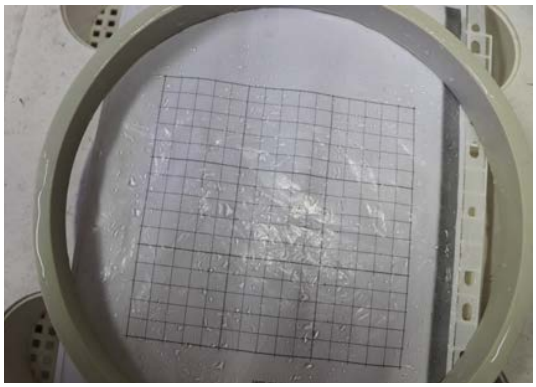
10. Convert number of units to microns according to magnification or scale used (objective size).
11. A petri dish marked with a grid of 1 cm^2 is used for viewing subsample under the dissecting scope.
12. For the petri dish subsample:
    - collect spat in a small diameter sieve (15 cm), and wash into petri dish using a lab wash bottle; and
    - count spat under microscope, using a clicker and working your way across and down the squares.

Note: The ocular micrometer or digital imaging must be calibrated for each objective lens and recorded. This is necessary only once for each magnification and each microscope.

7.1.6 PROTOCOL (continued)

Measuring spat >1 mm

13. Spat close to 1 mm shell height are visible with the naked eye as brown spots.
14. Collect one piece of substrate with spat or a subsample (if spat density is too high) from setting tank, and place into a 30–40 cm 120 µm sieve.
15. Working on the saltwater table, wash down spat from substrate using a gentle jet of filtered seawater (<10 µm) into a sieve.
16. Place sieve on top of a laminated 1 cm^2 grid.



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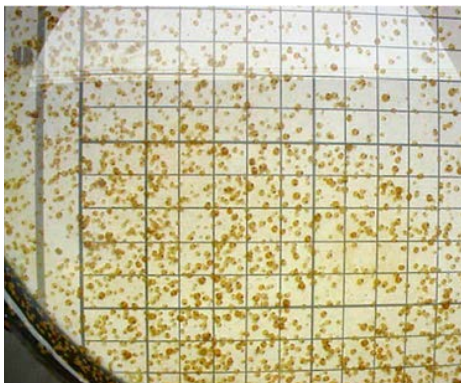
Laminated grid



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Measuring spat on 120 µm sieve placed on laminated grid

17. Using clicker, work your way across the squares of the grid (as you would with a Sedgewick-Rafter cell) and count.
18. Take care not to count the same spat twice.
19. Measure a minimum of 30 spat.
20. Alternatively, counting devices such as XperCount digitized bucket can be used.

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Counting spats

Weigh >1 mm spat

21. Using the same subsample measured, record weight by following the same procedure as Steps 1–12 in Protocol 7.2.7.
22. Estimate stocking density (see Section 7.2.6).

Expected Yesso set – The expected percentage set is as follows:

- Tank system – From Day-0 to 1 mm spat:
  Percentage set can range from 3 % to approximately 14 % when set at an initial average setting density of 2.8 $larvae.ml^{-1}$.
- Tank system – From Day-0 to 2.5 mm:
  2 % to approximately 5 % with same setting density.
- Raceway system – From Day-0 to 1 mm spat:
  16 % with initial setting density of 30 $larvae.cm^{-2}$.

The percent set and survival varies with respect to the seawater system and seawater quality, handling and food composition. Setting rates of up to 40 percent are reported for other species and the expected percentage set for the Yesso scallop may be increased through improved husbandry.

7.1.7 PROTOCOL: Changing from static to continuous spat culture tank system

MATERIALS

- Sieves for bottom drain
- Sedgewick-Rafter cell
- Compound or dissecting microscope

METHOD

Note: Using continuous flow vs static system is dependent on the heating capacity of the nursery. If heated seawater is not sufficient to remain constant during continuous flow of all spat tanks, post-larvae can be reared in a static system until 1.5 mm shell height (Day-50). Spat >1.5 mm are difficult to rear in a static system.

Changing to semi-recirculation system

1. Once the number of swimmers collected during water change has declined to zero, or those obtained are moribund, the setting period is ended; all remaining fixed larvae are referred to as spat.
2. Start the airlift system by transferring airlines in tank into vertical airlift pipes. Airlines should be all the way down to bottom of vertical pipe. Water should be seen to come out of ALL the holes in the transverse pipe. This recirculates water from bottom to top.
3. Filtration remains at 1 µm until 1.5 mm size.

Static tank system

4. Water exchange schedule is the same as that of larvae
5. Feeding is by batch or it can be introduced to all tanks simultaneously by pumping from a common reservoir with known amount of algae.

Continuous tank system

6. Outflow is from the bottom; a stand-up pipe is used to make sure there is no loss of larvae if incoming flow stops.
7. For added control, outflow can be additionally regulated using a top drain valve.
8. Incoming seawater is regulated by one-way valves and flows at the top of the tank. If there is concern of accumulated detritus, the valve for incoming flow can be connected to a pipe extending vertically close to bottom of tank.
9. For spat >1.5 mm, water is filtered to 10 µm. This supplements the food ration given by supplying naturally occurring algae and dissolved organic matter.
10. Adjust incoming flow rate according to spat biomass. Flow rate is increased as spat grow and stocking density increases, as follows:
    - Initial incoming flow rate is adjusted to provide a 50 % water exchange;
    - Incoming flow rate is gradually increased to 70 %, 100 % and >100 % throughout larval life based on stocking density; and
    - Care must be taken to ensure that residence time is long enough for food to be consumed by spat and NOT to be flushed out.

Daily morning checks: monitor water level, flow rates, oxygen, temperature and any other relevant parameters. Record values.

7.1.7 PROTOCOL (continued)

Flushing bottom detritus from tank system (frequency once a week)

11. Water is collected from the bottom of the tank. Use existing drain or siphon to flush bottom water.
12. Incoming water is regulated by a one-way valve connected to a 25 cm pipe extending to the bottom of the tank.
13. Do a water exchange for a period exceeding 100 %. For example, for a 10 000 L tank with an incoming flow of 6 $l.min^{-1}$, water exchange lasts 2 h.
14. Place a sieve with mesh size ranging from 80–120 µm under bottom drain valve to collect dead spat or debris.
15. Once flushing is complete, contents of sieve are washed into a 2 L beaker, and examined under microscope.
16. Record observations. Note other invertebrate larvae or organisms, state of culture, or dead spat.

If there are dead spat

17. Count and measure a subsample of 20. This will provide an indication as to timing of mortality and the cause may be explained by factors occurring during this period.
18. Record dead spat height and calculate mean.

Acclimating spat to ambient

19. Spat are to be acclimated to ambient prior to transfer to ponds or to sea, or if heating of seawater is not possible.
20. Acclimation takes place over a 5–10 day period.
21. Rearing temperature is decreased gradually by 1 °C per 24–48 h period.
22. Filtered ambient seawater is added to tank such that temperature decreases by 1 °C.
23. This process is repeated daily thereafter until ambient temperature is reached. Spat are kept at ambient for at least 48 h before transfer.

7.2 FEEDING SPAT IN LAND-BASED NURSERY

Providing a suitable amount of food to spat as they grow can prove challenging. Food is supplied in the form of live microalgae, commercially available diets and naturally occurring plankton in incoming seawater.

7.2.1 Spat food ration

Early spat (<2 mm) are fed microalgae, using the same species as for larvae. As spat grow and become more robust, commercially available substitutes to live microalgae are known to result in good growth and survival rates. Table 7.1 shows the food ration, expressed as algal $cells.ml^{-1}$ from day of set (Day-0) until >2 mm shell height. Thereafter, ration is based on dry algal weight and live (wet) weight of spat. The algal culturist will calculate the volume of algae to harvest based on the food ration; records of the quantity and composition of food are kept daily.

7.2.2 Feeding spat in raceway setting system

Day-0 to Day-14: The raceway seawater system is closed; live microalgae is mixed into the sump tank, and enriches the seawater supplied to each individual sieve.

Day-14 onwards: Once the system is changed to semi-recirculating with the input of new seawater, food ration is divided into two; half of the food ration is distributed via a

TABLE 7.1
Food ration for spat from day of set (Day-0) to >2 mm shell height

Spat size	Day after set	Food ration (cells.ml^{-1})	Food composition
<500 µm	0	19 000	1:1:1 (Flagellates:Diatom:Live Tetra)
	1–6	20 000	
	7–11	25 000	
	12	27 000	
	13	30 000	
	14–15	32 000	
	16	35 000	
	17	38 000	
	18	40 000	
	19	45 000	
	20	50 000	
	21	55 000	
600 µm	22	60 000	
600 µm –1.5 mm	23–36	60 000	**Option:** Substitute ⅓ of ration with Instant Algae 1:1:1 (Flagellates:Diatom:Instant Tetra)
	37	65 000	
	38	70 000	
	39	75 000	
	40	80 000	
	41	85 000	
	42	90 000	
	43	95 000	
	44	100 000	
	45	120 000	
	46	150 000	
	47–50	190 000	**Option:** Substitute with Instant Algae™ up to 60 % of total food ration
>2 mm	51–61	220 000	
	62	4–6 % wet weight of spat	**Option:** Substitute up to 85 % of total food with Shellfish Diet/Instant Algae or equivalent

Note: "Tetra" refers to *Tetraselmis* sp.

carboy which drip-feeds into the raceway, and the other half mixed into the sump tank. In this way, spat receive an adequate density of live microalgae, which is maintained over 24 h.

7.2.3 Feeding spat in tank setting system

Day-0 to Day-14: Microalgal ration is drip-fed over 24 h period from a carboy dedicated to each tank; live microalgae are kept in suspension in the tank through a gentle aeration to avoid disturbance to larvae which would limit settlement.

Day-14 onwards: Once the semi-recirculated system is started, a continuous supply of food is pumped from a food reservoir to the spat tanks. All live and commercial food is calculated and diluted with seawater in the food reservoir on a daily basis; the food reservoir often consists of a round flat bottom tank and is cleaned daily prior to the addition of new food.

7.2.4 Food ration and composition for spat in raceway and tank systems

Live microalgal ration for spat is initially calculated as for larvae, based on algal cell density and volume of rearing tank. Food ration for spat starts at 19 000 cells.ml^{-1} increasing to 120 000 cells.ml^{-1} by Day-45 after set; by Day-60 or when spat average 2 mm shell height, food ration totals 220 000 cells.ml^{-1}. Live microalgae is best suited for optimal scallop spat growth and is especially important in scallop spat <2 mm shell height.

Beyond 2 mm, spat biomass starts to increase exponentially, and maintaining growth requires a rapidly increasing amount of feed. At this stage, food ration is based on spat biomass (mg wet weight) per tank or per batch and averages 4 percent. High volumes of live microalgae are required at this time to satisfy the growing spat, equivalent to a daily ration of 4–6 percent dried algae per mg wet weight of spat; this can put substantial pressure on the algal production unit of the hatchery. In order to alleviate the demand for high quality microalgae, commercially available substitutes can be used, such as instant algae, or Shellfish Diet.

Food composition for spat >2 mm – Keeping a portion of the food ration as live microalgae benefits scallop spat growth; a minimum 15 percent of total food ration is recommended as live microalgae. Commercial substitutes are introduced gradually over a 7-day period to reach the targeted proportion of diet.

An example of food composition for Yesso spat reared in a land-based nursery is given in Table 7.2. Shellfish Diet can be substituted as early as Day-50, replacing Instant Algae, as shown in the example. If the operation's capacity for live microalgae is sufficient, there is no need for commercially available diets. See 7.2.5 for calculating rations when using commercial diets.

TABLE 7.2
Food ration for *M. yessoensis* spat from Day-1 after set to Day-100

Day after set	Live microalgae (%)	Instant Algae (%)	Shellfish Diet (%)
Day-1 to Day-45 (minimum)	100 1:1:1 Iso/Pav:Chaet/3H:Tetra	0	0
Day-46 to Day-66	40–50 % (minimum)	50–60 % (maximum) 1:1:1 Iso/Pav:3H:Tetra	0
Day-67 to Day-100	15	0	85 Shellfish Diet 1800®

7.2.5 PROTOCOL: Calculating food ration using commercial diets

MATERIALS

- Shellfish Diet 1800®
- Instant Algae
- L graduated beaker or graduated cylinder
- 20 µm sieve

METHOD

1. Use Table 7.2 as a guide for supplementing commercial diet.
2. Provide 100 % live microalgae at least until Day-45, preferably until Day-55.
3. Estimate biomass of batch on a weekly basis.
4. Calculate volume of Shellfish Diet as shown below.

Live microalgae

5. Estimate maximum capacity for live microalgae production for spat in facility.
6. Estimate maximum volume of live microalgae available per spat tank.
7. Count algae and calculate to provide food ration as per Table 7.2 until maximum capacity for live microalgae per spat tank is exceeded.
8. Once live microalgal capacity exceeds the requirements for 100 % live microalgae ration, give each spat tank calculated maximum live microalgae and supplement with commercial diet.

Calculating Shellfish Diet (SFD) to supplement food ration

9. Estimate total wet weight (or biomass) in grams for tank, batch or raceway to be fed.
10. See example below for calculations.

Example:
Live algal production has maximum capacity to supply 40 000 cells.ml^{-1} per spat tank
40 000 cells.ml^{-1} = 15 % of total food ration
SFD supplements 85 % of total food ration
Total spat biomass per tank is 750 g wet weight/tank

Calculate as follows:
for 100 % ration
ml of SFD needed = 750 g × 0.036 (manufacturer's instructions)
for 85 % ration
ml of SFD needed = 85 × (750 × 0.036) ÷ 100 = 23 ml

Preparing and distributing Shellfish Diet

11. Collect amount calculated in a graduated cylinder or beaker.
12. Pass through a 20 µm sieve before distributing into food reservoir.
13. **For batch feed:** pour SFD and dilute with 1 µm filtered seawater to top of food reservoir.
14. **For continuous feed tank:** divide food ration into 3 batches, administered over the 8 h day.
15. Fill feed tank to half with filtered seawater.
16. Pass ⅓ of Shellfish Diet required through sieve directly into tank.
17. Make sure tank is aerated vigorously and recirculate water to mix well.
18. Add live microalgae portion at this time and fill tank with filtered seawater.
19. Distribute food to spat tanks by pumping.
20. Repeat over the 8 h day with last ⅓ of Shellfish Diet added before end of day.

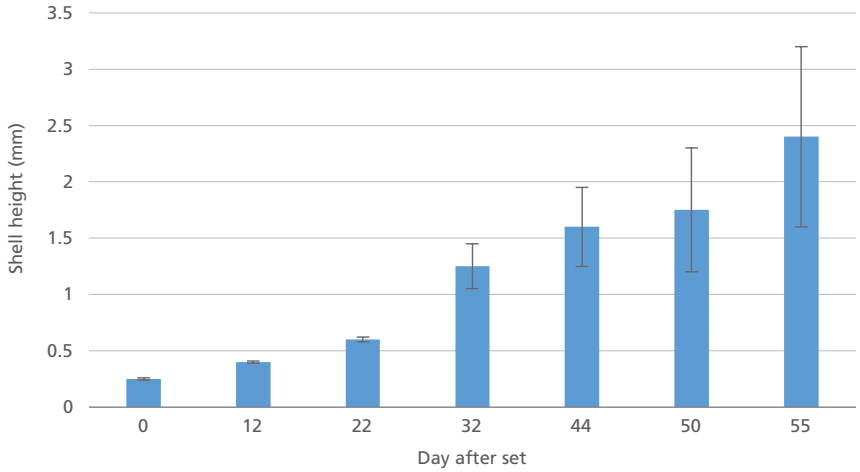
Calculating Instant Algae

Calculate as per live microalgae using manufacturer's information for cell density.

Expected Yesso spat growth – Spat growth for a good batch of *M. yessoensis* larvae set in 5 000 L round tanks at a density of 2 larvae.ml^{-1} at T = 13±1 °C and fed 100 percent live microalgae is illustrated in Figure 7.3. Average shell height >1 mm is attained by the first month of setting. Within two months, spat should grow to 2 mm and beyond. As a rule of thumb, spat grow approximately 1 mm per month. The increasing error bars in Figure 7.3 reflect the wide variation seen in growth rate among spat.

If spat are reared in a raceway setting, grading is done routinely to separate the larger and smaller size fractions.

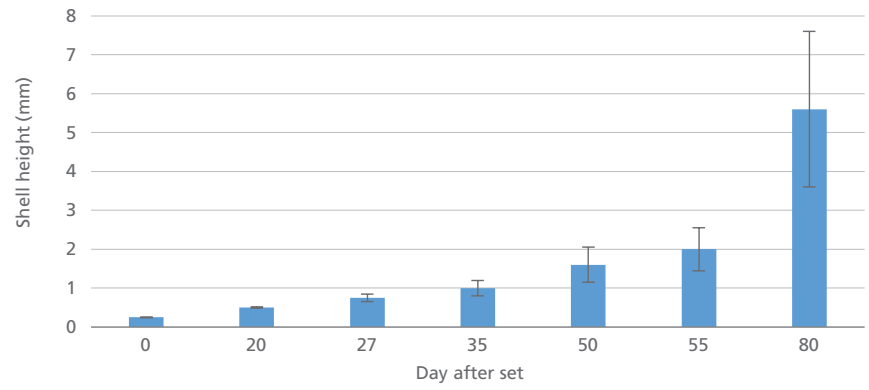
FIGURE 7.3
Expected shell height for Yesso scallop fed live microalgae from Day-1 to Day-55 after set (n = 50)



Shell height for Day-55 spat is slightly higher but comparable when fed a 100 percent live microalgae ration (Figure 7.3) and a mixed food ration of live microalgae and SFD as illustrated in Figure 7.4. Food ration in these examples is as given in Tables 7.1 and 7.2. For spat in Figure 7.4, by Day-46, more than 50 percent of the diet consisted of commercially available food; by Day-67, 85 percent of the diet consisted of instant algae and Shellfish Diet. Total food ration by Day-70 is equivalent to a live microalgal ration of 350 cells.ml^{-1}.

Spat kept in the nursery tanks until Day-100 after set with the same diet (15 % live microalgae, 85 % SFD) reached a maximum shell height of 9 mm.

FIGURE 7.4
Growth rate for *M. yessoensis* spat cultured in a setting tank system and fed a mix of live microalgae and commercial diet (n = 50)

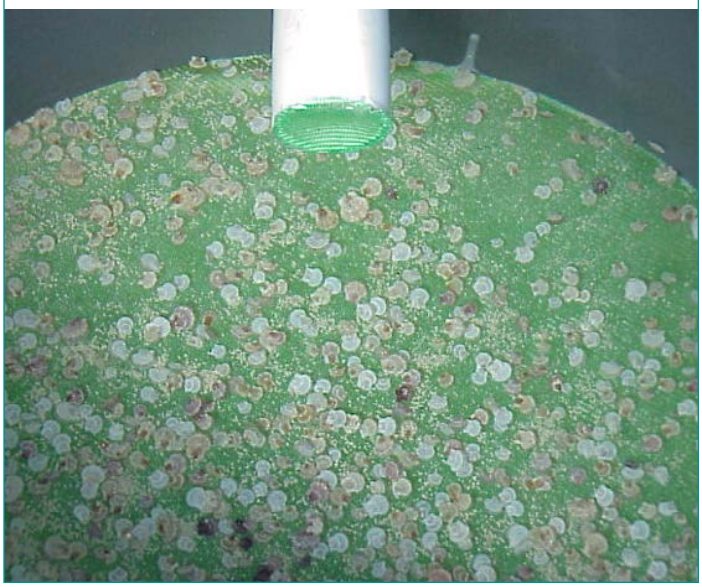


7.2.6 Stocking density and adjusting flow for seed biomass

Maintaining an optimal stocking density in a raceway or tank system is critical to successful seed production. Critical seed density in a nursery system is largely a function of the available surface area to the growing spat and the inflow rate of new seawater. At this stage, biomass becomes the measuring criteria rather than shell height.

Raceways – In raceways, scallops are reared one layer deep in sieves, at a density covering approximately ⅔ of the surface area of the sieve (Figure 7.5). As spat grow, the bottom of the sieve becomes fully covered and crowding occurs. To maintain an optimal biomass for continued growth, spat must be re-distributed at lower densities; this is referred to as "thinning". The objective is to provide sufficient space for spat as they grow. This becomes especially critical starting Week-3 after set when spat begin a rapid increase in growth.

FIGURE 7.5
Scallop seed (3–5 mm) reared in upwelling sieve in raceway, showing partial coverage of the meshed surface area with spat one layer deep



Thinning to the appropriate stocking density in sieves can be estimated visually by maintaining a 60 percent coverage on the surface area. If stocking density per sieve is not controlled, high mortalities and slow shell growth will ensue.

The size and volume of the raceway system dictates the total maximum stocking density supported by the raceway. Incoming flow to the raceway can be increased to adjust to a higher biomass in conjunction with semi-recirculation, but should not exceed 50 $ml.min^{-1}.g^{-1}$ of spat (wet weight); increasing the flow beyond the maximum will not retain added food long enough in the system to be consumed by spat.

The longer the spat are kept in the nursery, the higher the stocking density and the greater the number of raceways required to accommodate the increasing biomass.

The main limiting factor to stocking density in a raceway system is the total surface area of the meshed sieves. For larger operations, raceways may not prove sufficient to achieve the target seed production, if the goal is to rear seed >2 mm in size. In this case, the aquaculturist may achieve the target seed production by combining the raceway system with the nursery tank system. Alternatively, the aquaculturist can choose to transfer seed out of the nursery at a smaller size to an intermediate nursery.

Nursery tanks – Rearing spat in tanks may prove more advantageous for larger scale seed production or when space in the facility is limited. The use of substrates (bundles of mesh) increases the surface area available to spat (see Figure 7.2) and allows for increasing biomass as spat grow; in turn, the increasing biomass can be supported by increasing the incoming seawater flow.

In general, recommended stocking density for scallops is equivalent to 200 mg of biomass for 1 000 L volume of seawater. As spat biomass increases, incoming flow is increased to maintain the recommended stocking density; this gradually converts the spat tank up to twofold its volume. Table 7.3 provides an example of increasing flow rate in a 10 000 L nursery tank, and its effect on the resulting volume of seawater passed through spat. Accordingly, food supply rate is also adjusted to provide a continuous supply over a 24 h period. Residence time of food in the tank is partially ensured by semi-recirculation and by not exceeding a threshold in incoming seawater rate.

At the same time, food supply is supplemented by additional nutrients with incoming seawater when filtered to 10 µm (not 1 µm as during early set).

TABLE 7.3
Increasing a 10 000 L tank capacity with increasing incoming seawater flow rate to accommodate spat growth

Weeks after set	Mean shell height (mm)	Incoming seawater flow ($l.min^{-1}$)	Tank capacity as $l.h^{-1}$ (tank volume plus additional flow)
Week 4–5	1	7	10 420
Week 8–9	2	38	12 280
Week 12	5	380	22 800

"Thinning" to reduce spat biomass per surface area of substrate cannot be realistically implemented in a nursery tank system, as is done for sieves in a raceway. Maintaining growth and survival rates as spat grow in the nursery tanks, relies on an adequate initial setting density and on adjusting flow rate. A higher mortality rate can be expected in a nursery tank system compared to a raceway system and attaining production target relies on a higher volume of seed produced per given surface area of the nursery facility.

Initial setting density is a major factor in the resulting biomass per substrate in a tank system. An initial high setting density can result in a high yield of 2 mm seed, as is shown in Figure 7.6a. However, this will subsequently result in a high mortality rate, if young seed are not collected from the substrate and provided with a larger surface area in due time. Setting at lower densities will provide spat with more substrate area as they grow (Figure 7.6b); this facilitates the managing of stocking density and will allow to raise to a larger size in the nursery. This is a management decision and depends on production scale, personnel and space resources available.

FIGURE 7.6
Varying seed density for Yesso scallops on setting substrate suspended in nursery tanks – (a) high seed biomass on top section of substrate; and (b) low and medium seed biomass on substrate

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7.2.7 Calculating spat biomass in a tank setting

In order to calculate biomass for spat reared in a tank, total wet weight per tank is estimated. The following information is needed:

- number of substrate pieces used for setting per tank;
- average shell height; and
- average spat weight per substrate piece.

In the first year of operation, collecting data to determine the relationship between shell height and weight of spat throughout the nursery phase is recommended; this enables a rapid assessment of biomass for future batches and in the years to come. A subsample of substrate is collected, from which spat are washed onto a pre-weighed mesh, measured and weighed; data is graphed and used to estimate total spat weight in a tank. Protocol 7.2.8 describes the steps used for estimating spat settled on substrate; the procedure is the same for spat set on raceway sieves.

7.2.8 PROTOCOL: Estimating spat biomass on substrate

MATERIALS

- Screen with mesh of appropriate size
- Sedgewick-Rafter cell
- Ocular micrometer with compound microscope or digital camera fitted onto microscope for imaging
- Eppendorf pipette and tip
- Pieces of mesh (various aperture size)
- Analytical scale (±0.001 g)
- Petri dishes
- Dissecting Scope
- Scale (±0.01 g)
- 10 % formalin

METHOD

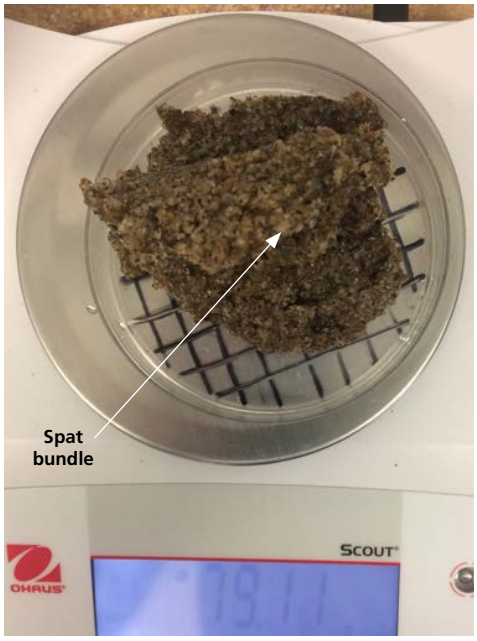
Weighing spat 1–1.5 mm

1. Prepare several pieces of 25 cm^2 700 µm mesh.
2. If spat are seen to be relatively dense on substrate; take a subsample of substrate piece (¼).
3. Make sure to carry substrate and spat in holding beaker or bucket.
4. Wash down spat from substrate into a small sieve using a gentle jet of filtered seawater.
5. Collect spat in 100 ml containers.
6. Cut an Eppendorf tip at the end, so that aperture is larger to collect and measure 30 spat using a Sedgewick-Rafter cell or a camera fitted on the microscope.
7. As spat grow, subsamples are collected on petri dish lined with a grid and measured under a dissecting scope.
8. Collect a sample of a minimum of 100 spat in a 100 ml container.
9. Tare 25 cm^2 mesh using analytical scale.
10. Carefully wash sample through mesh.
11. Blot mesh dry by placing on a piece of absorbent paper; paper only touches mesh not spat.
12. Weigh mesh and spat. Record value.
13. Calculate spat weight for 100 spat.
14. Repeat Steps 2–13 for 2 other pieces of substrate in the same tank, and for two other tanks.
15. Enter in Excel datasheet for plotting height vs weight graph.

7.2.8 PROTOCOL (continued)

Weighing spat >2 mm

16. Follow Steps 1–4 to collect spat in a small container.
17. Tare one piece of 25 cm^2 mesh on scale.
18. Place tared mesh over measuring cup and carefully pour your entire spat sample through the mesh, so that you collect all spat on the mesh.
19. Measure 30 spat under the dissecting microscope or with Vernier caliper if spat are >2.5 mm.
20. Blot mesh and spat dry with absorbent paper.
21. Weigh and record. If only ¼ piece of substrate was used for weight, multiply recorded weight by 4 to obtain total weight per 1 piece of substrate.
22. Repeat Steps 16–21 with two more samples taken from different pieces of settling substrate from same tank.
23. Repeat Steps 16–22 for two other setting tanks.
24. Enter in Excel datasheet to graph height vs weight.



Weighing spat on mesh

Estimate spat biomass per tank

25. Calculate:

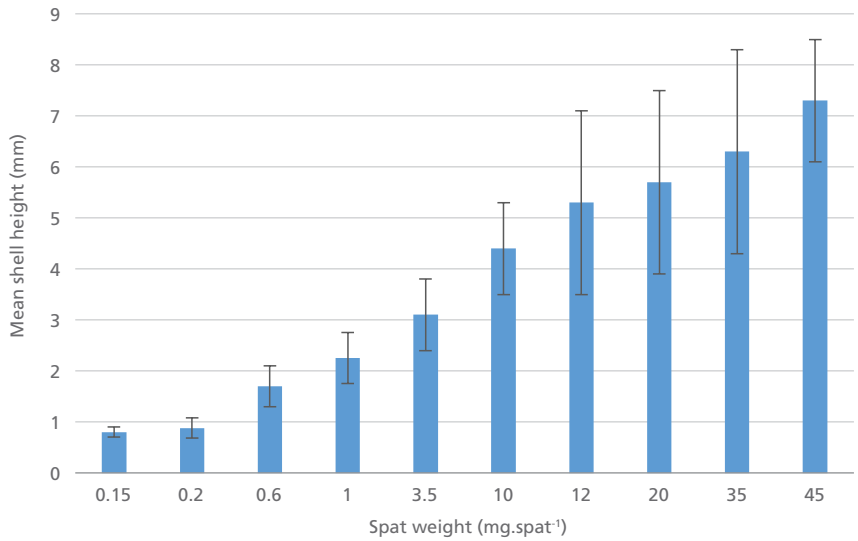
Average biomass/substrate × No. of substrate per tank

Estimate spat biomass for the whole batch

26. If calculated biomass per tank is similar among replicate tanks (Step 22), assume equivalent biomass for all tanks (this assumes equal number of substrate pieces per tank).
27. Calculate average biomass per tank × number of tanks for the batch.

Yesso spat height and weight – Data for height and weight of spat reared at 12±1 °C in a nursery tank system is given in Figure 7.7. At this temperature, 7 mm seed is expected to have a mean weight approximating 45 $mg.spat^{-1}$. Variations in shell height increase as spat grow reflecting a marked difference in growth within a spat batch. This relationship is dependent on nursery conditions, namely food composition and ration, and data given in Figure 7.7 should be validated against a new nursery system. Example below describes how to calculate total biomass per tank or per batch using the height/weight graph. The biomass data is also used for: a) calculating the food ration for the nursery tanks for seed >2 mm (see Section 7.2); b) distributing seed in bags and/or nets for transfer at sea (see Section 8.2); c) assessing total seed production per batch; and d) anticipating the number of bags, nets and longlines required for grow-out on the farm.

FIGURE 7.7
Relationship between Yesso spat shell height (mm) and weight (mg.spat^{-1}) from newly settled spat (<1 mm) to 7 mm seed reared in nursery tanks (n = 50 for height; n = 15 for 100 spat subsample weight)



EXAMPLE – Using a height vs weight graph to calculate stocking density

Using the height vs weight graph in Figure 7.7

Mean shell height = 800 µm (or 0.8 mm)
Total weight of all spat collected from 1 piece of substrate = 225 mg
Number of pieces of substrate in one tank = 50

From graph - Average weight per 0.8 mm spat is 0.15 mg

Calculation:

Total number of spat on 1 piece of substrate = Total weight (mg) ÷ Average weight per spat (mg.spat^{-1})
= 225 mg ÷ 0.15
= 1 500 spat on substrate

For a tank with 50 pieces of substrate
Total number of spat in tank = 1 500 × 50 = 75 000 spat

7.2.9 Rearing spat >2 mm

As spat grow greater than 2 mm shell height, they become increasingly tolerant to natural environmental conditions; they also become more costly to maintain and raise in a land-based nursery as their demand for higher food ration and seawater supply continues to grow exponentially. The rearing system in the land-based nursery remains similar; a 10 µm filtration is recommended, incoming flow rate is increased according to biomass and resulting stocking density, and there is often an increased reliance on commercially available food.

When to transfer spat out of nursery – The hatchery manager assesses the optimal time for transfer to outdoor intermediate nurseries (such as ponds, larger outdoor nursery tanks or upwelling raceways), or directly to the farm.

The size until which young seed is kept in a controlled land-based nursery is dependent on nursery parameters, but also on the physical, biological and chemical seawater characteristics of the farm site to which they need to be transferred. These will fluctuate seasonally and affect distinct spat batches differently. Acclimating nursery-produced spat to ambient conditions prior to transfer out of the nursery is a must; the duration of acclimation depends in great part on the temperature difference between the nursery and ambient conditions. For a continuous 12-month production, the aquaculturist will utilise several strategies to optimise timing and size of seed at transfer, based on seasonal conditions.

8. Nursery to farm

IN THIS CHAPTER – How to maximise cost-effectiveness in the nursery and how to transport seed from nursery to farm.

The nursery is costly in terms of food production, labour, seawater volume and treatment requirements and space. Ideally, the aquaculturist strives to transfer seed as soon as possible to the farm; but a balance has to be achieved between reducing the work load on land and optimising the growth and survival of seed transferred. The key is to coordinate spat or seed size at transfer with natural physical, chemical and biological conditions at the farm; if conditions are not suitable for the size of seed ready for transfer, it is recommended to hold the seed longer in the nursery or make use of an intermediate nursery. Successful rearing of Yesso scallops >2 mm and up to 10 mm shell height is doable in intermediate nurseries, if optimal stocking density is maintained.

Farm conditions for seed – The time of year and size at which spat are transferred is dependent on many environmental factors, including: temperature, primary production and natural recruitment of potential predators. These will affect the survival and growth of transferred seed. For example, moving young scallop seed to the farm during a crab recruitment period, can result in the trapping of crab larvae in scallop nets and subsequent predation of fast growing crab on the scallop seed. Information relating to the environmental processes at the farm is valuable and is important to obtain prior to setting up a farm site.

Production scale considerations – The production volume of spat is also a factor in selecting a strategy for transfer to farm. Hatchery operations which produce spat on a large-scale can opt to move very young spat (<1 month old) out of the nursery and transfer to the farm; they know that the survival rate will be low but this is counteracted by the large volume transferred. This approach is often referred to as a "remote nursery". On the other hand, smaller scale hatcheries will benefit from a longer nursery period to produce spat >2 mm as seed survival following transfer to farm increases with seed size.

8.1 REMOTE NURSEY

In cases where nursery operations are not cost-effective and high volumes or an excess volume of pediveligers are produced during the hatchery cycle, a "remote nursery" approach is an alternative. Using this methodology frequently results in high losses of competent larvae and/or young spat within the first month of grow-out on longlines; this subsequently leads to a reduced survival of spat to larger seed size. For this reason, it is only under circumstances described above, or if there is an excess of spat settled, that the culturist can afford to adopt such a cost saving protocol for the nursery operations.

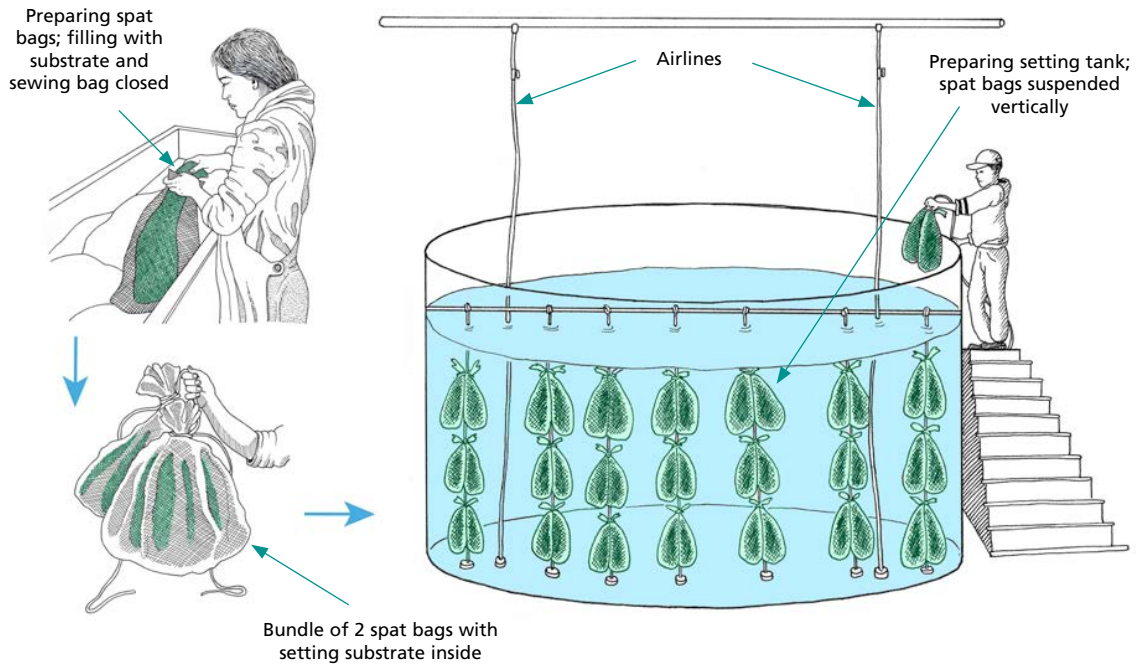
The general concept of a remote nursery is to minimise the nursery load by transferring spat recently set (<800 µm) from the land-based facility to the farm.

8.1.1 Use of spat bags for remote nursery approach

Setting for remote nursery approach – This approach is used at the time of writing in large-scale hatchery operations, routinely producing >50 million pediveligers per

spawn. Setting substrates are placed into fine meshed spat bags and suspended in a static aerated round or conical tank with gentle aeration (Figure 8.1). Spat bags come in various mesh sizes and sizes of 425–700 µm are used for the Yesso scallop. Sets of two spat bags are attached at regular intervals along a piece of floating rope tied to opposite sides of the setting tank; bags are suspended vertically at 1 m intervals to fill the tank with substrate from the surface to the bottom. Another option is to lay spat bags horizontally in lantern nets (3–4 mm mesh size). Two spat bags can be placed per layer of lantern net to maximise space on the longline at the farm. Competent larvae are introduced in the tank and allowed to set into the bags. The smaller the spat bag mesh size, the more likely larvae and young spat will be retained, and the higher the resulting density of spat settling per bag. Scallops will settle both inside the bag and on the outside of the bags; this means that an unknown percentage of scallops is additionally lost during transfer as spat dislodge easily during handling and will fall off the bags.

FIGURE 8.1
Remote nursery approach: spat bags suspended in nursery tank for setting and rapid transfer to longlines



Size of spat at transfer – Spat settled in substrate and bags are cultured at T = 12±1 °C to attain desirable sizes in 2–4 weeks. Minimum shell height for spat retained in 425 µm mesh bag is 600 µm and is attained within two weeks of nursery culture; for retention of spat in 700 µm bags, spat must exceed 1 mm in shell height, expected to be reached in 4 weeks of nursery culture.

Spat are gradually acclimated to natural seawater temperatures one week prior to transfer. Once ambient seawater is reached in the nursery tanks, spat bags are moved to longlines moored in a protected site for on-growing to juveniles.

Controlling fouling – Once transferred to the farm, maintenance of bags is critical to optimising survival and growth. Most importantly is the control of fouling on the spat bags; excessive fouling will reduce the water flow, poor growth and spat mortality will follow. For this reason, waiting an additional two weeks in the nursery and transferring

spat in the larger 700 µm spat bags is advisable; a large size mesh reduces the time in which fouling clogs the mesh aperture and the water flow supplying nutrients to young spat.

Routine maintenance includes scrubbing bags on a regular basis. Depending on the primary productivity level at the farm, scrubbing may be required monthly or more frequently; it will most likely cause additional loss of spat by removing and damaging spat fixed on the bag itself. This procedure can be very labour intensive, and ineffective in the operation of the farm. It is most suited to regions where labour is readily available and fouling is minimal.

Transfer to nets – Based on seed growth rates obtained for the Yesso scallop in northern temperate areas, it is anticipated that spat will be large enough to be transferred to 1 mm mesh bags within 1 month of culture on the longline. This can be a relatively simple and rapid procedure, dependent on the level of fouling of the substrate itself. In case of minimal fouling, the settling substrate with attached spat can be directly removed from a 700 µm bag into a 1 mm bag, without disturbing young seed.

For a substrate with heavy fouling and if seed are 2 mm+, scallops are washed off the substrate onto sieves and "thinned" for re-distribution in appropriate size mesh bags. A minimum 2 mm shell height is the advised size for handling spat in this way as smaller spat are too delicate and will incur high mortality if the aquaculturist attempts to remove these from the settling mesh.

The procedures used for retrieving seed from the settling substrate, thinning and re-distributing are from here on similar to those used for nursery-reared seed (see Section 8.2).

8.2 RETRIEVING NURSERY-REARED >2 MM SEED FOR TRANSFER AT SEA

The larger the seed, the hardier and more tolerant to handling it becomes; this minimises any potential mortality associated with stress during handling and transfer at sea. From the aquaculturist point of view, larger seed facilitates the retrieval of seed from the settling substrate and its distribution in grow-out bags or nets. This means a more labour and cost-effective process.

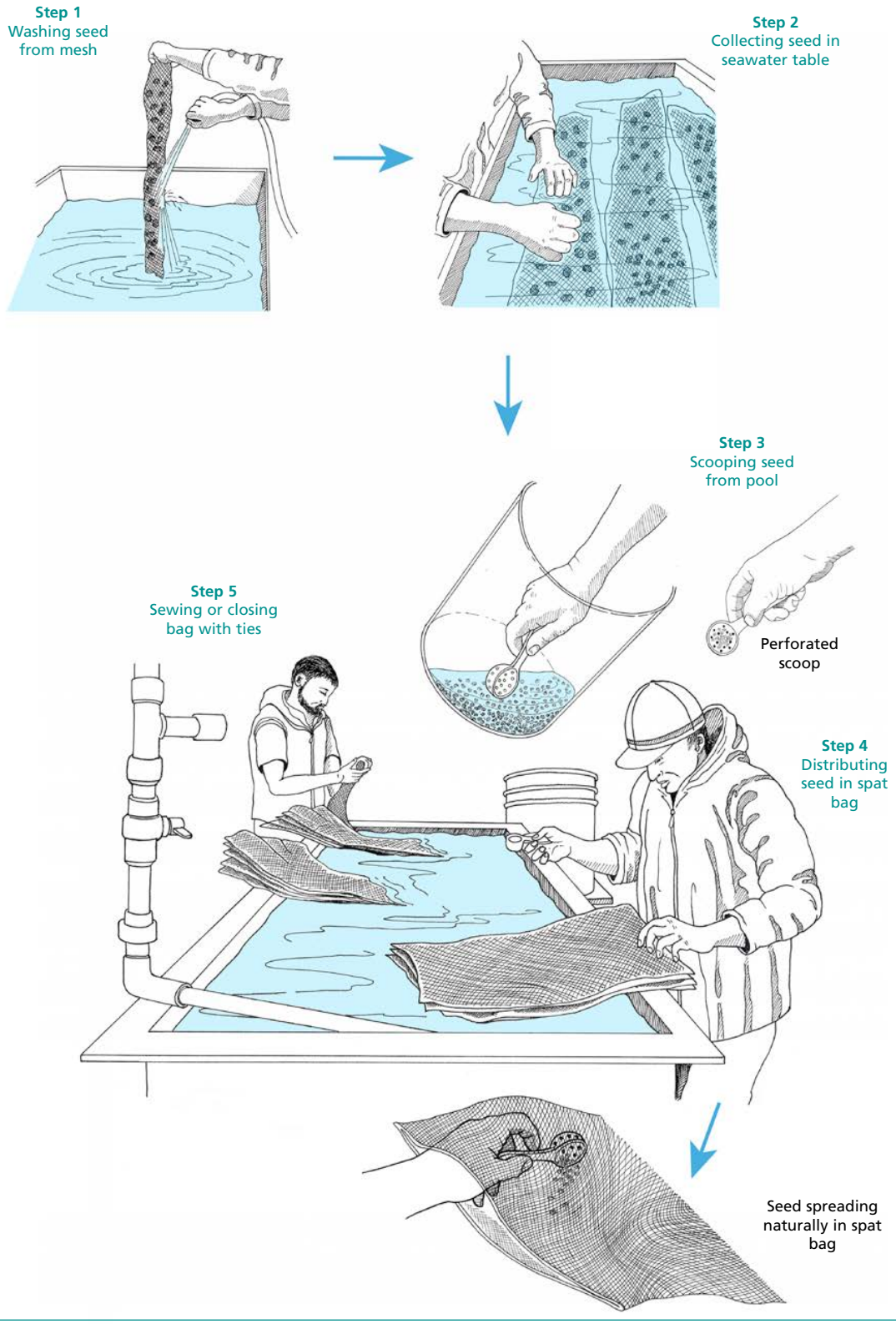
Raceway set – Spat set in sieves on raceways are easily washed off using a gentle jet of seawater into a holding bucket for distribution into pearl or lantern nets, prior to transfer to the farm.

Tank set – For spat set on substrate in a tank system, thinning is more easily and rapidly achieved when seed is free from substrate and collected from spat bags or nets. For this reason, if reared in the nursery, >2 mm seed can be washed off the settling substrate and distributed into spat bags in the nursery prior to transfer to the farm (Figure 8.2). It will be done more efficiently and thoroughly in the nursery rather than onboard the farm boat; it also reduces the workload of the farm crew for subsequent thinning at the farm by eliminating the handling of setting substrate and working with nets only.

Types of grow-out nets – There are three main methods for suspending seed in the water column: Pyramid-shaped pearl nets, round lantern nets, and square shallow scallop trays. Minimum spat shell height for use of nets is 2.8 mm (2 mm mesh). Pearl nets have a smaller surface area than lantern nets (0.12 m^2); they are generally used for 3 mm seed and up to 30 mm scallops. Unlike pearl nets, lantern nets are not tied one below the other but one lantern net is multi-tiered extending vertically. Round

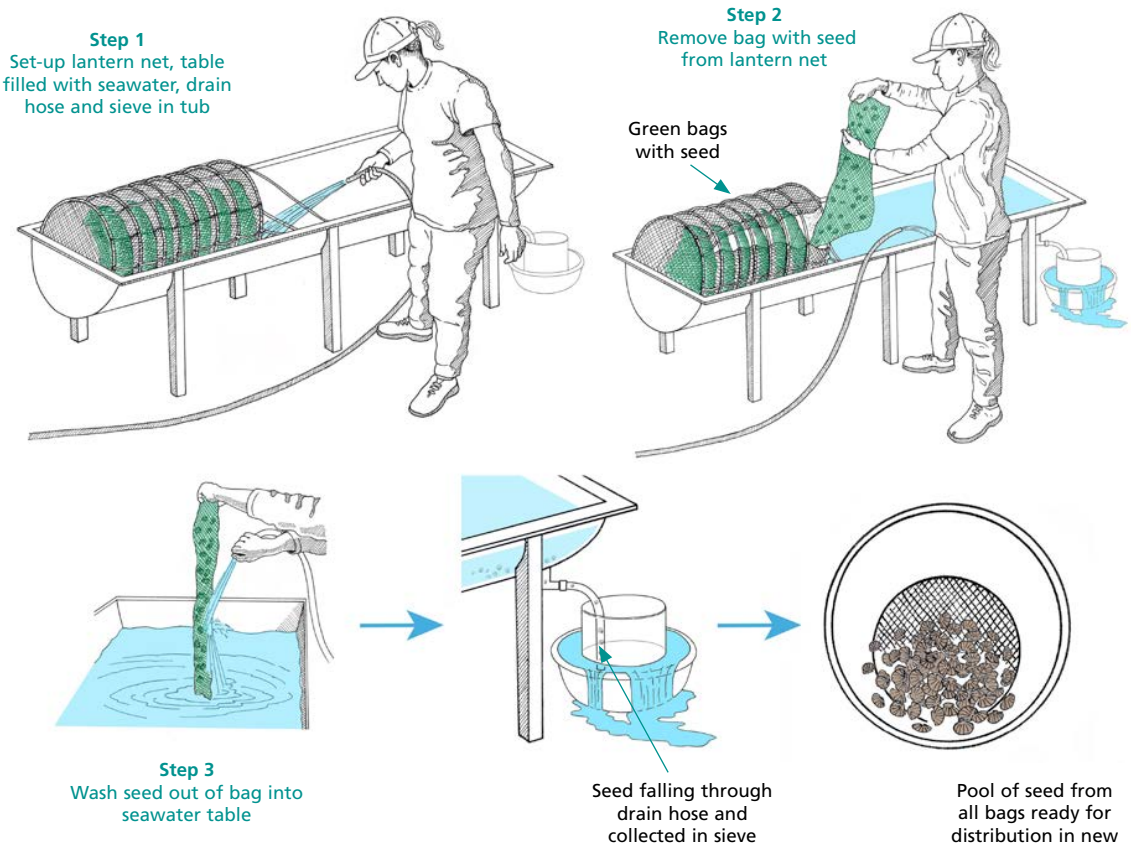
lantern nets consist of 5, 10 or 15 tiers with one seam running along side the entire net; diameters commonly are 30, or 50 cm (0.07 and 0.19 m^2 surface area respectively). Square shallow scallop trays approximate 30 × 30 × 10 cm (l × w × h); they can be tied in a stack and clipped on to a longline using a single point of attachment. As spat grow, they are gradually transferred to larger size mesh bags and nets.

FIGURE 8.2
Retrieving spat from settling substrate and scooping known density into spat bags



Thinning seed and stocking densities – The concept of "thinning" is similar to that applied to raceway setting in Section 7.2.6. It provides additional surface area to the scallops as they grow and it is applied throughout various stages of seed production: in the land-based nursery prior to transfer at sea, in the retrieval of seed from the remote nursery and during the juvenile farm stage of production. Methods used are similar whether they are implemented on land in the nursery or onboard the farm boat (Figure 8.3). Thinning involves the grading of seed according to size and methods are described in Protocols 8.2.1 and 8.2.2. The aquaculturist adapts his resources to facilitate the collection, grading and thinning of scallop seed. Protocols given are suggestions and other methods can be devised. The key is to minimise handling of seed, work quickly and reduce the exposure of seed to air.

FIGURE 8.3
Collecting seed from substrate onboard the farm boat for thinning and transfer into new nets



Because Yesso seed grow at a rate of 5–10 mm per month, thinning needs to be conducted every month for 5–6 months following transfer of seed from nursery to farm. The rate of thinning after this depends on the seawater temperature and the associated growth rate. The faster the growth rate, the more frequent the transfer into new nets at lower stocking densities, until the final density for market size scallops is reached. The reduced densities in nets provides the space required by scallops for optimal growth; this is one of the limiting factors in large-scale scallop culture; unlike other cultured bivalves such as mussels and oysters, free-swimming scallops suffer high mortalities and stunted growth if reared at high densities. They will spread out naturally in the bag or net (Figure 8.4). Table 8.1 gives suitable stocking densities in spat bags and nets for optimal growth during the first few months.

FIGURE 8.4
Grow-out of Yesso seed in spat bags – (a) sewing 2 mm seed in spat bags; (b) seed naturally distributed in bags; (c) 30 days later retrieved from longline (>5 mm)

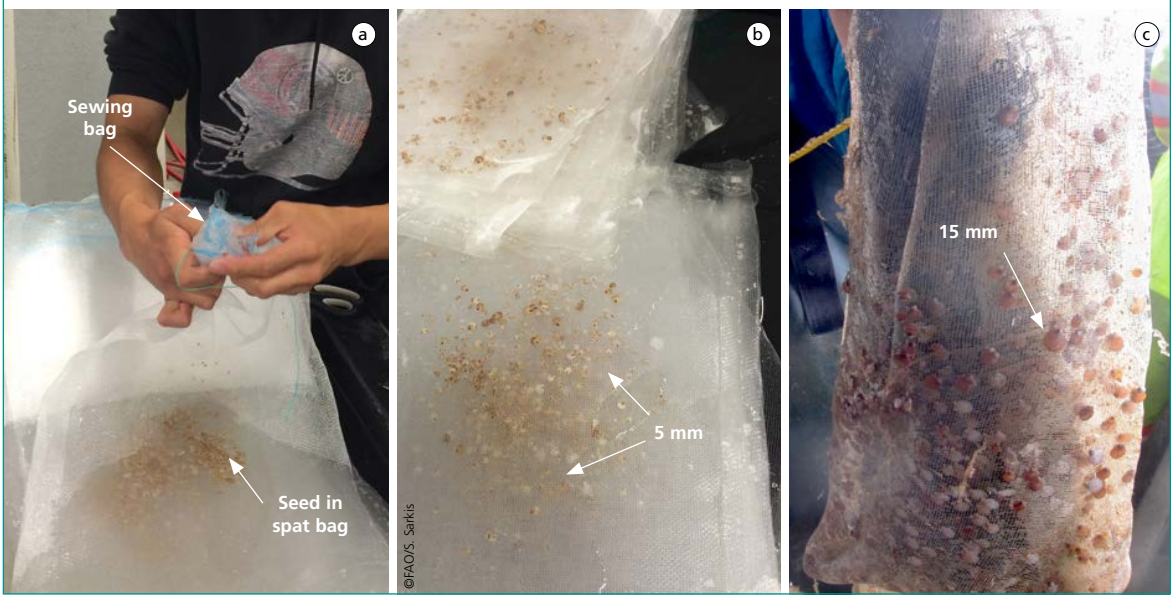


TABLE 8.1
Stocking densities for seed of increasing size adapted from various sources. Densities are given for 50 cm round lantern nets

Mesh size and type of net	Number of spat/bag	Spat shell height (mm)
700 µm bag	400	1
1 mm bag	400	1.5
1.5 mm bag	200	2
2 mm pearl net	150	2.8
3 mm pearl net	100–150	4
4 mm lantern net	100	5.5
6 mm lantern net	60	8

Preparing scoops for thinning – Scoops and plastic cups are identified for specific spat size and volume according to shell height and weight of spat. They are re-used at every routine thinning operation by nursery and farm crews. A procedure and example are given in Appendix III.

Grading seed on a large-scale – Aquaculturists devise various ways to simplify the grading of seed, especially in larger scale operations. A grading 'table' can be constructed which allows for grading a larger volume of seed at any one time than possible using a series of sieves. This grading table is a multi-layered structure with mesh frames secured one above the other. The largest mesh aperture is on top, and frames with decreasing mesh size secured underneath. The last mesh frame sits off the bottom, such that collected on the mesh do not touch a hard surface but are submerged in a shallow seawater tray.

Once scallops attain 8 mm shell height, they enter the farm system for juvenile grow-out to market size.

8.2.1 PROTOCOL: Grading seed

MATERIALS

- Grading sieves (400 µm to 4 mm)
- Seawater table
- Clean empty spat bags and lantern nets
- Labelling tape and magic marker
- Tubs for holding bags with seed

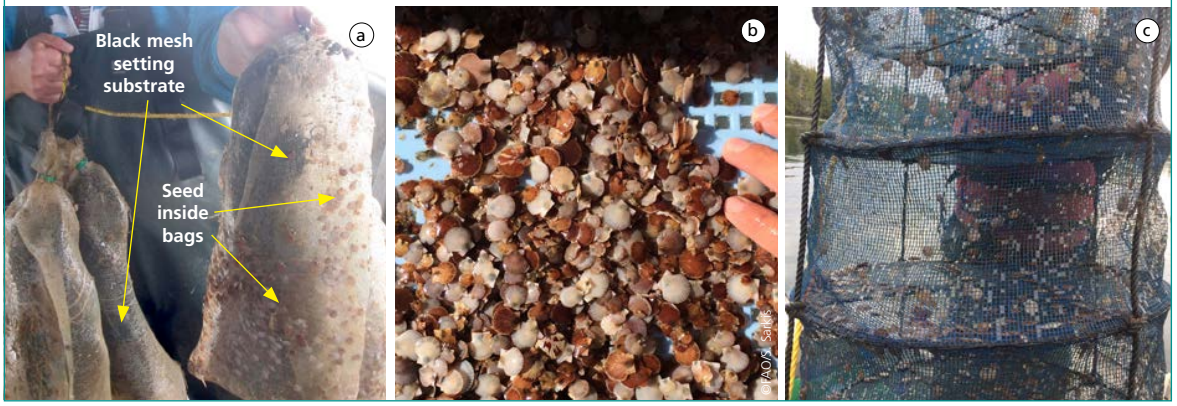
METHOD

Procedures described here apply to the land-based nursery or onboard the farm boat if it has a large enough deck (Refer to Figure 8.3).

Collecting spat from spat bags

1. Set up a spat collection station. This will be the area for washing spat from bags; each station has flowing seawater, a saltwater table, a space to stack new clean bags/nets.
2. Clean saltwater table and all equipment used.
3. Fit a draining hose to the drain plug of the saltwater table.
4. Place a sieve under the draining hose; with the sieve sitting in a tub filled with seawater. Seed will be washed from the table through the hose and collect on an appropriate mesh size.
5. Collect spat bags from longlines. Temporarily store in a large bin filled with seawater.
6. Start seawater flow in saltwater table.
7. Place one spat bag on saltwater table, remove mesh from bag
8. For seed <2 mm – Place mesh directly into a sieve; and wash spat into the sieve
9. For seed >2mm – Seed is washed into saltwater table and flushed through the drain hose.
10. Wash seed from bag using a gentle flow of seawater.
11. Wash spat bag thoroughly to remove any seed fallen to the bottom of the bag.
12. Repeat with all bags.
13. Seed can be pooled into one sieve until such time that there are too many layers of scallops in one sieve.
14. Grade collected seed by passing through 2 or 3 sieves stacked with larger mesh size at the top and lowest at the bottom.
15. Collect each size fraction in a separate tub (Figure 8.5). Label each tub with size fraction.
16. See Protocol 8.2.2 for distributing seed in appropriate densities.

FIGURE 8.5
Collecting seed from spat bags – (a) spat bag bundles with substrate and seed; (b) pool of seed collected in tub; (c) lantern net with good seed stocking density



8.2.2 PROTOCOL: Thinning seed

MATERIALS

- Seawater table
- Meshed bags (425 µm to 1.5 mm)
- Pearl or lantern nets (2 to 4 mm mesh)
- Labelling tape and permanent marker
- Tubs for holding bags with spat
- Perforated measuring scoops (for spat)
- Plastic cups (50 ml) or similar (for larger seed)

METHOD

This method is used for all spat sizes ready for transfer to bags or nets and is applied to the nursery or onboard the farm boat.

On-board thinning

Prepare all materials on land; this includes labelled scoops and cups for distributing seed (see Step 6).

Collecting seed for appropriate stocking density

Method 1 – Using a height:weight graph

1. Measure 30 spat from a subsample of each size fraction previously graded (see Protocol 8.2.1).
2. Check new stocking density (Table 8.1).
3. Pour spat in appropriate scoop or cup as previously identified (see Appendix III).

   If you do not have a height:weight graph, use Method 2 below:

2 mm seed graded

Method 2 – Counting spat

4. Collect a subsample of each size fraction graded previously.
5. Count the number of spat in each size fraction for the appropriate stocking density (Table 8.1).
6. Pour into appropriate size measuring scoop or cup and mark level to fill.
7. Label scoops and cups used for size fraction for future record.
8. Counting the number of spat to attribute a spoon or cup for each size fraction is only done once; the same spoons and cups are re-used in future thinning.

Distributing spat in new bags or nets

9. Work in saltwater table with running filtered seawater.
10. Place new bags and tub of collected seed nearby.
11. Using the appropriately labelled scoop or cup for new stocking density, collect seed from holding bin; fill to the level marked (as per Steps 4 to 6).
12. Empty the scoop or cup into the new bag or net; make sure bag is lying flat in flowing seawater; this makes it easier for smaller spat to distribute in the new bag. Procedure is similar to Steps 4 and 5 in Figure 8.2.
13. Sew or close bags and nets, and place in clean holding bin with flowing seawater dedicated to new bags.
14. Repeat until all seed for this fraction is distributed.
15. Bags and nets are either prepared for transport to the farm or are suspended directly on longlines if work is carried out at the farm site.

8.2.2 PROTOCOL (continued)

Securing bags to longlines

16. Place two bags per lantern net compartment, laying them flat.
17. Suspend lantern nets on longlines.
18. **Alternative:** Bags are suspended directly on longlines in single lines; they are tied to alternating side of a rope secured with an anchor and held vertical and taut by buoys.

8.2.3 Expected survival and growth rates post-transfer at sea

High survival rates can be expected when:
- environmental conditions are favourable,
- spat densities are maintained according to size; and
- nets are cleaned monthly.

Survival rates are given for a 30-day grow-out period between thinning and net change:
- Remote nursery: Survival rate of spat <1 mm on longlines is <2 %
- In 1.5 mm mesh bags: Survival rate of 2 mm spat averages 90 %
- In 2 mm nets: Survival rate of 3 mm seed averages 90 %
- In 4 mm nets: Survival rate of 5.6 mm averages 100 %

In order to achieve a 90 percent survival in 1.5 mm mesh bags, spat must be re-distributed after 4 weeks of grow-out; if not, clogging of the mesh will occur quickly. Survival rate can rapidly decrease from 90 to as low as 30 percent if transfer is delayed for 8 weeks and to a further 17 percent if transfer is delayed 10 weeks.

8.3 TRANSPORTING SPAT TO FARM

Scallop seed can be transported in bags or lantern nets using 1) a moist, insulated container; tolerated duration of transport is species specific (referred to as "dry transport"); or 2) a seawater-filled insulated container with adequate aeration and preferably continuous flow (referred to as "wet transport"). Alternatively, 3) if space onboard is limited, seed is transported in high densities and distributed in bags/nets at the farm site; this is done using small moist pouches such as coffee filters. Dry transport success depends on maintaining a constant and suitable temperature in holding bin for the duration of transport. The wet transport method minimises stress as scallops are maintained in well oxygenated water, but this requires a larger vessel. The "coffee filter" alternative minimises space required for transport.

8.3.1 Dry and wet transport of bags or nets

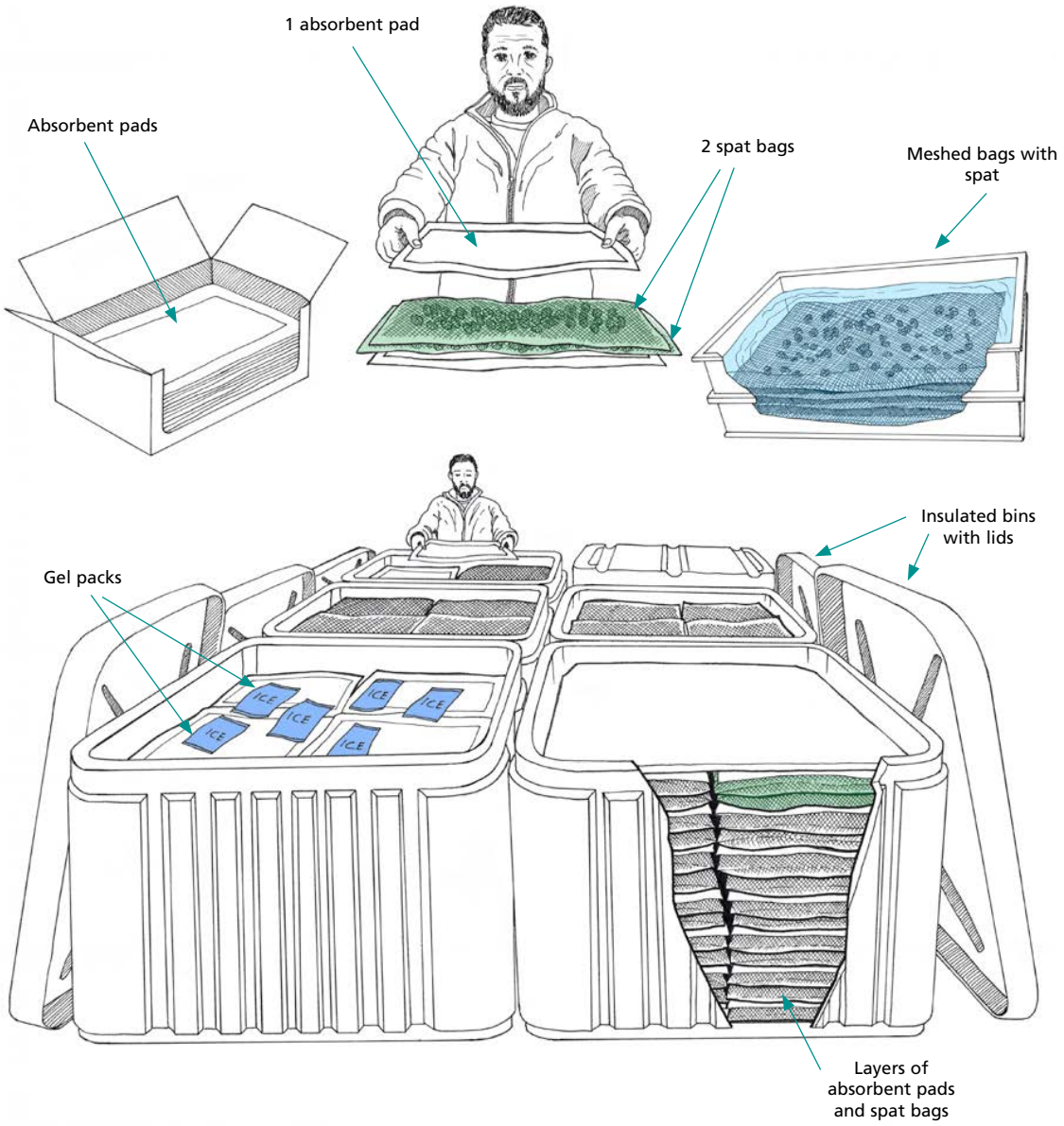
Both methods of packing and transporting (dry and wet) result in live spat, with no mortality seen at destination for transport durations of 6–8 h. Both methods use insulated bins, where nets or bags are packed loosely. A stepwise procedure is given in Protocol 8.3.2.

Dry transport – Dry transport is in fact the layering of spat bags in-between absorbent pads moistened with filtered seawater. Using an insulated bin, absorbent pads dampened with seawater sandwich spat bags and maintain seed moist. The layering pattern is repeated until the bin is nearly full; temperature is maintained cool for the duration of transport by the use of gel packs. The lid is placed tightly on for insulation and ready for transport (Figure 8.6).

Simulated nursery experiments showed that spat survival was near 100 percent during this type of transport, for a simulated transport period of 4 h. Post-transport stress was also monitored by recording survival of spat in nursery tanks one week after the simulated transport. No mortality was recorded for Yesso spat.

Wet transport – Wet transport also involves the layering of spat bags or lantern nets in an insulated container. The difference is that the container is filled with seawater and is supplied with continuous seawater flow throughout the duration of transport. This is possible with larger vessels, which have the ability to pump large volumes of water for an extended period of time while moving. For a shorter duration of transport, a static system of well-oxygenated insulated bins filled with seawater is suitable.

FIGURE 8.6
Packing spat bags for "dry" transport using absorbent pad layering



8.3.2 PROTOCOL: Packing spat for transport (dry and wet)

MATERIALS

- Insulated bins with lids
- Absorbent pads
- 20 L buckets

METHOD

Preparation

1. Hatchery manager to coordinate with Boat captain for transport of spat packed in bins.
2. Clean insulated bins used for transport.
3. Packs of absorbent pad and 2 or 3 buckets half-filled of filtered seawater are prepared close to packing bins for moistening pads.

DRY transport for spat bags

4. Two layers of spat bag are placed on bottom of tank.
5. Absorbent pad is moistened with seawater and laid over bags.
6. A second layer of absorbent pad is placed on top of first pad.
7. Repeat Steps 4–6 until bin is full.
8. On top of last absorbent pad, add an insulator material and gel pack(s) to maintain temperature cool (Figure 8.6). Make sure gel pad does not come in direct contact with scallop seed.
9. Close bin tightly with lid to maintain temperate constant (transport temperature must be assessed by aquaculturist).
10. Load bins onto farm boat.
11. Once at farm site, spat bags can be transferred into lantern nets before suspension on longline.

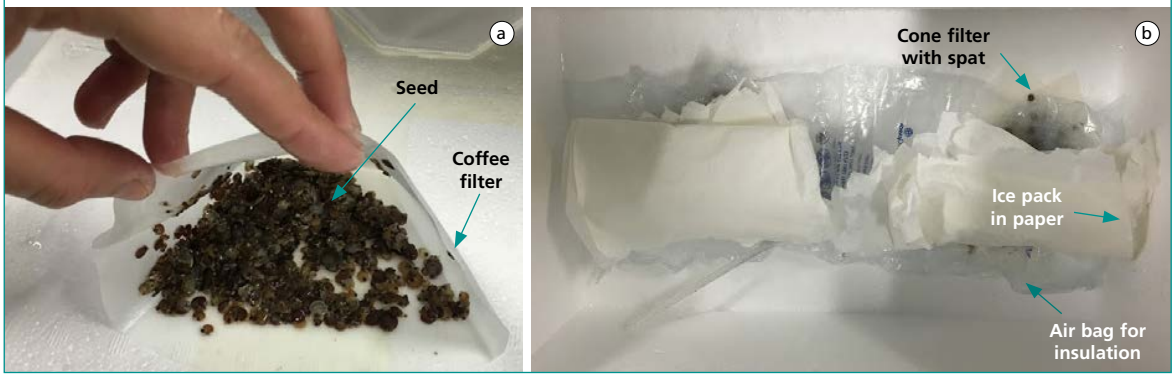
WET transport for bags or nets

12. Bags or nets with spat are placed flat in transport bin.
13. Cover with lid.
14. Load bins on farm boat.
15. Once onboard connect the bin with flowing seawater and fill.
16. If seawater cannot be continuously pumped during transport, aerate bin with onboard air compressor throughout transport.

8.3.3 High density transport of seed

Experimental studies show that Yesso scallop seed with an average shell height of 5.3±1.6 mm can be transported for up to 8 h in moist coffee filters, with no apparent stress observed and a survival rate of 100 percent following transport (Figure 8.7). Further monitoring after 30 days of grow-out on the farm, shows growth rate to be comparable to seed transported using more standard methods and a 100 percent survival. There are no published reports for transporting seed of smaller sizes using this method but the likelihood of success is high. This method is very space efficient and can be used for operations which have a farm boat limited in deck space, or if seed needs to be transported by air or by road. Procedures are given in Protocol 8.3.4.

FIGURE 8.7
Transporting seed at high density using the "coffee filter" method – (a) seed in filter; and b) top view of styrofoam transport container showing ice packs laid over insulated material and covering series of coffee cone filters with seed



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8.3.4 PROTOCOL: High density transport of seed, the "coffee filter" method

MATERIALS

- Conical coffee filters
- Seed scoop or measuring cup
- Insulated container (e.g. styrofoam box)
- Air bags for insulation
- Ice packs

METHOD

1. Scoop up to 1 800 seed using pre-measured spoon or cup (seed >4 mm).
2. Dampen coffee cone with filtered seawater and lay flat.
3. Transfer seed to cone.
4. Lay cone flat into insulated container one on top of the other.
5. Fill the container until all seed is used.
6. Make sure container is closely packed.
7. On top layer, add air bags or insulating separator.
8. Place ice packs on top to maintain temperature in container at 6 °C; make sure not to have direct contact with ice packs and coffee filters with scallops.
9. Secure lid on top of insulated container.
10. Once at destination, scoop out seed and distribute in appropriate densities in bags or nets.
11. Monitor survival and growth following 30 days of culture on farm site.

8.3.5 Seed monitoring on the farm

A comprehensive seed monitoring programme is advised during the first year of operation; this is valuable to both the nursery and farm managers. In subsequent years, a basic routine seed monitoring is sufficient to have a good understanding of the performance of the farm and allows to address any issues prior to high mortality.

A comprehensive seed monitoring programme involves monthly subsampling of seed from selected lantern nets. A vertical profile of water quality at the farm site is recorded at the same time. Procedures are given in the Protocol 8.3.6.

Growth rate of 2 mm seed during the first 4 months of transfer at sea attains 10 mm per month. Shell height of 8 mm is considered the size at which scallops enter the farm management protocols. Assuming a good farm management and favourable

environmental conditions, Yesso scallops grow from 8 mm to 40 mm shell height within 4 months; at which point they are robust with minimal mortalities seen until market size, assuming a good farm management and favourable environmental conditions.

8.3.6 PROTOCOL: Seed monitoring on the farm

MATERIALS

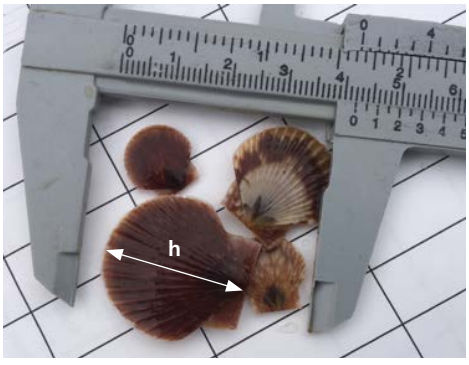
- Several shallow trays for collection of spat
- Two 10–20 L buckets
- 2–3 L beakers for counting/measuring
- Vernier caliper
- Sampling vials/formalin
- Data sheets/clipboard/field notebooks
- Seawater table or deep rubber bins for holding bags

METHOD

1. Create an Excel sheet to record seed batch growth and survival with following fields:
   - Date transferred at sea
   - Site and Longline
   - Mesh size of spat bag and/or net from which sample is taken
   - Date collected
   - Label of vertical spat bag line or net
   - For each vertical line or net record data from each depth, for e.g. in a 10-tiered net:
     - › Layer 1: no. of live scallops/no. of dead scallops
     - › Layer 1: shell height (mm) for 50 scallops
     - › Layer 5: no. of live scallops/no. of dead scallops
     - › Layer 5: shell height (mm) for 50 scallops
     - › Layer 10: no. of live scallops/no. of dead scallops
     - › Layer 10: shell height (mm) for 50 scallops

At the farm site

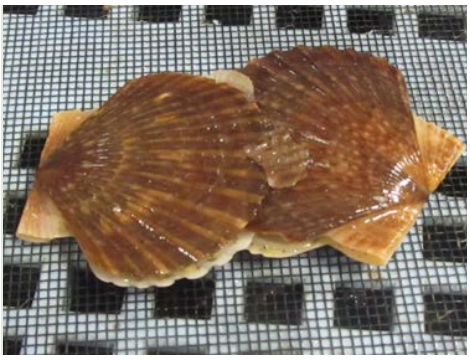
2. Collect a minimum of two vertical spat bag lines (remote setting) or two nets (lantern or pearl) representing each spat batch transferred to sea for sampling.
3. Label vertical spat bag lines and nets as follows:
   - batch number
   - date transferred
   - initial stocking density
4. From each vertical line, collect and label bags or nets from 2 or 3 different depths to reflect any changes in seawater chemistry within the water column. For e.g. if using a 10 or 15-tiered lantern net, sample top, middle and bottom layers.
5. Wash all of spat from bag or net into a dedicated small tray, keeping each sample separate. Label.
6. Count live and dead scallops for each sample. Record live and dead.
7. Record site, number of seed per bag and per layer.
8. Pool all seed from same batch collected for all layers into one tray.
9. Measure shell height of 50 seed using Vernier calipers (see photo).



Measuring seed with Vernier calipers

8.3.6 PROTOCOL (continued)

10. If there is no time to measure onboard, fix sample with formalin for later analysis.
11. Record any wear and tear of nets/bags, fouling, predation by crabs or other organisms and clamping (see photo).
12. Re-distribute seed in new net, keeping the same label; see Table 8.1 for stocking densities based on size and Protocol 8.2.2 for procedure on "Distributing spat in new bags or nets".
13. Return bag lines or nets to longline.
14. Repeat for two other bags or nets of same batch for triplicate measurements of growth and survival.
15. Do vertical water quality profile using YSI Pro meter (from 1m below surface to 1 m off the bottom).



Juvenile scallops clamping

In the office

16. Enter all data in Excel sheet.
17. Calculate mean and standard deviation (SD) of shell height for each sample.
18. Calculate survival rate from previous monitoring.
19. Record all observations on state of culture.

Further readings

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Hatchery culture of bivalves – A practical manual

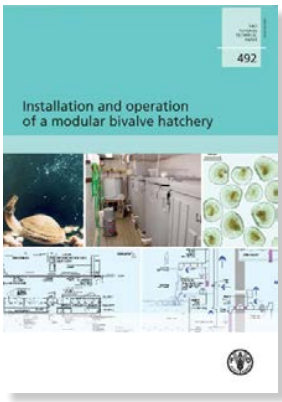
Michael M. Helm, M.M., Bourne, N. & Lovatelli, A.
FAO Fisheries Technical Paper 471, Rome, FAO, 2004

This manual describes the various aspects of bivalve hatchery culture and production from acquisition of broodstock to the stage at which the seed are of sufficient size to transfer to sea-based grow-out. Focus is on intensive methodology in purpose-built hatchery facilities rather than on more extensive methods of seed production in land-based pond systems. For a complete view, the intermediate nursery phase of production, which is the interface between the hatchery and sea-based growout, and the concept of remote setting are also described and discussed in some depth.

This manual provides the reader with a practical insight as to what is required in the way of resources and details of how to handle and manage the various life history stages of bivalves in the hatchery production cycle. Examples are largely drawn from the more commonly cultured temperate climate species including the Pacific oyster, *Crassostrea gigas*, the American (Eastern) oyster, *Crassostrea virginica*, the European flat oyster, *Ostrea edulis*, the Manila clam, *Tapes philippinarum* and a range of scallop species. Methods described are equally as applicable to bivalves of lesser significance in terms of worldwide production.

In addition to explanations of culture technology and methodology, the manual includes a brief discussion of the processes of identifying a suitable site for locating a hatchery and considerations in planning and designing the hatchery. It also includes advances that are likely to improve the reliability and economic viability of the hatchery industry in the near future, featuring topics such as polyploidy, the development of selected strains, cryopreservation of gametes and the need for novel, non-living foods.

Link English version: www.fao.org/3/a-y5720e.pdf
Link Spanish version: www.fao.org/3/a-y5720s.pdf



Installation and operation of a modular bivalve hatchery

Sarkis, S. & Lovatelli, A.
FAO Fisheries Technical Paper 492, Rome, FAO, 2007

Limiting factors such as minimal capital investment, lack of technical support or expertise and available physical space may put severe restrictions on setting up a hatchery. Not all investors have the means or the will to take the risk to support a large commercial aquaculture operation without substantial proof of its production capacity. For these reasons, the setup of an inexpensive modular hatchery may be a simpler option to the start-up of a large commercial operation, or may be sufficient to the needs of a smaller operation. This manual was written for those interested in establishing a bivalve hatchery, with minimal experience in this activity, limited technical support and restricted access to information. The manual stands as an entity, providing not only the technicalities of setting up and operating a hatchery, but also makes some of the scientific background, deemed useful to the aquaculturist, readily accessible. The manual is divided into chapters for each stage of rearing: broodstock conditioning, algal culture, hatchery, nursery, grow-out and economic considerations. The first five chapters include both the physical requirements and culture considerations and procedures for the relevant rearing stage. The final chapter on economic considerations provides an insight into the labour involved for each stage of production, along with a list of equipment and supplies, which may be used as a template for a new installation.

Link English version: www.fao.org/3/a0797e/a0797e.pdf

Glossary

Adductor muscle	Large muscle near centre of scallop that pulls the two valves together.
Algae	Aquatic plants that reproduce by cell division or spores.
Anterior	Front or head.
Banjo filters	In hatchery terminology, a ring meshed on both sides affixed to the outflow of a tank preventing larval loss through drain.
Bivalve	Mollusc of the Class Pelecypoda, having a shell of two valves that are joined by a hinge.
Byssus	Thread-like filaments used by bivalves to attach themselves to a substrate.
Diatom	A single-celled algae of the Class Bacillariophyceae; cells are enclosed in a siliceous shell called a frustule, cells can form chains.
Dioecious	male and female reproductive organs in separate individuals.
D-larva	The early veliger larval stage of bivalves, also known as straight hinge larva.
Downwelling	In hatchery terminology, a growing system in which the flow of water enters at the top of a spat holding container (compare with upwelling).
Embryo	Organism in early stages of development; in bivalves, prior to larval stage.
Eye spot	Simple organ that develops near centre of mature larvae of some bivalves and is sensitive to light.
Fertilization	Union of egg and sperm.
Flagellate	Group of single-celled algae characterized by having a locomotory organ called a flagellum.
Gamete	Mature, haploid, functional sex cell capable of uniting with the alternate sex cell to form a zygote.
Gametogenesis	Process by which eggs and sperm are produced.
Gastrula	The embryonic stage of development consisting of two layers of cells enclosing a sac-like central cavity with a pore at one end.
Gill	A leaf-like appendage that functions in respiration and filtration of food from water.
Gonadal Index	In this case the relationship of gonad weight to shell weight, reflecting gonad growth or depletion.
Gonads	The primary sexual organ: testis producing sperm or ovary producing eggs.
Grow-out	The process of growing seed to market size.
Inoculum	Culture of an organism (alga, rotifer), which is used as a starting point for another culture.
Larva	A stage of bivalves from the embryo to metamorphosis.
Mantle	The soft fold enclosing the body of a bivalve that secretes the shell.
Meiotic division	Process in which normal number of chromosomes (2n) is reduced to the haploid (n) number.

Metamorphosis	In bivalves, the period of transformation from the larval to the juvenile stage.
Microalgae	Small cell-size algae, either single celled or chain forming diatoms, cultured as foods for larvae and spat in a hatchery.
Muscle Index	In this case, the relationship of muscle weight to shell weight, reflecting muscle growth or depletion.
Oocyte	Cell, which develops into an ovum.
Pediveliger	Larval stage of molluscs that still has the swimming ciliated organ (velum) and sensitive foot needed for settlement and attachment.
pH	A measure of acidity.
Polar body	Minute cells released during meiotic division of the egg after the sperm has penetrated the egg; contains excess chromosomal material to produce a haploid egg.
Posterior	The rear, away from the head.
Prodissoconch	Bivalved shell formed by larva prior to metamorphosis. It may be possible to distinguish an earlier, smaller prodissoconch-I from a later, larger prodissoconch-II that encloses the entire animal.
Pseudofaeces	False faeces, waste material not taken into the digestive tract.
Seed	A young scallop with no specific definition to size.
Settlement	Behavioural process when mature bivalve larvae seek a suitable substrate for attachment.
Shell height	In scallops, the straight-line distance measured perpendicularly from the umbo to the ventral margin of the shell.
Shell length	In scallops, the straight-line distance from the anterior to the posterior margins of the shell.
Spat	A newly settled or attached bivalve (also termed post larval or juvenile in bivalves).
Spawning	Release of ova, fertilized or to be fertilized.
Trochophore	The first free-swimming planktonic stage of a mollusc larvae or bivalve embryo.
Umbo	Beak-like projections at the dorsal part of the shell; it is the oldest part of a bivalve shell (also called the umbone).
Upwelling	In hatchery terminology, a growing system in which a flow of water is induced through the base of a spat holding container (compare with downwelling).
Veliger larva	The larval stage of most molluscs, characterized by the presence of a velum.
Velum	Ciliated locomotory organ of the larva.
Ventral	Pertaining to the under or lower side of an animal.
Vitellogenesis	Formation of the yolk of an egg.

Appendices

Appendix I – **Datasheet sample for gonadic and muscle indices**

Site: .....

Date: .....

Rest = remaining soft tissues (less gonad and muscle)

GI = Gonad wet weight/shell dry weight × 100

MI = Muscle wet weight/shell dry weight × 100

Scallop #	Sex (M/F)	Total wet weight (g)	Gonad wet weight (g)	Muscle wet weight (g)	Rest (g)	Total soft tissue weight (g)	Shell dry weight (g)	Shell height (mm)	GI Wet	MI Wet

Notes: _____

Appendix II – **Broodstock feeding record**

Month:					
Date	**Time**	**Algae (total litres)**	**Shellfish Diet**	**AlgaMac**	**Operator initials**

Notes:

Appendix III – **Preparing measuring scoops for thinning**

1. Measure 30 spat from a subsample of each size fraction previously graded (Protocol 8.2.1).
2. Estimate the weight based on height, using the height:weight graph.
3. Check new stocking density (see Table 8.1).
4. Weigh spat for required new stocking density. Note: this is only done once for each size fraction.
5. Collect in a measuring scoop of appropriate size or mark the level to which scoop needs to be filled.
6. For larger seed, use a plastic cup and mark the level filled by spat as in photo.
7. Repeat steps 1–6 each time spat growth is monitored.
8. Label scoops and cups for each size fraction; use in nursery or on the boat during future thinning such that weighing in Step 2 is no longer needed.

Example as per steps above:

- Average shell height of spat = 1.5 mm
- Weight of 1.5 mm spat averages 0.5 mg per spat (see Figure 7.7)
- New stocking density = 400 spat per bag (see Table 8.1)
- Weight of 400 spat = 400 × 0.5 = 200 mg
- Weigh 200 mg of spat
- Pour 200 mg of spat into most suitable size container. Spat should fill the scoop or cup; if not, mark the level on container showing for 200 mg

Notes and observations

Scallop culture at a commercial scale lags behind that of clams and oysters in terms of number of operations and production volumes. The main reasons revolve around the natural free-swimming seabed bottom preference of scallops, their requirement for low-density culture, their sensitivity to seawater chemistry parameters and their short shelf life when sold live. These limiting factors translate into aquaculture challenges especially in large-scale farming or "grow-out" of market-size animals because of physical space demand, labour and cost. The Japanese scallop is one of the most attractive scallop culture candidate and offers several advantages; it generates a live and processed product, yields one of the largest scallop muscles highly prized as both a food and export product and more importantly, it attains market size in suspended cultures. The latter enables large-scale farming and harvesting, eliminates SCUBA-based labour as for bottom cultures, and allows for seed monitoring and harvesting of market-size scallops from a boat or raft. This manual describes well-tested hatchery-based techniques implemented at large-scale for all stages of seed production. It provides the potential to extend the hatchery cycle with a broodstock conditioning strategy, to rear and set pediveligers using different methodologies, to produce up to 10 mm seed on a large-scale in a land-based nursery and to achieve a 100 percent survival and growth for seed transported up to 9 hours to farm sites. This is a roadmap to the technical success of a commercial operation of the Japanese scallop. The financial sustainability of the operation will depend on a well-developed strategy, a sound business plan, a realistic target for production and on its management.

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