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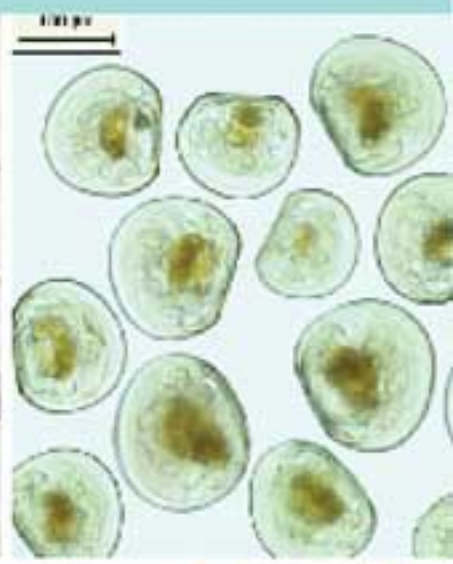
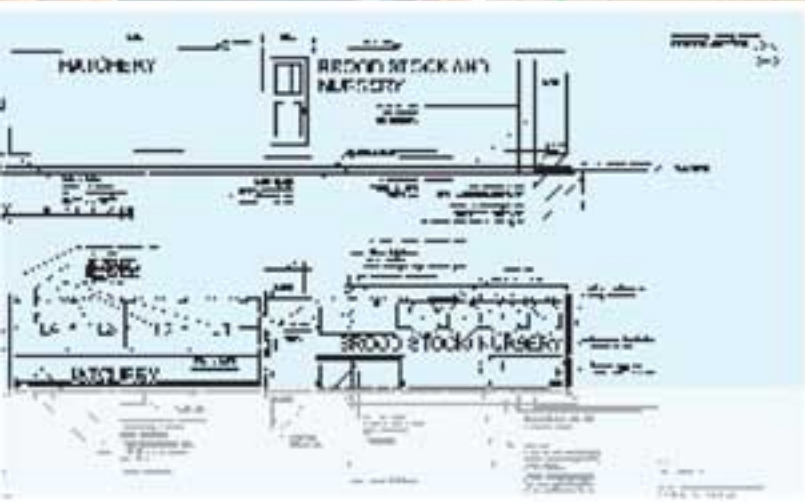
Installation and operation of a modular bivalve hatchery





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Cover photographs and drawings:
Clockwise from top left: A spawning female Manila clam (courtesy of Brian Edwards);
interior of a small bivalve hatchery; photomicrograph of six-day old sand scallop (*Euvola ziczac*) larvae;
technical layout plan of a modular bivalve hatchery; technical detail of a semi-recirculating
nursery raceway system (drawings by Souhaila Sarkis).

Installation and operation of a modular bivalve hatchery

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Preparation of this document

This manual is part of the publication programme of the Aquaculture Management and Conservation Service of the Fisheries and Aquaculture Department of the Food and Agriculture Organization of the United Nations (FAO) and complements the FAO Fisheries Technical Paper No. 471 entitled "Hatchery culture of bivalves: a practical manual" published in 2004.

This manual was written for those interested in establishing an aquaculture operation, with minimal experience in this activity, limited technical support and restricted access to information. It stems directly from one of the author's experience and differs from other manuals by its precision and amount of detail, going one step further in providing a practical template in the building of a hatchery which can be directly copied or modified if needed. Because it focuses on developing aquaculture in resource-limited areas, the manual has been written with the assumption that the user has little, if any, information sources. For this reason, 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 interest in producing this technical manual was generated by the efficiency of the modular hatchery developed and tested over the course of 4 years at the Bermuda Biological Station for Research Inc. (BBSR) for tropical scallop culture. This facility was designed around the facts that little space was available for the building of a hatchery, the budget was small, and proof techniques needed to be developed, prior to a substantial investment. This resulted in a cost efficient, compact, portable hatchery housed in insulated containers. It is a concept, which can be adapted to any region with, as its only requirement, access to "clean" seawater. This modular hatchery may be easily expanded or modified for other bivalve species.

This manual is therefore a technical guide enabling the replication of such a modular hatchery, and also includes detailed protocols pertaining to all aspects of scallop culture, as developed in Bermuda. Protocols reported here were initially written for unskilled personnel, providing a complete and basic outline of the procedures required. However, these protocols have also proved useful throughout years of operation, as they provide an easy routine to follow for all, including more experience personnel, preventing any careless mistakes easily made throughout the course of a chaotic hatchery day!

The manual is divided into chapters, each of them focusing on a rearing phase and providing: a) Technical drawings with descriptive text; b) Scientific background on biology and culture aspects; and c) Operational and culture protocols. The manual concludes with an economic summary of the set-up of such a modular hatchery and of the labour requirements based on operation in Bermuda. Although the costs given are specific to Bermuda, a detailed equipment list is provided as an appendix, presented as a template which can be used for the calculation of region-specific costs. Other appendixes provide details on required calculations, techniques, equipment list and templates to be copied for routine/maintenance checks.

Last but not least, all those involved in the development of this manual have to be gratefully acknowledged for their dedication, hard work, long hours and enthusiasm. Dr Neil Bourne was the first to provide us with the confidence to pursue our ideal. We also thank him for his constructive criticisms of this manual. Those involved in the

aquaculture technique developments and construction of the hatchery from its infancy, were: Doerte Horsfield, Paul Farrington, Mike Helm, Andrew Cogswell and Claudia Hohn. Data for the economic summary was compiled by Charles King.

The technical drawings were prepared by Souhaila Sarkis, registered Landscape Architect. She is gratefully thanked for her thorough and accurate work. The amount of detail given is a first in the description of an aquaculture facility. For printing purposes the original drawings have been reduced and are not to scale. Scaled drawings are available as PDF files in the enclosed CD-ROM at the back of the manual allowing the reader to print any of these for ease of use and consultation (original paper size format: 11”x17”). The CD-ROM also contains PDF files of all chapters.

A final thank you to Dr Sandra Shumway and Dr LeRoy Creswell, for putting in the time to ensure that this manual is scientifically sound and clear in its presentation. Preparation of the manual has been under the overall coordination of Alessandro Lovatelli, Fishery Resources Officer (Aquaculture), FAO.

The graphic layout of the manual was prepared by J.L. Castilla Civit.

Photos are courtesy of Mike Helm, Souhaila Sarkis and Samia Sarkis, unless otherwise noted.

Abstract

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 set-up of an inexpensive modular hatchery may be a simpler option to the start-up of a large commercial operation, or maybe sufficient to the needs of a smaller operation.

This manual is intended to stand on its own, as a guide for installation and operation of a bivalve hatchery. Based on years of experience in a resource-limited region, the need for optimal space usage coupled with a restricted budget, has resulted in a modular "portable" hatchery/nursery complex housed in insulated fiberglass containers. With its only requirement being access to "clean" seawater, this model may be easily adaptable to any region. Although the described facility is compact, it is by no means an experimental laboratory, but a hatchery geared towards production. Its functionality has been repeatedly tested over a four-year period, focusing on culture of subtropical/tropical scallops. The developed procedure is suitable for commercial production, the scale of which is dependent on the tankage capacity. In other words, the modular hatchery described here may be expanded by the addition of identical modules, increasing the number of tanks available and hence production.

The described hatchery comprises basic culture facilities for the rearing of bivalve species such as a dedicated seawater system providing a continuous supply of filtered seawater, a temperature control system for seawater, larval rearing tanks for closed or flow-through systems, and flexible usage stacked raceways for spat rearing. The detailed to scale drawings provide a clear guide intended for ease of replication of the facility. An accompanied written text provides further description of the physical facility. Nonetheless, it is not the intent of this guide to dwell into engineering details, but simply to describe a system that works.

This guide also considers the operation of the hatchery, and for this reason contains simple stepwise protocols. These protocols include both maintenance of the hatchery, such as the cleaning of raceways during spat rearing, and culture procedure, as spawning induction.

The modular hatchery is designed for flexibility and may be used for a range of bivalve species and some gastropods. However, protocols given for culture techniques are based on rearing procedures of subtropical/tropical scallop species developed in the pilot hatchery. For additional support, at times necessary in more isolated regions, concise scientific information is provided on various biological aspects of bivalve reproduction and growth.

The manual is divided into chapters for each stage of rearing: broodstock conditioning, algal culture, hatchery, nursery, growout and economic considerations. Every chapter is an entity, and the first five 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.

Keywords: modular hatchery, installation, operation, culture, bivalves, technical drawings, rearing protocols.

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Installation and operation of a modular bivalve hatchery.
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Contains a CD-ROM.

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Chapter 4 – Nursery: facilities and culture of post-larvae

Chapter 5 – Growout of juveniles: transfer from nursery to field

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List of technical drawings

***** "Pg" refers to the number of the technical drawing.

Technical drawing notes and pipe conversion

Please note:

1) All valves, pipes and other fittings are of PVC (Schedule 40) unless otherwise specified in drawings.

2) The subtropical scallop culture model for hatchery and nursery facilities represents an existing complex in Bermuda, built in accordance with the Bermuda environmental regulations. Installation in other countries is subject to local federal and regional environmental regulations and building code.

3) All drawings are metric. Please refer to chart below for imperial conversions.

Nominal NPT pipe size to nominal metric size conversion chart

Nominal pipe size (Inches/U.S.)	Nominal pipe size (Metric)
1/8″	6 mm
3/16″	7 mm
1/4″	8 mm
3/8″	10 mm
1/2″	15 mm
5/8″	18 mm
3/4″	20 mm
1″	25 mm
1-1/4″	32 mm
1-1/2″	40 mm
2″	50 mm
2-1/2″	65 mm
3″	80 mm
3-1/2″	90 mm
4″	100 mm
4-1/2″	115 mm
5″	125 mm
6″	150 mm

List of appendixes

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
Anoxia	deficiency or absence of oxygen in the blood and tissues
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
Blastula	a hollow ball of cells, one of the early stages of embryonic development
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
Cilia	hair-like structures whose rhythmic beat induces a water current in bivalves
Cleavage	the series of mitotic divisions, usually occurring with no increase in cytoplasmic mass that first transforms the single-celled zygote into a multicellular blastula
Ctenidia	leaf-like appendages that function in respiration and filtration of food from water (used interchangeably with the term gills)
Cultch	material used to collect bivalve spat
Detritus	fragmented or decomposing organic material from plant and animal remains
Diatom	a single-celled algae of the Class Bacillariophyceae; cells are enclosed in a siliceous shell called a frustule, cells can form chains
D-larva	the early veliger larval stage of bivalves, also known as straight-hinge larva
Dribble spawners	in this case used for scallops which do not spawn completely, but partially over a period of time
Dorsal	the back or part of an organism away from the ground
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)
Ectometabolites	a product of metabolism
Embryo	organism in early stages of development; in bivalves, prior to larval stage
Epiphytes	animals or plants living on the surface of the seabed or other substratum
Eye spot	simple organ that develops near centre of mature larvae of some bivalves and is sensitive to light

Fecundity	the potential reproductive capacity of an organism or population expressed in the number of eggs (or offsprings) produced during each reproductive cycle
Fertilization	union of egg and sperm
Flagellate	group of single-celled algae characterized by having a locomotory organ called a flagellum
Follicle	small sac-like structure in the ovary, a group of cells surrounding the oocyte and probably concerned with its nutrition
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 (see ctendia)
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
Growout	the process of growing seed to market size
Hermaphrodite	having both male and female reproductive organs in the same individual (animal)
Hypoxia	insufficient levels of oxygen in blood or tissue (short of anoxia)
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 which 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
Ovary	the sex organ which produces the egg or eggs in a female organism
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
Plankton	floating or weakly swimming aquatic organisms, can be phytoplankton (plants) or zooplankton (animals)

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
Primary oogonia	arising from primordial germ cells during the initial (premeiotic) stage of oocyte development, and differentiates into an oocyte in the ovary
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)
Spatfall	the settling or attachment of young bivalve molluscs, which have completed their larval stages
Spawning	release of ova, fertilized or to be fertilized
Statocyst	formed by invagination of the epithelium in bivalve larvae potentially providing the ability to detect gravity
Straight-hinge larva	early part of larval stage, sometimes termed D-stage
Testis	male reproductive organ
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
Zygote	diploid cell resulting from union of male and female gametes

Abbreviations, acronyms and conversions

AFDW	Ash-Free Dry Weight
ANOVA	Analysis Of Variance
BBSR	Bermuda Biological Station for Research Inc.
C	Control
CCMP	Centre of Culture for Marine Phytoplankton
CI	Condition Index
CO_2	Carbon Dioxide
DOPA	Dihydroxyphenylalanine
DW	Dry Weight
EDTA	Ethylene Diamine Tetraacetic Acid
Fisher's PSLD	Fisher's Protected Least Significant Difference
FT	Flow-Through
GF/C	Glass Fibre with particle retention of 1.2µm
GI	Gonadal Index
HCL	Hydrochloric Acid
ID	Inner Diameter
int-ext	Interior-Exterior Connection
LNSW	Low Nutrient Seawater
MI	Muscle Index
NPT	National Pipe Thread
MNPT	Male National Pipe Thread
No	Number
OD	Outer Diameter
PLSD	Protected Least Significant Difference
pers.obs.	Personal Observation
PPT	Parts Per Thousand
PUFA	Polyunsaturated Fatty Acid
PVC	Polyvinyl Chloride
Q-water	De-ionised Water
SA	Surface Area
SCUBA	Self-Contained Underwater Breathing Apparatus
SD	Standard Deviation
TFS	Typical Filtration System
USD	United States Dollar
UV	Ultra-Violet

Not all of the following abbreviations have been used in this manual. However, they are provided as reference when reading other documents.

<	less than
>	greater than
n.a.	not analysed or not available (also written as N/A)
µm	micron
mm	millimetre
cm	centimetre
m	metre
km	kilometre

inch	inch
ft	foot
yd	yard
mi	mile
ft²	square foot
yd²	square yard
mi²	square mile
m²	square metre
ha	hectare
km²	square kilometre
cc	cubic centimetre (= ml)
m³	cubic metre
ft³	cubic foot
yd³	cubic yard
µl	microlitre
ml	millilitre (= cc)
l	litre
µg	microgram
mg	milligram (milligramme)
g	gram (gramme)
kg	kilogram (kilogramme)
mt	metric tonne (1 000 kg) (also written as tonne)
oz	ounce
lb	pound
cwt	hundredweight [value differs in UK ('Imperial') and US units - see weight conversions]
t	ton [value differs in UK ('Imperial') and US units - see weight conversions]
psi	pounds per square inch
psu	practical salinity units
gpm	('Imperial' = UK) gallons per minute
mgd	million ('Imperial' = UK) gallons per day
cfm	cubic feet per minute
ppt	parts per thousand (also written as ‰)
ppm	parts per million
ppb	parts per billion (thousand million)
min	minute
hr	hour
kWhr	kilowatt-hour

Conversions

This section of the annex should be used in conjunction with the abbreviations section. Please note that the words gallon and tonne have different values depending on whether the source of the text you are reading is 'British' or 'American' in origin.

Length:

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 m	1 000 000 µm = 1 000 mm = 100 cm = 0.001 km = 39.4 inch = 3.28 ft = 1.093 yd
1 km	1 000 m = 1 093 yd= 0.621 mi
1 inch	25.38 mm = 2.54 cm

1 ft	12 inch = 0.305 m
1 yd	3 ft = 0.914 m
1 mi	1 760 yd = 1.609 km

Weight:

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 = 0.0353 oz
1 kg	1 000 g = 2.205 lb
1 mt	1 000 kg = 1 000 000 g = 0.9842 UK t = 1.102 US t
1 oz	28.349 g
1 lb	16 oz = 453.59 g
1 UK cwt	112 lb = 50.80 kg
1 US cwt	100 lb = 45.36 kg
1 UK t	20 UK cwt = 2 240 lb
1 US t	20 US cwt = 2 000 lb
1 UK t	1.016 mt = 1.12 US t

Volume:

1 µl	0.001 ml = 0.000001 l
1 ml	0.001 l = 1 000 µl = 1 cc
1 L	1 000 000 µl = 1 000 ml = 0.220 UK gallon = 0.264 US gallon
1 m³	1 000 l = 35.315 ft^3 = 1.308 yd^3 = 219.97 UK gallons = 264.16 US gallons
1 ft³	0.02832 m^3 = 6.229 UK gallons = 28.316 l
1 UK gallon	4.546 l = 1.2009 US gallons
1 US gallon	3.785 l = 0.833 UK gallon
1 MGD	694.44 GPM = 3.157 m^3/min = 3 157 l/min

Concentration – dissolving solids in liquids:

1 %	1 g in 100 ml
1 ppt	1 g in 1 000 ml = 1 g in 1 l = 1 g/l = 0.1%
1 ppm	1 g in 1 000 000 ml = 1 g in 1 000 L = 1 mg/l = 1 µg/g
1 ppb	1 g in 1 000 000 000 ml = 1 g in 1 000 000 l = 0.001 ppm = 0.001 mg/l

Concentration – dilution of liquids in liquids:

1 %	1 ml in 100 ml
1 ppt	1 ml in 1 000 ml = 1 ml in 1 l = 1 ml/l = 0.1%
1 ppm	1 ml in 1 000 000 ml = 1 ml in 1 000 l = 1 µl/l
1 ppb	1 ml in 1 000 000 000 ml = 1 ml in 1 000 000 l = 0.001 ppm = 0.001 ml/l

Area:

1 m²	10.764 ft^2 = 1.196 yd^2
1 ha	10 000 m^2 = 100 ares = 2.471 acres
1 km²	100 ha = 0.386 mi^2
1 ft²	0.0929 m^2
1 yd²	9 ft^2 = 0.836 m^2
1 acre	4 840 yd^2 = 0.405 ha
1 mi²	640 acres = 2.59 km^2

Temperature:

°F	(9 ÷ 5 x °C) + 32
°C	(°F - 32) x 5 ÷ 9

Pressure:

1 psi	70.307 g/cm^2

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 (see concentration above) 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.

Chapter 1

Scallop broodstock: facilities, reproduction and spawning

1.1 CULTURE FACILITIES

The model hatchery custom-built for rearing of subtropical and tropical scallop species at the Bermuda Biological Station for Research Inc. (BBSR), is shown in great detail in the technical drawings (labelled Pg 1-20). This was done in order to provide a clear understanding of the requirements for such a culture operation, and facilitate building of a similar facility; thus, details of equipment utilized, pipe diameter, valves, etc. is provided. Although this facility was built for rearing scallops, it is a flexible design

making it easily adaptable to the culture of other bivalves, and even to that of certain gastropod species. It is not the intent of this guide to dwell into the engineering of such a hatchery, but to provide enough information to allow replication of this modular facility and to facilitate its costing. There are publications available which provide a wealth of information on various engineering aspects such as materials selection, seawater flow control, heating and cooling, etc.; a recommended book for answers to such questions is that of Huguenin and Colt (2002).

In the described modular hatchery, all piping materials used were made of rigid polyvinyl chloride (PVC) schedule 40, unless otherwise specified. It is one of the most common materials used in seawater culturing systems; it is biologically acceptable, as it is resistant to seawater, has a smooth surface minimizing the burrowing or fouling by marine organisms in the seawater, and does not leach any toxic compound. Schedule 40 piping has thinner walls and is sufficient for a seawater system, such as the one described here. PVC can be joined by solvent or flanging. It has been found in Bermuda that it can be prone to cracking overtime, especially when exposed to sunlight and associated higher temperatures. PVC piping is readily available, easy to work with and relatively affordable. Care must be taken when costing out PVC materials, as fittings can be significant to the overall expenses.

The modular hatchery was built in Bermuda according to environmental laws of the country. Because of its size and its strict use for filter-feeding organisms (bivalves), effluent water was low in pollutants, and discharge was not considered an issue. However, any replication of this facility in another country must be adjusted to the regulations of the country. Please note that throughout the text, the terms "excess outflow" and "effluent" are used interchangeably.

The facility was designed to be flexible in its use, and of easy maintenance. For this reason, unions are used throughout the facility before and after valves or equipment to facilitate routine cleaning and replacement of parts when required. Cleanliness of the seawater supply is primordial in rearing of the early life stages, and procedures used for maintaining various parts of the seawater system are provided as appendixes or protocols throughout this guide.

Culture facilities of the described hatchery comprise:

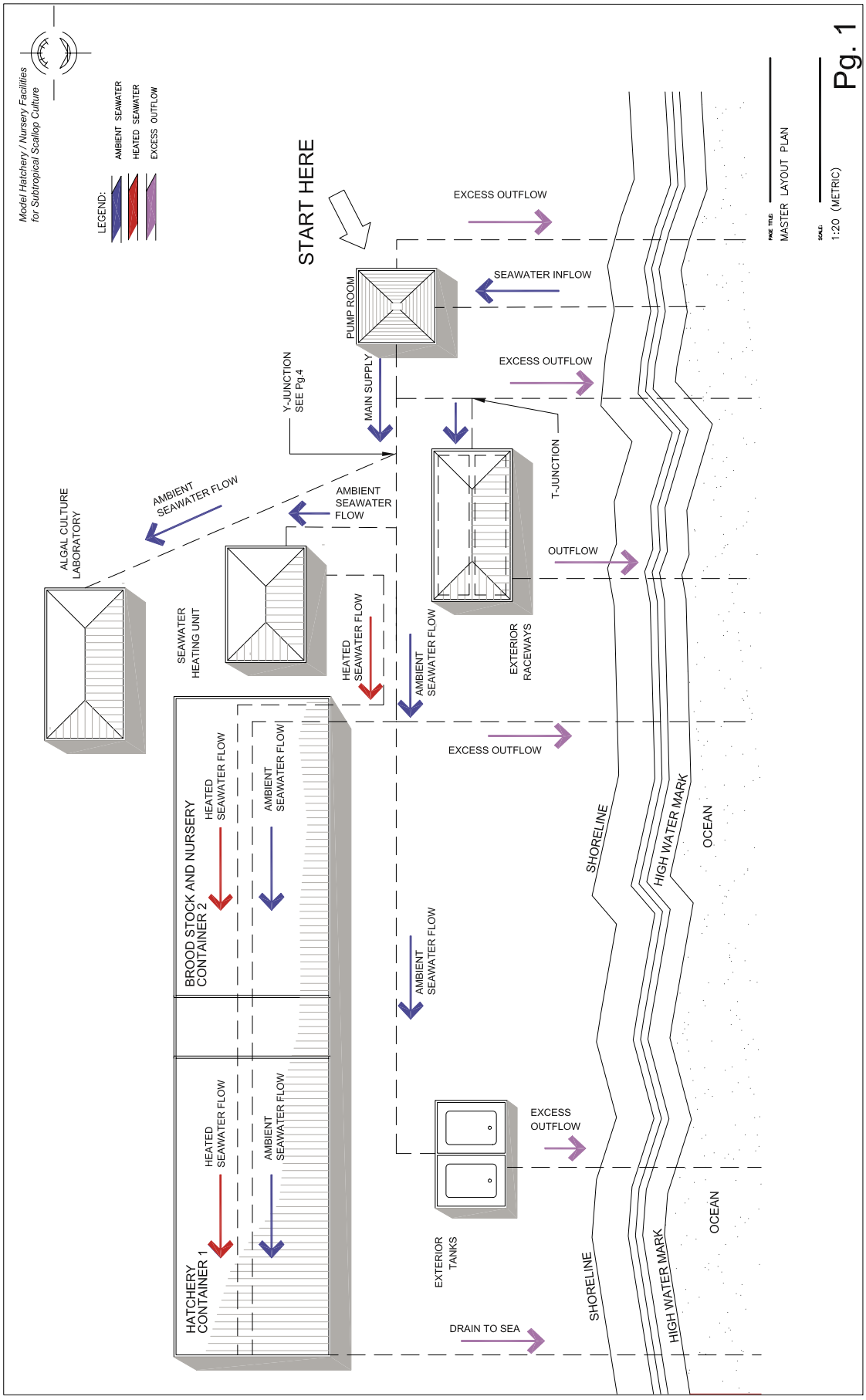
1. A dedicated seawater system providing a continuous supply of filtered seawater.
2. A temperature control system for seawater in support of broodstock conditioning and requirements for larval and juvenile rearing.
3. 1 000 litres insulated, polyethylene tanks for standard larval rearing in closed systems.
4. Flexible usage 450 litres round tanks, and stacked raceways for setting pediveligers and growing spat. Raceways may be connected either as, a single open-flow, a semi-recirculating system, two independent troughs, or holding tanks for a series of small, independent aquaria.

1.1.1 Master layout plan

Refer to Technical Drawing – page 1. The entire facility is comprised of a hatchery/nursery/broodstock complex housed in two fiberglass insulated air-conditioned containers, a separate container for algal cultures, a heating unit housed in a wooden shed providing heated seawater to the hatchery/nursery facility, and an exterior tank system providing additional space for maintenance of broodstock and spat. The pump unit located on the shoreline pumps in raw seawater and supplies the entire facility.

Technical drawing, Pg. 1

Master layout plan



General seawater flow is shown, including main ambient supply, heated seawater supply, and outflow. For ease of understanding, these are colour coded blue for ambient seawater, red for heated and purple for outflow. This colour code remains the same for all the technical drawings. Raw seawater is pumped from a 5 m depth and supplied via a main line to the hatchery complex; this was the maximum depth from which it could be pumped at this location, and is not a guideline for optimal pumping. Seawater is diverted from the main line to various pathways through T-junctions and regulated by one-way ball valves. Branching off from the main line, are supply lines to the exterior raceways, the heating unit, a Y-junction to the algal culture container, to the hatchery complex, and finally to the exterior holding tanks. A number of pathways are provided to allow excess water to flow out of the system back to sea; the first is found at the pump room. (Note: Throughout most of the operation, seawater supply exceeds the demand and excess water pumped in is sent to drain). Other outflow pathways are provided for the exterior raceway system, the hatchery complex, and the exterior tanks. These pathways also enable the regulation of water flow to various tanks, by altering the volume of water retained within the supply lines.

All tank systems receive ambient seawater from the pump room. The hatchery complex also receives temperature-controlled seawater from the heating unit via a second pipeline.

1.1.2 Seawater supply

The seawater system includes a primary pumping system with a main intake line (see technical drawing – page 2), a secondary pump linked to the main intake line (see technical drawing – page 3), and a secondary pumping system with a second smaller intake line linked to the secondary pump (see technical drawing – page 3). The pump house is housed within a 4 m^2 stone building with ventilation and a cement floor.

1.1.2.1 Primary seawater supply

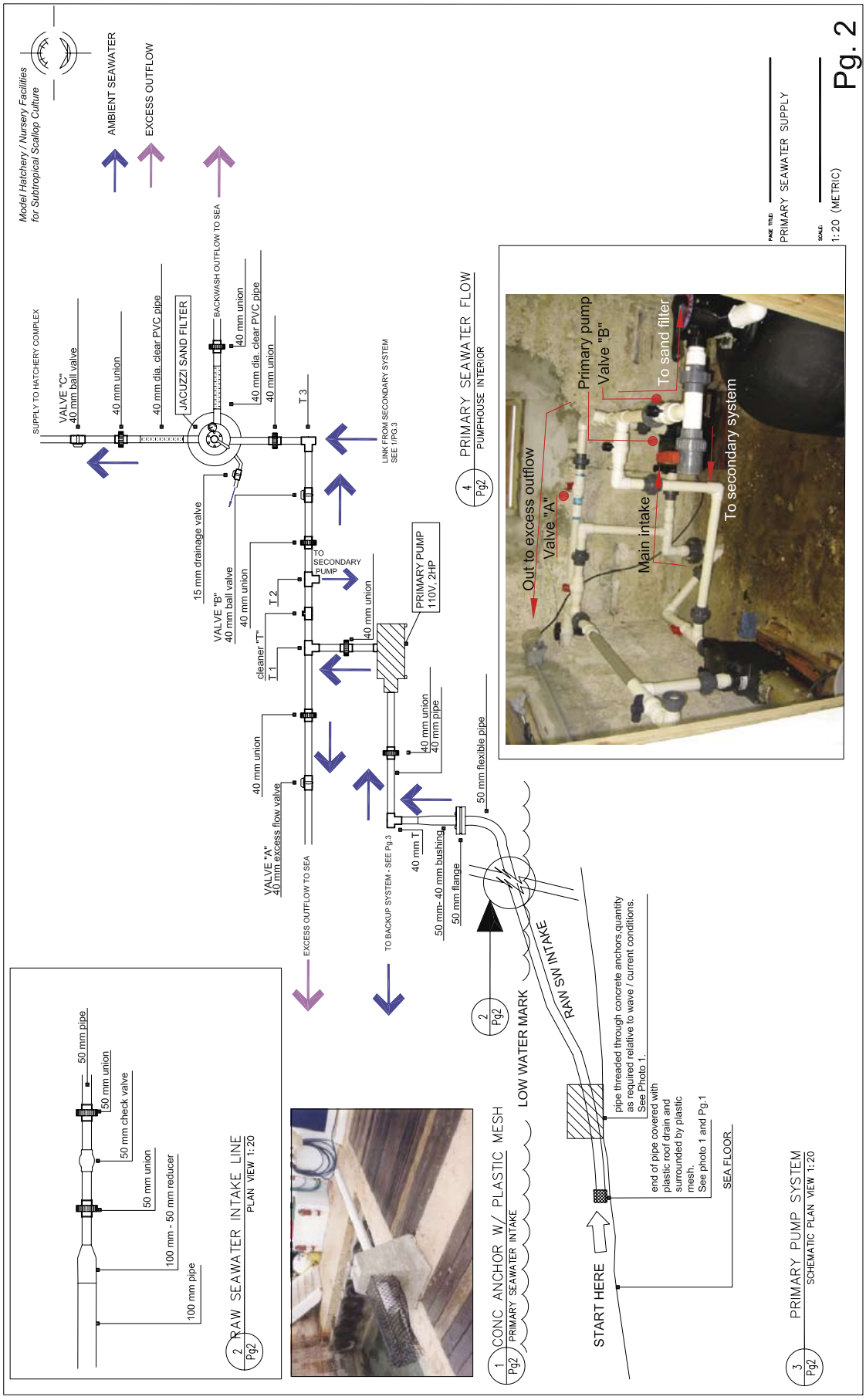
Refer to Technical Drawing – page 2. The main seawater intake is located at approximately 5 m depth. A 100 mm pipe is maintained in place by a 1 m high concrete anchor (1 m^2 base, tapering to 0.1 m^2 at top) (see technical drawing photo – 1/Pg2). The seawater intake is therefore approximately 1 m off the sea bottom; in areas where depth is greater, it is recommended to install the intake higher above the sea surface to avoid additional re-suspended sediments. A plastic roof drain is screwed into the end of the intake to prevent large objects and animals from being sucked in. In order to further ensure that the seawater pipe remains unclogged, a screen, made of 25 mm mesh, is tied with plastic cable around the intake. This mesh is changed once a year, prior to the beginning of the hatchery season.

The 100 mm intake line is weighted above the sea bottom with concrete blocks bridging the pipe. It is made up of 6.5 m pipe lengths glued together. Flange connections located every 30 m allow for dismantling of the pipe in case of blockages. Diagram 2/Pg2 (see technical drawing – page 2) shows details of the intake at the low tide mark, prior to connection to the pump in the pump house. The 100 mm pipe is reduced to a 50 mm diameter pipe, where a 50 mm clear check valve is installed to stop any loss of water in the pump in the event of a power blackout. Unions on either side of the check valve, allow for routine cleaning or changing of the valve. A flexible 50 mm pipe leads to a 50 mm flange for connection to the pump.

As seen in Diagram 3/Pg2 (see technical drawing – page 2), in the pump house, a 50 mm to 40 mm bushing reduces the diameter of the intake, to fit a 40 mm union connecting to the main pump. Seawater is pumped continuously at a flow of about 200 litres per minute from the primary centrifugal pump (Sweetwater PS-6 2 hp). Pumped water

Technical drawing, Pg. 2

Primary seawater supply



is passed through the first T-junction (T1) diverting the water either through the effluent (excess outflow) pipe to the sea, or towards the sand filter (Jacuzzi Sand Filter JF4) for removal of coarse material. A cleaner T fitting (T connection with threaded cap) provides the ability to clean the entire pipe system on a routine basis, by adding chlorine. A second T-junction (T2) links this main intake system to the secondary pump. Direction of flow is regulated by one-way ball valves (Valve B to sand filter, Valve A to excess flow). Unions installed before valves facilitate replacement and maintenance of system. Prior to the sand filter, a third T-junction (T3) links the secondary pump to the sand filter. Photo 4/Pg2 illustrates the system described.

The sand filter is set up for daily backwash; Appendix 6 provides detailed backwash procedure conducted every morning prior to any other routine operations, along with a sample check sheet. A 40 mm clear pipe aids in monitoring the clarity of the water supplied to the hatchery complex. Seawater supply from the sand filter to the hatchery complex is monitored by a one-way ball valve (Valve C) (see technical drawing – 2/Pg3).

The entire seawater pumping system, including the sub-surface pipeline is cleaned once a year, at the end of the hatchery/nursery season, in order to control marine biofouling. Some sites may have higher biofouling rates and may require more frequent cleaning. Huguenin and Colt (2002) outline various methods for controlling biofouling. The procedure used at the Bermuda hatchery is outlined in Appendix 5. At this time, complete drainage of the sand filter is done, and usually new sand is purchased for the following season. Cleaning of all parts, including the pipeline is done in Bermuda using commercially available chlorine (or bleach). The use of "pigs" is being considered; these are contractible bullet-shaped plastic parts, which are launched through a clean-out and pushed though the piping under pressure (<100 psi). These "pigs" clean the pipes by friction.

1.1.2.2 Secondary seawater supply – link to main intake line

Refer to Technical Drawing – page 3. In the event of a primary pump failure, the secondary pump may be utilized for supplying seawater to the hatchery complex by pumping water from the main intake line. As seen in Diagram 1/Pg3, this can be done by diverting water from T2 toward the secondary pump (Hayward self-priming centrifugal pump 1.5 hp), by opening Valve C and closing Valve B going through junction T4 (T4 allows the inflow of water to come from the main intake line or from the secondary line, see paragraph below). After passing through the secondary pump, water is passed through junction T5, and links either to the sand filter for supply to the hatchery complex, or to the excess outflow pipe. As a further option, junction T6 also provides a more direct route from the main intake line to the secondary pump, bypassing the primary pump. The pumped water then flows back towards the sand filter via T4. Photo 3/Pg3 illustrates the seawater flow for the secondary system.

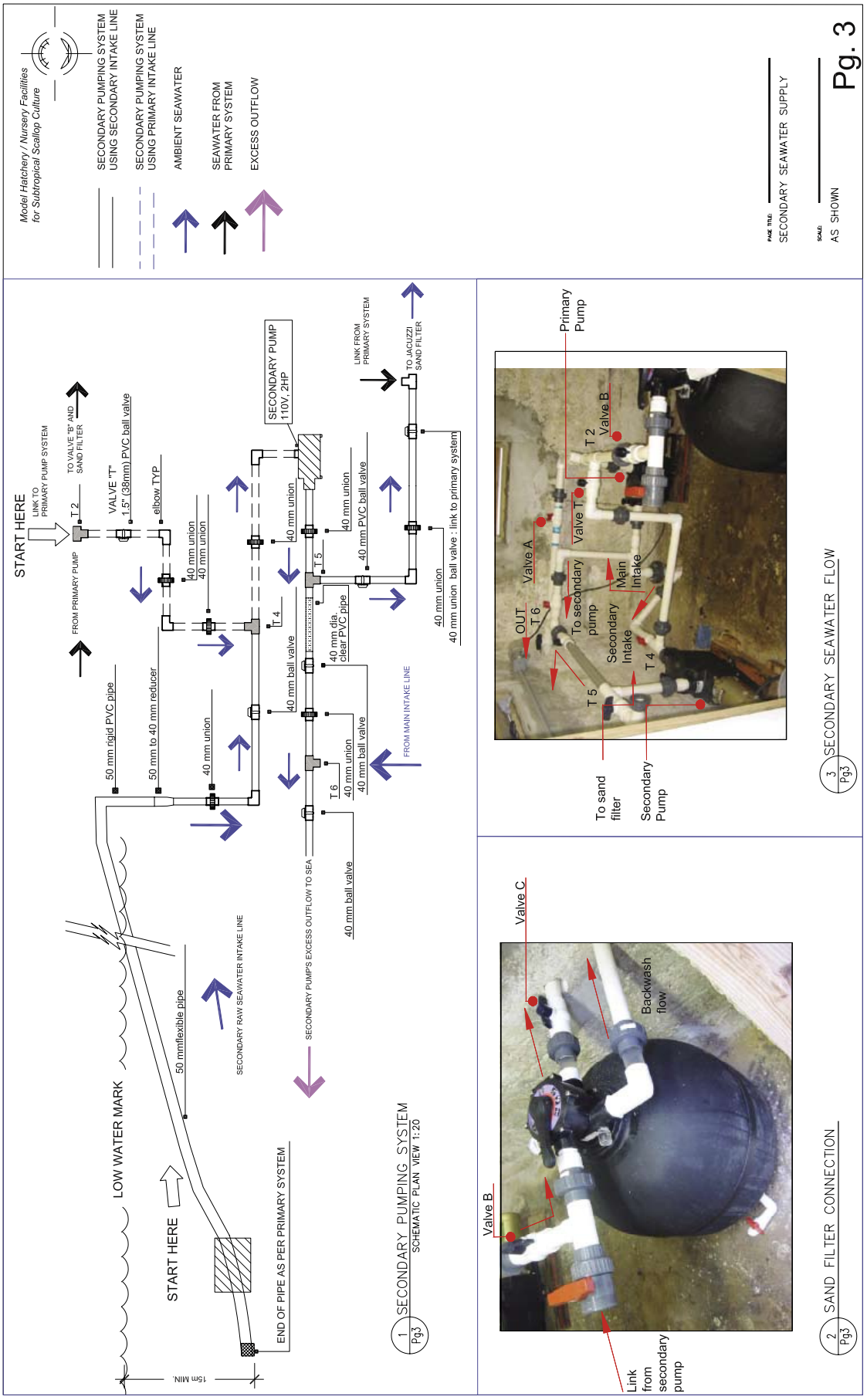
1.1.2.3 Secondary seawater supply – link to secondary intake line

Refer to Technical Drawing – page 3. A 50 mm line pumping water from a 1.5 m depth is installed as a secondary line; again this was the maximum depth of this location and is not a guide for optimal pumping depth. Ideally, the secondary line should parallel the main intake line. This was not possible in this case; hence a smaller line was set up in the event of clogging or other emergency rendering the use of the main intake line difficult. In practicality, during four years of operation, this line was used only to facilitate priming of the main pump; nonetheless, it is advised to have a back-up system.

This secondary intake line is connected to the secondary pump through a 40 mm pipe (Diagram 1/Pg3 and Photo 3/Pg3). At T4, water is directed towards the secondary

Technical drawing, Pg. 3

Secondary seawater supply



pump, flows through junction T5 and passed through the sand filter for supply. Any excess water is returned to sea through the excess outflow pipe connected at T5.

1.1.2.4 Main seawater supply to hatchery complex

Refer to Technical Drawing – page 4. The plan view on Diagram 1/Pg4 shows the main supply line to the entire facility via a 50 mm pipe, starting at the pump house and fixed alongside a land path leading to the complex. The supply line is made up of 6.5 m lengths segments connected using 50 mm straight, 45 °, or flexible rubber couplings, depending on the terrain. Drain valves are at low points in the supply line to ensure complete drainage of the line during cleaning. A clean-out Y-junction is installed at the beginning of the line after the sand filter, for chlorination purposes (see Appendix 5). All seawater passing through the main supply line is coarsely filtered through the sand filter, and is at ambient temperature. Any further filtration or temperature control of the seawater is conducted at specific points in the system when needed.

For ease of reading, water lines are colour coded – ambient supply lines in blue, and effluent (or excess outflow) in purple. At junction T7 sand filtered seawater is diverted to the outdoor raceway. This secondary line is also used for draining the entire line; for this reason, a one-way ball valve is installed immediately after T7, followed by a T-junction providing direct flow to the exterior raceway. One-way ball valves on either side of the T-junction control the flow rate. Excess seawater is sent to sea through the excess outflow pipe line.

Returning to the main supply line, the second main junction is a Y-junction providing seawater to the algal container. In this case, the algal container is located uphill of the hatchery complex. This provides a future option to gravity feed algae to the broodstock and spat. This option is not shown here. Seawater supply to the algal container is regulated by Valve Y. The Y-junction reduces the 50 mm supply line to a 40 mm line and connects to a typical filtration system installed on the exterior wall of the algal container. *Note: Details of the filtration system are provided in 1/Pg5A (see technical drawing – page 5A).* Algal supply line is drained by gravity through a reduced 25 mm pipe, and controlled by Valve Z. As this drain is used only following cleaning of the algal supply pipe, only chlorinated seawater or fresh water seep into the soil.

After the Y-connection, junction T8 on the main intake line, supplies coarsely filtered seawater to the heating unit for the temperature control necessary for larval rearing.

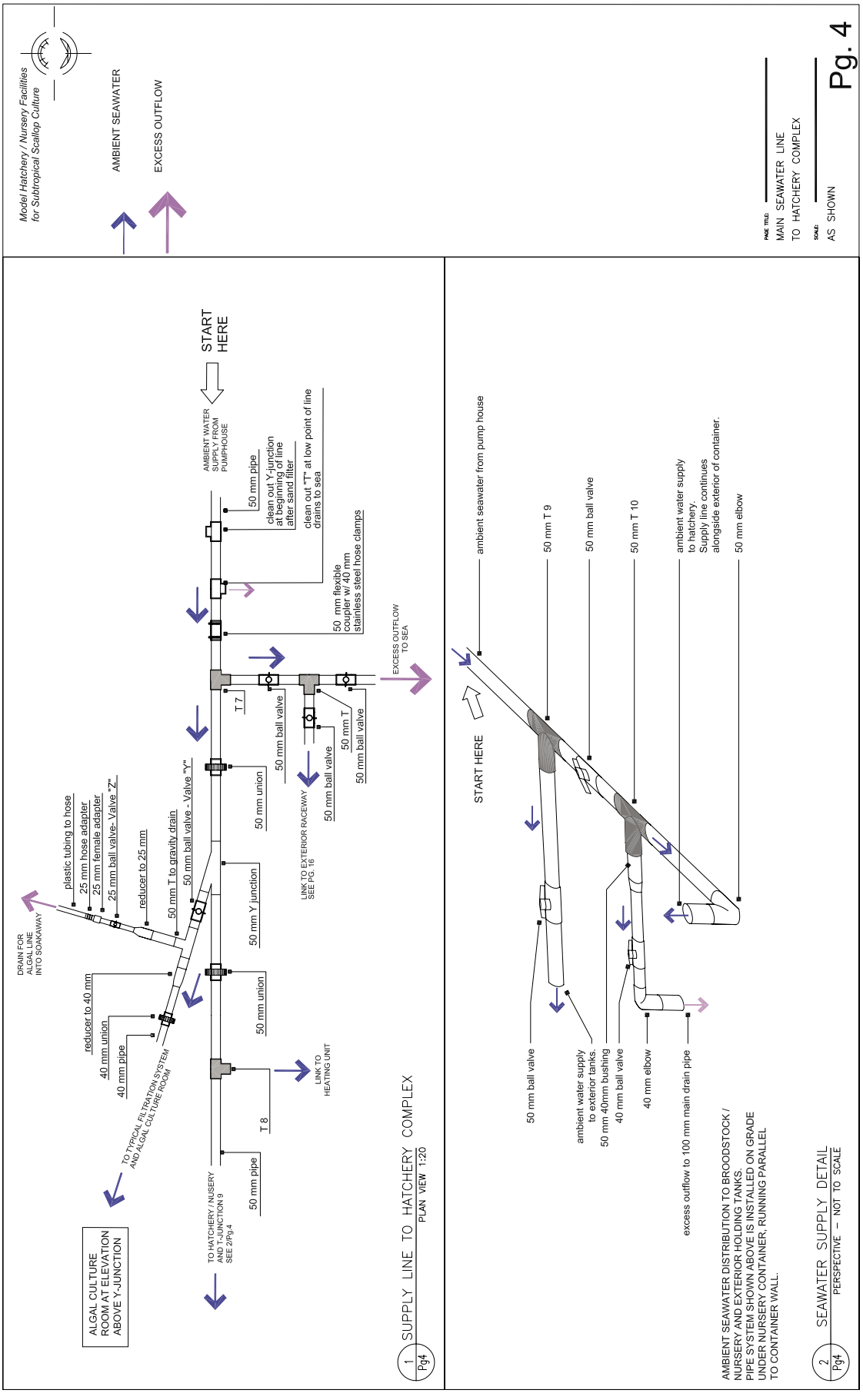
The distribution of seawater (both ambient and heated) to the hatchery complex and to the exterior tanks is shown in greater detail in the second diagram below (see technical drawing – 2/Pg 4). These junctions are located under the hatchery complex containers. Junction T9 provides ambient seawater to the exterior tanks; the flow of which is regulated by 50 mm one-way ball valves. Junction T10 is the last junction supplying ambient seawater to the hatchery complex, by a 50 mm pipe running alongside the wall of the container, and entering the complex at the top of the container, parallel to the ceiling. Flow to the hatchery is regulated inside the hatchery (see technical drawing Diagram – 1/Pg 8). The excess seawater is directed back to sea and regulated by 40 mm valve connected to T10.

1.1.3 Heating unit

Refer to Technical Drawings – pages 5A and 5B. Description of the heating unit is provided in technical drawing – page 5A; for ease of understanding, labelled photographs are given on technical drawing – page 5B. This heating unit is housed separately from the hatchery complex; mainly due to lack of space in the hatchery. A wooden shed of 2x2.6 m is used for this purpose (see Photo 1/Pg5B).

Technical drawing, Pg. 4

Primary seawater supply to hatchery complex



This unit consists of a square 1 000 litres tank, used for semi-recirculation of 1 µm filtered seawater; where, a continuous inflow of ambient seawater is heated and maintained at a set temperature through partial recirculation. Heated seawater is supplied to the hatchery on demand and is mainly required during water changes for larval rearing.

Ambient seawater, coarsely filtered by the sand filter, flows from the pump house through the 50 mm supply line (see technical drawing Diagram – 1/Pg5A). The line is reduced to 40 mm for connection to the filtration system, which is typical of that used throughout various points of the facility. A Hayward swimming pool filter (C250) removes coarser particles (to 25 µm). A 15 mm valve is fitted to the filter housing for cleaning and drainage after use. On the outflow side of the C250 filter, a reducer to 20 mm connected to two cartridge filters, 10 µm and 1 µm filters in that order. Unions before and after the coarse filter allow for replacement of parts and/or cleaning of the system when required. The inflow of 1 µm filtered seawater to the heating tank is done through a 20 mm pipe (Refer to Photo 3/Pg5B for illustration).

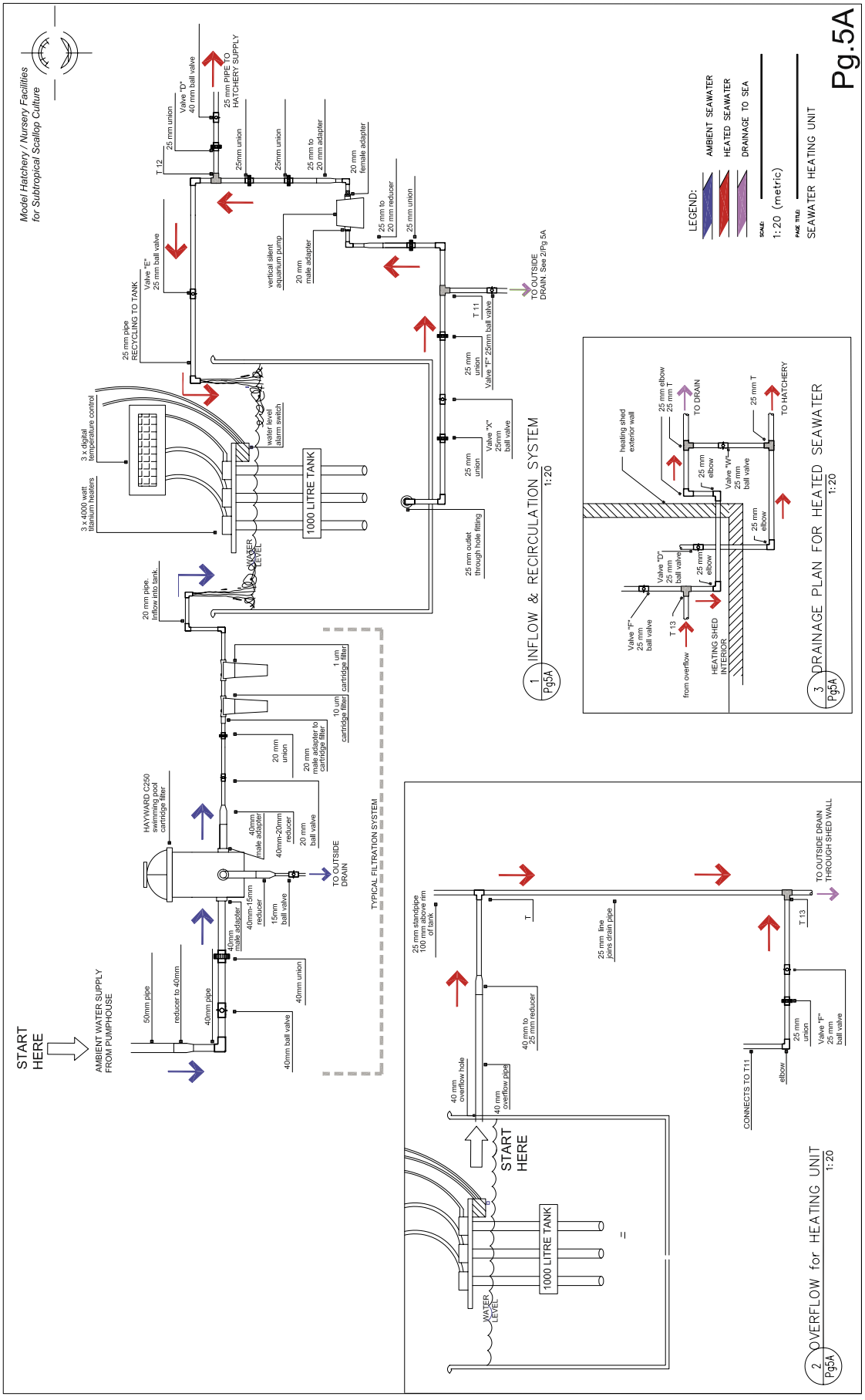
Within a 1 000 litres insulated BONAR tank are three titanium heaters (4 000 watts each) suspended so that maximum water level in the tank remains below the heads of the heaters. Each heater is connected to a digital temperature controller (one-phase), with an automatic function for turning the heater off when the desired temperature is reached. A water level alarm switch regulates the onset of the delivery pump; such that the switch closes contact and turns off the pump, when the water level drops (see Photo 2/Pg5B). This prevents the heaters from burning and the pump from running dry. The outflow is located at the base of the tank, and water flows through a 25 mm pipe; a 25 mm ball valve (Valve X) outside of the outlet controls the outflow of heated seawater. For delivery of heated seawater, Valve X is opened, distributing seawater through junction T11 prior to the pump. T11 allows for delivery of seawater to the pump when heated seawater is required or to outside drain via Valve F, when system is no longer in use. For heated seawater supply, flow is directed via T11 to a vertical delivery pump (Quiet One Centrifugal Pump), fitted with 20 mm adapters. (You may require yearly replacement of the pump and for this reason unions are fitted before and after the pump for ease of replacement). From the pump, the line is increased to 25 mm, and is passed through junction T12; this allows for partial recycling of the water to the heating tank (via Valve E), and for supply to the hatchery (via Valve D). In this way, water temperature is maintained constant through partial recirculation of heated water, and a continuous supply of heated seawater is available for spawning and larval rearing. Proper adjustment of valves D and E is critical to maintaining the balance between inflow of water-through the main supply and recycled pipe – and outflow to hatchery (refer to Photo 4/Pg5B).

A side view diagram of the tank indicates details of the overflow pipe (see technical drawing – 2/Pg5A). This is an additional security for preventing overflow of water over the sides of the tank, should the balance between inflow and outflow be disrupted. A 40 mm hole is drilled into the tank and fitted with a 40 mm pipe cemented into the tank, and reduced to 25 mm. A 100 mm standpipe is added to the top using a T-junction. The drain pipe from the overflow by-passes the heated outflow seawater pipe, and connects to a general drain pipe via T13.

Appendix 4 indicates the procedures used for setting up the heating unit, achieving the proper balance between inflow and outflow, and cleaning the system after use. Diagram 3/Pg5A (see technical drawing – page 5A) shows the heated seawater supply to the hatchery as it comes out of the heating shed, as well as the pathway for draining of the heated seawater system after use. The heating supply to hatchery is provided from

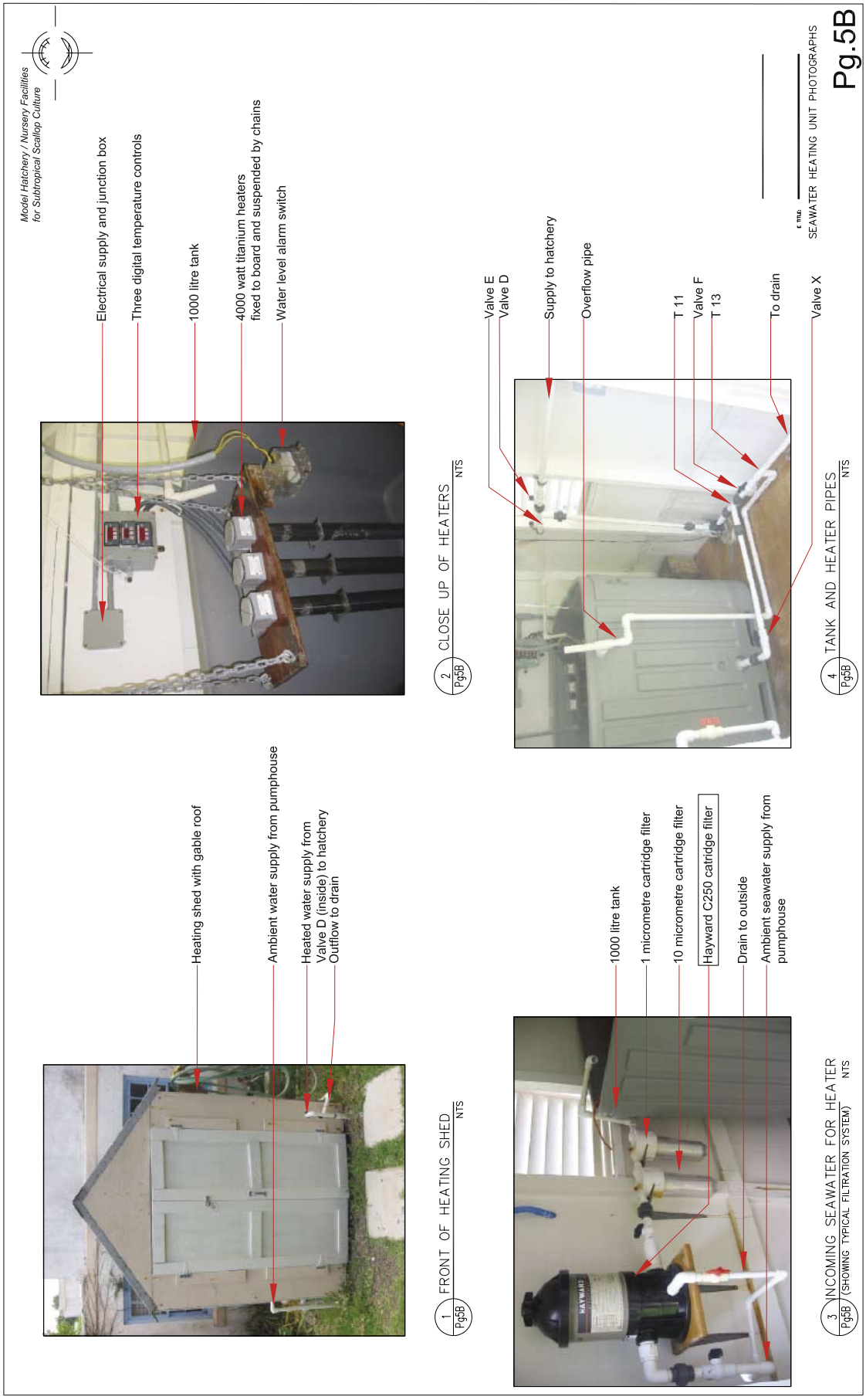
Technical drawing, Pg. 5A

Seawater heating unit



Technical drawing, Pg. 5B

Seawater heating unit: Photographs



Valve D; in-line to the hatchery is a T-junction allowing for drainage of hatchery heated seawater line after use; this is regulated by a one-way ball Valve W, which opens into a main draining pipe. This main draining pipe also collects water from the overflow of the heating tank, and from the bottom outlet (regulated by Valves X and F) when emptying of the heating tank is needed. Pipes for supply to hatchery and drain are buried in the ground.

This heating system allows for an 8 °C heating differential. It takes approximately 2 hours to fill the heating tank, and obtain the desired temperature. This design allows for the continuous supply of heated water during spawning procedures for maintenance of broodstock and gamete solutions at desired temperature, and supplies 4 000 litres of heated seawater for larval rearing.

1.1.4 Hatchery/broodstock/nursery complex

Refer to Technical Drawings – page 6A, 6B and 7.

1.1.4.1 Container layout plan

Refer to Technical Drawing – page 6A. Two reefer containers (6.5x2.5 m) are linked together to create the hatchery complex, comprising facilities for broodstock holding and conditioning, larval rearing and post-larval rearing (see technical drawing – 1/Pg6A and 2/Pg6A). Each container is installed on a concrete slab poured for each corner of the container. Containers are installed on a 2 percent slope, decreasing from the centre to the end, allowing for ease of drainage, namely during water exchanges to larval tanks; this also facilitates spraying clean the floor with a jet of freshwater to remove any debris and maintain cleanliness. Any water or debris washes under the container door into the side open gulleys installed on the outside of the containers. The floors of the containers are ribbed aluminium facilitating routine maintenance. The internal walls of the container are of glass-fibre, allowing for climate control and enabling the drilling of holes for seawater pipe entry. A small hallway is created between the two containers, closed in by doors on either side; such that the complex could be entered from either side. Large container doors are left on the end of each container facilitating movement of tanks or large equipment in and out of the containers. The total surface area of the complex is 33 m^2.

The container elevation diagram (see technical drawing – 1/Pg6A) also shows the two seawater supply lines, ambient and heated, running alongside the exterior wall of the containers, and entering the complex by an elbow through the wall parallel to the ceiling. The heated seawater line connects to the heating system drain via a T-junction for routine cleaning of the hatchery heated seawater line after use; drainage is controlled by Valve W as described on technical drawing – 3/Pg5.

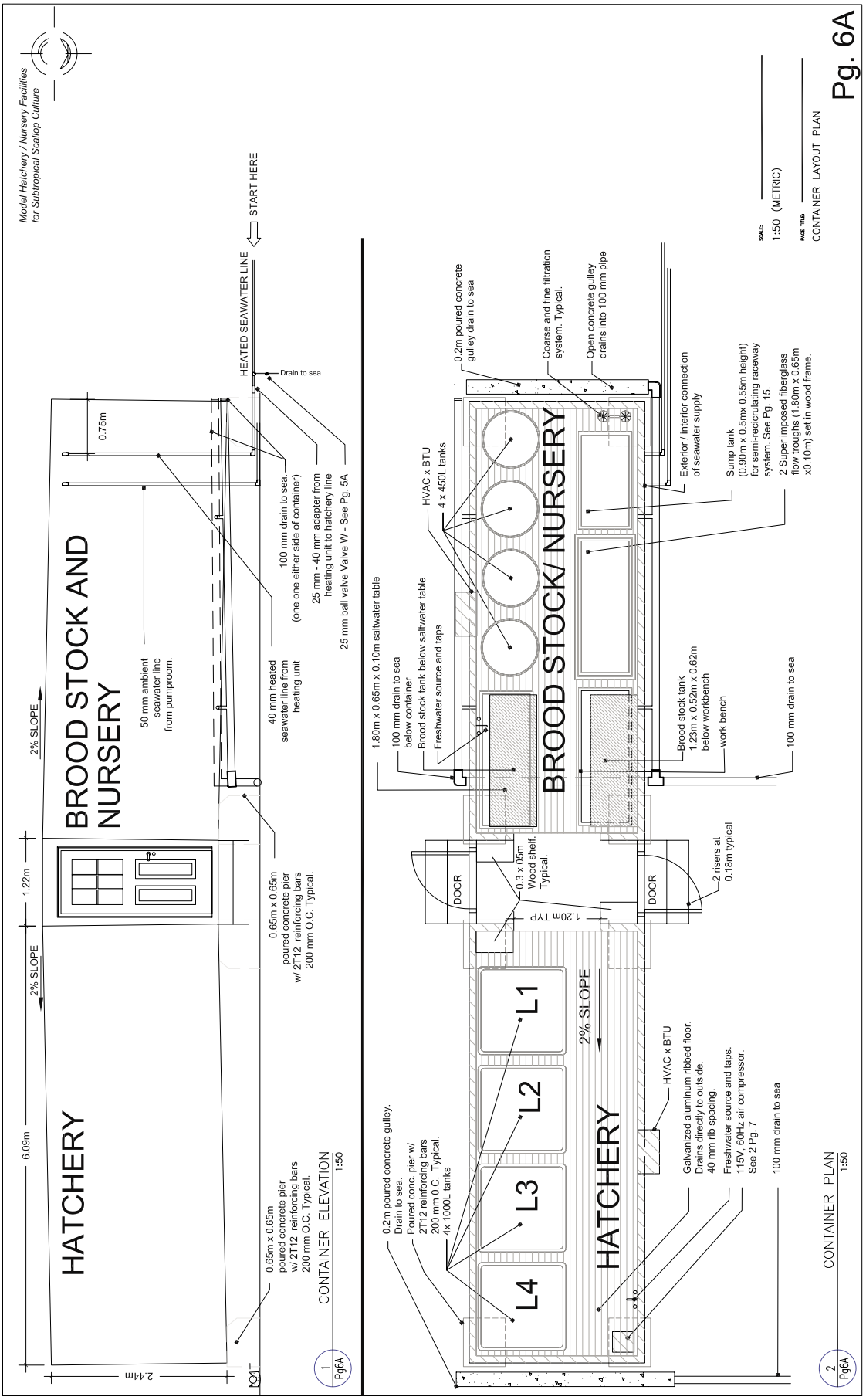
1.1.4.2 Container plan diagram

Refer to Technical Drawings – pages 6A and 6B. The overall plan of the container complex is shown separately (see technical drawing – 2/Pg6A). Photos are given on Page 6B for clarity. The left container consists of the hatchery facility with four 1 000 litres insulated BONAR tanks (see technical drawing – 2/Pg6B). Shelving is installed in the right hand corner of the container for storage of sieves used during water change. Other equipment used for hatchery purposes, such as trays, hoses and brushes are hung on the side of the container wall on hooks. One air conditioning unit in the hatchery maintains adequate climate control. Further shelving space, located in the hallway, stores spat sieves and other small equipment.

The right container houses the broodstock tanks, saltwater table, bench space, and nursery tanks and raceways (see technical drawing – 1/Pg6B). The seawater supply

Technical drawing, Pg. 6A

Container layout plan



Technical drawing, Pg. 6B

Hatchery & nursery: Photographs

lines enter the far right corner of the container and are affixed to the ceiling by plastic clips. The installation of the plumbing on the ceiling is fundamental, as not only are the pipes out of the way but safety is ensured within the facility. Both lines run down the middle of the whole complex, supplying all tanks. A typical filtration system is shown at the right hand corner of the broodstock/nursery container for filtration of ambient seawater to 1 µm, available to all tanks except broodstock tanks. A 220 litres sump tank, used in conjunction with the raceways, is located next to the filtration system (see technical drawing – Page 14 for detail). Two 200x60x15 cm deep flow-troughs are mounted on a sidewall, one above the other, acting as raceways. This system is extremely versatile; each raceway can be used independently as an open, closed, or semi-recirculating system. This allows for setting of larvae on sieves, conducting experiments in a series of independent aquaria, or using both raceways as one larger system with a total capacity of 510 litres, in, for example, the early nursery culture of spat.

Two broodstock tanks are located on the exterior of the container, one below the bench area, and the other below the saltwater table. Finally, space is available for 450 litres nursery tanks, used for the setting of larvae, and rearing prior to the transfer at sea. A second air conditioning unit is located on the side of the broodstock/nursery container to maintain climate control. The air compressor is located at the far left on a shelf, supplying air to all tanks.

Two concrete gullies are located on the exterior of the complex to deliver waste seawater from the hatchery/nursery complex to drain pipes. Main drain pipes are constructed of 100 mm perforated pipe to receive excess seawater from various sources. Two drainage pipes run alongside the broodstock/nursery container receiving outflow from the broodstock tanks, saltwater table, the round nursery tanks, and raceway system.

1.1.4.3 Hatchery and nursery ceiling plan

Refer to Technical Drawing – Page 7. The ceiling plan for both the ambient and heated seawater lines in the complex, and for the air supply are shown. The ambient filtered seawater line is coded blue; the heated seawater line is coded in red, and the airline in green. The raw seawater supply to the broodstock is dotted in blue, and bypasses the filtration system. In the hatchery, valves controlling seawater supply are also colour coded with respect to the temperature of the water – red for heated seawater and blue for ambient. This colour coding ensures clarity for all users, and minimizes any technical error which may be detrimental to larval and post-larval cultures.

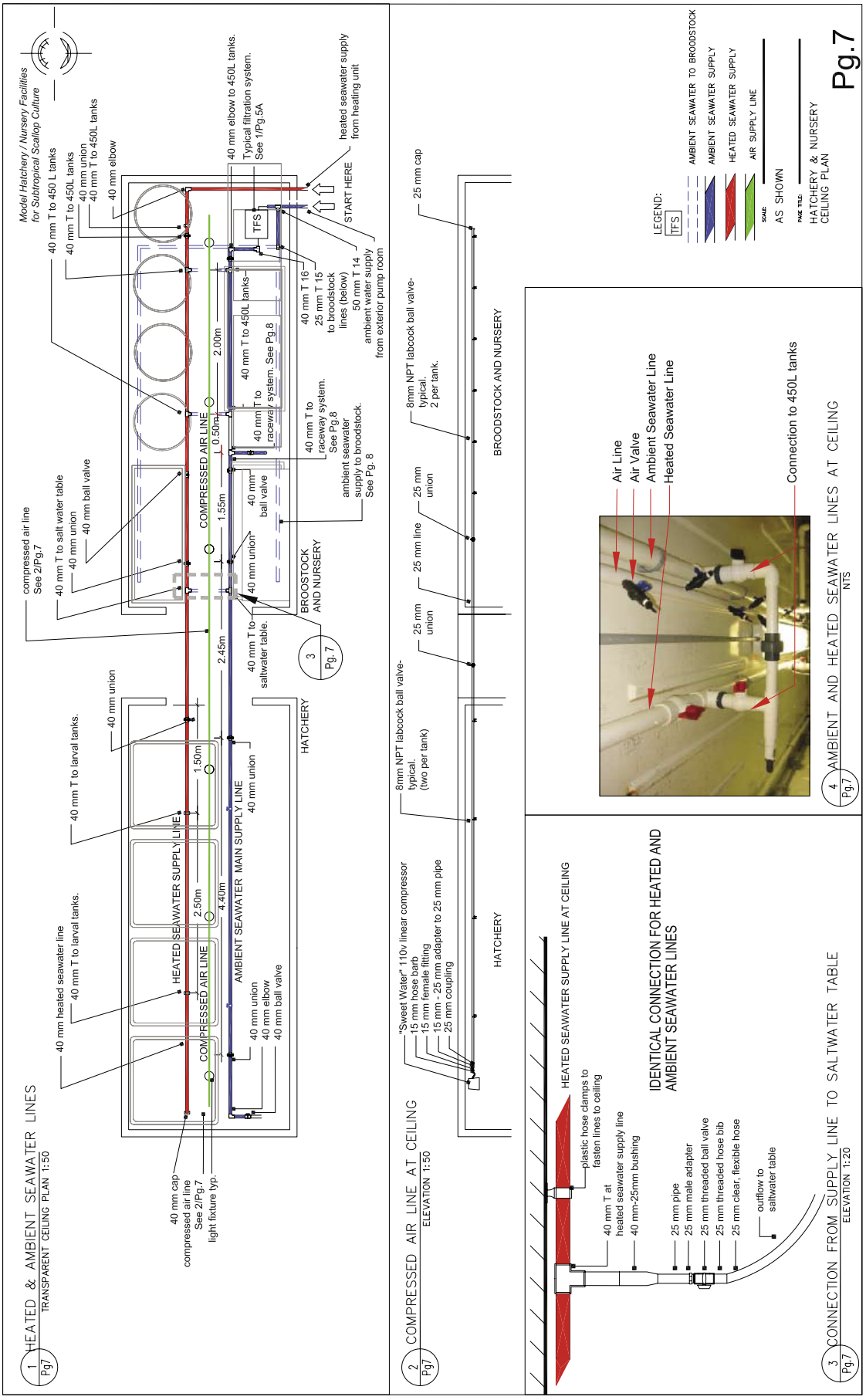
Heated and ambient seawater supply

Refer to Technical Drawing – 1/Pg7. The ambient seawater supply comes directly from the pump room, having passed through a sand filter before entering the hatchery/nursery complex. A 50 mm pipe is affixed on the exterior of the container, and passes through the wall of the container, parallel to the heated seawater line. Inside the container, a 50 mm T (T14) diverts the water into: a) A typical filtration system (TFS), supplying filtered seawater to all hatchery/nursery tanks and saltwater table; and b) Broodstock tank 1, Broodstock tank 2 via junction T15. *Note: Details for this main diversion junction (T14) are given in technical drawing – 1/Page 8.*

After passing through the typical filtration system the 1 µm filtered ambient seawater is distributed to all tanks via junction T16. The main (1 µm filtered) seawater line is affixed to the ceiling in the middle of the container parallel to the heated seawater line (see technical drawing Photo – 4/Pg7). T-junctions are fitted in-line for diverting filtered seawater to the remaining separate tank units; such that in the first container (nursery/broodstock area), there are three T-junctions supplying ambient (and heated)

Technical drawing, Pg. 7

Hatchery & nursery: Ceiling plan



seawater to the circular 450 litres tanks, one T-junction supplying ambient seawater to the raceways, and one T-junction supplying ambient seawater to the saltwater table. One-way ball valves regulate the flow to various areas. *Detail of the flow to the saltwater table is similar to that given for the heated seawater supply in technical drawing – 3/Pg7. Details of other junctions are given when appropriate in respective sections of the manual.* In the second container, specific to larval rearing, there is less need for ambient seawater; hence there is only one ball valve at the end of the water line for supply of ambient seawater, if needed.

Heated seawater line

The heated seawater line supply coming directly from the heating shed runs parallel to the filtered ambient seawater line. T-junctions are similarly fitted in line to supply heated seawater to the separate tanks. In the first nursery/broodstock container, three T-junctions supply heated seawater to the 450 litres tanks, and one to the saltwater table. *Connection to the saltwater table is described in technical drawing – 3/Pg7 and is installed parallel to that of ambient seawater. Similarly, details of connections to other tanks are provided in later sections of the manual*. In the second hatchery container, two T-junctions supply heated seawater to 4 larval tanks (one junction for two tanks). The heated seawater line is capped at the end with a 40 mm cap.

Air supply

Refer to Technical Drawing – 2/Pg7. The line for compressed air runs the length of the complex, affixed to the ceiling and parallel to the ambient and heated seawater lines, as shown in the ceiling plan. The location of a small compressor on a shelf at one end of the complex is shown on the diagram. Air is supplied through a 25 mm pipe to larval, broodstock, experimental and raceway tanks. Outlets are placed frequently, and airflow is controlled by labcock ball valves, to which a male barb is fitted for connection to an appropriate length of Tygon tubing (7 mm ID) to the tanks.

Details of seawater supply to saltwater table

Refer to Technical Drawing – 3/Pg7. Once diverted through the 40 mm T-junction at the main line, reduction of the pipe to 25mm is done with a 40 mm to 25 mm bushings. A 25 mm male adapter fits into a 25 mm threaded ball valve, which in turn is fitted on the outflow end with a 25 mm threaded hose barb. A 25 mm clear flexible hose supplies water to the saltwater table when needed.

1.1.4.4 Broodstock: tank and seawater supply

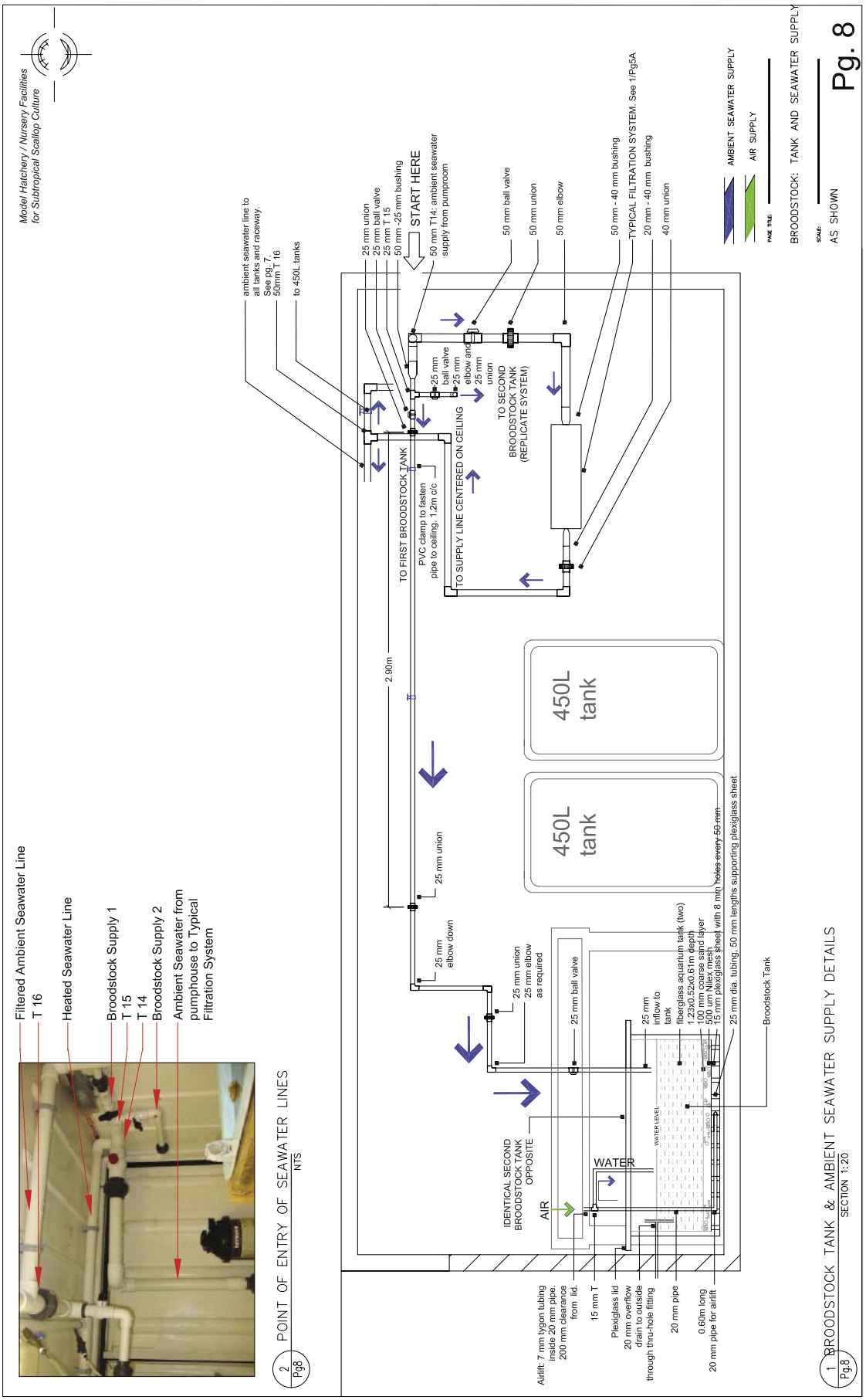
Broodstock seawater supply details

Refer to Technical Drawing – page 8. Sand filtered ambient seawater line entering the complex from the exterior, first passes through junction T14, located at the point of entry of the seawater pipe into the container (Diagram 1/Pg8). Water flow is directed to the filtration system and/or to a reduced 25 mm junction T15 for supply to broodstock tanks. The point of entry of both ambient and heated seawater lines is illustrated and labelled on the technical drawing photo – 2/Pg8.

For fine filtration of the ambient seawater, flow coming from T14 is regulated by a 50 mm one-way ball valve to a coarse Hayward filter (25 µm). The 50 mm pipeline is reduced 40 mm prior to the filter for proper fittings. Unions after the valve and on either side of the filter allow for replacement of parts and cleaning of system. A 10 µm and 1 µm cartridge filter housings are installed in-line following the coarse filter as detailed in the typical filtration system (see technical drawing – 1/Pg5A). From the 1 µm filter, the seawater is distributed to the raceways, tanks, and saltwater table, by a 40 mm line connected to the centre of the container ceiling using plastic clamps. Water

Technical drawing, Pg. 8

Broodstock: Tank and seawater supply



is then distributed to all tanks, via junction T16 along the supply line located on the ceiling of the complex.

For supply to the broodstock tanks, ambient seawater diverted through T14 flows through T15. This directs the coarsely filtered seawater (from sand filter in pump house) to Broodstock tank 1 and Broodstock tank 2. The flow for these tanks is regulated by a 25 mm ball valve for each tank. Unions (25 mm) located prior to elbows facilitate the necessary dismantling of pipes for cleaning. A second 25 mm ball valve located prior to the inflow into the tank allows for refinement of flow rate.

Broodstock tank

Refer to Technical Drawing – page 8. The two broodstock tanks are identical, 380 litres in volume (120x50x60 cm) and made of fiberglass with a clear viewing window in the front. A Plexiglas lid prevented any debris falling from above. A sub-sand filtration system is installed as follows: 25 mm diameter supporters are cut in 50 mm length and spaced out on the bottom of the tank; these supported a Plexiglas sheet drilled with 8 mm holes every 50 mm. On top of the Plexiglas sheet, is a 500 µm Nitex mesh covering the entire surface. Finally a layer of sand, previously washed and passed through a 1 mm screen, covers the bottom of the tank. Each broodstock tank is set for a semi-recirculation system, thus maximizing the residence time of added food, and allowing for some control in seawater temperature. Ambient coarsely filtered seawater (via the sand filter in the pump house) is supplied by a 25 mm pipe, and inflow is regulated by a 25 mm one-way PVC ball valve at the end of the line. Seawater flows in at the top of the tank. The semi-recirculation system is driven by an air lift, consisting of a 20 mm pipe placed on the bottom of the tank, and connected to a vertical pipe via an elbow. The airline (7 mm Tygon tubing) is inserted into the pipe. A T-junction at the top of the pipe allows for recirculated water to flow back into the tank. Outflow is achieved at the surface of the water, via a thru-hull fitting and hose discharging through the container wall and into the main drain pipes outside.

At certain times, during broodstock conditioning, temperature of seawater needs to be adjusted. Seawater within each tank can be cooled using a 1/5 HP chiller unit installed on a nearby shelf. The chiller is matched in size with the capacity of the tanks to allow for cooling through a 5 °C differential from ambient seawater temperature. For heating the ambient seawater, 250 Watts Ebo-Jaeger aquarium heaters were immersed in the tank; with a flow of 40 litres.h^{-1}, two 250 Watt heaters are sufficient to heat the water 5 °C above that of ambient.

Feeding of broodstock is done using one 20-litre carboy for each tank. Carboys are purchased with an existing spigot, located at the bottom of the carboy. This spigot is fitted with a stopcock valve and 7 mm tubing for a finer adjustment of algal flow to the tank. A fast drip-feed is usually adequate to supply algae from one carboy over 24 hours.

1.2 SCIENTIFIC BACKGROUND – NATURAL HABITAT AND REPRODUCTIVE CYCLE

1.2.1 Habitat

The scallop, *Euvola* (*Pecten*) *ziczac* (L.), also known as the sand scallop, zigzag scallop or Bermuda scallop, is a sub-tropical and tropical species (Figure 1.1). It is similar to other pectinids in that the right (lower) valve is very convex, whereas the left (upper) valve is usually flat but has been seen to slightly convex or concave in some cases. It has been fished recreationally and/or commercially in Brazil (Pezzuto and Borzone, 1997)

and along the Caribbean coast of Venezuela and Columbia (Velez and Lodeiros, 1990), and has also been seen off Florida (USA) as a by-catch in the calico scallop fishery. Its northernmost distribution is Bermuda (Lodeiros *et al*. 1989).

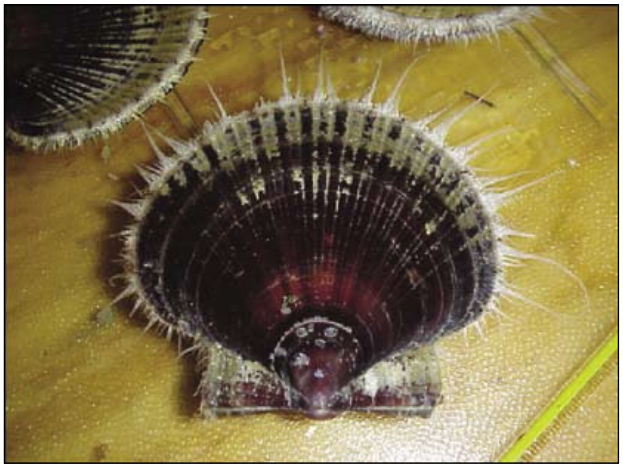


Figure 1.1: Photograph of a live *E. ziczac*, the sand scallop or zigzag scallop.

In Bermuda, the sand scallop inhabits protected inshore waters, lying on grassy, sandy bottoms, ranging in depth from 2–10 m (Sterrer, 1986). In its natural state, this scallop is recessed in the sand with the rim of its outer left valve showing. It will swim when disturbed, but does not cover great distances. It has also been observed to bury completely 5–10 cm into the sand when faced with unfavourable conditions. Maximum shell height recorded in Bermuda is 130 mm (Sterrer, 1986). Its life span is thought to approximate 5 years. Worldwide, population numbers of *E. ziczac* have been reported to be low, with a decline seen in the 1990's. In Bermuda, it was known to occur in relatively large abundance during the 1940's and 1950's, and was recreationally fished until the early 1970's. It since has been recorded here and there, with no real evidence of a self-sustaining population. To our knowledge, there is currently no existing commercial hatchery for this species.

The calico scallop, *Argopecten gibbus* (L.), is largely restricted to the sub-temperate and tropical waters of the western North Atlantic with major stocks distributed from Cape Hatteras, North Carolina (USA) to the Cape San Blas areas of the northeastern Gulf of Mexico (Waller, 1969). Calico scallops have also been collected from the Greater Antilles, Bermuda, and the western portions of the Gulf of Mexico (Waller, 1969). Commercially important stocks are located off North Carolina and northeastern Florida (USA), where it supports a small and transient fishery (Figure 1.2) The calico scallop has two convex valves, although the right is slightly more convex than the left (Sterrer, 1986). The upper valve is usually mottled with a combination of brown, red, purple, yellow and white. It is found lying on top of the seabed in sandy, rocky and grassy substrates, and has been recorded in Bermuda in several inshore waters, as well as on the more exposed North shore of the Island. It attains a maximum height of 70 mm in Bermuda (Sterrer, 1986). This species, like the sand scallop, was a commonly found bivalve in Bermuda and supported a recreational fishery at one time. Population numbers are at present very low in Bermuda (Sterrer, 1986). There is no commercial hatchery for this species at present.

Figure 1.2: Map indicating the distribution of the calico scallop, *A. gibbus*.

A generalized diagram of a pectinid is shown below (Figure 1.3) outlining some

shell characteristics and scallop organs. A photograph of a dissected calico scallop is also inserted to show the colouration of the gonad when ripe, the muscle and gills.

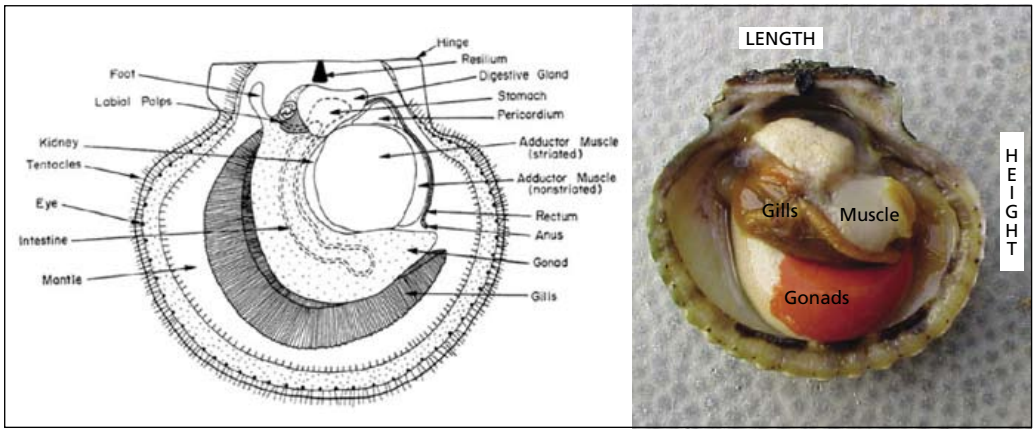


Figure 1.3: Generalized diagram of a pectinid (taken from Bourne, Hodgson and Whyte, 1989) alongside an open calico scallop specimen showing major organs.

1.2.2 Reproductive cycle

1.2.2.1 The sand scallop, Euvola (Pecten) ziczac

The reproductive cycle of *E. ziczac* in Bermuda has been determined based on histological analyses, gonadic index determinations and natural spatfall (Manuel, 2001). This species is a simultaneous hermaphrodite with whitish-yellow testis and orange ovaries when mature. According to Manuel (2001), gametogenesis is initiated in autumn and gonads mature during the colder winter months. High gonadic indices were determined by Manuel (2001) when seawater temperature was below 20 °C; this concurs with the observed peak spawning activity at the Bermuda hatchery between December and March. There was no major synchronized spawning recorded for this species in Bermuda waters. Animals tend to spawn sporadically subsequent to any small environmental changes, and very often were seen to release gametes partially rather than completely. This led to their classification by Manuel (2001) as "dribble spawners".

Lodeiros and Himmelman (1994) found that *E. ziczac* in Venezuela attained full sexual maturity at a shell height of approximately 44 mm; this shell growth is obtained in Bermuda in the first 10–11 months, and allows for a first gametogenic cycle to concur with the first winter period (Sarkis, 1995). Latitudinal differences in the timing of the reproductive cycle are seen with this species as in the more southern region of the Venezuelan coast, where two major spawning events have been recorded for the sand scallop (Velez, Sotillo and Perez, 1987); the first occurring in April/May and the second during August/September, when sea temperatures range from 23–26 °C. Temperature and phytoplankton abundance are two environmental factors which have been cited as influencing gametogenesis and spawning in the sand scallop (Lodeiros and Himmelman, 2000; Velez, Alifa and Freites, 1993). Fecundity of *E. ziczac* when stimulated to spawn in Bermuda at the hatchery averaged 5 million eggs per female; similar results were recorded by Velez, Alifa and Freites (1993).

1.2.2.2 The calico scallop, Argopecten gibbus

The reproductive cycle of *A. gibbus* in Bermuda was determined using gonadic index and histological analyses. This species is also a simultaneous hermaphrodite with whitish testes and bright orange ovaries when mature (Figure 1.4). For ease of understanding, a description of the oocyte developmental stages is given in Appendix 1.

Figure 1.4: Calico scallop, *A. gibbus*, showing gonads with both mature ovaries (bright orange) and sperm (white).

Timing for the initiation of gametogenesis and maturation of gametes is similar to the sand scallop, where gametogenesis is initiated in September and maturation reached during the winter months. The calico scallop in Bermuda exhibits a spawning period over the winter months, as for *E. ziczac*, associated with colder water temperatures and lower food availability. The difference between the two species is a more defined spawning period in *A. gibbus*, where release of gametes is complete and synchronous among the population, as observed in Bermuda. This is in agreement with Blake and Moyer (1991) who report, that a large percentage of any sub-population of calico scallops in Florida (USA) waters normally spawns over a 1–3 week period. This definition is reflected in the specific peaks seen in gonad weight between February and April (Figure 1.5) and in the high percentage of ripe cells (70–90 percent) determined in histological sections of the ovary (Figure 1.6). Histological analyses suggest that the spawning period of *A. gibbus* in Bermuda ranges from December to possibly May. In the more southern waters of Florida (USA), Moyer and Blake (1986) reported two spawning periods for the calico scallop, the first in late spring (April to June), and the second in autumn. These latitudinal differences may be explained in part by environmental differences, namely those of food and temperature (Barber and Blake, 1983). The lack of a clear trend in muscle weight determined for *A. gibbus* in Bermuda suggests a direct dependence on food supply for gonadal development and maturation. Blake and Moyer (1991) found that the required threshold temperature for the same species in Florida (USA) was of 19–20 °C. Their

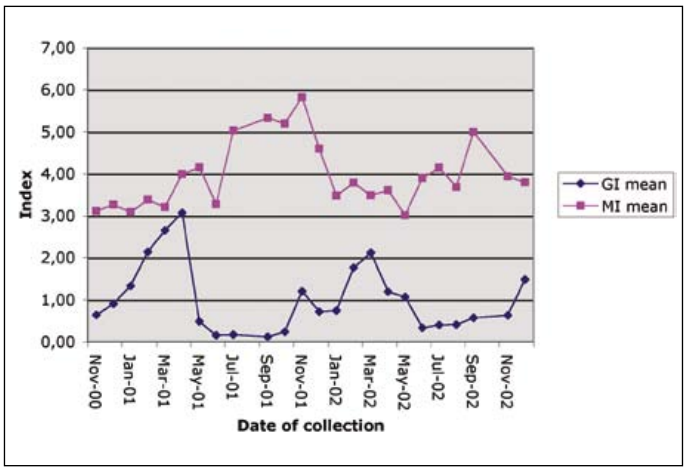


Figure 1.5: Gonadal indices and muscle indices for calico scallop, *Argopecten gibbus* in Bermuda waters (GI= Gonadal Index; MI= Muscle Index).

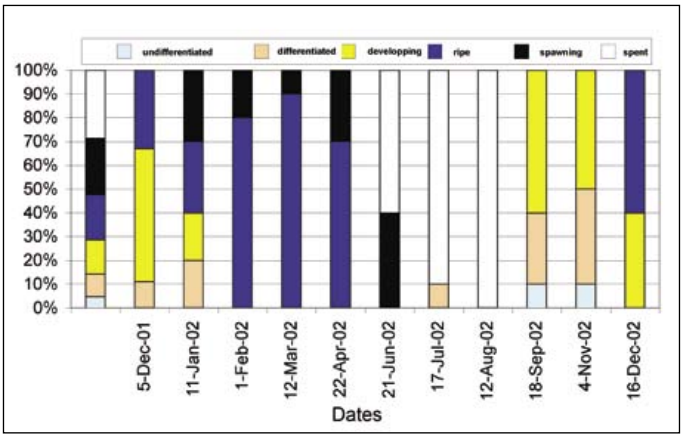


Figure 1.6: Reproductive patterns in cultured *A. gibbus* from Bermuda.

further conclusion that at temperatures above 22 °C maturation apparently stopped and spawning did not occur, concurs with results seen in the histological work and at the Bermuda hatchery, where a lack of gametogenic activity was seen during the warmer months, and spawning terminated in late spring (T=22±1 °C).

It has been noted by Blake and Moyer (1991) that an individual scallop normally reproduces for the first time at an approximate age of 6 or 12 months depending upon season of spawn. In Bermuda, 8 month old scallops have been observed to undergo gametogenesis and become reproductively mature within their first year of life (mean shell height of 47.4±2.2 mm). Each scallop is thought to spawn 2 or 3 times during its 18–24 months life span in Florida (USA) waters (Blake and Moyer, 1991). Although this has not been scientifically assessed in Bermuda, one cohort has been observed to spawn twice a year for a maximum period of three years. Fecundity of *A. gibbus* when stimulated to spawn in the Bermuda hatchery averages 6.26 million (n=5).

1.2.3 Life cycle

A generalized diagram of the life history of a scallop is provided below in Figure 1.7. Size shown for each stage is general, and differs among species. As seen in the above section, gamete release from ripe adults into the water, allows for external and controlled fertilization of the oocytes. Fertilized eggs or embryos are allowed to divide and develop into trochophore and early veliger larvae in larval tanks. The early larval stage is often referred to as D-larvae, as they take on a characteristic "D" shape, or straight hinge larvae, or Prodissoconch-I stage. Rearing of larvae is continued in these tanks, for development into umboned or Prodissoconch-II larvae. This hatchery stage lasts until larvae are mature and reach the pediveliger stage. At this time, larvae alternate from a swimming state to substrate-search behaviour by use of a newly developed foot. They may attach to various surfaces by secreting a byssus acting as temporary holdfast. Larvae are then ready to undergo metamorphosis, a critical time in their development. High mortalities may be seen at this time. Metamorphosed larvae settle, and are termed spat. They are reared in a nursery system until they are strong enough to be transferred to the field. Juveniles are reared in enclosures in the natural environment until adult size. Descriptions of techniques for larval, post-larval and juvenile culture are given in Chapters 3, 4 and 5.

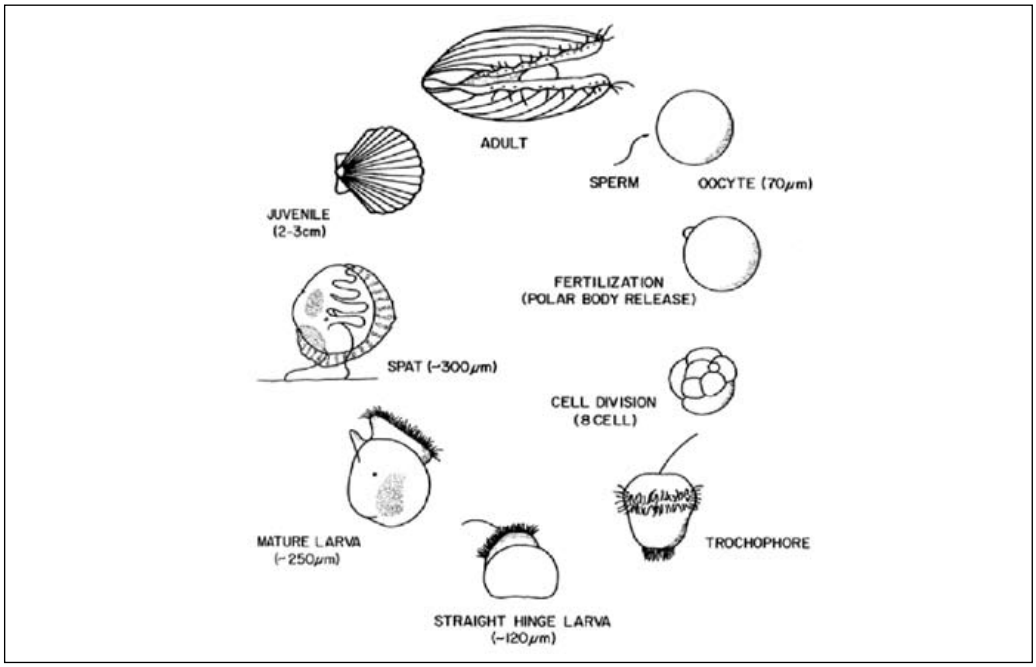


Figure 1.7: Generalized life history of a pectinid (taken from Bourne, Hodgson and Whyte, 1989).

1.3 TECHNIQUES – BROODSTOCK

1.3.1 Gonadal and muscle indices

The use of gonadal indices as an indication of reproductive activity is a simple, fast and inexpensive procedure in a hatchery. It provides reliable quantitative information required prior to the stimulation of spawning. The verification conducted at the Bermuda hatchery by comparisons with simultaneous histological analyses, confirmed the usefulness of gonadal index determination. Although there are various definitions of gonadal and muscle indices, all are based on the weight change of the tissue with reproductive maturity. Lucas and Beninger (1985) critically reviewed several of these indices, concluding with that best representing bivalve static condition. In the present work, gonadal and muscle indices are calculated separately; the former for reflecting gonadal development, and the latter as an indication of nutrient mobilization and utilization associated with gonadal development (Gabbott and Bayne, 1973). The step-by-step procedure for determining both indices is given in detail below in Protocol–1; a sample data sheet used during analysis is given in Appendix 2.

Gonads and adductor muscles are dissected and dried to constant weight at 80 °C. Indices are calculated as follows:

$$\frac{\text{Dry weight of organ}}{\text{Empty shell weight}} \times 100$$

A high gonadic index is indicative of mature gonads, and a lower index reflects the onset of gametogenesis or spent gonads.

For some pectinid species, visual observation of the gonad is sufficient to determine the spawning condition as for *Patinopecten yessoensis*, the Japanese scallops (Bourne, Hodgson and Whyte, 1989); however, visual observations have been misleading for the calico scallop studied in Bermuda. Although this species exhibits a change in colouration as the gonad matures from whitish-yellow to bright reddish-orange as observed by Roe, Cummins and Bullis (1971), it has been observed in Bermuda that gonads become bright orange prior to maturation and coincidental with sub-maximal gonadic index values. It is therefore advised that gonadic index determinations be made to assess the reproductive status of these species.

PROTOCOL–1

DETERMINING GONADAL AND MUSCLE INDICES

Preparation for procedure

1. Collect 15 scallops from the grow-out site. Record date and site of collection.
2. Place scallops in a tank supplied with flowing filtered seawater. Do not feed and allow 24 hours before dissecting to ensure gut clearance.
3. Use a 2 or 3 decimal point balance, if possible. Switch on and leave to warm up for at least 15 minutes.
4. Bring scallops in a bucket of seawater to laboratory.
5. Set up dissection kit with scissors, forceps and scalpel. You will also need Vernier calipers for measuring scallops and a laboratory book to record all information.

6. Take about 1 meter of absorbent paper and lay it on the bench. Number the paper with the number of scallops for dissection, i.e. if there are 10 scallops, Label 1, 2, 3…10, in equidistant spacing along the paper. Lay each scallop by their number on the paper, and the scallop then becomes this number. In this way, it avoids confusion. Also you will find that as scallops start to gape for air they are much easier to work with!
7. Tear small squares of foil paper (6x6 cm); two for each scallop.
8. Record date and site of collection, and date of dissection in lab book.
9. In the lab book, organize columns for scallop number (see step 6), shell height, total wet weight, gonad dry weight, and muscle dry weight.

The dissection procedure (wet weights)

1. Measure scallop height with Vernier calipers (see Figure 1.3). Record data on the lab book next to appropriate scallop number.
2. Blot dry each scallop with absorbent paper, and place on balance for total wet weight. Record data on the lab book next to appropriate scallop number.
3. As scallops begin to gape open, take one and keep it open with thumb and forefinger. Use scalpel to cut the adductor muscle from shell. This will allow opening the scallop completely, laying it flat for dissection.
4. Take a piece of prepared foil, label as "G" for gonad; write matching scallop number next to it directly on the foil paper. Such that each piece of foil is labelled G1 for scallop no. 1, G2 for scallop no. 2, etc. Tare the balance, and weigh foil. Record as foil weight under proper label.
5. Remove the gonad, using scissors and tweezers. Place the gonad on the matching piece of foil, and weigh. Record as gonad wet weight with foil (gram). Fold the foil around the gonad and place it to one side.
6. Take the next piece of foil for muscle, and label as "M" for muscle; write matching scallop number, such that similar to the gonad, each piece of foil is labelled as M1 for scallop no. 1, M2 for scallop no. 2, etc. Tare the balance and weigh foil. Record as foil weight under proper label.
7. Remove the muscle, carefully removing all the muscle from the ventral shell using a scalpel. Place on foil and record muscle wet weight with foil. Wrap the muscle in the foil and put it to one side.
8. Scrape all other tissues into a waste container.
9. Blot empty shells dry with a piece of absorbent paper. Tare the balance, and weigh and record the weight of the empty shell. Discard shells.
10. Repeat the dissection procedure for all remaining scallops. At least 10 scallops are required for reliable determination of Gonadal Index (GI) and Muscle Index (MI).
11. When all scallops have been dissected, put all labelled gonads and muscles wrapped in foil into a drying oven at 80 °C. Dry until constant weight; this should take 48 hours.
12. Clean all equipment thoroughly, especially dissecting tools and Vernier calipers to prevent any salt corrosion.

Dry weights

1. After about 48 hours, take gonads and muscles out of the oven. Make sure that constant weight has been reached after this time period.
2. Switch balance on and leave to stabilize according to manufacturer's instructions (usually about 15 minutes).
3. Weigh each organ with foil. Record on lab book.
4. Repeat until all organs weighed.

5. Transfer data into an Excel Spreadsheet (see Appendix 2). This will allow you to calculate gonad and muscle weight without foil, and gonadic and muscle indices as follows:

To calculate Gonadal Index (GI):

$$\frac{\text{Gonad dry weight}}{\text{Empty shell weight}} \times 100$$

To calculate Muscle Index (MI):

$$\frac{\text{Muscle dry weight}}{\text{Empty shell weight}} \times 100$$

Calculate the mean and standard deviation of the GI and MI for each collection date.

6. Keep all gonadal and muscle indices conducted in one spawning season in one Excel workbook. Assess gonad development on a routine basis. Keep yearly records for comparison.

1.3.2 Maintenance and conditioning of broodstock

The main goal in maintaining a broodstock in the hatchery is to ensure a healthy stock of reproductively mature and ripe adults. Depending upon species, this goal may be easily achieved or not. Conditioning broodstock, by manipulating environmental factors, such as temperature, food, photoperiod, allows for control of the gametogenic cycle, or parts of, and provides an aquaculturist with a tool for management of spawning periods and larval rearing and production in the hatchery. Again, depending on species, conditioning may be easily achieved or not. It has also been reported that conditioning of the broodstock affects lipid content of the egg, and subsequently larval survival (Gallager and Mann, 1986); these authors found that variations in broodstock conditioning protocol induced large fluctuations in egg lipid levels of two bivalve species, and suggested that strict attention should be paid to conditioning if optimal culture potential is desired. In other cases, difficulty in obtaining ripe broodstock, has prompted development of hatchery conditioning protocols, as for *Pecten fumatus*, in Australia (Heasman, O'Connor and Frazer, 1996); these authors found that the rapid conditioning of *P. fumatus* was possible by controlling water temperature and feeding to satiation; conditioned scallops exhibited a better fecundity than those spawned immediately upon collection from the wild.

For *E. ziczac*, Velez, Alifa and Perez (1993) found that sexual maturation and spawning could be induced out of the regular spawning season, by maintaining a temperature of 26–29 °C up to a total of 400 °C days; they furthermore found that the number of oocytes released was significantly higher in scallops maintained at a higher temperature for a longer number of day degrees. In Bermuda, no attempt was made to condition scallops out of their regular spawning season; however, efforts were made to ensure ripe broodstock at specific periods, thus optimizing use of the available hatchery and nursery space in this compact facility. As is seen below, working with two different species resulted with two different strategies.

In Bermuda, broodstock was maintained in the tanks described on Page 8 of the technical drawings. It was found that 50 calico scallops of mean shell height 57.2±2.7 mm could be comfortably held in one broodstock tank (equivalent to 80 scallops per m^2). Due to the larger shell size and recessing nature of the zigzag scallop, a maximum of 20 scallops only (approx. 75 mm in shell height) were kept in one broodstock tank (equivalent to 30 scallops per m^2).

1.3.2.1 The sand scallop, Euvola (Pecten) ziczac

Unlike results reported by Velez, Alifa and Perez (1993), where conditioning was conducted successfully out of season, it was found that the sand scallop was not easily maintained in the hatchery in Bermuda, even during spawning season. Keeping apparently ripe animals in a broodstock tank with a daily supplement of algae for any length of time, yielded poor spawns in terms of number of eggs released (Fecundity= 1.4±1.1 million eggs per female as opposed to the norm of 5 million eggs per female) and poor subsequent survival to D-larval stages (1.6 percent as opposed to 60 percent). Moreover, this species was found to spawn spontaneously following any type of stress, such as handling on the boat, transport to the hatchery, etc. The strategy for ensuring ripe zigzag scallops thus became one for collection of scallops when ripe, and prevention of spontaneous spawning. Protocol–2 provides a detailed description of the procedure. In short, scallops are collected directly from the field. Their reproductive state is assessed visually on board the boat. Scallops are considered ripe when the following three criteria apply: 1) well rounded gonads 2) female colour orange 3) digestive tubule invisible, covered by gonads. Ripe scallops are transported to the hatchery with great care. In order to avoid release of gametes due to boat movement, scallops are transported "dry". Zigzag scallops, as most pectinids, gape open when exposed to the air; in order to avoid dehydration, rubber bands are quickly placed around the two shells upon collection to prohibit them from opening their valves. Spawning is induced upon arrival to the hatchery.

PROTOCOL–2

COLLECTING AND HOLDING OF SAND SCALLOP BROODSTOCK

Preparation for procedure

1. Collect 30 specimens from the grow-out site by SCUBA using collector bags.
2. Assess gonadal state on board for as many animals as possible. Place ripest scallops aside (see criteria Section 1.3.2.1).
3. Quickly fasten rubber bands around shell of ripe scallops to prohibit valves from opening.
4. Place scallops gently in a cooler on a bed of seaweed, or layers of polyethylene mesh previously moistened with seawater.
5. Total exposure out of water for this species should not exceed 45 minutes.
6. Upon arrival at the hatchery, transfer scallops to a cold-water bath previously prepared for spawning induction (see Protocol–4).
7. If not possible to induce scallops on the same day, transfer into one of the broodstock tanks at ambient seawater, and induce the following day.
8. Do not feed 24 hours prior to spawning induction if maintained in hatchery.

1.3.2.2 The calico scallop, Argopecten gibbus

Gonadal indices are determined monthly to assess the stage of reproduction, as visual observation of the gonad was found deceiving in this species (see Section 1.2). Two strategies for ensuring a ripe broodstock in this species are used in Bermuda.

The first strategy relies strongly on the natural gametogenic cycle of the calico scallop. When a gonadal index reaches 2 or above, a sub-sample of scallops is collected from the grow-out sites and brought into the hatchery. Animals are kept in a broodstock tank at ambient seawater temperature, and fed on a daily basis a mixture of algal species. Diets consist of *Isochrysis galbana*, *Chaetoceros muelleri* and *Tetraselmis chuii*. Algal food is

supplied to the broodstock via a 20-litre carboy fitted with a drip-feed. This carboy is filled in the morning and in the evening, such that scallops receive a continuous flow of food amounting to 40 litres of algae for 50 animals or 14 litres of algae per kg total wet weight. This yields levels of 380 cells.g^{-1} wet tissue weight. Although, this is a relatively low level of food compared to others (Bourne, Hodgson and Whyte, 1989; Neima and Kenchington, 1997), it seems adequate for development of gonads to maturity as seen in results obtained following a 2–month conditioning period (see below). Feeding is stopped 24 hours prior to spawning induction. Ripe scallops are usually induced to spawn 1 or 2 weeks following collection.

The second strategy involves conditioning of broodstock to accelerate the later stages of gametogenesis, and thus advance the commencement of the spawning period to an earlier date. Several studies were conducted at the hatchery on conditioning of the calico scallop broodstock. Conditioning regime consists of two phases: Phase 1 involves exposure of scallops to a temperature lower than ambient, stimulating the differentiation of gametes; a temperature differential of 3 °C was sufficient. Phase 2 involves transition of scallops to ambient temperature, and exposure to a gradual increase in temperature to 4 °C higher (for the acceleration of gamete maturation). The evaluation of conditioning is done by routine determination of gonadal indices.

A series of conditioning studies were conducted at the BBSR hatchery, and provides the basis for the standard protocol followed for the calico scallop. These studies demonstrate that the required length of the conditioning regime depends on the gametogenic state of the scallops upon collection. For scallops with an initial gonadal index <1, both phase 1 and 2 have a duration of 30 days; this regime yields an index approaching 3 by the end of the 60–day conditioning, with 100 percent ripe oocytes (determined by histological analyses) following Phase 1. For scallops with an initially higher gonadal index (>1), maintaining scallops in lower than ambient temperature has shown to accelerate gametogenesis within the first two weeks, exceeding the developmental rate of scallops from the wild. Gonadal index doubled in this time. However, for these scallops, Phase 2 should probably be shortened as atretia (or resorption) of oocytes was observed during the latter part of this phase. Spawning induction results show an increased fecundity for conditioned scallops, if atretia is avoided.

The conditioning protocol followed at the Bermuda hatchery, therefore, allows for advancement of the spawning period, by acceleration of the later stages of gametogenesis. Use of gonadal indices is found to be a practical tool supplying reliable information on the reproductive stage of the calico scallops, and may be used as a basis for the timing of spawning induction in the hatchery. In Bermuda, it has been found that a gonadal index of 2 is required for successful spawning induction of calico scallops.

The procedure used in Bermuda is given in detail in Protocol–3.

PROTOCOL–3

CONDITIONING OF CALICO SCALLOP BROODSTOCK

Preparation for procedure

1. Collect 50 scallops from the grow-out site, and transport back to the hatchery in coolers filled with ambient seawater.
2. Upon arrival at the hatchery, transfer 40 scallops to pre-chilled broodstock tank (T= 15 °C)

3. Remainder 10 scallops are maintained in holding tank at ambient seawater for 24 hours. Use this sub-sample for assessment of gonadal and muscle indices the following day.
4. Feed broodstock with a mixture of two or three algal species, depending on availability. Clean 20-litre carboy with jet of fresh water and chlorine, taking extra care to clean drip-feed set up (stopcock valve and tubing). Fill 20-litre carboy and open drip-feed.
5. Record temperature before leaving the hatchery.
6. Do daily checks, recording temperature, flow rate, algal ration supplied and record in data sheet as sampled in Appendix 3.
7. Two weeks later, collect a second sub-sample of 10 scallops from conditioned broodstock for gonadic and muscle indices. Keep scallops in holding tank, not fed for 24 hours prior to dissection.
8. If gonadic index assessment results in GI<2, maintain broodstock at T= 15 °C for another two weeks. Skip to 10.
9. If GI>2, transfer broodstock into an ambient seawater tank. Following 24 hours, increase temperature by 1 °C every two days, so as to reach 22 °C within one week. Maintain high temperature for one week and induce scallops to spawn.
10. From scallops in 15 °C tank, collect another sub-sample of 10 scallops after two more weeks of conditioning (or 4 weeks after start of trial) for gonadal index assessment. GI should be close to 2.
11. Transfer the broodstock into an ambient seawater tank. Following 48 hours, increase the temperature by 1 °C every two days, so as to reach 22 °C.
12. Maintain the broodstock at 22 °C for a period of 30 days. Continue the same feeding regime. Following 30 days, collect a sub-sample of scallops for determination of gonadal indices. At this time, gonadal index should be above 2.
13. Stop feeding for 24 hours. Induce spawning.

1.3.3 Spawning induction of scallops

To date, thermo-stimulation is agreed to be the most efficient method for inducing sperm and ova release (Monsalvo-Spencer, Maeda-Martinez and Reynoso-Granados, 1997), especially in pectinids where a critical temperature range or minimum threshold temperature has been most frequently implicated in the initiation of spawning (Moyer and Blake, 1986; Barber and Blake, 1983). This induction of gamete release by temperature proved most efficient for the calico scallop in Bermuda; it yields a relatively rapid (90 minutes following induction) and efficient (70–90 percent) response to release gametes, compared to responses seen in other species Monsalvo-Spencer, Maeda-Martinez and Reynoso-Granados, 1997). This same method proved even more efficient when applied to the sand scallop, when 90 percent of the animals released both male and female gametes within 20 minutes induction.

The protocol utilized at the Bermuda hatchery differs slightly for both scallop species. Differences lie mainly in the degree of thermal shock provided, and length of shock required. *E. ziczac* is more sensitive to stress, and hence requires a lower thermal differential than *A. gibbus*. For both species however, the strategy is the exposure to an initial cold shock, followed by an exposure to a warm shock. Both species release gametes when exposed to warmer seawater temperatures.

As mentioned in the previous section, scallops are induced to spawn when observed to be ripe or close to ripe following methods outlined above. It must be added in this section that the sand scallop responds readily to a thermal shock by releasing gametes; although this response is caused by stress, and release of gametes may occur at times when gametes are not mature. On the other hand, the calico scallop is found to be more

tolerant of stress, such that although gametes may be determined to be ripe (GI>2), release does not always occur following thermal stimulation. This was seen on several occasions in the hatchery over a 4-year period. Figure 1.8 outlines the number of attempts made from February 20 to April 22 in 2002, with scallops showing a GI>2. As can be seen, only 2 out of 6 attempts resulted in release of eggs within 2 hours of induction. It has also been observed that response can also be very slow with the calico scallop, and that a time of up to 5 hours in a warm water immersion may be necessary for gamete release. Nonetheless, this lack of consistent response for animals known to be ripe renders further investigating of what cues initiate spawning, worthwhile. This is especially true for commercial aquaculture applications, where control of timing of spawning events is necessary.

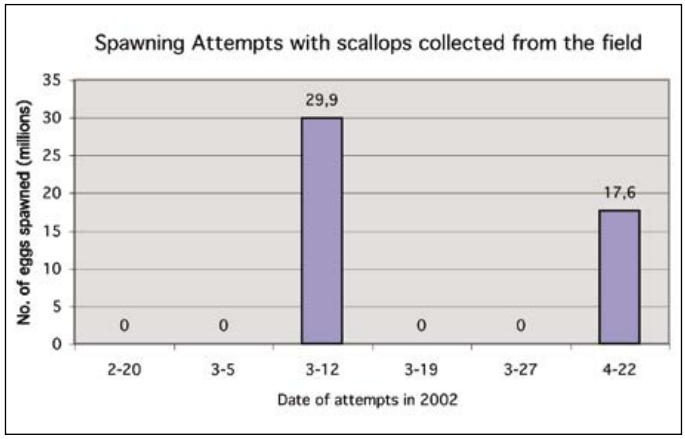


Figure 1.8: Spawning attempts with calico scallops, *A. gibbus*, collected from the grow-out sites.

Protocol–4 outlines in point form, the procedure followed for spawning induction of both species in Bermuda. Generally, extreme care is taken to ensure cleanliness of all equipment and seawater lines used during spawning and larval rearing. All equipment is washed with commercial grade bleach, rinsed abundantly with fresh water, and given a final rinse with filtered seawater (1 µm). Seawater used for spawning baths and for collection of gametes is filtered twice to 1 µm. Set up of the heating system is primordial, as it may take approximately 2 hours to obtain heated seawater for filling of rearing tanks. A saltwater table and bench space are prepared for collection of gametes and counting of eggs prior to distribution into tanks. In both species, sperm release usually occurs first, appearing as a milky white stream. Care must be taken when spawning a hermaphroditic species to avoid self-fertilization. For this reason, attributing a number to a scallop, and keeping the same number as it switches from male to female is important. Scallops are labelled as they begin to spawn; such that the first male is labelled as male 1 on beaker. As soon as egg release is noted (orangey-pink in colour), the scallop is removed from the beaker, the contents of which are discarded, the scallop rinsed, and transferred to a new beaker, labelled as female 1. In this way, sperm from other scallops is taken to fertilize this female. Scallops are changed regularly into new beakers with clean seawater as they spawn, as solutions become very cloudy, and it becomes difficult to observe a change in gamete release (Figure 1.9). As animals are transferred to new beakers, care must be taken to also transfer the label. Sperm or egg solutions are pooled into a larger beaker or bucket for use at a later date. As the spawn continues, initial sperm may be discarded, as it is advised that sperm <30 minutes old be utilized for fertilization. Two or three males may be pooled, and such a pool may be

Figure 1.9: Isolating sand scallops, *E. ziczac*, once gamete release is initiated.

used for fertilization, ensuring that the sperm utilized does not correspond to females of same individual. Calico scallops can continue releasing sperm for as long as 3 hours, but normally switch as females after approximately1 hour.

In order to further avoid self-fertilization, addition of sperm to an egg solution is done quickly; as soon as release of eggs is constant and solution in the beaker appears pink. This precaution is advised as scallops may switch back to male spawning unexpectedly, and result in self-fertilization. The volume of sperm added must be noted, as a lower ratio of sperm to eggs has been found favourable to subsequent fertilization and development rate (Gruffyd and Beaumont, 1972). Fertilization rate is enhanced by gentle mixing, using a homemade plunger. In Appendix 8 details of plungers used are shown. Egg counts are made using a Sedgewick-rafter cell, on pools of fertilized eggs. Development to D-larval stage is optimized, by eliminating debris from the egg solutions and distributing the egg solution to larval tanks prior to multi-division stage. As seen in Figure 1.10 a fertilized egg can be recognized by the presence of a fertilization membrane surrounding the entire egg. When fertilized, the egg undergoes meiotic division, at which time two polar bodies are released. For both the zigzag and calico scallop, a round membrane showing successful fertilization is seen approximately 15 minutes after the addition of sperm. The sequence of events was timed for *E. ziczac*. The first polar body is seen approximately 25 minutes after fertilization. The second polar body is observed 15 minutes later. Cell division, two- and three-celled stages, occurs approximately 75 minutes after the addition of sperm (Figure 1.10). From here on, division continues rapidly. This sequence and timing of events is similar to that reported by Costello *et al.* (1973) for *A. gibbus*, and concurs with the observations made at the BBSR hatchery for this species.

Distribution of eggs in the culture tanks is preferably done at the time of the two-celled division stage. Fertilized egg solutions are passed through a 150 µm sieve prior to suspension to eliminate debris and/or large clumps of eggs. A more complete procedure for removal of debris is the rinsing of eggs within 20 minutes of fertilization; eggs are passed through a 105 µm screen and collected on a 35 µm screen. The eggs are rinsed with filtered seawater while on the smaller screen to remove additional debris and excess sperm. The smaller screen should be submerged in a tray of water, so that the eggs are not pressed against the screen, as they are collected. After the fertilized eggs are rinsed, they are re-suspended in a known volume of filtered seawater. Sub-samples are collected to estimate the number of eggs collected. Care must be taken that the distribution of eggs into the larval tanks must be done prior to the multi-

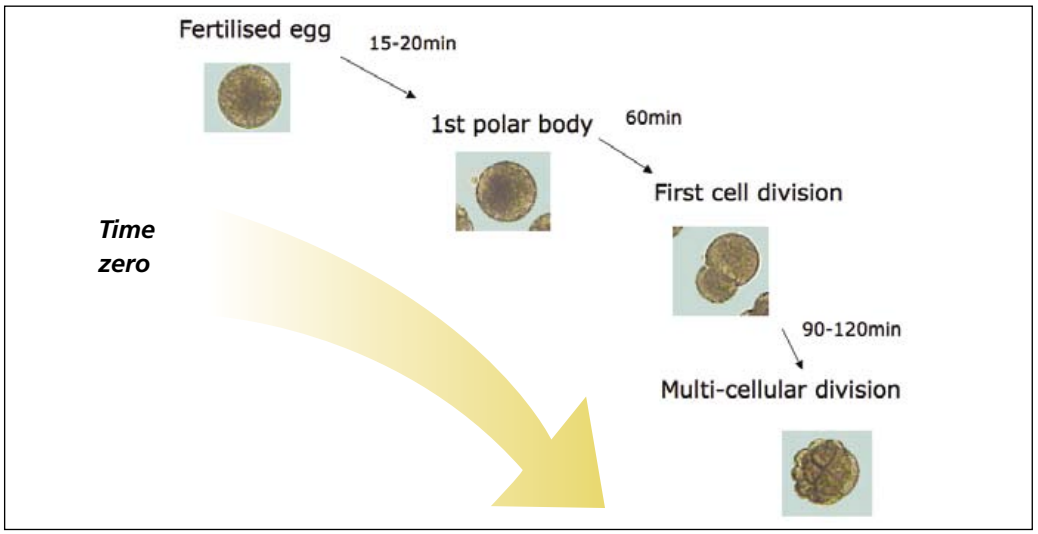


Figure 1.10: Sequence of events following fertilization of *E. ziczac* eggs.

cellular division stage. At this point, eggs become more fragile, and development may be hindered if eggs are passed through a sieve. It is therefore advised that should distribution occur later on, the process of passing eggs through a sieve be eliminated.

Finally, the keeping of records for all procedures, including transfer times between baths, first release of sperm, egg counts, etc., is important in understanding any problems arising at a later date.

PROTOCOL–4

SPAWNING INDUCTION

1. Set up heating tank (see Appendix 4).
2. Install a second 1 µm filter cartridge and housing inline with 20 mm ID reinforced hose for ease of supply to seawater baths and receiving beakers.
3. Install a 1 µm filter cartridge and housing inline to larval tanks.
4. Fill three trays with double filtered 1 µm seawater.
5. Prepare one cold-water bath (14±1 °C for sand scallop, 11±1 °C for calico scallop). Fill 1 litre zip-lock bags with ice cubes, and place in seawater tray. Once temperature is reached, remove all bags except one to maintain temperature constant.
6. Prepare two warm water baths by filling with 1 µm filtered seawater and immerging 1 250 W aquarium heater in each tray (see Figure 1.11).
7. Prepare beakers for collection of gametes. Fill as many trays as possible (at least 4) to 1/3 with heated 1 µm filtered seawater. Place one heater per tray so as to maintain temperature at 25 °C.
8. In each tray, place a maximum number of 2 and 3 litres beakers filled with heated seawater.
9. 4 litres beakers and/or 1 000 ml graduated cylinders are kept empty for pooling of gametes.
10. Once trays and beakers are ready, start filling larval tanks with double filtered 1 µm seawater.
11. **1st cold-water shock** – 30 scallops are selected for spawning. All scallops are placed in the cold-water bath for a period of 30 minutes. Record time and temperature
12. **1st warm-water transfer** – Scallops are divided into two and transferred to a warm-water bath for a period of 1 hour. (For sand scallop, response usually occurs within 20 minutes of this first warm water bath).
13. **2nd cold-water shock** – Following 1 hour, transfer animals back to cold-water bath. Make sure that water is clean (free of detritus or faeces), and temperature is same to initial cold-water bath. If necessary, prepare a new bath while scallops are in warm water. Record time and temperature. Similarly this cold-water shock only lasts 30 minutes.
14. **2nd warm-water shock** – Transfer the batch of scallops to the warm-water bath for a second time, and leave for a period of at least 2 hours. Record time and temperature. Calico scallops usually respond following the first hour of warm-water bath. Should no response be seen, attempt a 3rd cold-water shock, and a 3rd warm water shock; start flow of water in warm-water bath, and leave scallops. Check for gamete release for next 5 hours.
15. Once a scallop is seen to release gametes, leave for a few minutes in water bath. This may trigger spawning in other individuals. Remove the scallop, rinse with 1 µm filtered seawater to clean off sperm or eggs from shell, and thus preventing self-fertilization. Place scallop in a beaker, label and allow spawning to continue.

16. Once solution is cloudy, transfer scallop to new beaker. Keep sperm until no longer required.
17. Once scallop switches to female, rinse scallop and transfer to new beaker. Discard previous solution if suspecting self-fertilization. Label female with same number as initially given to male.
18. When solution becomes orange-pink, add a mixture of sperm using a Pasteur pipette to egg solution – 1 ml of sperm: 1 litre of eggs.
19. Mix sperm with egg, by a gentle up and down motion with plunger (see Appendix 8 for details).
20. Remove scallop from egg solution, and place into new beaker when solution becomes too thick.
21. Pool two or three egg solutions into a 10 litres bucket.
22. Count eggs from pools – Take a known volume of egg solution with an Eppendorf pipette preferably; 100 µl aliquot is usually sufficient and place on a Sedgewick-Rafter cell. Count using a compound microscope. Start at one end of the cell, scanning up and down, to avoid counting the same egg twice. Do triplicate counts (see Figure 1.12).
23. Calculate number of eggs as follows: (number of eggs.ml^{-1} x volume (l) of beaker or bucket) x 1 000.
24. Distribute calico eggs at 15 eggs.ml^{-1} and zigzag eggs at 10 eggs.ml^{-1} to larval tanks. No aeration is required for the first 24 hours.
25. For distribution to the rearing tank pass fertilized eggs gently through a 150 µm sieve before they reach the multi-division stage.
26. Once all eggs are distributed in tanks, transfer spawning animals to outdoor holding tank. Ensure a high water flow. Allow complete release of gametes.
27. Wash all equipment with commercial bleach solution and rinse with fresh water.
28. Heating tank unit and hatchery pipelines are cleaned following the procedure in Appendix 7.

Figure 1.11: Warm water bath set-up for spawning induction of scallops.

Figure 1.12: Measuring eggs or larvae on a Sedgewick-Rafter cell.

Chapter 2

Algal cultures: facilities and techniques

2.1 ALGAL CULTURE FACILITIES

The algal culture facility is a vital part of an aquaculture operation. Extreme care must be taken to ensure the production of healthy monocultures of selected algal species. Algae are harvested from large vessels whose production is reliant on a reservoir of small master or stock cultures. It is crucial to maintain this reservoir free of contaminants and excessive bacteria. Contamination of algal cultures may occur via the seawater supply, air supply and cross-contamination from nearby algal cultures. For this reason, master cultures are maintained in a separate unit from the larger culture containers. Cleanliness and careful transfer techniques cannot be overemphasized in maintaining a functional and healthy algal culture operation. The following section describes the facilities set up for larger cultures, and the smaller incubation unit used for master cultures. The largest

tanks used in Bermuda for algal cultures are 100 l vessels; these are relatively small compared to commonly used tanks of 1 000 to 5 000 l. Techniques, however, remain similar and those used in Bermuda are described in detail in Section 2.2.

2.1.1 Algal culture container

Large scale algal cultures are reared in a 3.7x2.4 m fiberglass container. Four concrete piers make up the supporting corner structures for the container. Steps are constructed with concrete blocks for access to the front door. Coarsely filtered ambient seawater is supplied from the pump house, and passes through an independent filtration system, affixed to the external wall of the algal container.

2.1.1.1 Elevation and connection to outside

Refer to Technical Drawing – page 9. Sand filtered incoming seawater, diverted from the main supply line by a Y-junction (see technical drawing – 1/Pg4) and regulated by Valve Y, passes through a 40 mm pipe that connects to the filtration system. Valve Q (40 mm), located prior to the 25 µm coarse filter, allows a further control of seawater flow. The fine filtration system depicted here is typical of the system used throughout the complex and described in detail on the technical drawing diagram – 1/Pg5A. It consists of a coarse filter (25 µm) and two in-line cartridge filters of 10 µm and 1 µm; unions are fitted on either side of the cartridge housings, for ease of cleaning and replacement. Seawater supply line is reduced after the 25 µm coarse filter and prior to the finer cartridge filters. Filtered seawater (1 µm) is passed through a 20 mm pipe fitted through the container wall for supply to the algal culture vessels. Elbows in the line are fitted when necessary, and unions are added to facilitate cleaning.

Details of the connection of the incoming water through the container wall and to the interior of the containers are shown in the int-ext connection window on the same page (see technical drawing diagram – 2/Pg9). A 20 mm hole drilled through the container wall is fitted with a thru-hull fitting and made water tight with caulking adhesive. Immediately after entry into the container, a 20 mm union is fitted for ease of cleaning. The incoming seawater pipe is fitted with a 20 mm to 40 mm bushing, leading to a 40 mm coupling and pipe for connection to a Lifeguard vertical UV sterilizer. On the outflow side, the UV sterilizer is fitted with a 25 mm threaded female, connecting to a reducing 25 mm male adapter (25 mm NPT x 20 mm hose barb). In this way a 20 mm flexible hose may be adapted for supply of UV disinfected water to 4 l flasks and 100 l vessels. *Details of the UV connection are illustrated in the technical drawing diagram – 3/Pg10.* It is found that the UV sterilizer improves water quality for algal cultures by eliminating potential bacterial contamination; as efficiency of "sterilization" or disinfection is related to the speed of water flow, care is taken in providing a slow water flow through the UV sterilizer.

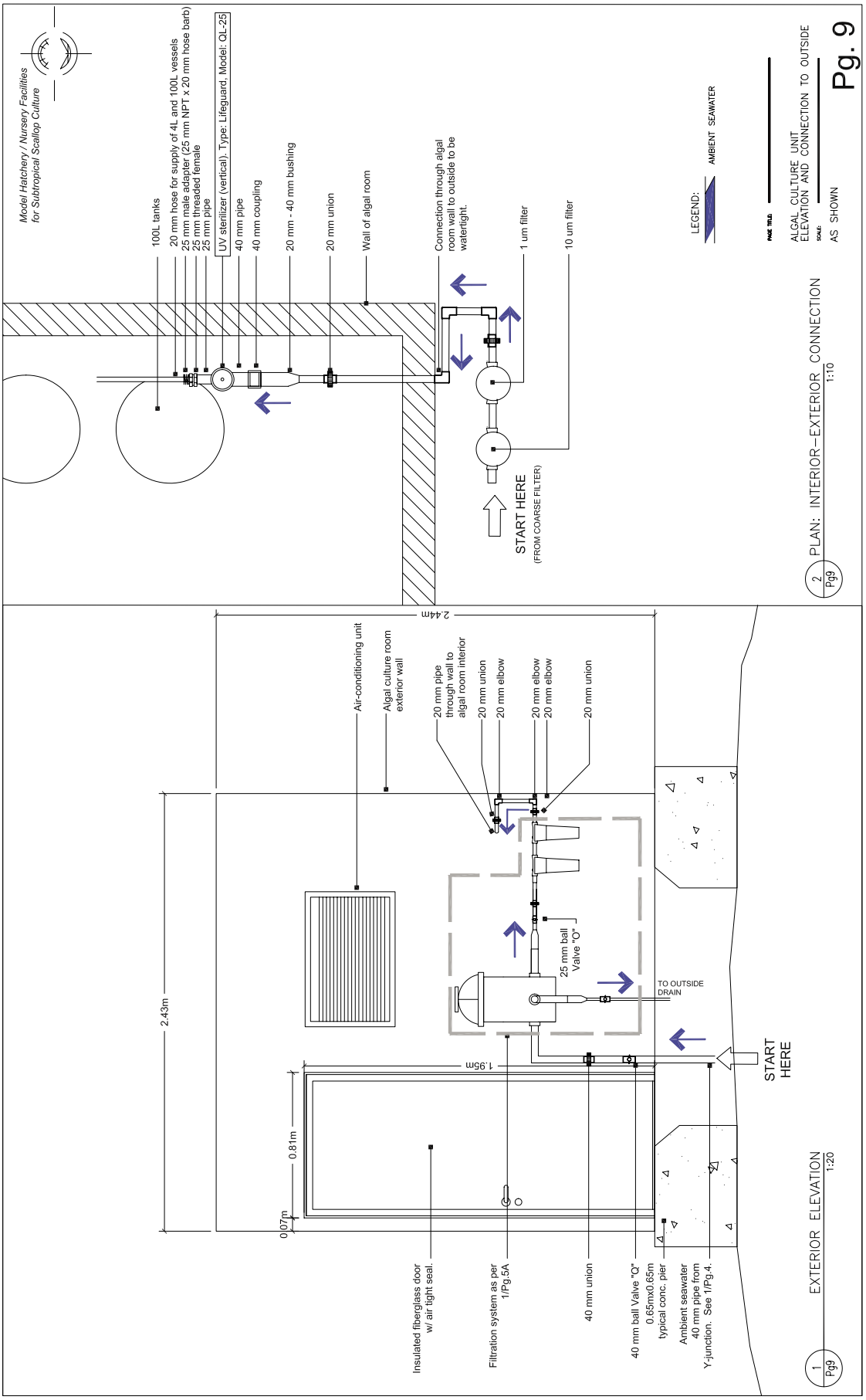
2.1.1.2 Floor plan

Refer to Technical Drawing – page 10. Climate control in the algal culture facility is maintained using an air condition unit affixed to one of the walls. This area must remain dry at all times to minimize proliferation of bacteria and molds. When required seawater and fresh water are supplied through flexible hoses adapted to their respective outlets. When not in use, both hoses are dismantled, leaving a clean and dry area.

The unit floor plan shows the position of the equipment necessary for serial inoculations from 500 ml cultures to large-scale algal cultures. In brief, 500 ml flasks are used as inoculum to 4 litres flasks, which in turn are used to start-up or boost 100 l cultures. Basic requirements are access to fresh water for cleaning of culture vessels, a sink for draining of fresh water or seawater, and UV sterilized seawater. Various

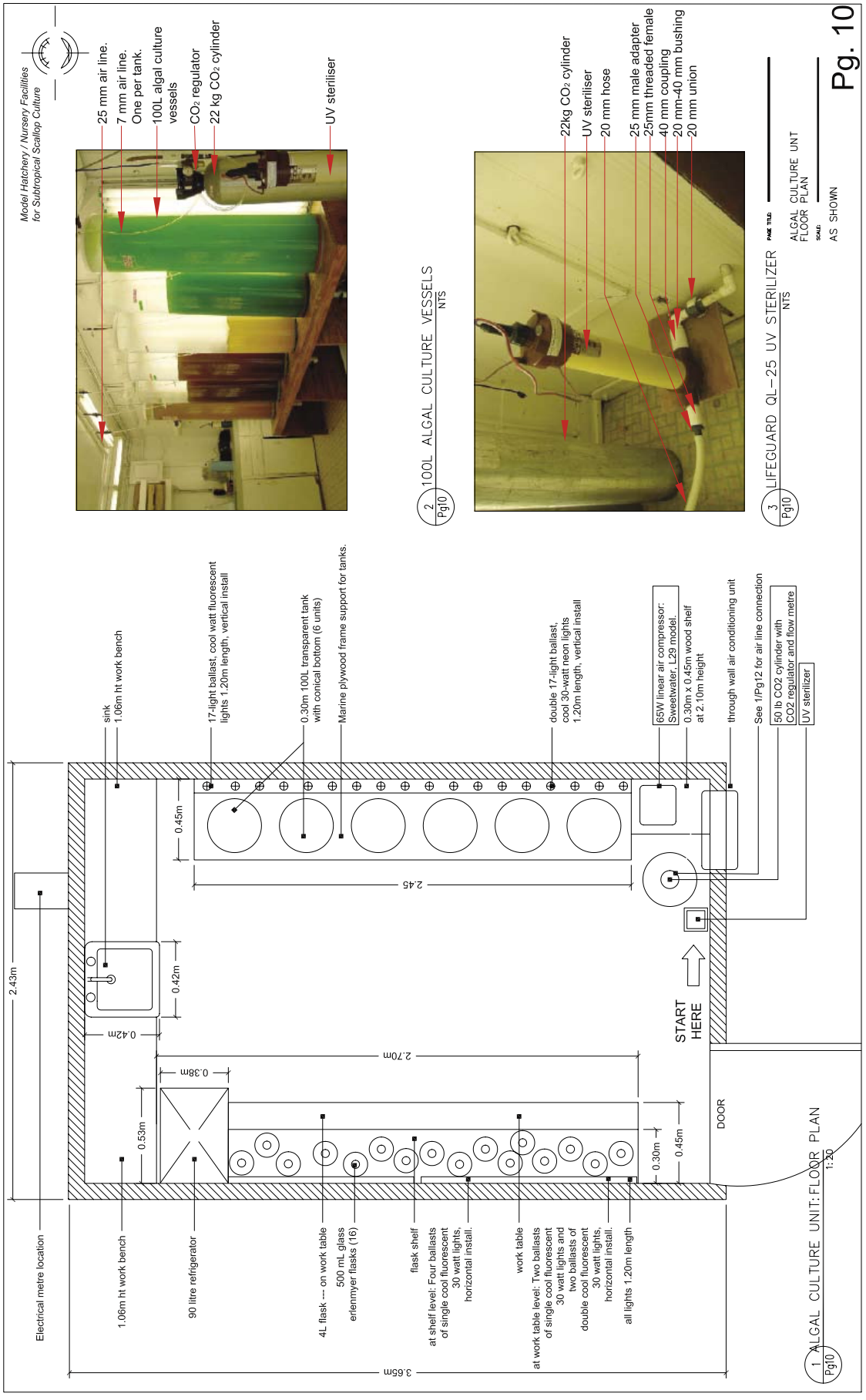
Technical drawing, Pg. 9

Algal culture unit: Elevation and connection to outside



Technical drawing, Pg. 10

Algal culture unit: Floor plan



methods are used for algal cultures; at the BBSR hatchery, it is found that aeration of cultures with an addition of CO_2, for maintenance of constant pH, yields best results. For this reason, an airline is present providing a mixture of air and CO_2 to every algal container.

At the entrance of the facility (on the right) is situated the UV sterilizer affixed on a 0.09 m^2 shelf to the container wall. The flexible hose (20 mm ID) used for supplying incoming seawater should be long enough to provide seawater the entire facility. Above and to the right of the UV sterilizer is a second shelf, supporting an air compressor (Sweetwater, 65 W); it supplies air to the culture vessels in the facility. Below the air compressor, on the floor, is a 22 kg CO_2 cylinder for the addition of carbon dioxide to the air supply. The connection of the CO_2 tank to the airline is shown in detail in technical drawing – page 12. Six 100 litres tanks are located along the right wall of the facility. These transparent tanks with conical bottom are placed on a wooden frame, with access to the drainage valve below the frame (see technical diagram – 2/Pg10). *Details of the drain are in the technical drawing diagram – 3/Pg12.* A work-bench, useful for inoculating small cultures (500 ml and 4 l) is located at the far end of the container. In the middle of the counter is a sink allowing for drainage or overflow of seawater when not in use. Going counter clockwise, a small refrigerator is used for storage of chemicals needed for algal cultures. On the left hand wall, a counter top, with drawers beneath it for storage of small materials, supports 4 l flasks. Above it a 30 cm wide shelf is fixed to the wall for the holding of 500 ml flasks. Lighting is used 24 hours a day for rearing of algal cultures in Bermuda. A light bank is fixed to the wall behind the 100 l tanks, consisting of 17 vertical ballast of cool 30 Watt fluorescent light of 1.2 m length. For the 500 ml flasks, 4 ballasts of single cool fluorescent 1.2 m lights are installed horizontally. For the 4 l flasks, a total of 4 ballasts, 2 with double 1.2 m lights, and 2 with single 1.2 m lights are installed horizontally.

2.1.1.3 Ceiling plan

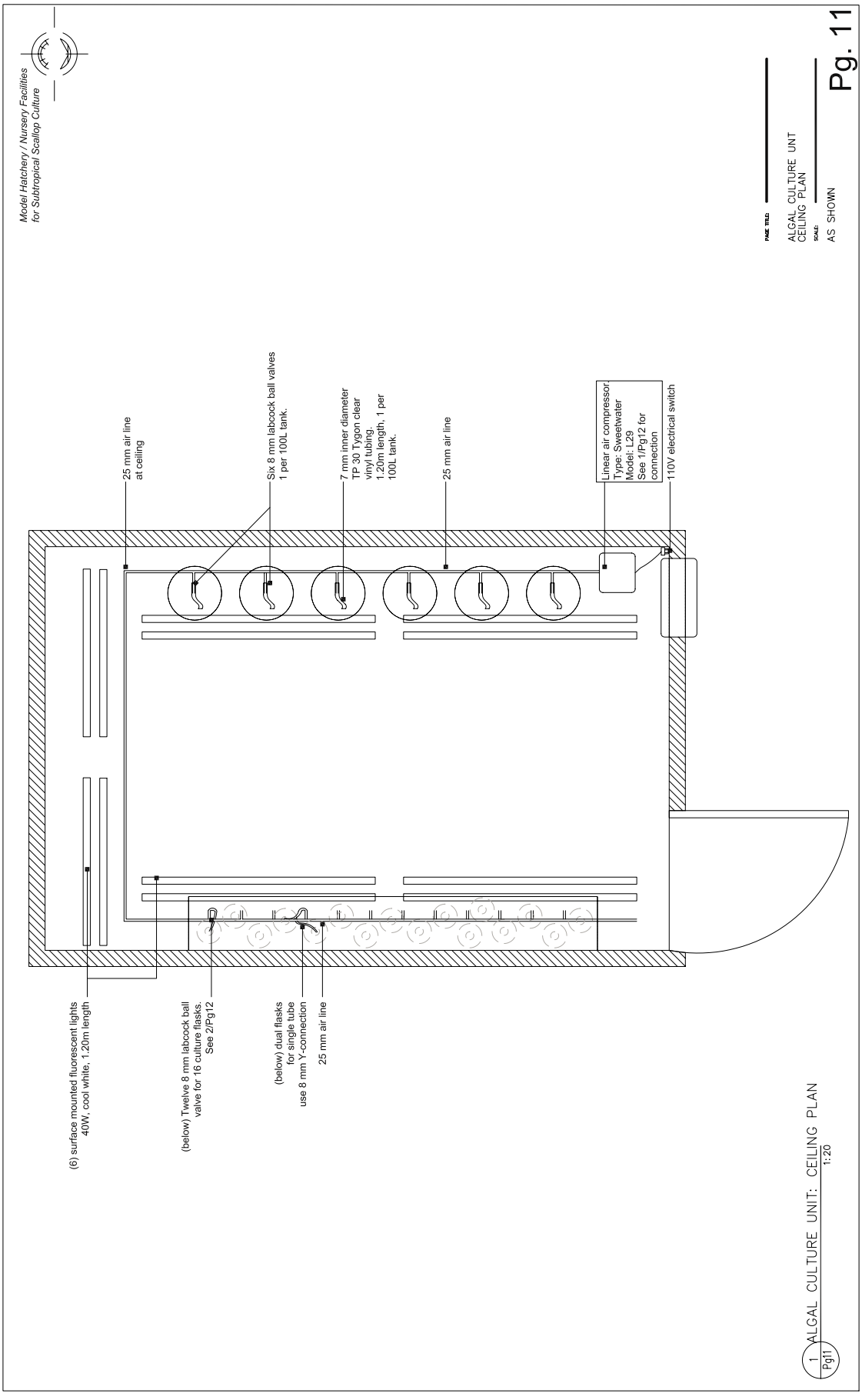
Refer to Technical Drawing – page 11. The ceiling plan outlines the contour of the 25 mm airline. Elbows are glued at the corners of the airline. The airline is secured to the ceiling by plastic clamps. In the airline, 8 mm holes are drilled and threaded with an 8 mm NPT tap drill bit and 8 mm NPT thread cutting tap. To these, an 8x8 mm NPT nipple is connected to an 8 mm labcock ball valve. This is similar to air connections described for the hatchery/nursery complex in Chapter 1 (see technical drawing diagram – 2/Pg7). There is one connection for every 100 l tank; for the 500 ml and 4 l flasks, one connection serves two flasks, joined together using a Y-connection (8–6 mm). *Details of the air connection for the flasks are shown in the technical drawing – page 12.* A total of eighteen air connections are available, allowing for twelve 500 ml flasks, twelve 4 l flasks, and six100 l tanks, respectively.

2.1.1.4 Details of air supply and 100 l culture vessels

Refer to Technical Drawing – page 12. All flasks and tanks should be supplied with a mixture of air and CO_2. In the technical drawing diagram – 1/Pg12, mixing of CO_2 with air is shown, and is further illustrated in technical diagram – 4/Pg12. Fittings for the Sweetwater air compressor are included with this model; the outlet is fitted with 15 mm flexible hose, secured with aluminium rings, and connecting to the 15 mm airline pipe with a 15 mm hose nipple and female fitting into a 15–25 mm bushing. From this bushing, air is directed through to a 25 mm elbow for supply throughout the container via a 25 mm airline. The CO_2 cylinder, located nearby, is fitted with a regulator including a flowmeter, and controls CO_2 input into the airline. From the flowmeter, a 7 mm ID Tygon tube connects to an 8 mm ID. tube leading to a hose barb x 8 mm male adapter fitting. The male adapter is threaded into the air supply via the 25 mm elbow depicted; in this way, CO_2 is mixed with the air supply from the

Technical drawing, Pg. 11

Algal culture unit: Ceiling plan



compressor. The technical drawing photo – 4/Pg12 shows the air/CO_2 connection. The general rule is that the air supplied should consist of 0.5–5 percent CO_2. It is found that one 22 kg bottle of CO_2 lasts 5–7 days of continuous bubbling for all tanks.

Technical drawing diagrams – 2/Pg12 and 5/Pg12 show the air supply to individual culture vessels. Air mixture to each culture vessel is regulated by an 8 mm labcock ball valve (shown in the technical drawing photo – 4/Pg7). From the labcock valve, an 8 mm NPT x 7 mm barb male adapter connects to a 7 mm ID Tygon tube, directly supplying the algal culture. Bacteria filters are placed inline prior to entry into the flask, and help minimize air-borne bacterial contamination. The technical drawing photo – 5/Pg12 shows labels for these connections. There is sufficient pressure from a single valve to supply air to two flasks; the line can be divided if needed, by use of a Y-junction (8–6 mm). To ensure a tight connection, the smaller inner diameter tubing should be heated using a propane torch to fit the Y-junction.

Details of the 100 l vessels in technical drawing – 3/Pg12 describe the fitting of each tank for support, air supply and drainage. A wooden frame, made of marine plywood, is cut for the fitting of all tanks and is supported by concrete blocks on either end, for all tanks. Air is supplied to each tank using a similar connection to that described for the small vessels above, and Tygon tubing is connected to the bottom of the cone into an 8 mm barbed tube fitting. A drainage system is fitted to the bottom of the cones for harvesting of algae, and cleaning of tanks. A 50 mm male adapter is threaded into the base of the tank; an 8 mm hole is threaded into a 50 mm pipe for inflow of air and CO_2 mixture. The 50 mm pipe is reduced to 20 mm and connects to a one-way 20 mm ball valve. A 30 cm diameter lid prevents detritus from falling into the vessel.

2.1.2 Chamber unit for master cultures

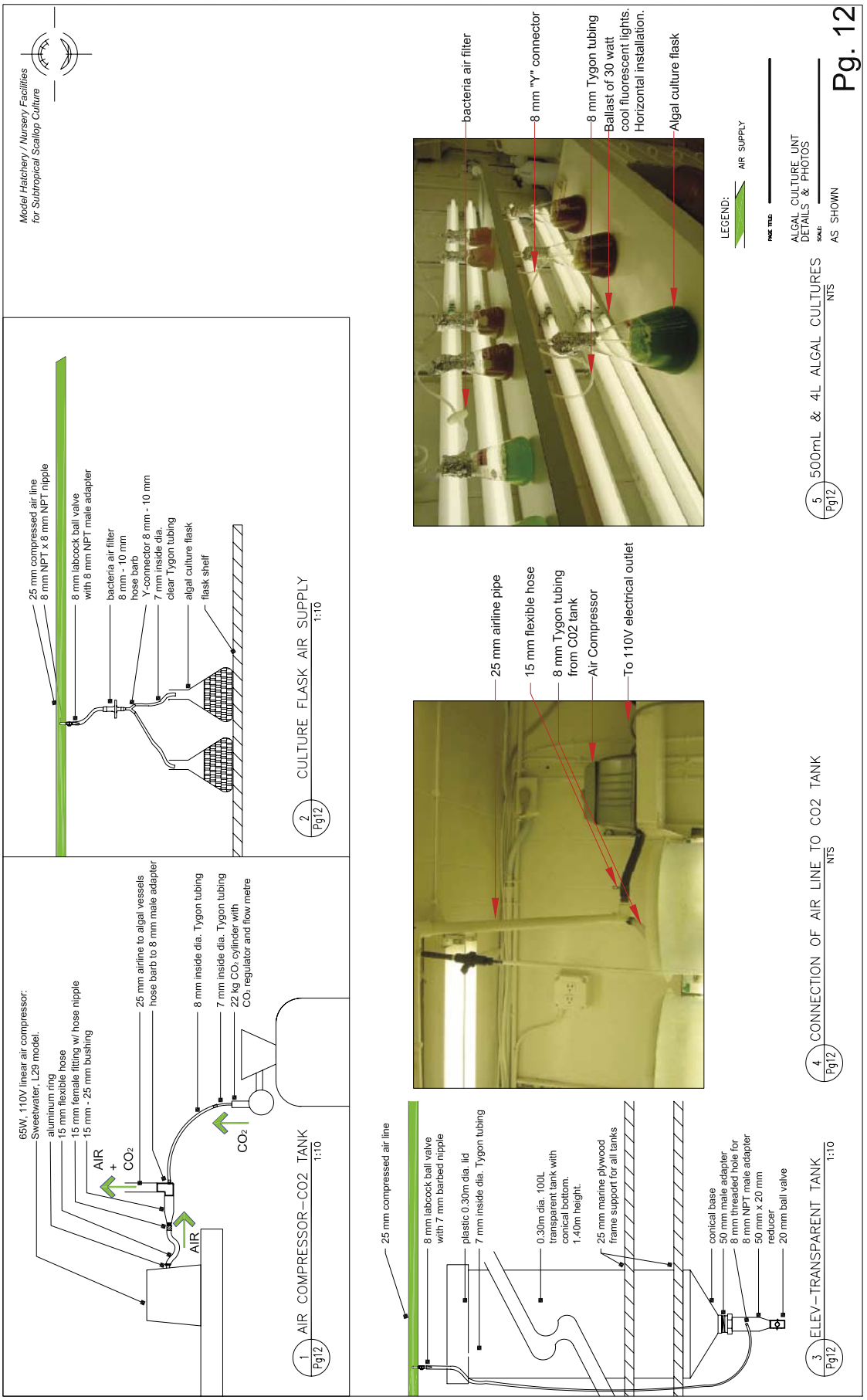
Master or stock cultures received from commercial phytoplankton suppliers arrive in 15 ml test tubes. For start-up of 15 ml test tube cultures and subsequent 125 ml Erlenmeyer flask cultures, an incubation chamber (Dual Program Illuminated Incubator 818 from Precision Scientific) is set at T= 25 °C and placed on a 12 hour illumination cycle. Next to the chamber, is an autoclave used for sterilizing small volumes of seawater, from 125 ml to 500 ml. The incubation chamber is maintained in a separate location from the large-scale culture unit. It allows for maintenance of clean stock cultures, independent of any contamination, which may occur in the larger cultures. Access to a workbench, and fresh water sink nearby allows for inoculation of cultures on a regular basis, and cleaning of culture flasks thereafter. Seawater supply differs for the stock cultures; low nutrient (and in this case "Sargasso") seawater is used for master cultures, and treated as described below; whereas, 1 µm hatchery filtered seawater is used for 500 ml cultures and above.

2.2 SCIENTIFIC BACKGROUND – LIVE ALGAE AS FOOD

The algal culture facility is a most important part of a bivalve hatchery. Adequate quantities of high quality food must be available at all times for successful operation, and a failure in the algal culture facility can be catastrophic to the hatchery. Larval and adult scallops feed on unicellular phytoplankton. Although studies have shown that it is possible to grow bivalve juveniles on a non-algal diet (Langdon and Siegfrid, 1984; Chu *et al.* 1987), the manufacture of artificial diets for bivalve hatcheries is still in the experimental stage. Micro-encapsulated diets (Langdon and Onal, 1999; Davis and Campbell, 1998) have been only partially successful in replacing live unicellular algae, which remain the major source of nutrition for filter-feeding organisms. In a compact

Technical drawing, Pg. 12

Algal culture unit: Details & photos



and space-restricted facility such as the one described here, the heaviest load for algal culture production is the broodstock and older spat requirements. For this reason, dry algae are purchased from Reed Mariculture, for the feeding of broodstock and 2–5 mm spat at the BBSR facility. Dry algal use is discussed in Section 2.3. Live algal culture techniques used at BBSR are described below.

2.2.1 Algal growth and composition

Growth of unicellular algae is by simple cell division, i.e. a single cell divides to form two cells, which then divide to form four cells, etc. Under normal conditions, an algal culture goes through three phases of growth: lag, exponential and stationary phases (Figure 2.1). The lag phase occurs when the culture is started and little increase in cell density is observed. In healthy cultures this period is quite short. In the exponential phase cell division occurs rapidly and cell density increases geometrically. Growth is limited only by the time required for cell division in this phase. In the stationary phase, the rate of growth (cell division) declines because some factor, such as nutrients or light, has become limiting and cell density remains relatively constant. During these different phases of growth the biochemical content of the algae differs. It has been shown that higher energy levels are found in the stationary phase for most species, the diatom *Thalassiosira* being an exception (Whyte, 1987). In a hatchery situation, a balance between high cell density and optimal energy content is strived for. For this reason, the strategy at the Bermuda hatchery is to maintain cultures in the exponential phase of growth, thus remaining healthy and continuously dividing, and harvest them at the beginning of the stationary phase, when energy content peaks. Cultures are harvested within 3 days, thus remaining for a short time in the stationary phase, and preventing the presence of a large number of dying cells within the culture.

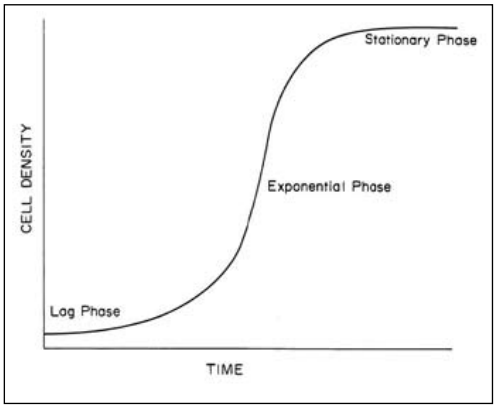


Figure 2.1: Theoretical growth curve of typical algal culture showing lag, exponential and stationary phase (taken from Bourne, Hodgson and Whyte, 1989).

2.2.1.1 Selecting algal species

Unicellular marine algae are widely used as food in the hatchery production of commercially valuable fish and shellfish. Of the many species of algae occurring in the world's oceans, only a handful are routinely used for their nutritive quality in hatcheries. Their success as food species depends not only on their nutritional quality, but also on their tolerance to temperature, salinity and light. Because of this, algal species will exhibit different growth performance depending on the hatchery site. Knowledge of the optimal growth responses of microalgae under local conditions is of great benefit, but a first selection can be based on the literature, as much has been reported on biochemical constituency of microalgae with respect to environmental factors (Brown *et al*., 1997; Volkman *et al*., 1989; Brown *et al*., 1993; Whyte, 1987). The first criteria, in selecting a range of microalgal species is thus dependent on the environmental conditions of the hatchery site itself.

Culture conditions such as, nutrient media used, temperature, light, quality of seawater, and the phase of growth (Brown, 1991; Moal *et al.,* 1987; Wikfors, Twarog and Ukeles, 1984; Dortch, 1982; Fabregas *et al*., 1986), affect the total concentrations of protein, lipid and carbohydrate in microalgae. Selecting the right algal species is of importance, as the performance of larvae and juveniles in terms of growth and survival is dependent on the content and nature of biochemical constituents in the algal food.

Despite variations among species, protein is usually the major organic constituent, followed by lipid and then by carbohydrate. Whyte, Bourne and Hodgson (1990) stress the importance of carbohydrate in providing a balanced diet for effective conversion of dietary macronutrients to tissue and energy reserves; according to these authors nutritional condition of the larvae correlated in their study with the content of dietary carbohydrate rather than dietary lipid or protein. Microalgal species used in bivalve culture can be divided into two groups: diatoms and flagellates. Whyte (1987) found that *Chaetoceros* sp. and *Thalassiosira* diatoms had a reduced organic content compared to flagellates, such as *Isochrysis* and *Tetraselmis*; on the other hand, diatoms in general do contain higher levels of carbohydrates than flagellates. Considering the total caloric content, Whyte (1987) found that *Isochrysis* species were major sources of energy followed by *Chaetoceros calcitrans*, *Tetraselmis suecica*, *Thalassiosira pseudonana* and finally *Chaetoceros* sp. All of these species have been shown to promote excellent growth for larval and juvenile oysters (Enright *et al*. 1986) and scallops (Bourne, Hodgson and Whyte, 1989). The second main criteria in selecting algal species, is based on the species of bivalve cultured and its specific requirements. Nutritional requirements for bivalves not only vary among species, but also among the various life cycle stages (larval, post-larval, juveniles and adults); for example, size of an algal cell, presence of spines, or cell wall thickness may preclude use of an algal species as larval food. Extensive research has been conducted for some bivalve species (Davis and Guillard, 1958; Epifanio, Valenti and Turk, 1981; Wikfors, Twarog and Ukeles, 1984). Bourne, Hodgson and Whyte (1989) give a detailed account for each life stage for the Japanese scallop, *Pactinopecten yessoensis*. These authors note that young larvae may not be capable of ingesting some of the longer chained algal species, such as *Skeletonema costatum*, or larger algae such as *T. suecica*; the siliceous spines of some *Chaetoceros* species (such as *Chaetoceros gracilis*), have also been shown to be unsuitable for younger larvae. However, *Chaetoceros* sp. are used in larger larvae, as well as juveniles and broodstock; in fact, the biochemical composition of *C. gracilis*, makes it the best single algal diet for conditioning broodstock and juveniles.

Generally, in order to obtain optimum performance in bivalve culture at any stage, a mixed diet of two or three algal species, is found necessary to provide all of the essential constituents. Protein is required for tissue production, carbohydrate for metabolism, and fatty acids for lipid storage and metabolic requirements. The type of fatty acid is also an important consideration, where the polyunsaturated fatty acids (PUFAs) 20:5w3 and 22:6w3 produce the best growth for juvenile oyster and scallops (Enright *et al*. 1986; Whyte, Bourne and Hodgson, 1989). More specifically, Bourne, Hodgson and Whyte (1989) conclude that for complete complements of fatty acids for larval scallops, a mixed diet of any of the diatoms with Tahitian *Isochrysis* is optimal. It therefore becomes clear that algae with more balanced proportions of micronutrients should be of higher food value to bivalves, assuming other nutritional factors are comparable such as, cell size, digestibility, and freedom from toxins or growth inhibitory metabolites.

Species listed in Table 2.1 are commonly used in bivalve hatcheries worldwide and are recognized as suitable species in terms of ease of culture and nutritive value. Coutteau and Sorgeloos (1992) found Tahitian *Isochrysis* used in 72 percent of hatcheries, while only 37 percent of hatcheries reported using *C. calcitrans*. Despite the favourable results in several studies (O'Connor and Heasman, 1997; Peirson, 1983; Nell and O'Connor, 1991), *Pavlova lutheri* was used by only a quarter of hatcheries surveyed (Coutteau and Sorgeloos, 1992). Observations have shown a limited tolerance of this species to temperatures above 27 °C, which may explain its limited use, especially in the tropics.

Table 2.1: Commonly used species of micro algae in bivalve hatcheries.

Algae	Cell diameter (µm)
Diatoms	
Skeletonema costatum	6
Chaetoceros calcitrans	2.5
Chaetoceros gracilis	6
Phaeodactylum tricornutum	5
Thalassiosira pseudonana	5.5
Chaetoceros muelleri	3
Flagellates	
Tetraselmis suecica	8.5
Isochrysis galbana or T-Iso	5
Nannochloris occulata	2
Dunaliella tertiolecta	6.5
Pavlova lutheri	5
Tetraselmis chuii	8.5

The algal species cultured at the BBSR hatchery over the course of four years for the rearing *of Euvola ziczac* and *Argopecten gibbus* are: Tahitian *Isochrysis*, *Isochrysis* sp., *C. calcitrans, C. gracilis, Chaetoceros muelleri*, *T. pseudonana* (clone: 3H), *T. suecica* and *Tetraselmis chuii*. Several studies were conducted at the Bermuda hatchery, evaluating growth performance of algae. From these in-house trials, it was found that *Isochrysis* sp., *C. muelleri* and *T. chuii* were best suited to Bermuda's culture conditions. Furthermore, these species yield satisfactory growth rates for larval and post-larval stages. Due to space limitations in Bermuda, it is found more manageable to culture a few species, rather than overextend the algal culture facility by rearing a larger number of algal cultures.

2.2.1.2 Requirements for algal cultures

Several factors are necessary for algal growth. As with all plants, algae must have sufficient nutrients to support growth. For rearing high concentrations of algae, as in hatcheries, type and amount of nutrients added is crucial. There are several types of nutrient media which may be used; they can either be made using purchased chemicals, or can be purchased ready-made from aquaculture companies. The Culture Centre for Marine Phytoplankton (CCMP) website provides recipes for various culture media (F/2, L1); the Conway medium recipe is provided in Appendix 9 and was initially used at the BBSR hatchery. It is no longer used as the preparation is lengthy, although it does support excellent growth for bivalves (Sarkis, 1987). To minimize the work-load, commercially available media is purchased by the BBSR hatchery; F/2 solution is easily obtained from aquaculture suppliers; supplement solutions of vitamins and sodium metasilicate are made at the hatchery, Details for the preparation of all culture media can be found in Appendix 9. Following preparation, culture media is autoclaved when used for smaller volume inoculations. Tris buffer may be added to prevent the precipitation of some chemicals during autoclaving. It also buffers the pH of the culture media during algal growth. *Note: With the F/2 purchased by BBSR, the addition of Tris buffer is not found critical.*

Factors, such as light, temperature, salinity, seawater quality, mixing and cleanliness are essential to algal growth. Attempting to achieve as constant culture conditions as possible will favour optimal algal growth, which will furthermore affect their biochemical composition. As mentioned previously, this is an important consideration in growth and survival of bivalve larvae and juveniles. Ensuring that harvest of algae is conducted at the same phase of growth, is a second constant to be achieved during routine culture procedure. Some of the more important criteria for successful algal growth are reviewed below, but more detailed accounts can be found in the literature.

Light is normally provided by fluorescent lamps. The most commonly used are "cool white", but little difference has been reported when using others such as gro-lux or full spectrum (Bourne, Hodgson and Whyte, 1989). Increasing the light intensity usually means better growth and faster division of algal cells. Lamps also generate heat, and

hence climate control of the algal culture housing facility is important. Most types of algae grow well between 17–22 °C. Tropical species are chosen at the Bermuda hatchery, as climate control is most easily achieved at 23–25 °C. Above 27 °C, most types of algae will die. Lower temperatures will reduce the growth rate. Ambient salinity is used at the BBSR hatchery (36 ppt), which is relatively high; nonetheless, all algal species, except for diatoms, which are reared at 25 ppt, fare well. Generally salinities between 25–30 ppt are best for the cultures of flagellates, and between 20–25 ppt for the culture of diatoms. Lower salinities can be obtained by diluting seawater with tap water or Q-water (de-ionized water). Salinity can be measured with a hydrometer or refractometer. Seawater used must be clean of unwanted types of algae and other contaminants which may feed or compete with the algae. For this reason, seawater is filtered finely and sterilized or pasteurized. There is a wide range of suitable equipment commercially available for this purpose. Chemical sterilization can be easily achieved for large volumes, if no equipment is available (see Appendix 10). Carbon dioxide is provided to algal cultures for faster growth and maintenance of high densities. Carbon dioxide is supplied from compressed gas cylinders; very little is required (0.5–5 percent) in the air supplied to the culture. The CO_2 should be passed through a flowmeter to facilitate the monitoring of delivery levels; the aim is to maintain a constant pH of 7.5–8.5 in all algal cultures, as algae divide and use up CO_2 in the culture water. The pH can be checked with indicator papers or a pH meter if available. Both the air and the CO_2 should be filtered through an in-line filter unit of 03–0.5 µm before entering the culture, as this helps to prevent other, possibly contaminating, organisms from getting into the cultures. Finally, mixing the cultures using air supply, allows all cells to be exposed to light and nutrients. Air can be supplied from a compressor or an air blower, and acts as a carrier gas for carbon dioxide. Not all aquaculturists keep smaller volume cultures mixed by bubbling. However, at the Bermuda hatchery, it is found that best results are obtained with mixing.

2.3 TECHNIQUES – GROWING ALGAE

The following sections provide the protocols used at the BBSR hatchery for culture of algae from stock to 100 litres vessels used for harvest. In order to ensure a contaminant-free culture, sterile microbiological techniques are used during sub-culturing or start-up of solutions. These techniques rely on the flaming of all equipment prior to use, and the continuous working by the flame during addition of nutrients, transfer of cultures, etc. At the Bermuda hatchery, a propane bottle, fitted with a nozzle is used as a flame source. Prior to opening of any culture flasks or nutrient bottles, the torch is turned on, placed on the workbench, and left on throughout the inoculation process. In this way, equipment is continuously flamed and "sterilized" when in use.

2.3.1 Master cultures

All algal culture system require a set of "stock" or "master" cultures; these are usually of about 125–250 ml in volume, and provide the reservoir of algal cells from which to start the larger-scale cultures used for feeding. Several centres, which specialize in the culture of algae can provide inoculum for stock cultures. However, it is also possible to isolate algal species from a specific body of water, and attempt to rear them under controlled conditions for feed. The isolation of singles cells of a species from natural live phytoplankton samples can be done using a capillary pipette and/or via a series of dilutions; this allows the separation of a selected species into a culture chamber with nutrient media. Thereafter, algae are cultured as detailed below.

At the BBSR hatchery, master algal cultures are purchased or received from various laboratories. The Culture Centre for Marine Phytoplankton in Bigelow (CCMP) has an extensive list of algal cultures, which may be purchased. Information may be

obtained online at the following address: www.ccmp.bigelow.org. Cultures are sent in 15 ml plastic test tubes and shipped by courier, to minimize transport time and ensure a large number of healthy cells upon arrival. The procedure for starting algal cultures and growing them to high density in 125 ml flask is described below. The following section describes the start-up and sub-culturing of 500 ml, 4-litre and 100-litre cultures.

Prior to reception of purchased cultures, 125 ml Erlenmeyer flasks are cleaned and prepared with adequate salinity seawater (see Protocol–5). The seawater used is classified as low nutrient seawater (LNSW), collected from the Sargasso Sea by oceanographers at BBSR. It is collected in 5 percent HCL cleaned 50 litres carboys from a depth of 5 m using Niskin bottles. At the laboratory, it is left to age for about 2–3 weeks to help strip out any inorganic nutrients, and then subsequently filtered through a sterile 0.2 µm mini capsule filter (Pall Corp., Item #12122). Following this procedure, no detectable nitrogen (<0.04 µm) or phosphorus (<0.03 µm) is found. Erlenmeyer flasks (125 ml) are autoclaved with seawater, for sterilizing of both flasks and seawater. Full strength salinity (36 ppt) is used for all cultures, except for the diatoms, *Chaetoceros* species and *T. pseudonana*, which fare best in reduced salinity seawater (25 ppt). Once the autoclaving process is completed, cooled flasks are ready for inoculation. LNSW is used because it is available; however, finely filtered (0.2 to 1 µm) and autoclaved seawater should be adequate.

PROTOCOL–5

PREPARATION OF CULTURE FLASKS (125 ml – 500 ml)

1. Wash glass flasks, Pasteur pipettes and rubber stoppers with glass rods in 10 percent HCl (hydrochloric acid) bath.
2. Rinse 3 times with fresh water and do a final rinse with Q-water (de-ionized) and let dry.
3. Fill flask with appropriate volume of filtered seawater using a graduated cylinder.
4. For reduced salinity, dilute seawater with Q-water. Use the following equation to calculate the volume of Q-water required to reduce the salinity:

$$X \text{ (ml seawater)} = Y \times \frac{\text{Total volume (ml)}}{\text{Full strength salinity}}$$

where:

X = volume of seawater added, and

Y = new salinity

Volume of Q-water required = Total volume (ml) - X

so that if full strength salinity = 36 ppt, and total volume in flask is 50 ml, and a new salinity of 25ppt was required

X = 35 ml of seawater, and

Volume of Q-water = 15 ml

5. Label flasks with reduced salinity.
6. Close flask loosely with cotton plug and aluminium foil. Cotton plugs are made with cheesecloth material tied around absorbent cotton.
7. Place flasks in autoclave and start cycle following manufacturer's directions.
8. Wrap all pipettes, stoppers and 4 aeration rods and stoppers separately in aluminium foil. Label clearly on the foil the contents and put an arrow pointing to the fragile thinner end of the pipettes as an indicator. Make sure contents are completely wrapped in foil as they will not be sterile if there are any gaps.

The transfer of purchased stock cultures from 15 ml test tubes to 125 ml Erlenmeyer flasks is described in Protocol–6. Culture media used is F/2, and preparation for small culture volumes is outlined above (Protocol–5). Microbiological sterile techniques are used to transfer master algal stocks from 15 ml test tubes to 125 ml flasks. Depending on the density of each stock culture, one test tube is used to inoculate two flasks, yielding duplicates of each stock. Addition of media to the cultures is done using sterile microbiological techniques. For diatom species, an addition of autoclaved 3 percent sodium metasilicate is added for growth of the siliceous frustule. Flasks are closed with a cotton plug and wrapped with aluminium foil. They are maintained in the incubation chamber at T= 25 °C and on a 12-hour light cycle. Flasks are swirled once a day to prevent settlement and sticking of algae on the bottom; as well as to distribute the culture media throughout the solution. These are hereon referred to as master cultures. These master cultures must be sub-cultured frequently; some hatcheries do this weekly. At the BBSR hatchery sub-cultures are done monthly. Sub-culturing involves inoculating some cells from an old stock culture intro fresh culture medium. In this way cells can continue to grow and divide ensuring a healthy culture. If sub-culturing is not carried out, the algal cells in the stock culture will eventually die. It is important to take precautions to prevent contaminants from the air entering the stock cultures when sub-culturing. In this way, master cultures can be maintained indefinitely.

To start a new master culture, 10–20 ml of algae inoculum (depending on density) is taken from a master culture for inoculation of new flasks with new seawater and media. In the first instance, when cultures are inoculated with purchased stock, the lag phase is long, and it is found that an average of 1 month is required to achieve a density capable of inoculating a larger volume of seawater, as density of purchased stock is usually low. Protocol–6 describes the procedure utilized in the first inoculation of 125 ml master cultures with purchased stock, and the subsequent monthly sub-culturing for maintenance of master cultures.

PROTOCOL–6

INOCULATION OF 125 ml MASTER CULTURES

Preparation of flasks

1. Two 125 ml flasks for each start-up culture are cleaned as outlined in Protocol–5.
2. Flasks are filled with 50 ml of seawater with adequate salinity requirement.
3. Flasks and seawater are sterilized using autoclave procedures.
4. Culture media is prepared and sterilized as outlined in Appendix 9.

First inoculation of 125 ml flasks using purchased stock cultures

1. Upon receipt of stock cultures, open package and let cultures stand upright in incubation chamber, awaiting inoculation. Depending on state of received cultures, they can be left as received for 24 hours.
2. Prepare a work area, with a Bunsen burner close at hand, and F/2 solution mixed with vitamins and sodium metasilicate. Working under a hood is best.
3. Stock cultures usually come in 15 ml test tube with screw caps. Unscrew cap, keeping opening of test tube close to flame; discard cap. With other hand, hold 125 ml flask, remove cotton plug, keeping it in palm of hand.
4. Transfer stock culture to 125 ml flask without mouths of either container touching and remaining close to flame. Place cotton plug immediately back onto flask. Discard tube.

5. Label flask with algal species and date. This will allow you to maintain a tight schedule of re-inoculation.
6. Working close to flame, remove cap of culture media mixed with vitamins. Place cap on clean surface area and maintain media container close to flame. Using a sterile 1 ml pipette, remove 0.5 ml of nutrients from container. Flame mouth, flame cap and close, keeping pipette tip close to flame. Take 125 ml flask with inoculum, remove cotton plug and keep in palm of hand and add 0.05 ml of nutrients to algal inoculum (1 $ml.l^{-1}$). Replace cotton plug immediately back onto flask. Discard pipette. Swirl flask to mix nutrients and algae.
7. Using same technique, add sodium metasilicate (2 $ml.l^{-1}$) if species is a diatom.
8. Place inoculated flask with nutrients in incubation chamber. Swirl daily.

Maintenance of master cultures – monthly sub-cultures

1. Once a month, prepare the same number of 125 ml Erlenmeyer flasks as already inoculated.
2. Fill flasks with 75 ml of seawater adjusted to adequate salinity.
3. Autoclave and let cool.
4. Prepare a workbench as above.
5. Do all transfers using sterile microbiological techniques.
6. Using a sterile 10 ml graduated pipette and bulb, pipette 10 ml of culture, maintaining pipette close to the flame.
7. Pick up one flask (check salinity) with other hand, remove foil and cotton plug, placing it on clean area, and add inoculum close to flame.
8. Quickly close flask with cotton plug after flaming the mouth of the flask and the cotton plug. Be careful not to put cotton plug too close to flame, it will burn.
9. Repeat 2 or 3 times with same culture and same pipette, so as to add an inoculum of 20–30 ml into the new flask.
10. Label with species and date.
11. Add nutrients accordingly.
12. Swirl and store in incubation chamber.
13. Swirl on a daily basis until next inoculation.

Once master cultures are established, larger volumes of algae can be cultured using this reservoir of various algal species. There are many different ways of culturing algae. These can be divided as batch culture, semi-continuous culture and continuous cultures. Batch culture is the most traditional method used for large-scale culture in bivalve hatcheries. Large volumes of algae are grown and harvested fully once desired cell density is achieved. Each new batch is inoculated from working culture flasks. It is a simple method, and a variety of containers can be used, ranging from 20-litre carboys to 3 m diameter tanks. Semi-continuous cultures refer to a system where part of the culture is harvested and used as food, and the amount taken is replaced with fresh culture medium (clean seawater and nutrients). After allowing 2–3 days for the remaining cells to grow and divide, the process is repeated. Semi-continuous cultures may be operated for up to 7–8 weeks. These types of cultures can crash because of a build-up of contaminants, bacteria or mismanagement. Minimizing contamination from any source is critical in semi-continuous cultures. Various containers can be used for semi-continuous cultures. The most common type of container is a sterile polyethylene bag. The bags are sealed and the inside is sterile. They can be inflated with sterile air to form the shape required before filled with seawater. The bags need to be supported by a frame. At the BBSR hatchery, 100 l vessels, described in the facilities Section 2.1 are used for semi-continuous cultures.

Continuous cultures may be maintained by using turbidostat culture, and by chemostat culture. In the former, the number of cells in the culture is monitored, and as cells divide and grow, an automatic system keeps the culture density at a pre-set level by diluting the culture with fresh medium. In the latter, a flow of fresh medium is introduced into the culture at a steady pre-determined rate. The surplus culture overflows into a collecting container, from which it can be taken and used as food. These systems are not commonly used in commercial hatcheries because they are expensive, very sensitive, and difficult to install and maintain.

Depending on the requirement for algae, they can be cultured using closely controlled methods on the laboratory bench top, for a few litres of algae to less controlled methods in outdoor tanks relying on natural light conditions, and producing thousands of litres. At the BBSR hatchery, several steps are taken to ensure a daily harvest of algal food of optimal quality to larvae and post-larvae scallops. Algae are first cultured in batches of 500 ml flasks and 4 l flasks; these 4 l cultures are used in turn to inoculate 100 l vessels, reared in a semi-continuous method and used for daily harvest.

2.3.2 500 ml batch cultures

Once 125 ml cultures achieve higher densities of algae (6 000 cells.ml^{-1}), inoculation to 500 ml flasks can be performed. These 500 ml cultures are in turn used as inoculum for 4 l cultures. Procedures for preparation, first inoculation, and maintenance of 500 ml flasks are outlined in Protocol–7. Flasks are filled with 250 ml of 1 µm filtered seawater (collected from the hatchery). Similar salinities as to those used for 125 ml flasks are used. Seawater and flasks are autoclaved. All procedures including transfer of inoculum, addition of media, or aeration pipette are conducted using sterile microbiological techniques. The inoculum of 35 ml is transferred using sterile microbiological techniques to the 500 ml flask. The exact amount is dependent on the master culture density. The denser the culture, the smaller the volume of inoculum required. The remaining volume in the master culture is used to re-inoculate a new 125 ml master culture flask (see Protocol–6). Culture media (F/2) and vitamins are added depending on volume of seawater inoculated; sodium metasilicate is added to diatom cultures. In 500 ml flasks, aeration is provided for mixing of culture media and algae, maintenance of algae in suspension and addition of CO_2 for pH stability. For this reason, sterilized Pasteur pipettes are added prior to closure of flasks with cotton plug and aluminium cover. Flasks of 500 ml are connected to the airline in the algae container. In the first instance of inoculating a 500 ml from a master culture, approximately 2 weeks are required for a high-density culture, as lag phase is longer. At this time, 200 ml of the culture is transferred to a new 500 ml flask filled with 200 ml of sterile seawater. These are allowed to grow until cell density approaches 10 000 cells.µl^{-1} (1-2 weeks). Thereafter, once cultures are well established and dividing rapidly, 500 ml flasks are re-inoculated twice a week; this high frequency insures a healthy culture in continuous exponential phase. Maintenance of 500 ml flasks is achieved by transferring approximately 50–100 ml of algal culture from one 500 ml flask. The remaining 250–300 ml is used to inoculate a 4 l volume.

2.3.3 4 litres batch cultures

Algal cultures are reared to 4 l volumes for the purpose of obtaining a large inoculum required for the start-up of the semi-continuous 100 l cultures. Four litre Erlenmeyer flasks are too large to be sterilized in the existing autoclave at the Bermuda hatchery. For this reason, 1 µm filtered UV disinfected seawater is used. In the described facility, the seawater supply for the algae room needs to be set up, prior to inoculating 4 l flasks. Appendix 11 indicates the step by step procedure for obtaining 1 µm filtered UV disinfected seawater. *Note: If UV disinfected seawater is not available, it is possible to sterilize seawater chemically, by using sodium hypochlorite (or commercially available*

chlorox), and neutralize it with sodium thiosulfate (1N solution). Appendix 12 provides the protocol for chemical sterilization. This was initially used at BBSR, and yielded satisfactory results.

Flasks are filled to 3.5 litres with UV disinfected seawater and closed with a rubber stopper (no. 10). One half of the 500 ml culture is used to inoculate this volume. Similarly culture media, vitamins and sodium metasilicate (for diatoms) is added to the new solution. For aeration of 4 l flasks, rubber stoppers are fitted with (diameter) glass rods. One rod equates the length of the flask, nearing the bottom used for bubbling, and the other is shorter, acting as a vent. Aluminium foil is used to cover the stoppers. Four litre flasks are connected to the airline in the algal container. Sub-inoculation of 500 ml flasks and 4 l flasks are conducted on the same day at the BBSR hatchery, twice a week (Monday and Thursday). In this way, healthy cultures in the exponential phase of growth are always available for inoculating larger 100 l cultures. Four litre cultures are allowed to grow for a period of 3–5 days before use as inoculum for 100 l cultures.

PROTOCOL–7

INOCULATION OF 500 ml FLASKS

Preparation of flasks

1. Two flasks for each algal species cultured are cleaned in a 10 percent HCl bath. They are rinsed three times with fresh water and have a final rinse with Q-water.
2. For a first inoculum from 125 ml master cultures, fill flasks to 200 ml. Use 1 µm filtered seawater.
3. Close flask with cotton plug and foil, made as described in Protocol–6.
4. Sterilize flasks and seawater in autoclave.
5. For maintenance and sub-inoculation of 500 ml algal cultures, fill flasks to 350 ml using 1 µm filtered seawater, adjusted to required salinity.

First inoculation of 500 ml flasks with 125 ml master cultures

1. When master cultures increase in density, inoculate 500 ml flask (filled with 200 ml of seawater) with 35 ml of master culture. *Note: Remaining volume of master culture is used to re-inoculate a new 125 ml flask. See Protocol–6.*
2. Use microbiological sterile techniques for transfer.
3. Prepare a work area, with a Bunsen burner close at hand, F/2 solution, vitamin solution and sodium metasilicate solution. Keep 5 and 10 ml sterile pipettes and 3-way pipette bulb nearby.
4. Using a 10 ml Pasteur pipette and bulb, transfer 30–35 ml of master culture to 500 ml flask, remaining close to flame at all times. Do not touch mouth of either flask with pipette. Plug flask quickly after transfer.
5. Label flask with algal species and date. This will allow you to maintain a tight schedule of re-inoculation.
6. Working close to flame, remove cap of culture media mixed with vitamins. Place cap on clean surface area, and maintain media container close to flame. Using a sterile 1 ml pipette, remove 0.5 ml of F/2 from container (nutrients added need to equate 1 ml of nutrient per litre of culture). Flame mouth, flame cap and close, keeping pipette tip close to flame. Take 500 ml flask with inoculum, remove cotton plug and keep in palm of hand and add nutrients to algal inoculum. Replace cotton plug immediately back onto flask. Discard pipette. Swirl flask to mix nutrients and algae

7. Using same technique, add sodium metasilicate if species is a diatom at 2 ml of sodium metasilicate per litre of culture. In 500 ml flasks, 1 ml of sodium metasilicate is added.
8. Remove one Pasteur pipette, from autoclaved packet and keep close to flame. Take 500 ml flask in one hand, remove cotton plug and maintain in palm of hand, place Pasteur pipette in flask and plug.
9. Take flask to lightbank shelf and connect Pasteur pipette to airline. Regulate air bubble so as to have good mixing.

Maintenance of 500 ml cultures

1. 500 ml cultures initially inoculated with master cultures will take some time to reach required densities (2–4 weeks depending on strength of inoculum).
2. Once required densities are obtained, 500 ml cultures are inoculated twice a week, always maintaining cells in exponential phase of growth.
3. Prepare 500 ml flasks as above, but filling with 350 ml of seawater.
4. Transfer inoculum from 500 ml flask directly from flasks using microbiological techniques, working by the flame and avoiding for the mouths of the flask to touch, thus preventing contamination.
5. Label flasks with algal species and date.
6. Add nutrients using techniques described above, but with higher volumes For F/2 add 1 $ml.l^{-1}$, and for sodium metasilicate add 2 $ml.l^{-1}$.
7. Add Pasteur pipette for aeration using techniques described above.
8. Connect Pasteur pipette to aeration. *Tetraselmis* sp. has a tendency to stick to the bottom, if not well aerated, so more vigorous aeration is usually required for this species.

PROTOCOL–8

INOCULATION OF 4 LITRES FLASKS

Preparation of flasks

1. Clean two flasks for each algal species cultured using commercial grade bleach (5 percent chlorox). If needed, soak in seawater and bleach solution. Rinse well in fresh water.
2. Fill flasks at time of inoculation with 3 litres of 1 µm UV disinfected seawater. If salinity needs to be adjusted, adjust using fresh water (see Protocol–5).
3. Wrap rubber stoppers fitted with glass rods, acting as aerating tubes, in foil and autoclave.

Inoculation of 4 l flasks with 500 ml cultures

1. When algal density in 500 ml flasks reach density >10 000 $cells.ml^{-1}$, use 2/3 of culture to inoculate new 4 l flask. *Note: Remaining volume of 500 ml culture is used to reinoculate a new 500 ml flask. See Protocol–7.*
2. Use microbiological sterile techniques for transfer.
3. Prepare a work area, with a Bunsen burner close at hand, F/2 solution, vitamin solution and sodium metasilicate solution. Keep 5 and 10 ml sterile pipettes, and 3-way pipette bulb nearby.
4. Take 500 ml flask in one hand, remove cotton plug, keeping mouth of flask close to flame. With other hand, flame mouth of 4 l flask.
5. Transfer inoculum from 500 ml flask directly into 4 l flask; avoid the mouths of the flask to touch, thus preventing contamination.

6. After transfer, replace cotton plug quickly on 500 ml, even if discarded later. This ensures you to maintain a clean area, and prevent cross-contamination.
7. Flame stopper (used while cleaning of flask) and mouth of 4 l flask. Close flask quickly.
8. Label flasks with algal species and date.
9. Add nutrients, by pipetting required volume from stock solution, continuously ensuring that pipettes, and flasks are close to the flame. Quickly cap bottles, and transfer nutrients to 4 litres, flaming the mouth of the flask, before addition of nutrients and after. In between addition, keep flask closed with stopper. If you need to put stopper down, make sure it is put on a clean area. For F/2 add 1 $ml.l^{-1}$, and for sodium metasilicate add 2 $ml.l^{-1}$.
10. When nutrient addition is complete, unwrap rubber stopper with aerating rods, and flame. Replace plain rubber stopper with aerating stopper.
11. Connect aerating stopper to aeration tube on shelf by lightbank. *Tetraselmis* sp. has a tendency to stick to the bottom, if not well aerated, so more vigorous aeration is usually required for this species.

Remember:

Always use a different pipette for each algal culture species to avoid cross-contamination.

Use a different pipette for each nutrient solution (F/2, vitamin, sodium metasilicate).

Note: For ease of inoculation, it is best to inoculate one species at a time. Do 500 ml sub-inoculation on same day as 4 l. For example: Start with T-Iso, sub-inoculate two new 500 ml and two 4 l. Add nutrients, aeration, connect to air supply. Clean bench, discard pipettes, and work with second algal species.

2.3.4 100 litres cultures: semi-continuous method

The volume of algae required in hatchery operations, mainly for larval, post-larval, and broodstock purposes, is harvested daily from 100 l vessels. The set-up at the BBSR hatchery yields approximately 120 l of algae a day.

Preparation and inoculation procedure for 100 l vessels are given in Protocol–9. Similarly to 4 l flasks, 100 l vessels are filled with 1 µm filtered UV disinfected seawater. These large-scale cultures are reared on a semi-continuous cycle. This yields a culture in a continuous exponential phase of growth, and minimizes labour. As a culture is harvested daily, and decreases in volume, new water and nutrients are added; this boosts the culture growth, such that algal densities reach 12 000 $cells.ml^{-1}$ within three days. A first inoculum is given initially, and thereafter, addition of new water and nutrients done on a regular basis allows the culture to be maintained for at least one month or more depending on cleanliness of the culture and of the techniques.

Details of the procedure are given in Protocol–9. For a first inoculum, vessels are filled to 25 litres or 50 litres depending on the strength of the inoculum. The lower the strength, the smaller the volume of new seawater inoculated. A 4 l flask is used for inoculum. Culture media, vitamins and sodium metasilicate are added dependent on volume. Algal cultures are allowed to grow for a period of 5 days. At this time, addition of seawater is done, and vessels are filled. Cultures are allowed to grow for a period of 3–5 days; at which time, daily harvest of cultures for the hatchery complex is possible. One hundred litre vessels are harvested down to 25 litres in approximately 4–5 days. When 25 litres of culture remain, 75 litres of new 1 µm UV disinfected seawater is added with culture media. It usually takes approximately 3 days to reach harvest density. In this way, semi-continuous cultures can be maintained on the average 4–6 weeks depending on cleanliness of techniques.

PROTOCOL–9

INOCULATION AND SEMI-CONTINUOUS CULTURE OF 100 LITRES VESSELS

Preparation of vessels

1. Allocate two vessels to each algal species.
2. Clean vessels outside using a fresh water hose and commercial grade bleach. If needed, muriatic acid can be used. Care must be taken when using muriatic acid. Special attention is given to the bottom of the cone, valve area, and rim of the cone and other edges when cleaning these vessels. If needed, the bottom of the cone is soaked in seawater and chlorox solution for a few days.
3. Rinse well with fresh water.
4. Fill vessels with UV disinfected seawater prior to inoculation.

Inoculation of 100 l vessels with 4 l cultures

1. Connect 100 l vessel to airline and turn slight airflow on. It is important to do this, prior to addition of water or algae, as if there is no airflow, water will pass through the Tygon tubing and soak the bacteria filter. If this occurs, the bacteria filter needs to be replaced.
2. For a first inoculation, fill vessel to 25–50 litres, depending on the density of the inoculum. The denser the 4 l culture, the greater an initial volume of water can be used.
3. When density in the 4 l flasks reaches an algal count >10 000 cells.ml^{-1}, use culture to inoculate 100 l vessel. *Note: Take care not to use the very bottom of the culture, as it often contains some precipitate and detritus*.
4. At this time, sterile microbiological techniques are no longer required. A 4 l culture is simply poured into a 100 l vessel. Also, diatoms appear to grow well in full salinity in these large volumes, and there does not seem any need to adjust the salinity.
5. Label vessel with date and algal species.
6. Add nutrients using 25 ml graduated cylinder or 10 ml pipettes. For 100 l vessels, F/2 solution is taken directly from purchased containers; equal parts of A and B are mixed, according to manufacturer's instructions.
7. When nutrient addition is complete, place lid on top to avoid any detritus from falling and adjust aeration so as to create gentle mixing of nutrients throughout. Again, more vigorous mixing is needed for *Tetraselmis* species.
8. Allow for algal cultures to grow until dense. At this time, fill vessel with UV disinfected seawater. If volume of seawater added is 75 litres, add nutrient volume in accordance to 75 litres volume. For example: in Bermuda, 14 ml of nutrients in total is added to 100 l; during semi-continuous culture, if 75 ml of new seawater is added, only 10.5 ml of nutrients would be added.
9. Allow algal cultures to grow. When densities required are reached begin harvesting using valve at the bottom of the cone. Culture should be harvested within 6–7 days. Do not harvest below 25 l.

Semi-continuous culture method

1. Once culture volume has decreased to 25 litres, add 75 litres of new UV disinfected seawater and adequate nutrient volumes.
2. Label with date of added water.
3. Allow cultures to grow for 3 days before harvest.
4. Note: Cultures can be boosted at any time; if a culture of 50 litres volume needs to be reboosted, add 50 litres of seawater and according nutrients.
5. Although sterile techniques are not used for these large cultures, cleanliness is a must to avoid cross-contamination between vessels.

2.3.5 Monitoring of algal cultures

Algal cultures should be examined daily for clumps or aggregations of cells on the bottom. Colour of the culture is most important, and with experience, one can quickly determine if a culture is healthy. Microscopic examination of the algal culture should be done routinely using a compound microscope. Cultures should have cells of uniform size that are not clumped together, and are actively swimming if the species is motile (for e.g. *Isochrysis* sp., *Tetraselmis* sp.). If the cells are clumped, cell walls broken, more than one species present, or a species other than algae present, or if the culture is badly contaminated with bacteria, it should be discarded. The culture vessel should be well cleaned before next use. A bad odour emanating from a culture vessel usually indicates bacterial contamination. Algal cultures used in bivalve hatcheries are not axenic (bacteria-free). In order to have a healthy algal culture, bacterial levels must be kept under control, since they can depress growth of the algae and cause cultures to crash before reaching harvestable densities. Cultures with high level of bacteria should not be fed to larvae but should be discarded; they could be used for broodstock, if needed. If a severe bacterial contamination occurs in stock cultures, every effort should be made to clean the culture with antibiotics, or a new culture should be ordered from an algal culture centre. There are various methods for determining levels of bacteria contamination. Appendix 12 describes bactopeptone testing.

At the BBSR hatchery, a daily routine check of the algal cultures, giving results of visual inspection, is reported on an "Algal culture check" list (see Appendix 13). Monitoring of algal cultures under the microscope is done during periods of sub-culturing or harvesting. During the latter, algal cell density is estimated to calculate the volume required for feeding. There are two methods used most commonly in hatcheries to estimate algal cell density, haemocytometer and coulter counter. Coulter counters are expensive but useful machines. Sometimes used machines can be purchased from hospitals or factories. The time saved and the accuracy of the counts is superior to that when using the haemocytometer. At the Bermuda hatchery, funds were not available for a coulter counter, and a haemocytometer cell is used. This cell was initially developed to count blood cells, and consists of a thick glass slide with two chambers. A special coverslip is placed over these two chambers giving a total volume of 0.1 mm^3 per chamber. The chambers are divided into a grid, to aid in counting cells within the area (Appendix 14). Before counting motile algal species, 1 or 2 drops of 10 percent formalin should be added to a 50 ml sample. The coverslip is mounted over the chambers, and the chambers are filled with the algal sample using a Pasteur pipette. Care is taken not to introduce any air bubbles, as the number of algae estimated is dependent on the exact volume of the chambers. Protocol–10 outlines the procedure used for estimating the number of algal cells using a haemocytometer cell.

PROTOCOL–10

ESTIMATING CELL DENSITY USING A HAEMOCYTOMETER CELL

1. Collect 10–20 ml of algal culture in a scintillation vial.
2. Add 2–3 drops of 10 percent formalin to culture if flagellate species to stop it from swimming. Mix sample thoroughly.
3. Mount cover slip on haemocytometer cell.
4. With a Pasteur pipette, retrieve 1 ml of sample, and introduce a drop into the chamber at the edge of the 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. Allow 1 or 2 minutes for cells to settle out on bottom of counting chamber.
7. Using a counter, count number of algal cells in at least three of the 25 squares. Count all cells lying within the square or overlapping the lines on the right-hand or bottom sides. Each square measures 0.2x0.2 mm.
8. Calculate the average number of cells per square.
9. To obtain the cell density: Average number of cells/square x 250. This gives you number of algal cells per µl.
10. To obtain the number of cells per ml multiply above number by 1 000.

Explanation:

Each cell is 0.004 mm^3
Average number of cells is per 0.004 mm^3
To obtain average number of cells per mm^3, multiply by 250. 1 mm^3= 1 µl
There are 1 000 mm^3 in 1 ml. Multiply average by 1 000 to obtain number of cells per ml.

2.3.6 Alternate feed for spat

The production cost of microalgae using conventional phototrophic means, as described above, is high, ranging from 20–50 percent of hatcheries' operating costs (Coutteau and Sorgeloos, 1992). Nutritionally adequate alternatives have been sought that may be more cost-effective than on-site algal production. Some of those tested include spray-dried, heterotrophically grown microalgae (Langdon and Onal, 1999), and microencapsulated artificial diets (Laing, 1987). Although these show potential in future rearing of bivalve spat, they are not commercially available. Other off the shelf alternatives that show more promise include microalgal concentrates; these are produced by centrifugation and refrigerated at 2–4 °C for 1–8 weeks. They have been used successfully as part of mixed or complete diets for larval or juvenile bivalves (Heasman *et al*. 2000). Such microalgal concentrates can either be prepared by hatcheries with existing infrastructure to produce microalgae and concentrates on-site, or by large specialized algal production facilities for sale to hatcheries (Brown and Robert, 2002). It was shown that not all algal species lend themselves to the process of concentration, notably the flagellates, *P. lutheri* and *Isochrysis* sp. (T-Iso) are easily damaged and deteriorate rapidly (Heasman *et al*. 2000). Another alternative is the use of dry, non-live microaglae which can be purchased commercially; studies conducted by several authors (Langdon and Onal, 1999; Davis and Campbell, 1998) found that a mixed live algal diet of T-Iso and *C. calcitrans* supplemented with spray-dried microalgae enhanced juvenile mussel growth; and that a mixed spray-dried algal diet of *Schizochytrium* and *Spirulina* contained the biochemical constituents necessary to satisfy the nutritional requirements of mussels.

In light of the restricted capacity of the algal culture facility at BBSR, replacement of live algal diet with commercially available dry algal mixture was tested on scallop spat. Results of a short study on growth of *E. ziczac* show that food ration composed solely of dry algae meet the nutritional requirements of larger zigzag spat (>3 mm); shell growth was seen to increase by 1.2 mm per week in the first three weeks. This growth was comparable or better to that of spat fed live algae. On the other hand, smaller spat (<3 mm), seemed to fare better in the long term when fed live algae. These results led to the routine use of commercially purchased dry algae for older spat, prior to transfer to grow-out sites. Dry microalgae are purchased from Reed Mariculture (www.instant-algae.com).

Chapter 3

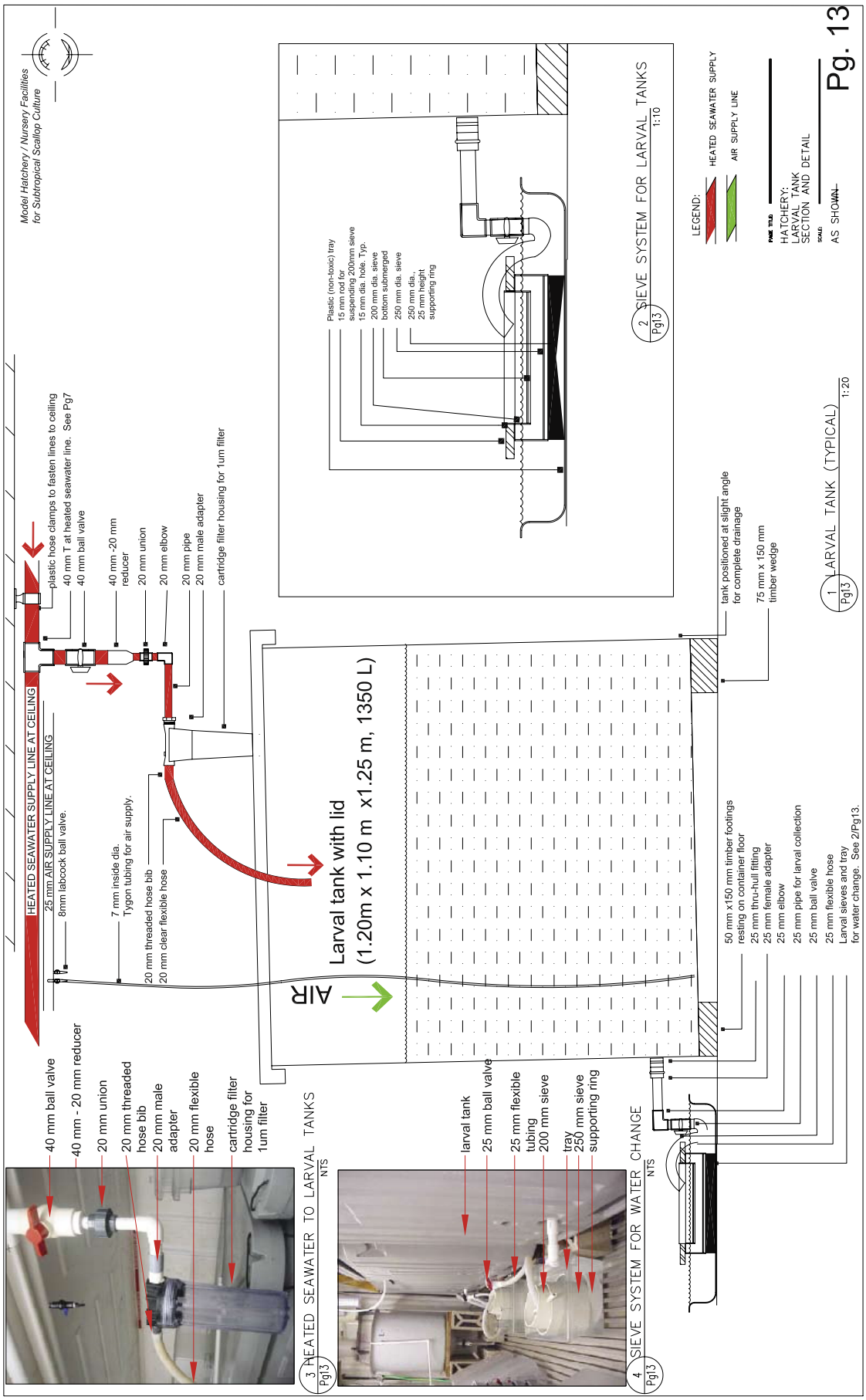
Hatchery: facilities and techniques for larval culture

3.1 HATCHERY FACILITIES

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

Technical drawing, Pg. 13

Hatchery: Larval tank section and detail



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

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

3.1.1 Larval tanks

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

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

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

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

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

3.2 SCIENTIFIC BACKGROUND – EMBRYONIC AND LARVAL DEVELOPMENT

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

3.2.1 Embryonic development to D-larvae

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

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

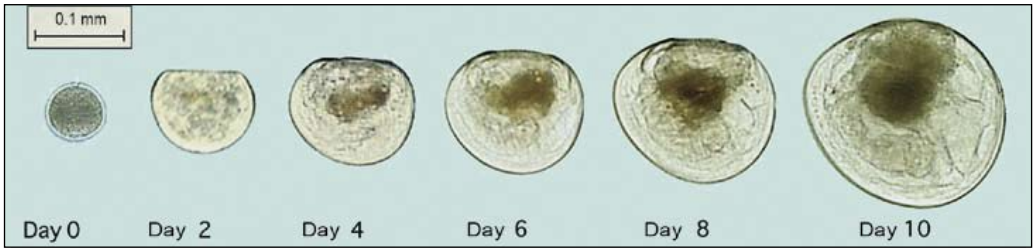


Figure 3.1: Developmental changes of sand scallop larvae to metamorphosis.

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

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

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

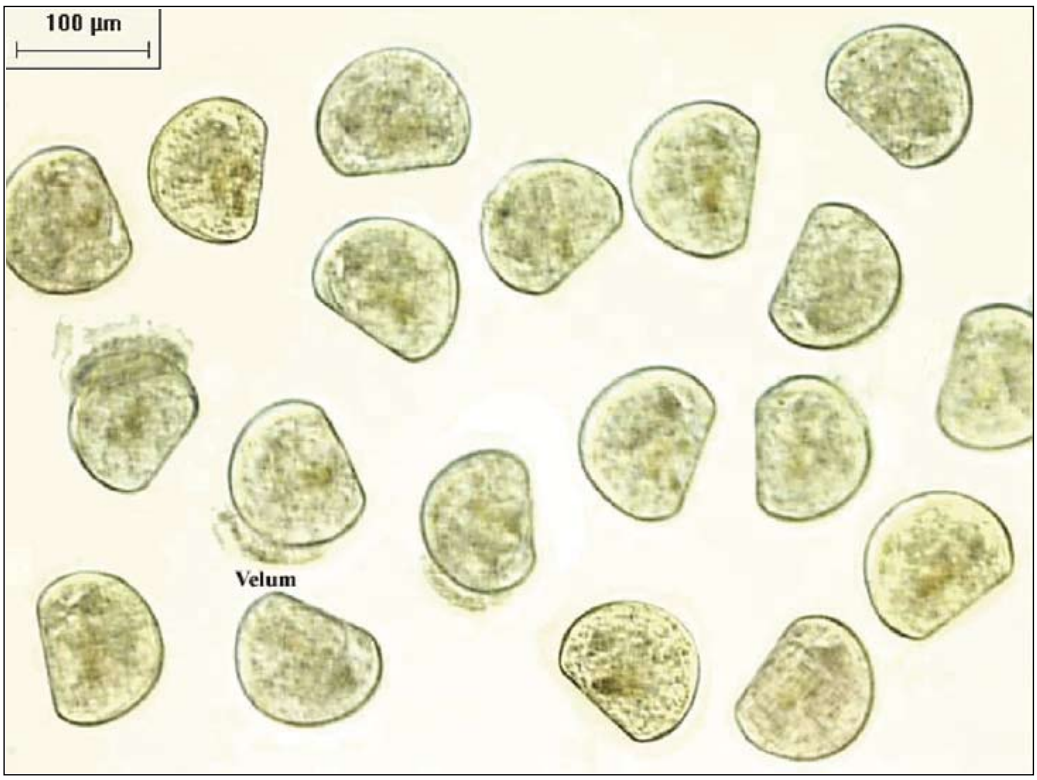


Figure 3.2: One-day old *E. ziczac* veligers showing extended velum.

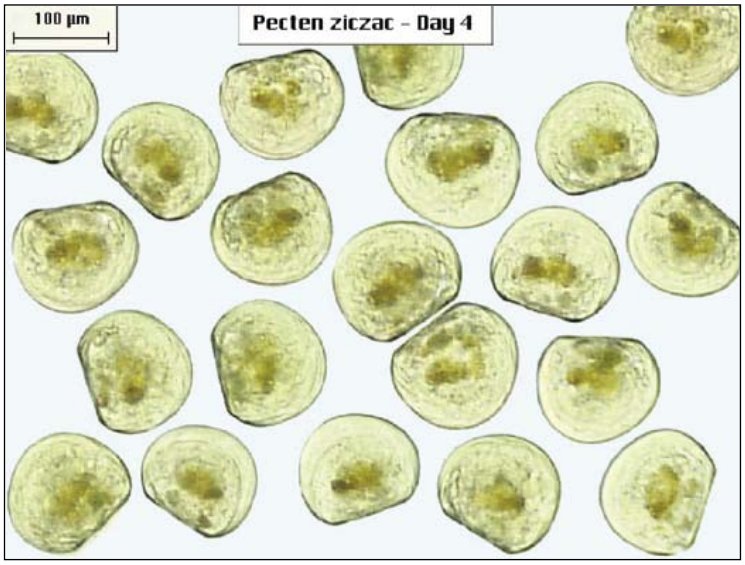


Figure 3.3: Straight-hinge or D-larvae stage of *E. ziczac*.

3.2.1.1 Fertilized eggs: characteristics and developmental requirements

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

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

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

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

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

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

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

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

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

3.2.2 Larval development

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

3.2.2.1 Veliger larvae

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

3.2.2.2 Pediveliger larvae

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

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

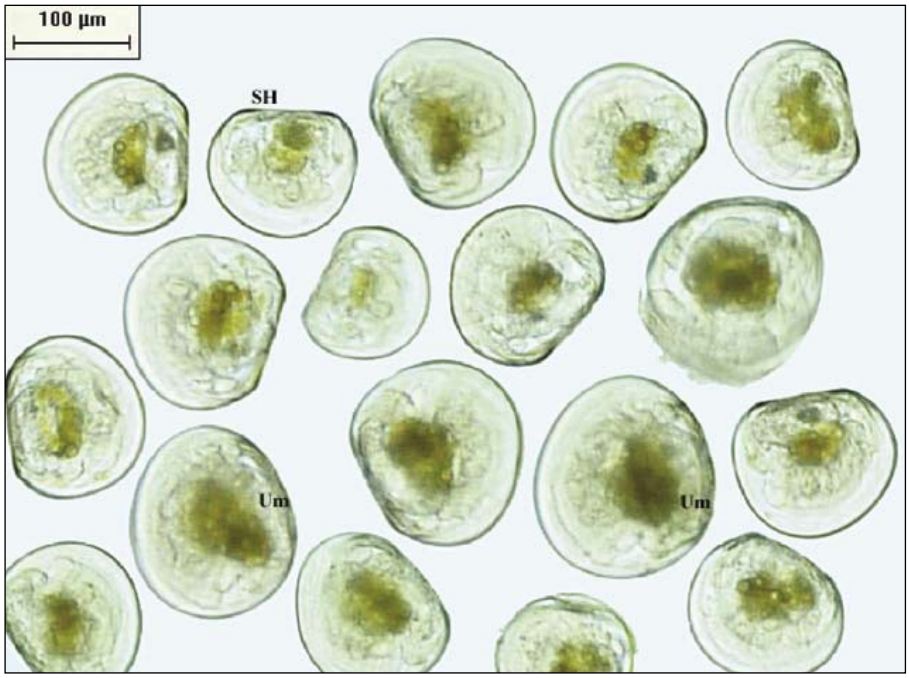


Figure 3.4: Day-6 sand scallop larvae showing initial development of umbones (Um) compared to straight-hinge characteristic (SH) of D-larvae.

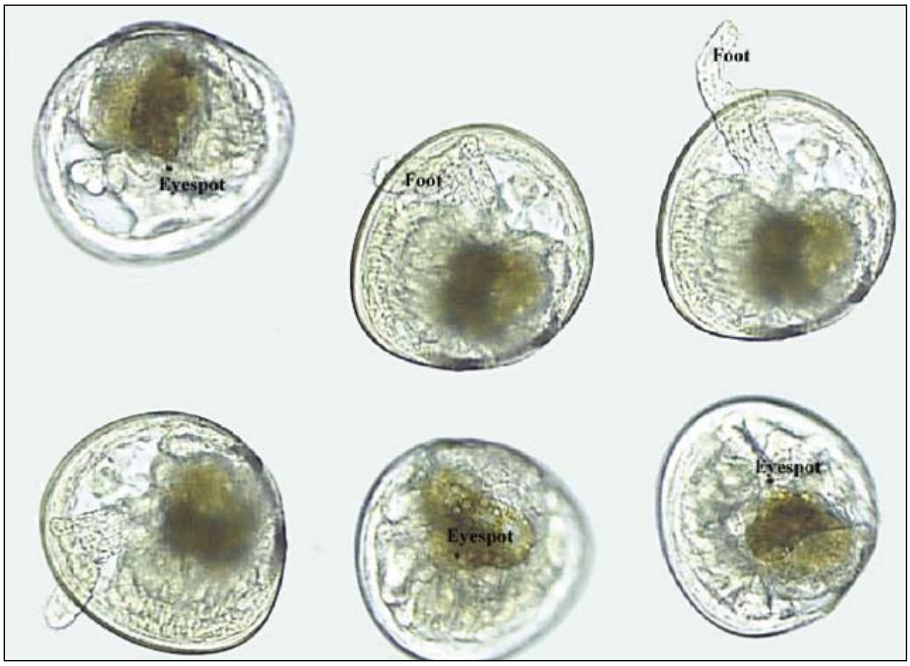


Figure 3.5: Day-11 pediveligers of *E. ziczac* showing eyespot and a well-developed foot in and out of the shell.

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

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

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

3.3 SCIENTIFIC BACKGROUND – FACTORS INFLUENCING LARVAL REARING

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

3.3.1 Temperature

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

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

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

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

3.3.2 Density

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

3.3.3 Salinity

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

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

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

3.3.4 Food ration

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

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

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

3.3.4.1 Effect of food ration on calico scallop larvae

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

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

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

3.3.5 Culture systems: flow-through vs. static

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

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

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

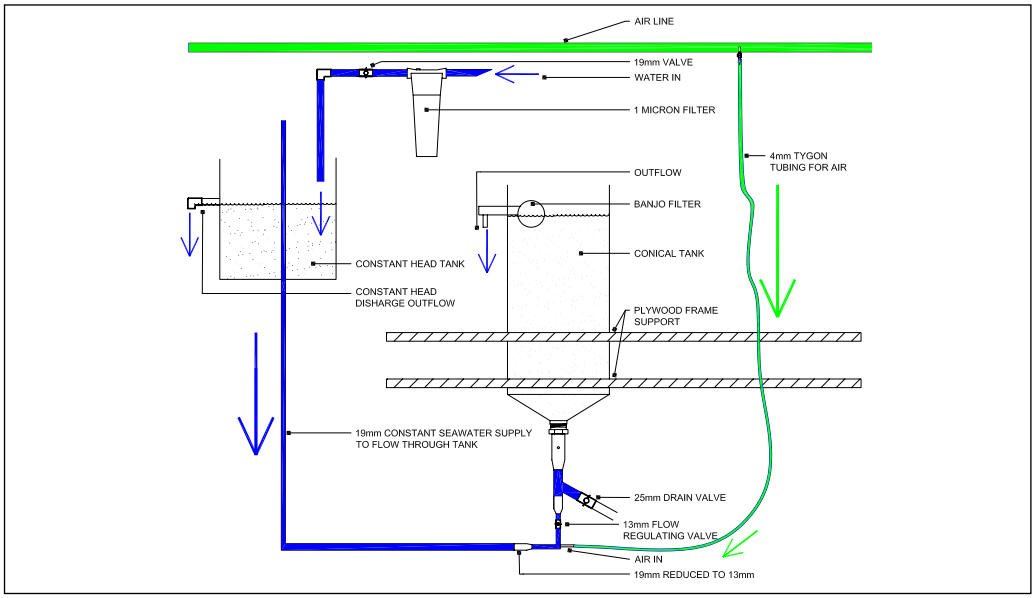


Figure 3.6: Conical tank modified to a flow-through system for larval rearing.

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

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

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

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

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

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

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

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

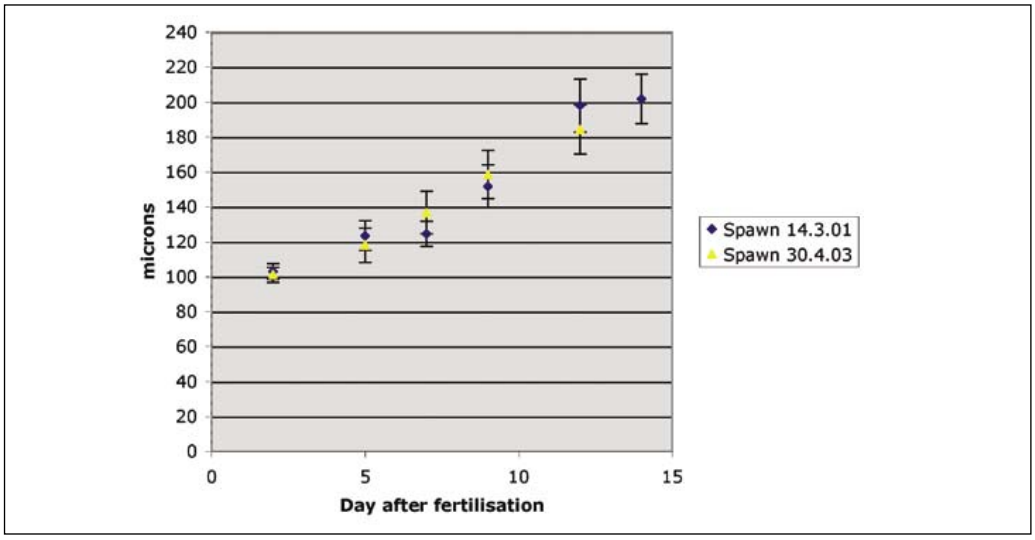


Figure 3.7: Results of shell growth (length) for calico scallop larvae reared in Bermuda. Two curves show maximal and minimal range obtained over 4 years of operation.

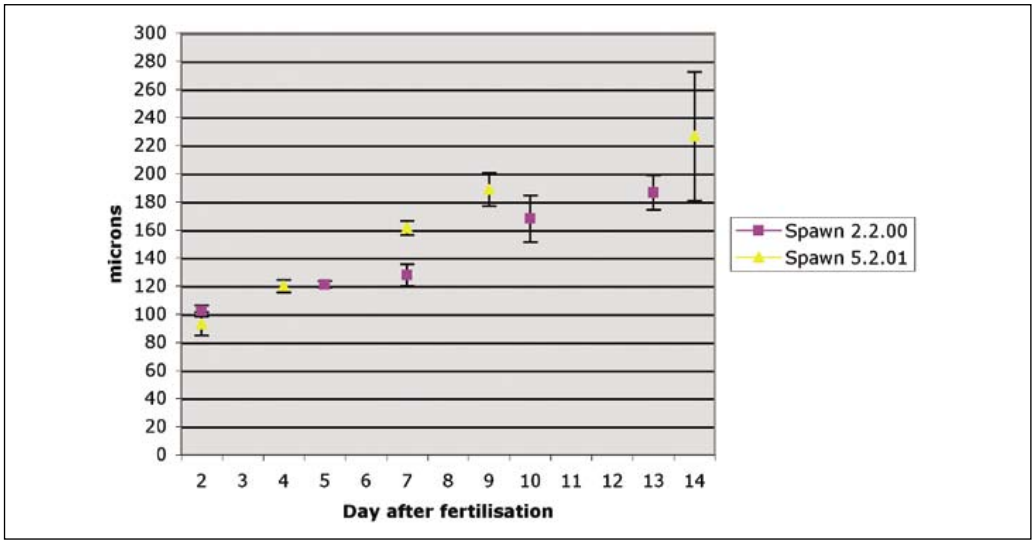


Figure 3.8: Shell growth (length) for zigzag scallop larvae reared in Bermuda. Two growth curves show maximal and minimal length obtained over 4 years of operation.

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

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

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

3.4.1 Larval rearing procedure

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

3.4.1.1 Water change

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

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

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

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

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

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

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

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

PROTOCOL–11

TAKE-DOWN OF LARVAL TANKS: LARVAL COLLECTION AND REDISTRIBUTION

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

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

3.4.1.2 Standard rearing density

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

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

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

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

3.4.1.3 Standard food ration

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

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

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

3.4.1.4 Counting larvae and determining survival rate and shell growth

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

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

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

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

3.4.1.5 Setting of larvae

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

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

Criteria used for initiating settlement at BBSR are:

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

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

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

Chapter 4

Nursery: facilities and culture of post-larvae

4.1 NURSERY FACILITIES

Two methods of settling larvae are used at the model hatchery/nursery in Bermuda. The first involves the setting of pediveligers on a downwelling system of sieves in a raceway and continued rearing fixed spat until they reach at least 2 mm in shell height;

spat may be kept in this system until they reach 5–10 mm shell height. This method requires continuous monitoring in the nursery and is labour intensive; nonetheless, it maximizes survival of spat to the juvenile stage. The second system, involves the setting of larvae on cultch (or artificial substrate) submerged in a circular tank for a shorter rearing time until spat reach 1 mm shell height. In this latter system, spat are transferred to the natural environment on a suspended culture system of longlines within one month of settlement. This method, maximizes space in the hatchery, however, survival to juvenile stage (5–10 mm shell height) is reduced. The method chosen depends on the specific strategies selected by the hatchery and on conditions prevalent in the field at the time of transfer. Facilities for both methods are shown here as they are both used in Bermuda. However, should one be chosen over the other, modifications can be easily made to the facility.

4.1.1 Semi-recirculating raceway system (indoor)

Refer to Technical Drawing – page 14. Colour codes are similar to that of other diagrams, in that ambient seawater is coded blue, outflow is coded purple and semi-recirculating flow is coded white.

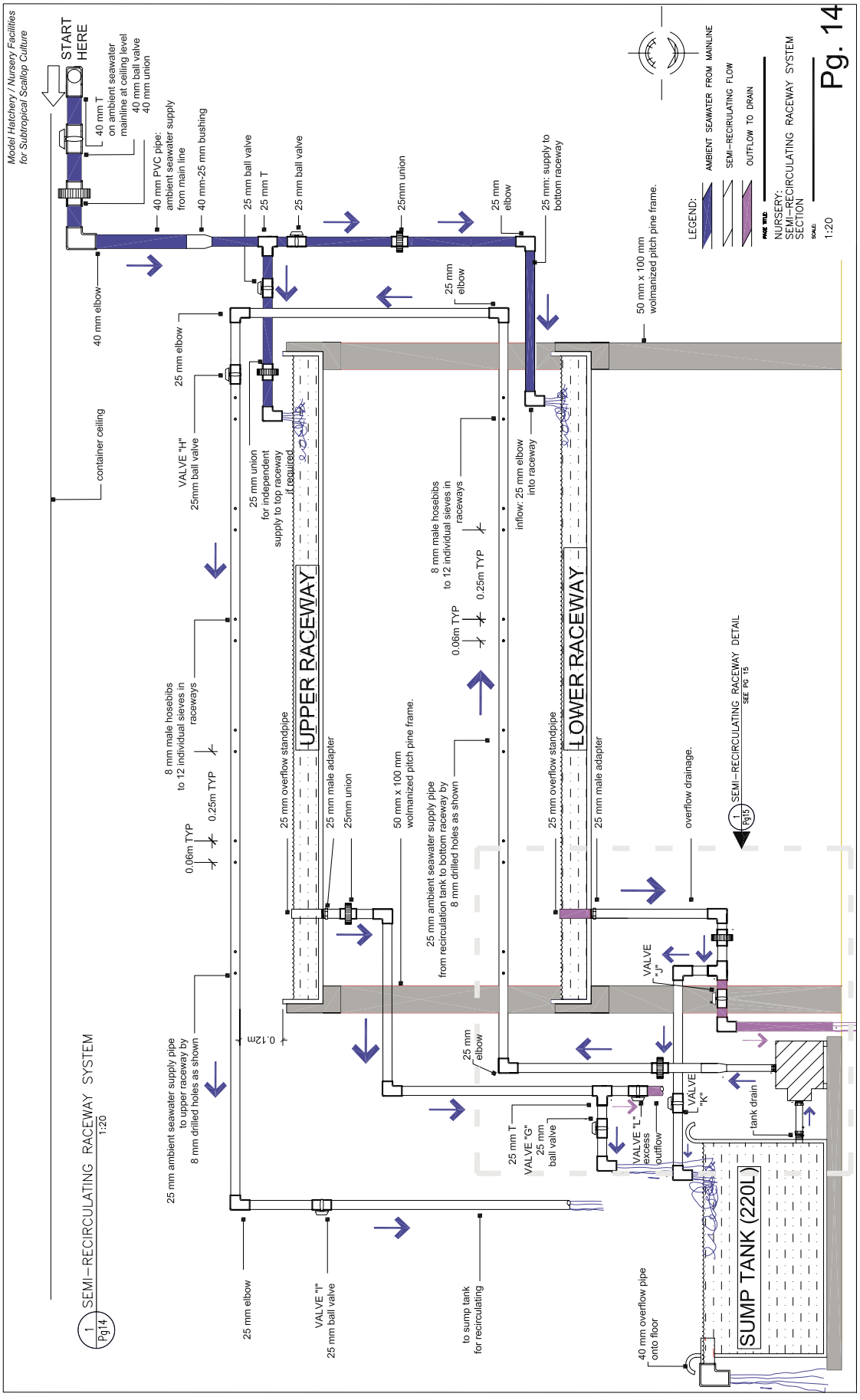
The raceways are two 200x60x15 cm deep fiberglass flow-troughs, with blue gel coated interior and grey pigmented exterior (Red-Ewald Inc.), mounted on a side wall, one above the other, and supported by a wooden frame (60x130 cm). For protection against saltwater the wooden frame is coated with several layers of epoxy. Each raceway has a 25 mm drain hole into which a 25 mm male adapter can be threaded and fitted with an overflow standpipe.

Seawater for the raceway is diverted from the mainline fastened to the ceiling, containing ambient seawater filtered to 1 µm. Diversion is created by a 40 mm T. Flow to the raceway system is regulated by a 40 mm one-way ball valve. A 40 mm union allows for dismantling and cleaning of system. The 40 mm pipe is reduced to 25 mm using a 40 mm to 25 mm bushing. Seawater flow is divided between the top and bottom raceway as it passes through a 25 mm T. Flow is regulated individually to each raceway through a 25 mm ball valve. The fitting of 25 mm unions after each ball valve facilitates cleaning of the pipes at the beginning and end of season. A 25 mm elbow directs the flow into the raceway. This therefore provides ambient seawater to the raceways. For the rearing of spat, the system is usually connected as a semi-recirculating system, where a constant inflow of ambient seawater is provided and partial recirculation of seawater is set. This minimizes the waste of algal food cells provided to the spat and can also be used to control seawater temperature, if needed, during the settlement phase. *Note: seawater control can be affected by manipulating seawater temperature in the sump tank.*

For the semi-recirculation system, a 220 litres sump tank is located on the floor next to the bottom raceway. Overflow of the sump tank is at the top through a 40 mm pipe and spills onto the floor. Discharge rate can be easily monitored using this overflow. Seawater is pumped from the bottom of the tank by a quiet vertical pump (P 95V). *Details and dimensions are given in technical drawing – 1/15A.* Water pumped from the bottom of the sump tank passes through a 25 mm pipe affixed to the wall of the container above the raceways. The recirculation pipe follows the length of the lower raceway, bends upward towards the upper raceway through two elbows, and passes through a 25 mm valve H. This valve is an additional control to the flow of water in the raceways. The 25 mm seawater line continues above the upper raceway and runs to the end of the raceway where a 25 mm elbow directs the flow of water through a one-way ball valve (Valve I) to the sump tank. This recirculated water is supplied to the raceways through 8 mm holes drilled into the pipe running the length of the raceway

Technical drawing, Pg. 14

Nursery: Semi-recirculating raceway system section



sand fitted with male hose barbs. Tygon tubing connected to these hose barbs are cut in pieces long enough to reach individual sieves suspended in the raceway.

Flow for each raceway is therefore twofold: 1) Incoming flow from the ambient seawater line at the right hand side of the raceway, which flows directly into the raceway trough and empties into the sump tank via the drain; and 2) Seawater flow from the sump tank system supplying each sieve separately. A constant water level is maintained in each raceway by the overflow standpipe. Each standpipe is connected to a 25 mm line fitted under the raceway and connected to a 25 mm union for dismantling and cleaning of pipes. A T-junction diverts the water back to the sump tank for recirculation or directly to the outside through the container wall draining into the external 100 mm drain system. The system is identical for both raceways. For the upper raceway, control of flow to drain is regulated by Valve L; for the lower raceway, it is controlled by Valve J.

The degree of semi-recirculation is controlled by Valves K, G, H and I regulating flow into the sump tank, and by Valves J and L, regulating the degree of water discarded from the raceway overflow. To achieve equilibrium, valves are opened in the following manner: Valves H and I are wide open; Valves G and K are 3/4 opened, and valves J and L are fully closed. Any excess water flows out of the sump tank through the overflow pipe. The last two valves J and L are only opened for cleaning of raceways.

4.1.1.1 Details of sump tank

Refer to Technical Drawing – page 15A. Details in Diagram 1/Pg15A show a close-up of connections required. The sump tank drain is a thru-hull fitting (20 mm) connected to the vertical pump via a 20 mm pipe and male adapter. This pump is supplied with 20 mm fittings. The outflow of the vertical pump is conducted through a 20 mm female adapter. Water passes through a 20–25 mm bushing increasing the pipe size to 25 mm. A union facilitates the removal of the pump at this end, and the adapter fittings on either end of the pump aid in its replacement when needed.

The seawater line from the pump is taken back to the wall of the container, and runs parallel to the container wall to supply the raceways (see technical drawing photo – 3/pg15A). Once water has passed along raceway contours (as described in the technical drawing – page 14) it is returned to the sump tank. Additional recirculation is provided from the overflow pipe of each raceway, where seawater is passed through a 25 mm T-junction and controlled by Valve G for the upper raceway, and Valve K for the lower raceway. For cleaning of the raceways and complete drainage, Valves G and K are closed, and outflow is diverted through Valves L and J (upper and lower raceway respectively) for discard (see technical drawing photo – 2/pg15A).

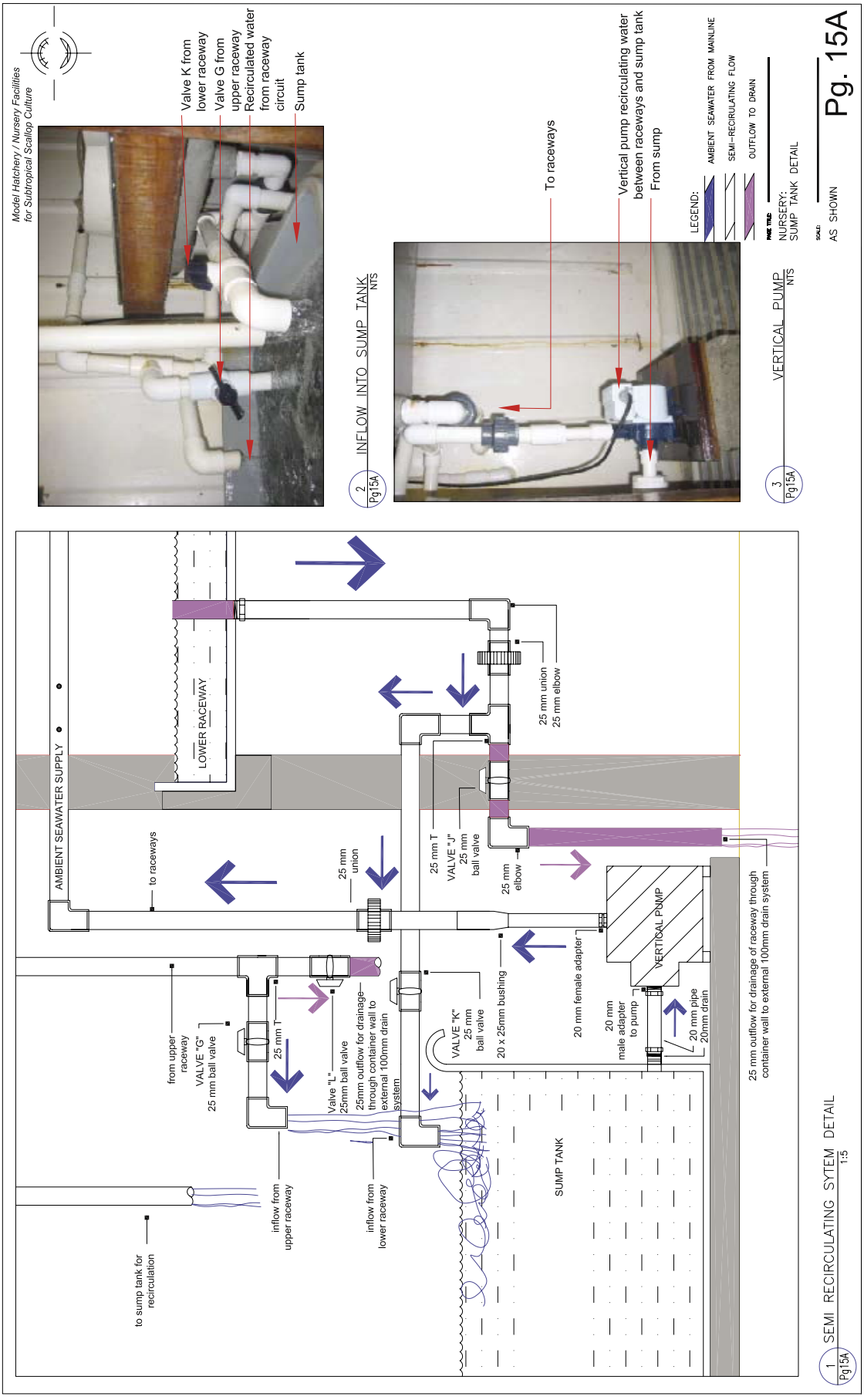
Refer to Technical Drawing – page 15B. The sump tank with overflow discharge and incoming flow is illustrated in Diagram 1/Pg15B. The relative positioning of both raceways and sump tank is seen in Diagram 2/Pg15B. Finally, the sieves used for post-larval rearing and suspended in the raceway, are set as a downwelling system in Diagram 3/Pg15B with incoming seawater flowing into each sieve. Two 25 cm diameter sieves can be placed widthwise and with a total of 12 sieves per raceway. Sieves are suspended by 15 mm transverse pipes resting on the top of the raceway.

4.1.2 Outdoor raceway

Refer to Technical Drawing – page 16A. An experimental outdoor raceway is used to grow 2 mm spat to >5 mm, a size suitable for transfer to 3 mm mesh pearl nets for growout. This is found beneficial as survival following transfer at sea is observed to be dependent on size of spat at transfer. In this outdoor nursery spat are reared in sieves, similar to the indoor raceway, but of slightly larger diameter (30 cm) and of larger mesh

Technical drawing, Pg. 15A

Nursery: Sump tank detail



Technical drawing, Pg. 15B

Nursery: Semi-recirculating system photographs

size (1.2 mm). The system is an upwelling system as opposed to the indoor raceway which is a downwelling system. Seawater and algal supply are supplied at one end of the raceway and passed through the bottom of each sieve flowing out at the top of the sieve. It is found that older spat have better growth and survival when reared in such an upwelling system. In this way spat are not trapped with faecal or detritus material present in the water.

Technical diagram photo 2/Pg16A illustrates the outdoor raceway. It is built on a concrete slab of 3.35x2 m. This support pad is made of a 150 mm reinforced concrete slab on grade. The raceway is constructed of 20 mm, exterior grade plywood coated with polyester resin on all surfaces in contact with seawater. The construction is strengthened by a middle timber brace made of 130x60 cm marine plywood. The entire plywood construction is seated on 4 concrete block series at each corner; raceway channels are thus at waist level and facilitate working. A rooftop, made of corrugated plastic, and fixed onto a wooden frame and 4 wooden posts (130x60 cm), bolted into the trough system, provides shade and protection from debris and rain to the raceway. For further protection for the sides of the raceway, drop-down, fly screen panels are fitted to the canopy.

Refer to Technical diagram – 1/Pg16A. The raceway is 2.56 m in length and is divided lengthwise into 3 sections: a 15 cm wide central drainage channel and two completely separate 43 cm wide raceway channels. These interconnect with the drainage channel via six 2.5 cm diameter drain holes. Coarsely filtered ambient seawater is supplied directly to the raceway from the pump house. T-junction (T7), diverting water from the main line to the exterior raceway is also shown in the overall layout of the facility in the technical drawing – page 1 (Chapter 1). Seawater flow, diverted from the mainline, is regulated by a 50 mm one-way ball valve (Valve M). This point on the line is also one of the lowest points on the entire line and has a 50 mm drain pipe going into the sea for emptying the entire line at the beginning and end of the season. In this drain line, a T-junction diverts the water directly to the outdoor raceway and a 50 mm union valve (Valve N) placed directly afterwards is opened completely when the outdoor raceway is used. The third valve (Valve O) on the other side of the T-junction is only opened for cleaning of the seawater system pipes.

A 50 mm line is laid around the periphery of the cement pad, using 50 mm elbows when required, and clean-out valves are located at the ends to facilitate cleaning of the pipes. On the diagram, clean-out T's shown are associated with bends following the contour of the land not seen in the diagram. Direct inflow of seawater to each raceway channel is provided through a 40 mm pipe. Each raceway channel and central drainage channel has a 40 mm drain, at the opposite end of incoming flow, made of a 40 mm male adapter fitted with an overflow standpipe. Effluent water is then discarded through a 100 mm drain pipe laid on the concrete pad. Seawater supply to the raceway is illustrated and magnified in the technical drawing diagrams – 1Pg16B and 2/Pg16B; for additional clarity a front elevation drawing is given in technical drawing – 2/Pg 17.

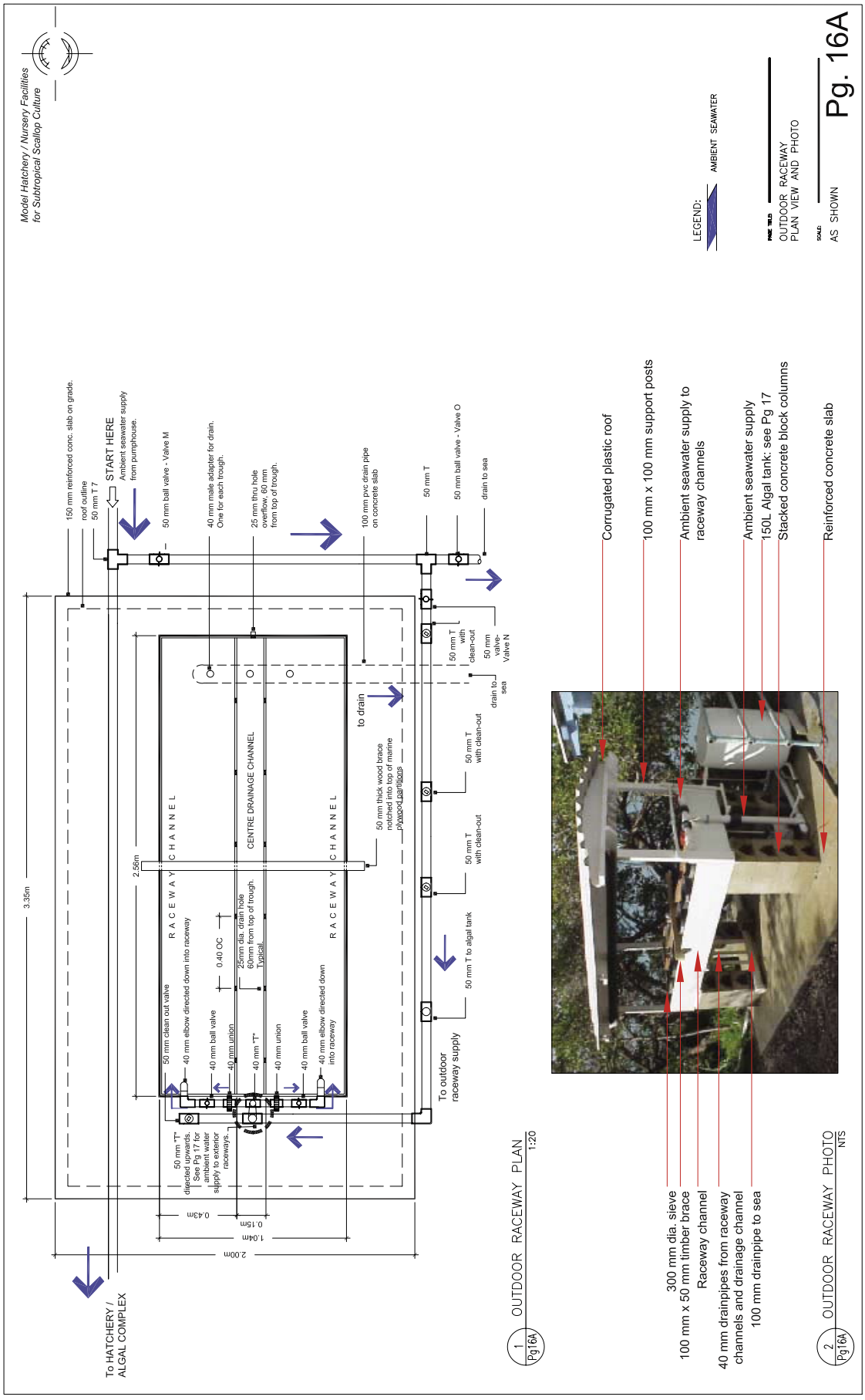
The outdoor raceway is thus an open seawater system, with supply coming from the pump house, flowing into each raceway channel from the left hand side of the diagram and flowing out at the opposite end of the raceway, where it is discharged.

4.1.2.1 Seawater supply to outdoor raceway

Refer to Technical Drawings – pages 16B and 17. Seawater inflow to raceway is magnified in the technical drawing diagrams – 1/Pg16B and 2/Pg16B. From the T-junction, water is equally distributed to both raceway channels; a 40 mm union is connected on both sides of the T-junction and flow to the raceways is regulated

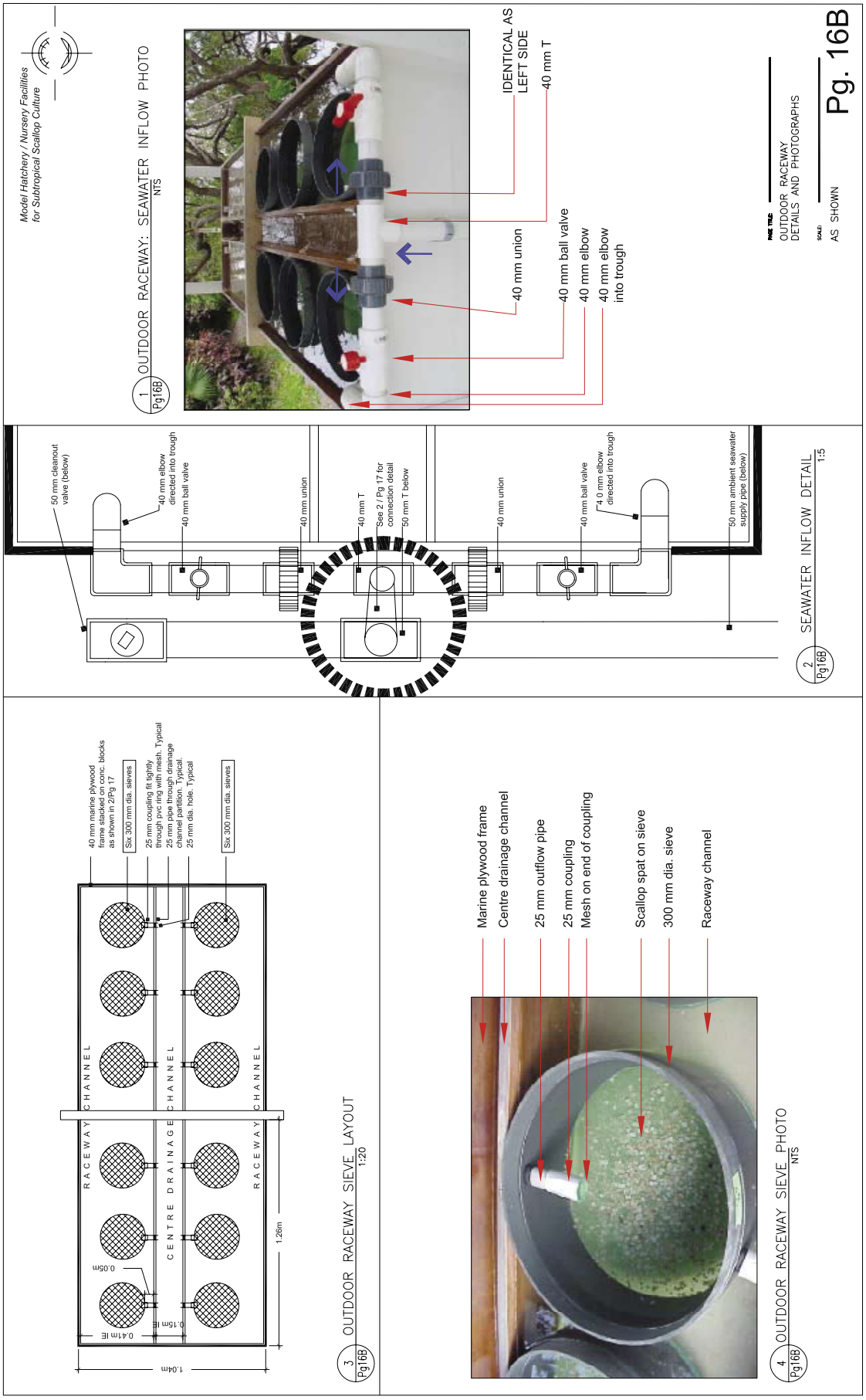
Technical drawing, Pg. 16A

Outdoor raceway: Plan view and photos



Technical drawing, Pg. 16B

Outdoor raceway: Details and photographs



through 40 mm ball valves. An elbow pointing downwards directs the flow into the raceway. Unions fitted in line allow for replacement of parts and cleaning of pipes.

The front elevation of supply to the raceway is depicted in the technical drawing diagram – 2/Pg17. From the main seawater supply running on the ground along the periphery of the concrete pad, flow is directed upwards via a T-junction capped with clean-out valve for maintenance purposes. A 50 mm union is fitted in-line for ease of dismantling followed by a 50–40 mm bushing reducing the seawater supply line to 40 mm. The line runs in the middle of the raceway frame to the top of the channels; at this point, seawater is equally diverted to both raceway channels via a 40 mm T-junction.

4.1.2.2 Sieve layout

Refer to Technical Drawing – 3/Pg16B and 4/Pg16B. Each 25 mm drainage pipe connecting the raceway channel to centre drainage channel is fitted with a 25 mm coupling and pipe connected to each sieve. Sieves are 30 cm diameter, 10 cm high with a 1.06x0.72 mm (1.2 mm diagonal) mesh bonded to its base. This provides for a total of 12 spat holding sieves when the raceway is fully utilized.

The procedure for making sieves is similar to that described in Appendix 17. The mesh used to line the bottom is the same as that used for green collector bags with an aperture size of 1.2 mm on the diagonal. Fitting of the sieves to the raceway and outflow system differed from the indoor raceway system. A 25 mm hole is drilled onto the top of the sieve, through which a 25 mm pipe is tightly secured, extending on either side of the sieve wall by 50 mm. A 25 mm coupling is fitted into the 25 mm pipe in the interior of the sieve. The opening of the coupling is closed by a piece of 1.2 mm mesh to prevent any spat from flowing out. The overflow pipe is secured into the wall of the trough by tightening it into the drilled hole (see technical drawing photo – 4/Pg16B). Sieves are suspended off the bottom; and water flow is directed so as to move through the bottom mesh and out at the top of the sieve into the centre drainage channel, causing an upwelling movement.

Note: The system can readily be adapted for a downwelling flow by inputting water into the central channel and discharging the waste water through 40 mm diameter drainage pipes at the outflow end of each raceway compartment. In this case, the flow enters the sieve at the top and flows vertically downwards through the mesh of the sieves.

4.1.2.3 Outdoor raceway elevations and algal supply

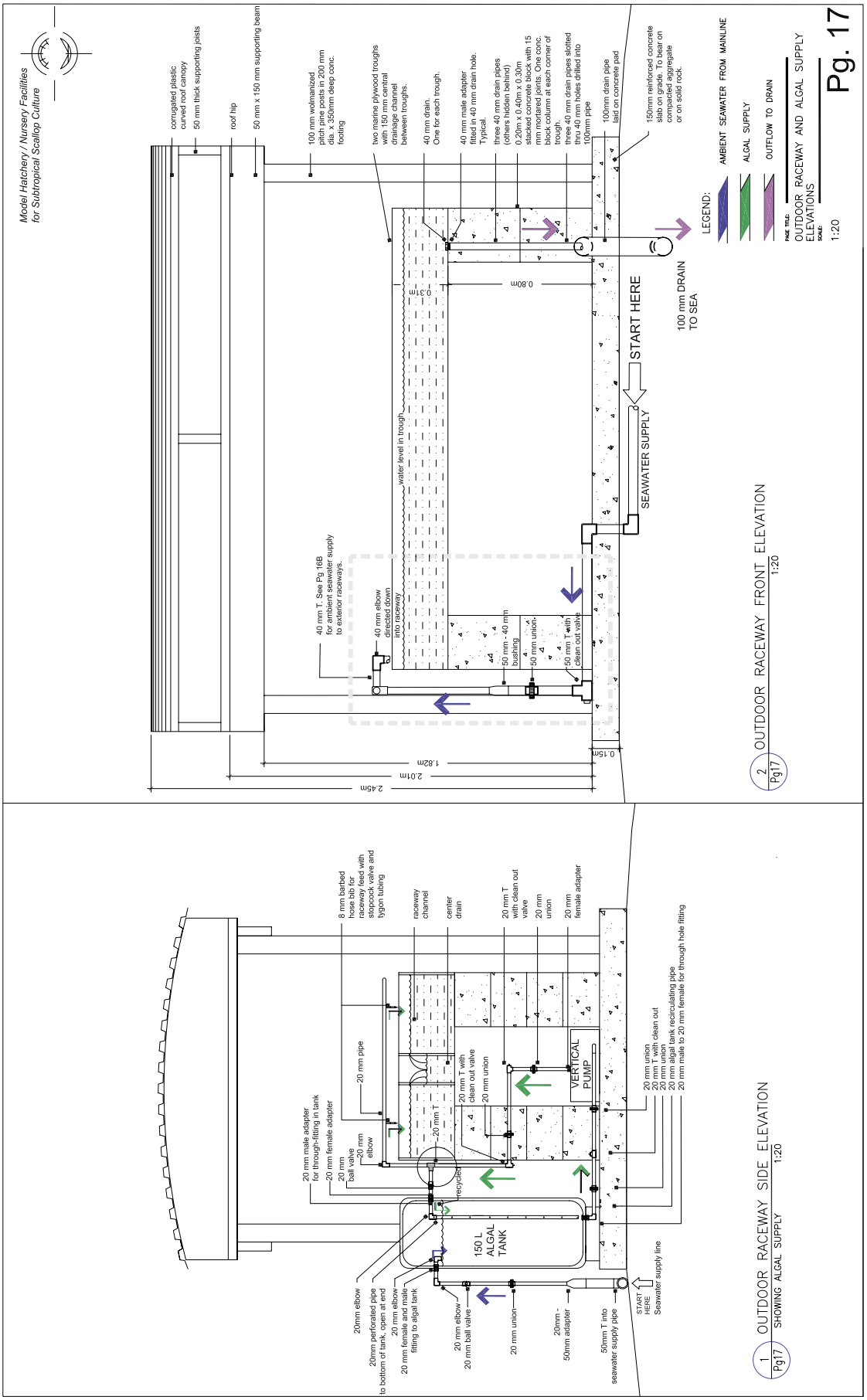
Refer to Technical Drawings – page 17 and page 18). Colour codes for seawater are blue and purple for ambient supply and outflow; additionally, algal supply is coded in green.

The side elevation diagram (see technical drawing – 1/Pg17) depicts the plastic cylindrical tank used as an algal reservoir (56 cm diameter, 75 cm high tank; Volume of 150 l). In order to allow for supply or outflow of algae and seawater, three 20 mm holes are drilled into the wall of the tank. Two holes, on opposite sides of the tank, are close to the top and one is at the bottom. *Position of the holes can be seen in the photos (see technical drawings – 1/Pg18 and 2/Pg18).* The holes are sealed with an O-ring and fitted with a 20 mm female adapter to 20 mm male adapter to make a watertight connection.

There is a small demand for seawater supply to the algal tank on a daily basis for diluting of algal ration and cleaning of tank. For this purpose, the ambient seawater line coming from the pump house is first diverted to fill the algal tank (see technical drawings – 1/Pg17 and 1/Pg18). It is immediately reduced to 20 mm by a 50 mm to

Technical drawing, Pg. 17

Outdoor raceway and algal supply elevations



Technical drawing, Pg. 18

Outdoor raceway: Algal and seawater supply photographs

20 mm adapter. A 20 mm union fitted in line allows for removal of tank and cleaning of pipes when necessary. Flow is regulated by a 20 mm ball valve; seawater passes through the tank wall, and is directed downward via a 20 mm elbow. *Note: A bleed line (7 mm Tygon tubing) for the seawater supply, is put in as a precautionary measure, but was actually never needed; it is not recorded in these diagrams, but can be seen in the technical drawing photo – 1/Pg18).*

For algal supply to the raceway channels, algal solution is pumped from the bottom of the 150 litres tank and runs through a 20 mm pipe parallel to the ground. Two 20 mm union are fitted in line close to the algal tank for daily cleaning of the tank. A 20 mm T-junction, capped on one end for cleaning, directs the flow to a quiet vertical pump Fittings for the pump are typical, as described in the technical drawing – page 15, and are supplied with the pump. Algal solution is passed through a 20 mm pipe alongside the raceway wall. Each end is capped by a cleaner valve, facilitating cleaning using a pipe brush. The algal line follows the contour of the raceway channels, running along the width of the channels. For each raceway channel an 8 mm male NPT fitting (hose barb) is threaded into an 8 mm hole. Algal feed is provided through Tygon tubing (7 mm) and regulated by a stopcock valve. Algae are thus continuously injected into the raceway channels at the point of seawater inflow. As pressure from the pump is high, and flow of algae restricted into raceway, an overflow valve is fitted following a T-junction (at the top of the algal tank) in the algal supply line; excess algae is thus passed through a 20 mm pipe through the tank wall for recycling. Any excess algae is mixed into the tank, as it is forced through a 20 mm perforated pipe running the length of the 150 litres tank, stopping 25 mm off the bottom.

The elevation view for the entire raceway also depicts the roof of the raceway and its wooden framework supporting the canopy, the stacked concrete blocks elevating the raceway to waist level, the concrete pad and seawater inflow and outflow. The framework supporting the canopy drawn is a sound engineering design and differs to that quickly constructed at the Bermuda hatchery (see technical drawing photo – 2/Pg16).

Technical drawings photos – 2/Pg18 and 3/Pg18 clearly illustrate the location of the algal supply line relative to the seawater supply line.

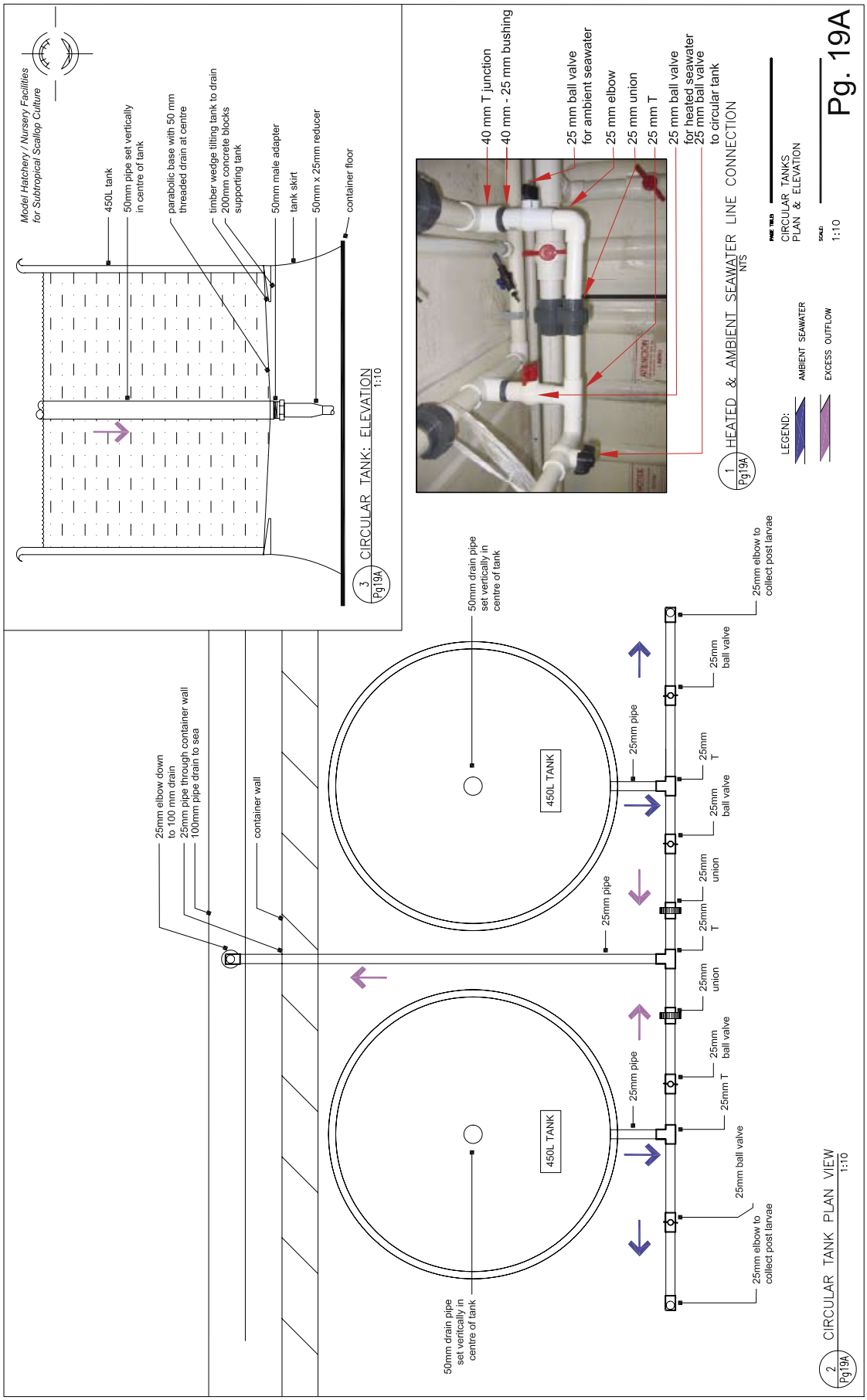
4.1.3 Circular tanks

Refer to Technical Drawings – page 7, 19A and page 19B. The seawater and air supply lines for all tanks including the circular tanks described in this section are shown in a general ceiling plan in technical drawing – page 7. As a reminder, ambient seawater lines (coded blue) and heated seawater lines (coded red) run parallel to each other along the length of the container.

Refer to technical drawing – 1/page 7. Circular tanks are connected to both the heated seawater line and ambient seawater line via three connections. Ambient seawater, filtered to 1 µm, is diverted from the main line via a 40 mm T-junction; for the first connection, seawater is directed to the left, for the other two connections, seawater is directed to the right). The end of the 1 µm filtered seawater line is capped). All connections are similarly fitted, and details of one connection are shown in the technical drawing photo – 1/Pg19A. The connections are identical for both heated and seawater line. In-line of main supply pipes a 40 mm T diverts the flow of water downwards towards the circular tank. A 40 mm to 25 mm bushing reduces the circular tank line to 25 mm. The flow of seawater is regulated by a 25 mm one-way ball valve, glued to an elbow. This elbow allows for ambient seawater to flow into the circular tank. A 25 mm union is glued in line for cleaning and/or replacing of parts. After the union is a 25 mm T-junction directing the water flow to the circular tank and connecting this inflow

Technical drawing, Pg. 19A

Circular tanks: Plan and elevation



pipe to the heated seawater line. Inflow is regulated by a 25 mm valve and water is distributed at surface of tank.

Refer to technical drawing – Page 19A. The tanks used are of 1 m inner diameter fiberglass with a 10 cm wide top lip, 68 cm high and a 15 mm tapered bottom with a 50 mm centre threaded drain. A 30 cm skirt was added by the company (Red Ewald Inc.). See technical drawing diagram – 3/Pg19A. Interior of tanks was coated with blue gel coat and exterior was of grey pigment. A 50 mm standpipe of 80 cm length is used for overflow. The total capacity of the tank is 450 litres. For drainage a 50 mm drain is fitted with a 50 mm male adapter glued to a 50 mm elbow directing the drain pipe parallel to the floor. The remainder of the line is reduced to 25 mm with a 50 mm to 25 mm bushing. The plan view (see technical drawing – 2/Pg19A) shows the remainder of the connection. The 25 mm pipe extends throughout a ready-made hole in the skirt of the tank and connects to a 25 mm T-junction. This T-junction allows either for collection of swimming non-fixed spat during water exchange, or diverts the outflow to an exterior 100 mm drain system. For the collection of swimming spat, flow is controlled by a one-way 25 mm ball valve fitted with an elbow. A flexible hose is attached to the elbow during collection. For drainage to the outside a mirror system on the opposite side of the T-junction is installed; similarly a one-way 25 mm ball valve regulates the outflow. A 25 mm union allows for cleaning of the system and connects to a T-junction. This junction is the intersection of drainage pipe for two 450 litres tanks. Water is sent to the exterior drain through the container wall.

The 450 litre tanks can be set up as an open, semi-recirculating or recirculating system, useful during spat settlement. Technical drawing photo – 4/Pg19B shows the set-up for an open system where seawater is continuously supplied via a 25 mm pipe; for this, outflow valves are opened completely, and collecting valves closed. Technical drawing photo – 5/Pg19B illustrates the set-up for a semi-recirculating or recirculating system. Four 25 mm pipes of the same height as the tank are vertically placed at opposite ends of the tank and connected together using two T-junctions and four 25 mm transverse pipe lengths in the centre. Additionally, four 100 mm long standpipes are fitted to each vertical pipe via a 25 mm T-junction. The centre 25 mm transverse pipes are drilled with 8 mm diameter holes. An airlift system is achieved by a 7 mm ID Tygon tube connected to the airline and inserted into a vertical pipe for its entire length. In this way recirculation of water is created, where water from the bottom of the tank is driven by air up the vertical pipe, and flow out through the holes of the transverse pipe. It is found that two airlines inserted in two vertical pipes provide sufficient airlift.

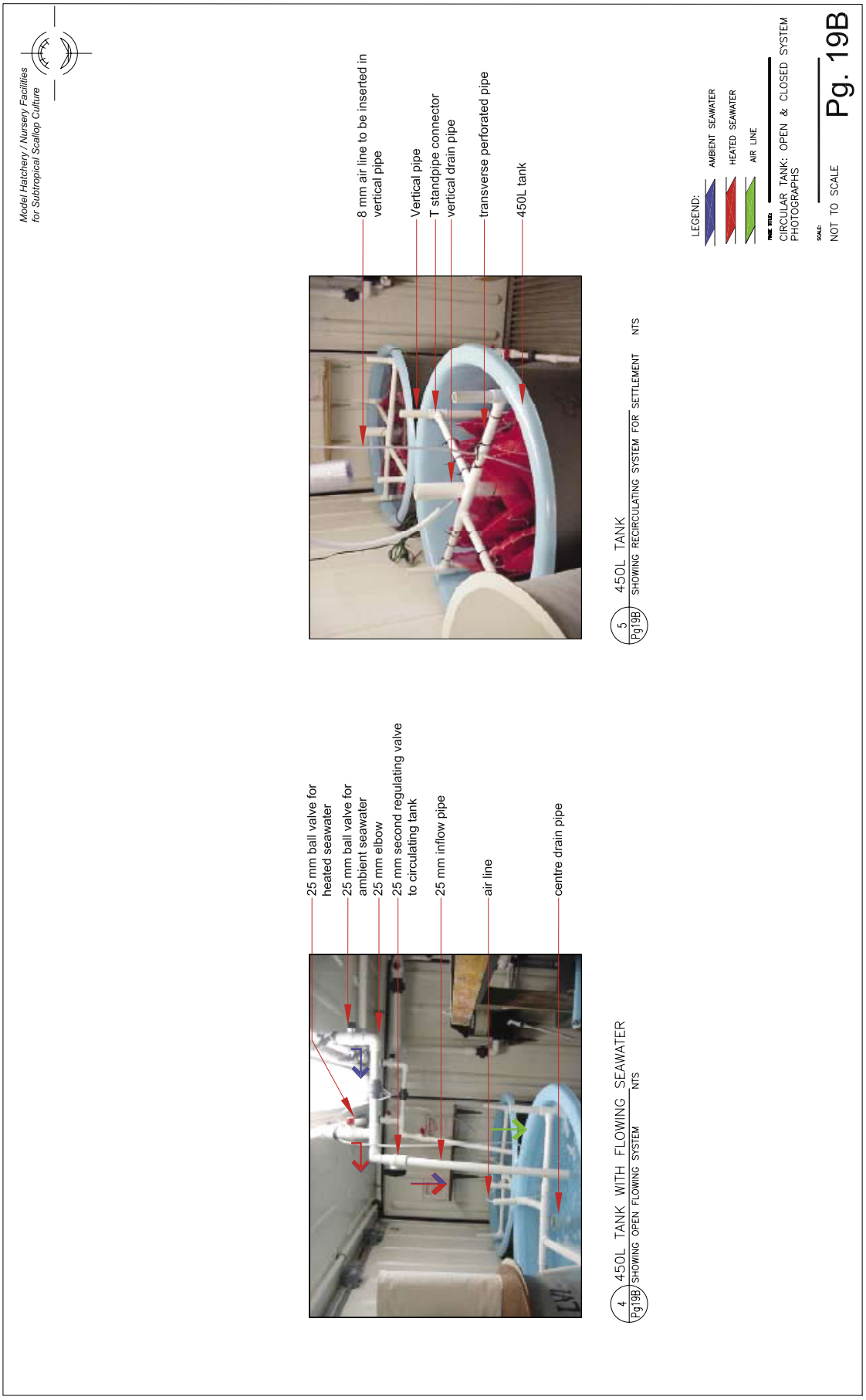
4.2 SCIENTIFIC BACKGROUND – SETTLEMENT AND METAMORPHOSIS

The goal of the nursery is to grow large quantities of juvenile scallops quickly from setting size (200 µm) to approximately 5 mm shell height. At this time, they can be transferred to the natural environment for growth to market size (60 mm). The nursery phase in Bermuda can be divided into two stages. The first stage, referred to here as the indoor nursery, involves settlement of mature larvae and post-larval growth of scallops to approximately 2 mm shell height. The second stage, the outdoor nursery, is optional and involves the further rearing of scallops from 2 mm to a minimum of 5 mm and up to 10 mm.

The first nursery stage allows for enhanced survival of juveniles when spat, rather than newly settled larvae are transferred to the field; as has been reported in the literature, survival rate of juveniles in the field is dependent on size at transfer. For example, Bourne and Hodgson (1991) show that immediate transfer of mature larvae to the

Technical drawing, Pg. 19B

Circular tanks: Open and closed system photographs



field results in a 2.6 percent survival to 1.5-3.5 cm juveniles. This low survival implies costly hatchery operations and a minimal survival to 10 percent is recommended for economic viability by these authors. The second nursery stage where spat are reared for a longer time period, not only further enhances survival following transfer but also results in a reduction of labour and time required to reach adult/market size; this, therefore maximizes cost-efficiency of a growout operation. In this instance, however, costs in the nursery are increased to rear to a larger size.

4.2.1 Factors affecting settlement and metamorphosis

The process by which marine invertebrate planktonic larvae transform to become bottom dwelling juveniles can be divided into two stages; settlement, a repeatable behavioural stage, and metamorphosis, an irreversible physiological stage. Many mature invertebrate larvae have been induced to metamorphose in response to specific environmental cues – i.e. chemical, photic and tactile cues which may indicate the presence of a substratum or habitat suitable for juvenile life (Crisp, 1974; Hadfield, 1977; Burke, 1983).

In order to develop an efficient nursery system and obtain a reliable number of juveniles per larval batch, optimizing the number of larvae setting and metamorphosing into post-larvae is critical. This phase in the culture cycle is most probably the one where aquaculturists have the least control. It is a critical stage in the life of scallops and high mortalities can occur at this time, as early juveniles are extremely fragile. Bourne, Hodgson and Whyte (1989) report that for the Japanese scallop highest mortalities occur at 0.4–0.6 mm shell height, immediately following metamorphosis. The causes for mortalities seen in the nursery are unknown and are attributed to poor nutrition or improper handling. In an attempt to induce metamorphosis and enhance the number of mature larvae setting, several methods have been investigated for commercially important bivalves. Chemicals, such L-DOPA, norepinephrine and serotonin have been tested on Japanese scallop, *P. yessoensis* mature larvae (Bourne and Hodgson, 1991). Other cues such as cold temperature shock have also been tested on the same species (Kingzett, Bourne and Leask, 1990). Greatest increase in percent metamorphosis (17–19 percent over controls) was obtained with norepinephrine. The procedure is relatively simple and involves exposure of mature larvae to a diluted solution of the chemical (10^{-4}M-10^{-6}M) for a short time period (60 minutes) prior to settlement. Other authors have obtained complete metamorphosis within 12 h of exposure to glycine and theophylline (Naidenko, 1991 – on *Swiftopecten swifti*). Physical stimuli such as a sudden decrease in water temperature may also influence the onset of settlement, as demonstrated by Bourne and Hodgson (1991) for rock scallop, *Crassadoma gigantea*, larvae when chilled from 15 °C to 5 °C.

Generally, successful settlement and metamorphosis for bivalves only occur when larvae are "healthy". One of the most critical factors is the accumulation of reserves throughout the larval life, related to food ration in both a qualitative and quantitative sense (Farias, Uriarte and Castilla, 1998). It has been shown that rearing conditions affect storage and utilization of biochemical components, and hence metamorphosis and settlement (Gallager and Mann, 1981). Rearing techniques described in Chapter 3 for sub-tropical pectinid species have proved adequate in yielding mature larvae with necessary biochemical reserves. This is certainly true for larvae reared in conventional static system, as well as in low-density experimental flow-through system, (Sarkis, Helm and Hohn, 2006). The effect of rearing techniques on post-larval settlement was seen in several instances, namely that of adequate food ration. The standard diet used at the BBSR hatchery has proved adequate over the years for ensuring good spat settlement and food ration is calculated on the basis of water volume within the tank. However, this approach was not found correct for a flow-through system supporting

an increased density of larvae. Sarkis, Helm and Hohn (2006) demonstrated poor survival to post-larval stage for larvae reared in a high-density flow-through; this study has furthermore identified the need to base food ration on biomass rather than water volume for such a system.

4.3 SCIENTIFIC BACKGROUND – POST-LARVAL DEVELOPMENT

Post-larval development has been well described for some scallop species (Sastry, 1965; Cragg and Crisp, 1991). Following the conspicuous appearance of the foot, the behaviour of the veliger larva changes and may be considered as the beginning of the process of metamorphosis and preparation for settlement. The appearance of the post-larval organs and the actual attachment take place in a relatively short time, but the process of metamorphosis is not an abrupt change from a pelagic larva to an attached post-larval life. In scallop larvae, as in other bivalve larvae, metamorphosis involves changes in the nature of shell secretion, loss of some organs, greater development and/or relocation of others. The principal organs lost at metamorphosis are the velum, the velar retractor muscles and the anterior adductor. Accounts on the loss of the velum vary, but most state that the velum is lost at the end of the pediveliger stage. The veliger actively swims with the extended velum, but as the velum begins to reduce in size the swimming activity decreases. Prior to the conspicuous development of the foot, the larvae alternately swim and rest on the bottom. At this time, veligers are often collected from the bottom of larval tanks rather than the surface.

There is a general migration, relative to the axes of the shell of those organs which survive metamorphosis. This results in the mouth migrating from its posterio-ventral larval location to the adult anterior-dorsal position, the foot becomes ventral rather than posterior and the posterior adductor migrates to the centre of the valve. After metamorphosis, the gill filaments increase in length and number. Outer filaments of the adult gill system appear after metamorphosis. The time at which the gill becomes capable of filter feeding is uncertain. Some studies have shown that efficient filter feeding does not occur for spat <460 µm (Bourne, pers. comm.). The shape of the foot gradually changes after metamorphosis, becoming narrow with cilia on the free end. The foot is projected to the outside of shell and acts as a locomotory organ. The glands present in the pediveliger foot become better developed and the byssus secreted changes abruptly to a more sticky form after metamorphosis. The statocysts and eyespots are retained.

Immediately after attachment, the shell grows rapidly. The thin and fragile post-larval shell (dissoconch) is sharply demarcated from the thicker and more homogeneous larval shell. The shell margins extend rapidly, resulting in a complete change in the outward form of the newly settled larval scallop. The most important feature of dissoconch growth is the formation of the byssal notch and the gradual change in the shell to adult form. The byssal notch appears as an indentation on the right valve below the hinge line. The byssal notch narrows to form a groove; teeth appear on the byssal groove and are retained even in the adult shell. Toward the end of dissoconch development, the shell margins are semicircular in outline; the colour appears as small dashes on the shell and spreads over the entire shell by the end of dissoconch stage. Both valves of post-larval and pre-adult scallops show pigmentation.

At the end of the dissoconch stage, the pigmentation has extended over the entire shell. The young spat can now be easily recognized as a scallop. The only difference in the external shell morphology from the adults is the absence of ribs and prominent ears. Furthermore, the plications (ribs) appear on the shell at an average size of 1.175 mm.

The true ears characteristic of adults make their appearance as indentations on the anterior and posterior margins below the hinge line. The only difference at the end of post-larval development with the adults is the absence of gonads.

A series of photos showing the development of *Euvola ziczac* spat is given in Figure 4.1.

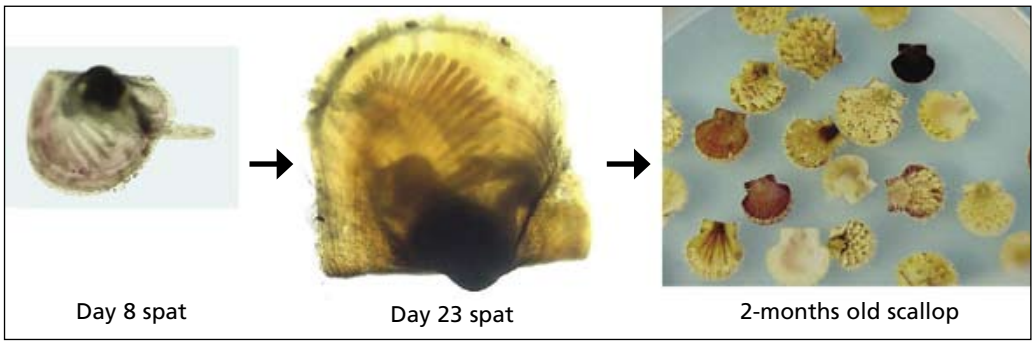


Figure 4.1: Development of sand scallop, *E. ziczac*, following settlement, showing dissoconch in Day-8 scallops, byssal notch formation and pigmentation in Day-23 scallops and similarity to adults in 2 months old scallops.

4.4. TECHNIQUES – SETTING SYSTEMS AND PROTOCOL

The protocol chosen for setting depends partly on space available in the nursery, the period required to remain in the nursery prior to transfer to the field and of the species itself. Furthermore, setting is influenced by several factors such as, the type of setting system and setting density.

Features in setting systems, such as cultch, bacterial films and water flow are believed to stimulate metamorphosis. Cultch is an artificial substrate which increases the amount of surface area in a setting system; if no material is provided larvae can only settle on sides and bottom of tanks, as scallop pediveligers form a byssus attachment to surfaces. It has been found for some bivalve species that if a suitable substrate is not located, larvae can delay metamorphosis and may die (Bayne, 1965). Scallop larvae are believed to be selective about the type of substrate to which they attach before beginning metamorphosis. Several types of material have been tested as cultch for scallop larvae; monofilament, scallop shells polypropylene line, Kinran and Vexar are a few examples. Bourne, Hodgson and Whyte (1989) found Kinran, an artificial fibre made in Japan, to provide optimal results. In addition, the presence of a biofilm on cultch seems to increase settlement, as demonstrated by Parsons, Dadswell and Roff (1993) on *Placopecten magellanicus*. For this reason, it became standard practice at the Bermuda nursery to soak cultch in filtered flowing seawater for a minimum of 5 days prior to its use for setting.

Other hatcheries use "Chinese hat" collectors for scallop settlement, which are commonly used in the oyster industry (Neima and Kenchington, 1997). These collectors consist of 30 cm plastic disks stacked 6–8 cm apart on a central pipe. A total of 15 collectors can be placed in 1 400-litre tanks. Other hatcheries have devised horizontal panels with laminar flow for the setting of *P. magellanicus* larvae (Dabinett, Caines and Crocker, 1999).

4.4.1 Calico and zigzag scallop settlement

Little work has been conducted on the chemical or physical induction of metamorphosis for *Argopecten gibbus* and *E. ziczac*. In Bermuda, spontaneous metamorphosis is allowed to take its course and setting is initiated by providing a suitable substrate.

Mature larvae are identified as per criteria outlined in Chapter 3 (see Section 3.4). Set is usually initiated on Day-12 or Day-13 after fertilization for both calico and zigzag scallop species. Mature larvae are collected on sieves ranging from 150 µm to 80 µm as conducted throughout larval rearing (see Protocol–11). If any are collected on 80 µm, health of the culture is visually assessed and culture is either discarded (if mortality is high) or maintained for another two days as larval culture. Spat collected on 150 µm and 120 µm sieves are kept separately; they are suspended in 2 litres beakers and counted. At this stage, larvae are very fragile and fix rapidly on the surfaces of the holding beakers; continuous but gentle plunging is required to avoid fixation of larvae on the surfaces and minimize shell damage. A sample of larvae is kept for determination of shell growth. Counts are done microscopically as outlined for eggs and larvae using a Sedgewick-Rafter Cell (see Protocol–4; Chapter 1). Volume required for setting system is calculated as outlined in Protocol–12 and gently siphoned using a 7 mm Tygon tubing fitted with a stopcock valve into graduated cylinder. Mature larvae are placed into setting systems to undergo settlement, metamorphosis and development into juveniles.

Two methods for setting have been tested at the BBSR nursery. The first, referred to as rapid transfer approach, minimizes the time spat are kept in the nursery to allow transfer to the field within one month of setting; this strategy reduces labour required in the nursery and frees up space for subsequent larval batches. This strategy is useful in areas like Bermuda, where physical space is restricted and larval cycle is short (12–14 days); in this way, production is enhanced by conducting frequent spawning inductions, resulting in a quick turnover of larval batches. One important consideration in rapid transfer of spat to the natural environment is that nursery and ambient temperatures coincide, minimizing stress at transfer. The second method of setting is more conventional and used widely, where spat are set in raceways, on individual sieves, and may be reared up to 5 mm in the same system. This approach (Raceway set) facilitates control of food supply and water flow to spat as well as allows for routine monitoring of spat growth and survival. Although it is more labour intensive with respect to nursery work than the first approach, it allows for a more controlled production of older spat and enhances survival of spat following transfer to the natural environment.

A description of each method is given below.

4.4.1.1 Rapid transfer approach

A detailed procedure (Protocol–12) is given below. Circular fiberglass tanks (450 l) are filled with cultch made of black 3 mm polyethylene mesh, acting as substrate for pediveligers. This mesh comes as a roll and is cut off in 30 cm lengths; fifteen of these strips are tied together and soaked for one week in 1 µm filtered flowing seawater prior to use as settling substrate. All surfaces of the setting tank are covered with bundles of cultch and others are suspended so as to fill the centre of the tank (Figure 4.2). A total of 8 strings are suspended on average. This provides a large surface area for settlement of larvae. Temperature is maintained similar to larval rearing temperature (24±1 °C). Setting (or stocking) density of larvae placed in a setting system is critical. If density is too high, rate of settlement, metamorphosis and subsequent post-larval survival declines. Studies on *P. yessoensis* have shown that low

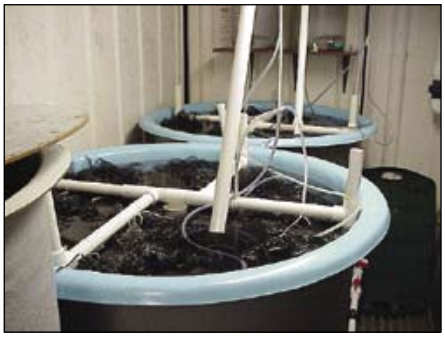


Figure 4.2: Cultch made of 3 mm black polyethylene mesh filling 450 litres tanks used for set.

density yields better settlement; satisfactory results were obtained with densities of 0.5-2 larva.ml^{-1} when set in tanks (Bourne, Hodgson and Whyte, 1989). On the other hand, Neima and Kenchington (1997) found satisfactory results when setting 500 000 larvae in a 1 400 litres tank (equivalent to 35 larvae.ml^{-1}). Stocking density in tanks was not determined specifically for the two scallop species in Bermuda and densities did not exceed 4 larvae.ml^{-1}.

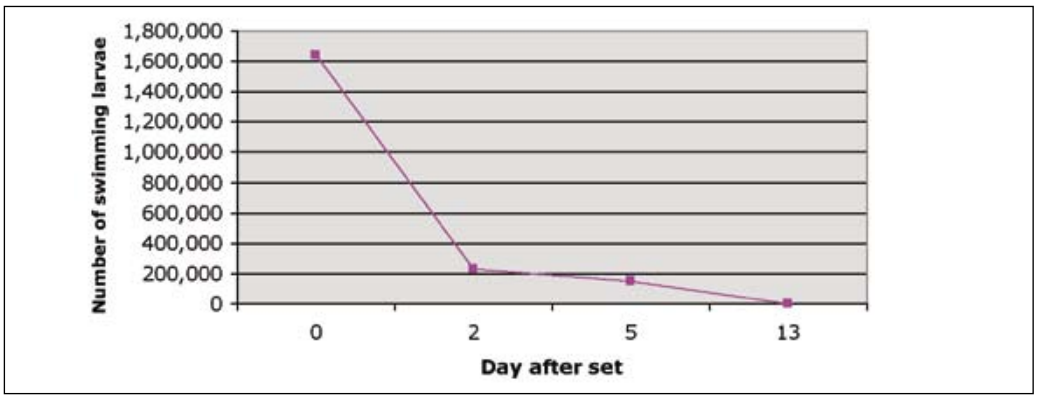


Figure 4.3: Evaluation of calico scallop, *A. gibbus*, set in 450 litres tanks.

The duration of the setting period, where the maximal number of pediveligers metamorphoses and becomes fixed, is determined by assessing the culture over time. During this time, setting tanks are treated as static larval tanks. Aeration is provided through 7 mm airlines (2 per tank) and algal ration is provided in batches. Larvae are initially fed a mixed algal diet amounting to 18 cells.µl^{-1} starting day of set; food ration is gradually increased to 24 cells.µl^{-1} as the number of larvae metamorphose and settle. A detailed table of daily food ration is given in Table 4.1 (see Section 4.5.1).

Although water is exchanged three times a week as for larvae, the procedure differs in that setting larvae are not removed from the setting tank, and as they need to be continually submerged, the water level in the tank is maintained constant. A 20 mm inner diameter flexible hose is connected to a supply of 1 µm filtered heated seawater to provide continuous flow to the setting tank. Water is passed via the outgoing valve at the bottom of the tank and any swimming larvae (or not fixed) are collected on a sieve in a submerged tray as for larvae. The valve is opened to provide a similar outflow to the inflow supplied to the tank; in this way, water level remains constant throughout the water exchange. This water exchange lasts for a period of 1 hour in order to ensure complete water exchange of the whole tank. Larvae collected on the sieve are washed into a beaker, counted using a Sedgewick-Rafter cell and measured with an ocular micrometer using a compound microscope. Larvae collected are for the most part those which had not fixed onto the substrate and are classified as "swimmers". Collected larvae are re-suspended into the setting tanks, unless a high percentage of dead larvae are seen in the sample. Setting period is considered complete when swimmers are no longer seen in the collected sample.

Towards the end of the setting period (sixth day), temperature acclimation to ambient seawater is achieved by decreasing set temperature by 1 °C every two days. Once maximal number of larvae are set (or 10 days after set for these species), the setting system is changed to an open flow seawater system, where seawater is filtered to 1 µm and supplied continually to the tanks; an air-lift driven recirculation system (illustrated in the technical drawing diagram – 5/Pg19B) enhancing water exchange within the tank, is initiated. Algal food supply is provided continuously over 24 hours by drip-feed. Young spat are reared in this way for a further 20 days. After which, they are ready for transfer to growout sites.

For both scallop species reared in Bermuda, a period of 10 days is selected as standard for duration of the setting period. As seen in Figure 4.3, monitoring of 450 litres sets has shown the absence of "swimmers" in the collected sample by Day-13. It is assumed that larvae are either fixed or dead by this time. *Note: shell length of larvae collected during setting did not change (198±10.2 µm) implying that larvae collected are those with slow development.*

PROTOCOL–12

SET OF MATURE LARVAE IN 450 LITRES TANKS – RAPID TRANSFER APPROACH

1. Five days prior to anticipated setting day, prepare cultch. Cut 30 cm strips of black 3 mm polyethylene mesh and tie in bundles of 15. Soak in 1 µm filtered flowing seawater to develop a biofilm.
2. Fill 450 litres tanks with bundles of 3 mm polyethylene strips. Cover the bottom of tank, and use re-circulation pipe system to suspend bundles so as to fill centre and top of tank.
3. Fill tank with heated double 1 µm filtered seawater. Temperature in 450 litres tank should be same as larval rearing temperature (24 °C).
4. Prepare aeration as for larvae using two 7 mm Tygon tubing connected to airline and reaching to bottom of tank. Place two lines on opposite side of tank.
5. Setting Day referred to as Day-0 of set.
6. Collect mature larvae on 150, 120 and 80 µm sieves. Only set those collected on 150 and 120 µm. Suspend separate fractions in 2 litres beakers. Keep larvae in suspension by using a gentle up and down motion with plunger. Motion for mature larvae has to be continuous during sampling and prior to setting as they will attach to sides of beaker quickly.
7. Count mature larvae using a Sedgewick-Rafter Cell. Calculate number of larvae so as to distribute in 450 litres tank at density not exceeding 4 larvae.ml^{-1}.
8. For calculation: Do Triplicate counts and take average count (if culture very dense, take 500 µl aliquots instead of 1 ml).

   Example:

   - *Average count: 151 eyed larvae in 500 µl = 302 larvae in 1 ml*
   - *Total volume of pool (in beaker or in cylinder) =3 litres = 3 000 ml*
   - *Total number of larvae in pool: 302 x 3 000 = 906 000 larvae*
   - *Setting in 450 litres tanks at a density of 1.5 larvae per ml:*
   - *Total number of larvae in 400 litres = 400 x 1 500 = 600 000 larvae*
   - *Since 302 larvae in 1 ml, for 600 000 larvae need to siphon:*
     600 000/302 = 1 986 ml

9. Pass mature larvae over 300 µm sieve. Keep size fractions separate if possible (150 and 120 µm) and set in different tanks.
10. Maintain temperature in tanks to 24 °C by two immersion heaters.
11. Feed set larvae as per larval rearing, as batch feed.
12. Water exchange conducted every two days as for larvae (Monday, Wednesday and Friday).
13. For water exchange: Prepare shallow tray and sieve (dependent on size set – for 120 µm set use 120 µm sieve; for 150 µm set use 150 µm sieve) to collect any swimming larvae. Connect 20 mm flexible hose to outgoing valve of 450 litres tank and place into sieve. This is same procedure as for collecting larvae outlined in Protocol–11.

14. Connect 20 mm ID flexible hose to treated seawater line (double 1 µm filtered seawater) and start gentle flow.
15. Partially open collecting valve of 450 litres tank and ensure flow of water is into sieve.
16. Ensure to maintain larvae and substrate in water by adjusting incoming flow to be similar to outgoing flow.
17. Exchange water for 40 minutes.
18. At the end of exchange, close collecting valve and stop incoming flow.
19. Count number of larvae obtained in sieve using Sedgewick Rafter Cell; record number of dead and swimmers.
20. If live larvae outnumber dead larvae in collected sample, re-suspend in tank, passing over 500 µm sieve to eliminate any debris. If not, discard.
21. Feed daily as per Table 4.1. Food ration calculated as:
Ration (ml) = (cells.µl^{-1} to supply/total algal count in cells.µl^{-1}) x water volume (l) x 1 000
22. On Day-6, begin decreasing temperature of seawater so as to reach ambient seawater temperature by Day-10. Decrease by 1 °C every two days.
23. Continue rearing in this system until Day-10.
24. When maximal number of larvae is considered fixed (Day-10), begin re-circulating system and change static system to flow-through system. Connect hose to incoming ambient 1 µm filtered seawater supply and open drain valve.
25. Algal feeding supply now provided through 20-litre carboy placed on drip-feed. Carboy cleaned and filled daily.
26. Maintain in open and re-circulating system until transfer to field.

4.4.1.2 Setting density for raceway system

A second approach to setting makes use of raceway systems. Mature larvae are set on sieves, meshed with 120 and 150 µm Nitex material; sieves are suspended in raceway troughs as shown in the technical drawing diagram – 3/Pg15B. Density of larvae/spat per sieve is critical at all stages of this procedure; it is monitored weekly in terms of biomass (grams wet weight.sieve^{-1}) and re-adjusted if needed. In order to optimize percent settlement, initial stocking density for mature larvae set in sieves was investigated for *E. ziczac* at BBSR nursery. Three densities were tested: 25 000, 50 000 and 100 000 pediveligers per sieve. As expected, decreasing initial density results in an increased percentage of fixed spat; it is evident that a balance needs to be achieved at the nursery between an optimal spat yield and usage of space. As a stocking density of 25 000 is not a practical solution in a space-limited hatchery like the BBSR modular hatchery, standard stocking density in Bermuda averages 50 000 larvae per 25 cm diameter sieve. Stocking density never exceeds 60 000 larvae per 25 cm sieve or 30 larvae.cm^{-2}.

4.4.1.3 Raceway set

The standard protocol for setting spat on sieves in raceways is given in Protocol–13. The calculated number of pediveligers is quickly transferred into prepared 150 µm or 120 µm sieve following counting (Figure 4.4). Once again mature larvae are allowed to set with minimal flow and no other disruption for ten days. Food supply is provided from 20-litre carboys, placed on 24 hours drip-feed; this accounts for 50 percent of the ration. The second half of the ration is distributed in the sump tank and distributed through the re-circulating system. Sieves are suspended in the raceway, set on a semi-recirculating system (described in Section 4.1), with inflow of 1 µm filtered seawater at a rate of 3 l.min^{-1}. Semi-recirculation ensures that all algae supplied is consumed, and allows for some temperature control of seawater. Similarly to the procedure for 450 litres

Figure 4.4: Mature larvae set on meshed sieve suspended as downwelling system in raceway.

tank set, mature larvae are set at a temperature of 24 °C as during larval rearing. Temperature is maintained by two immersion heaters placed in the sump tank used for recirculation.

Monitoring of several larval batches has shown that mature larvae set within 10 days of being provided with a substrate, as seen in section on 450 litres tanks above. Following this time period, any larvae collected are dead or poorly developed. Following this setting period flow and food ration are increased and the protocol for post-larval rearing in terms of cleaning, monitoring and thinning sieves for optimal post-larval yields is initiated. Food ration throughout this stage in the indoor raceway system is provided in Table 4.1. After Day-10, flow of raceways is set according to 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. *Note: Flow can be adjusted depending on the amount of live algae available; if fewer algae are available, decrease the flow so as to decrease volume of water*. Keeping a record of biomass and maintaining adequate biomass per sieve and in the system becomes critical towards the third week (Day-22) following set when spat begin a rapid increase in growth. If this biomass per sieve, and per system, is not controlled, high mortalities will ensue coupled with slow shell growth.

Spat are maintained in this system up to 2 months, or until they reach 3.5 mm in shell height. At which time, they are ready for the secondary nursery stage – i.e. transfer to the outdoor raceway for further growth to a minimum of 5 mm in shell height. Adherence to a strict protocol (see later Section 4.5.3) facilitates maintenance of the entire system on a weekly basis, ensuring adequate biomass for growth. Collection of spat for the next stage is simply done by washing them off the sieve with a gentle jet of seawater.

PROTOCOL–13

SETTING MATURE LARVAE IN RACEWAY – MAINTENANCE AND CARE

Setting Day = Day-0 of set.

1. Clean 30 cm sieves by wiping off sides and mesh with cloth and chlorinated water. Rinse thoroughly with fresh water using a strong jet on mesh to clear all openings.
2. Clean raceway thoroughly following directions in Appendix 19.
3. Fill raceway system (including sump tank) with 1 µm filtered seawater.
4. Once filled, turn off incoming flow and place raceway on re-circulating system only.
5. Place two immersion heaters in sump tank to increase temperature to 24 °C. Monitor temperature.
6. Pool larvae and pass through 150 and 120 µm sieves. Re-suspend each fraction in a graduated container and count as per Protocol–12.

7. Calculate volume required as per Protocol–12.

   Example:

   Average count: 302 larvae per ml

   For Setting in 25 cm diameter sieve at a density of 50 000 larvae per sieve: 50 000/302 = 165 ml
8. Distribute larvae into 150 or 120 µm raceway sieve dependent on size at a density not exceeding 50 000 larvae per sieve.
9. Partially open incoming seawater flow so as to obtain a flow of 3 $l.min^{-1}$. This should provide a total volume of 1 200 litres for entire raceway system.
10. Restrict flow (drip-like) to individual sieves on setting day so as to leave larvae to settle without any surface agitation.
11. Calculate food ration (Protocol-10 and 12). Distribute 50 percent in sump tank. Distribute remainder in 20-litre of carboy and top up, with 1 µm filtered seawater. Adjust flow of carboy to raceway pipe to create 24 hours drip-feed. Use Table 4.1 for food ration.
12. Fill 1 carboy per raceway. If two carboys are used, total volume of algae required is divided into two.
13. Record number of larvae set in sieve in raceway check sheet (see Appendix 18) and label sieve with date and species.
14. Conduct daily check of flow, temperature and algal ration provided on raceway check sheet.
15. On Day-2 – Increase flow slightly too fast drip.
16. On Day-6 – Start decreasing temperature to ambient seawater temperature (1 °C every two days).
17. On Day-8 – Clean raceways. Transfer sieves to saltwater table filled with heated 1 µm filtered seawater. Clean raceway as detailed in Appendix 19. DO NOT CLEAN SIEVES; DO NOT DISTURB SETTLING LARVAE.
18. On Day-10 – Assess number of swimmers: Prepare a tray (clean with chlorinated water and rinse); fill to 1/4 with filtered seawater. Remove sieve from raceway and gently wash inside mesh and sides into beaker. DO NOT BLAST POST-LARVAE OFF SCREEN; this will damage and cause breakage of shell and mortality. Place sieve in tray so that fixed spat remain submerged. Count swimmers and/or dead larvae using 1 ml aliquots with Sedgewick Rafter Cell. If many swimmers, re-suspend in sieve. Put sieve in raceway and adjust flow to maximum. Collected sample should contain few swimmers and mainly dead larvae. It is difficult to estimate the number of spat set at this time due to small size.
19. From Day-10 on begin routine cleaning of raceway system once a week (see Appendix 19).
20. By Day-22, some crowding may be observed, and spat are seen climbing up the sides of the sieve. Thinning of sieves should be initiated (Table 10).

4.5 TECHNIQUES – POST-LARVAL REARING REQUIREMENTS

4.5.1 Food ration for spat

Observations suggest scallop spat may use an alternate type of feeding between the time the velum is lost and the gill filaments are able to take over the feeding process. During this time, spat may not be able to filter feed and instead may feed on particulate material adhering to surfaces. The foot may be used for this feeding process. For this reason, culturists differ in their feeding protocols at time of setting. For *P. yessoensis*, Bourne, Hodgson and Whyte (1989) fed setting larvae similarly to other larvae, at a

level of 20–25 cells.µl^{-1} with a mixed diet of algal species (*Isochrysis* sp., *Chaetoceros calcitrans* and *Thalassiosira pseudonana*); these authors report only a slight removal of algal cells from the water column, implying little algal consumption.

Investigating optimal food ration for fixed larvae has been attempted several times at the hatchery in Bermuda. It has proven difficult to assess optimal food ration with newly settled spat. Difficulties lie in the assessment of spat without causing shell damage or mortality, and in setting-up an experimental system, which mirrors a larger nursery system. The method for assessing the effect of food ration in spat was developed over the course of several studies; it was found that due care must be taken for the even distribution of food supply throughout the experimental system. Preliminary studies seem to imply that increasing food ration has little effect on shell growth in the first 14 days of post-larval life. A 14-day experiment, using beakers, was conducted with newly settled spat (Day-10 after set). Some difference was seen, namely in survival rate of post-larvae, with food ration; where, high food ration is associated with low survival rate. On the other hand, no trend was seen between shell growth and food ration. It appears that food ration is not critical to growth of settling post-larvae, however, several subsequent studies indicated that older post-larvae of 1.7–3.0 mm in shell height (approximately 1 and 2 months old spat, respectively) do benefit from an increased food ration. They demonstrated that spat >1.5 mm in shell height benefit from an increased daily food ration, reflected in increased shell and tissue growth, and an associated high survival. The point where food ration supplies exceeds the filtering and ingestion capacity of scallop spat was not reached in these experiments. Nonetheless they provided the basis for the food ration protocol used at the BBSR nursery.

4.5.1.1 Standard food ration protocol for calico and zigzag scallops

Standard food ration given for calico and zigzag scallops follows the schedule given below. As seen above, food ration does not need to be high in the first 7 days of growth; it is steadily increased as spat grow. An approximative seeding schedule is given in Table 4.1; this is dependent on batches and may vary slightly with different larval cycles.

Table 4.1: Standard food ration for rearing of calico and zigzag spat.

Spat size range	No. of cells.µl^{-1}	Approximate days after set
<500	18	0–6
500 µm – 1 000 µm	40	7–21
1 mm – 1.5 mm	60	22–29
1.5 mm – 2 mm	150	30–39
2 mm and up	220	40

4.5.2 Strategy for efficient use of space in rearing spat

The main protocol in rearing post-larvae makes use of the raceway system, as opposed to the 450 litres tanks. The reason for this is related to ease of handling of spat as they grew; it proves more labour intensive to remove spat from cultch than to rinse them down from the sieves. Although this is true for both *E. ziczac* and *A. gibbus*, it may not be for other species, which may detach more easily. Furthermore, there are studies showing the successful use of chemicals for detaching of spat from cultch. The setting of mature larvae in 450 litres tanks is therefore done in Bermuda when there is an overflow of pediveligers, which the raceway system cannot accommodate. It is however, anticipated that the nursery in Bermuda will remove the 450 litres tanks and replace them with a second indoor raceway system.

Due to the small size of the hatchery in Bermuda and hence of limited larval production at any one time, the goal of the nursery area is to ensure optimal juvenile production

by enhancing survival rate during the following three stages: 1) From settlement to 3 mm shell height; 2) Transfer to the field; and 3) In the first month of growout in the natural environment. In this way, efficiency is maximized despite the small production capacity of the facility. Survival and growth following settlement to 3 mm size seem to be very dependent on initial stocking density, food ration, available surface area and minimal handling. On the other hand, for older spat, biomass per sieve and associated water flow, appears most important. Finally, in order to minimize mortality during and following transfer to the natural environment, it has been observed that increased size of spat at transfer enhances survival. For these reasons, an additional raceway was built outdoors (see Section 4.1), maximizing available space for growing of spat to a larger size prior to transfer to the field. When spat reach 5–7 mm in shell height, they can be directly transferred to 3 mm pearl nets, and hung on longlines. Survival rate after one month of transfer averages 90 percent. The additional exterior raceway system has for its main purpose the rearing of spat from 2 to 5 mm.

4.5.2.1 Characteristics of outdoor raceway

The outdoor raceway system differs to that of the indoor raceway system in the following: Sieve characteristics, seawater treatment and algal food composition. Sieves used are of a greater surface area (696 cm^2) than those used indoor to accommodate a larger biomass of spat. They are meshed with green collector bag mesh (1.2 mm on diagonal), ensuring greater water flow. Sieves are set on an upwelling system, reducing clogging of mesh with detritus.

Seawater supply comes directly from the pump house (see technical drawing – page 16A) and is only filtered through the sand filter; this allows supply of additional nutrients to the spat, such as naturally occurring algal species.

Algal food supply is provided from dry algae cultures, purchased at Reed Mariculture Inc. Three species of algae are provided daily by weight of algae. Appendix 21 gives the procedure for preparation of algal food ration for outdoor raceway. The dry algal mixture is diluted in a small volume of seawater and transferred to a large 150 litres reservoir filled with seawater and connected to the outdoor raceway. Algal supply is administered via the seawater to each raceway channel; algal mixture is supplied to each sieve via an upwelling system. This system most probably does not provide uniform supply of algae to each sieve, dependent on the position of the sieve in the raceway. For this reason, sieves are rotated weekly to ensure uniform supply of food (Protocol–15).

There is less control in this outdoor raceway system than in the indoor raceway, in terms of food composition and seawater treatment. This does not seem a deterrent to successful rearing, as with age spat become more tolerant to varying environmental conditions.

The role of the outdoor raceway is to maximize growth enabling the transfer of spat directly into 3 mm pearl nets and enhance subsequent survival in the natural environment. For this reason, several studies were conducted to investigate the optimal size for transfer of spat to the outdoor raceway, and procedures for optimal shell growth in this system. Zigzag scallop spat of two size fractions (<3.5 mm and >3.5 mm) were reared in the two different raceway systems for a period of 4 weeks. Preliminary results indicate that the smaller size fraction fared best in the indoor raceway system; this system is more controlled in terms of water filtration, distribution of algal ration, temperature and quality of algae provided. On the other hand, as spat grow, they become more tolerant to varying environmental conditions; this was seen in the shell growth rate of older spat reared in the outdoor system. In fact, the indoor system

appeared limiting to the growth of larger spat, and these showed a faster growth rate in the outdoor system. This may be related to nutritional requirements and the lack of fine filtration in the outdoor system, which may provide a nutritional supplement to the spat. On the other hand, the outdoor system did not support good survival of spat >10 mm; this is most probably also due to increasing nutritional requirements exceeding the supply provided in this system.

The efficiency of the raceway system in rearing spat tolerant to subsequent transfer to the natural environment for growout was also seen during this study. No subsequent mortality was recorded following transfer to the field in 3mm Japanese pearl nets.

This preliminary experiment was designed to give an indication of growth and survival of spat in the raceway systems; although it is not comprehensive, it does help in defining a strategy for the overall nursery system and allocate space logically. From thereon, it was concluded that spat larger than 3.5 mm would be either moved to outdoor raceway for rearing to 10 mm prior to transfer to the field, or transferred to the field in a tray system, depending on hatchery capacity at the time.

4.5.2.2 Density effect on spat growth

Rearing scallops differs from the rearing of other bivalves, namely oysters and mussels, in the required surface area for optimal growth. Scallops do not cluster as some other species do. This explains observations of scallop post-larvae seen "climbing" the sides of the sieve as they grow, distributing themselves in such a way that they are not on top of one another. As space appears to be a factor for growth, effect of density on shell growth of older spat (>3.5 mm) was investigated. Density was expressed as grams wet weight.sieve^{-1}, as this is a more practical assessment than counting of spat. The latter can be useful at times, however, and is discussed later. Densities tested were selected on the basis of percent coverage of sieve; three densities were tested 7.2, 21.6 and 43.2 grams wet weight.sieve^{-1}. Although survival was not affected within the density range tested, scallop spat reared at lower densities clearly benefit in shell and tissue growth. Practically, low density is difficult to sustain in a compact environment, as is the modular hatchery. As can be seen from Table 4.2, the lowest density translates into 56 spat per sieve at the end of week 4. As it is not cost efficient to culture spat at this density, a balance between space availability and optimal growth needs to be achieved.

Table 4.2: Relationship between density tested and number of spat per sieve.

	Initial No. of spat	Initial density spat.cm^{-2}	Week-4 No. of spat	Week-4 density Spat.cm^{-2}
D1	1 044	1.5	56	0.1
D3	3 132	4.5	450	0.6
D6	6 264	9.0	700	1.0

4.5.3 Raceway weekly maintenance

The studies conducted on post-larvae and older spat, although not comprehensive, do provide baseline data for management of the nursery area. From these stemmed a protocol, strictly adhered to, in order to maintain correct biomass within raceway systems, thus enhancing survival in the early spat stages, growth rate, and subsequent survival following transfer in the field. Monitoring is done in terms of biomass rather than number of spat, as obtaining wet weight is less time consuming than counting spat per sieve. Nonetheless, at times it is useful to translate production into number of spat, and Protocol–14 gives the procedure used for counting spat directly on a sieve. A grid was made at the BBSR nursery and laminated to be somewhat waterproof; this grid is placed under a sieve of spat for counting (see Appendix 20). Within the nursery

routine, a schedule for cleaning of raceways is incorporated, as cleanliness for post-larvae is as critical as when rearing larvae.

Raceways, associated tanks and sieves are cleaned once a week following the protocol outlined in Appendix 19. The first cleaning following set is done only on the raceway itself (see Protocol–13). Sieves are transferred into a holding area filled with 1 µm filtered seawater and left undisturbed during the period of cleaning. On the second week of set, a regular schedule of cleaning raceways and individual sieves is initiated; sieves are cleaned with a gentle jet of seawater, care is taken to not damage the shells, but detritus is removed to allow for better flow through the mesh. Cleaning of raceways is conducted on Tuesdays allowing for routine hatchery operations on Monday, Wednesday and Friday. Daily monitoring of raceway system and post-larval culture requirements are checked and recorded on a raceway check sheet, a sample of which is given in Appendix 18.

As post-larvae grow, total biomass in the raceway system increases; adjustments in algal food ration, water flow and available surface area are required. Food ration is discussed above and given in Table 4.1 for the indoor raceway. Total seawater volume for the indoor raceway, when used as a semi-recirculated system, is maintained at 2 320 l per day, irregardless of biomass; this is calculated by incorporating incoming flow of 3 $l.min^{-1}$, volume of reservoir (220 l) and overflow discharge of 1.25 $l.min^{-1}$. At this stage, algal food ration is based on volume of water, rather than on biomass. On the other hand, seawater flow for the outdoor raceway, set as an open system, is based on spat biomass in the system (50 $ml.min^{-1}.g$ wet $weight^{-1}$, equating 2 l min^{-1} for each raceway channel). Care is taken to minimize flow so as to avoid too rapid a discharge of algae. Food ration is provided as commercially purchased dry algae, diluted with seawater in a 150 litres tank and pumped to each raceway channel. Protocol–14 provides details on procedures followed for rearing of spat in the outdoor raceway.

Weekly cleaning of raceway is combined with biomass check starting Day-22 after set. This protocol also allows for monitoring of growth, and size fractionation of spat in the latter stages of the nursery.

PROTOCOL–14

REARING SPAT IN OUTDOOR RACEWAY

1. Clean raceway once a week (generally on Tuesday)(see Appendix 19).
2. Clean one channel at a time; transferring sieves to other channel during cleaning.
3. Use chlorox and a scrub brush for removing all detritus. Use a bottle brush for cleaning pipes, inserting it through cleaner valves.
4. Clean algal system by chlorinating 150 litres reservoir and pumping chlorinated water through algal supply lines. Be careful not to pump chlorine into raceway channel with spat.
5. Wash sieves gently with saltwater so as to remove silt. Do this twice a week (Tuesday and Friday). System clogs up more quickly as water is not fine filtered.
6. Rotate sieves once a week (Tuesday) so that first three sieves are moved to last three positions to ensure equal supply of food for all.
7. Maintain 25 grams biomass per sieve. Check on weekly basis (Wednesday)(see Protocol–15).

8. Daily, ensure that algal tank does not drain completely and that pump does not run dry. Check algal level before leaving in the evening. It should be 1/3 down. If not, increase algal supply to raceway.
9. For feeding: Turn pump off. Drain algal tank by opening union at bottom of tank (see technical drawing – 1/Pg17) and clean algal tank thoroughly with chlorox and fresh water. Rinse tank with ambient seawater. Close union and fill tank with ambient seawater. Turn pump back on once tank is filled to 1/3.
10. Remove prepared aliquots of dry algae (use a mixture of 3 species) and dilute in 1 litre of seawater. Let sit, and add to 150 litres tank. See Appendix 21 for preparation of dry algae.
11. Adjust supply to raceway using stopcock valves. A fast drip is usually adequate to empty 150 litres tank in 24 hours.

4.5.3.1 Maintaining a critical biomass

The terms "thinning" and "grading" are used throughout the following section. "Thinning" refers to assessment of biomass (total wet weight) per sieve, and redistribution of spat. "Grading" refers to fractionating sizes of spat using various mesh size.. There are mechanical graders available commercially, and are useful for large operations. In smaller hatcheries, graders can be made using a series of 30 cm diameter sieves, meshed with polyethylene material of various apertures. In Bermuda, collector bags used for natural spat collection in the field, and Japanese pearl nets used for growout were cut and used as mesh for graders. Table 4.3 shows the aperture of each mesh size used; aperture size was measured diagonally as it is the largest opening through which spat may pass.

Table 4.3: Aperture size, measured diagonally, for each mesh used in grading of spat.

Mesh type	Aperture size
Green collector bag	1.2 mm diagonal (0.6x0.72 mm)
Red collector bag	2.0 mm diagonal
Black polyethylene mesh	2.7 mm diagonal
Japanese pearl nets	4.9 mm diagonal

A general schedule for thinning and grading of early spat, reared in the indoor raceway system, is given in Table 4.4, and the procedure itself is outlined in Protocol–15. Thinning of spat, or first control of biomass, is usually required by Day-22 after set. Spat are washed off the sieve using a gentle jet of filtered seawater and collected into a tarred mesh square of appropriate size. At Day-22, it may be difficult to detach all spat from sieve; it is best to leave those that stick on, rather than damage by detaching. Collected spat, enclosed in the mesh, are blotted dry and weighed on a Sartorius balance (±0.01 grams) (Figure 4.5). Spat from same set (and size) are divided into

Table 4.4: Procedure for maintenance of biomass in raceway system and transfer to outdoor raceway. Sieve size for indoor (532 cm^2) with 150 or 120 µm mesh. Sieve size for outdoor (696 cm^2) with 1.2 mm (green) mesh.

Days after set	Procedure	New biomass per sieve (grams)	Sieves used for grading	Raceway
22	Thinning	9		Indoor
29	Grading & Thinning	Small: 9 Large: 25	Red & 120 µm	Indoor
36	Thinning	Small: 9 Large: 25	Red & 120 µm	Indoor
43	Grading & Thinning	15 to 25	Black & green	Outdoor for larger
50	Grading & Thinning	25	Blue & red	Outdoor and field

9 grams wet weight aliquots and re-distributed into sieves. A 9-gram biomass at Day-22 is equivalent to 60 percent coverage of the sieve; it was found that this provided adequate surface area for growth of spat, and this percent coverage became the standard objective for re-distribution at every grading/thinning session. At Day-22, size fractionation is not found necessary. On the other hand, by Day-29, size range of spat increases, and pooled spat are passed through a red mesh sieve and 120 µm sieve. Those collected on the larger red sieve are ready for transfer to the outdoor raceway. On average, this amounts to approximately half of spat. Spat are weighed and redistributed into sieves; biomass per sieve does not exceed 9 grams for the smaller size fraction (indoor raceway), and 25 grams for the larger size fraction (outdoor raceway). The next thinning and grading session is usually two weeks later (Day-43), where all spat become large enough to be transferred to the outdoor raceway at a density of 15–25 grams per sieve. On Day-50, or <2 months after set, spat are graded using black and red sieves; those spat collected on black mesh are ready for transfer to pearl nets. Others are re-distributed into sieves at a density of 25 grams per sieve.

Figure 4.5: Weighing spat on a Sartorius balance (±0.01 gram).

PROTOCOL–15

WEIGHING AND COUNTING OF SPAT FOR THINNING AND GRADING

1. Clean raceway once a week (Tuesday)(see Appendix 19).
2. Thin and grade sieves once a week (Wednesday) starting Day-22.
3. Preparation: Clean 20 mm hose and connect to ambient 1 µm filtered seawater.
4. Clean saltwater table with chlorinated sponge, rinse thoroughly with fresh water and 1 µm filtered seawater.
5. Let filtered seawater flow in table.
6. Place measuring grid (Appendix 20) on saltwater table and place sieve on top.
7. Identify 10 squares (each 1 cm^2) for counting. Ensure that selected squares represent all areas of the sieve (corners, centre, borders).
8. Count number of spat per cm^2. Average number of spat per cm^2. Estimate total number of spat as follows:
   Total number of spat in sieve = Average (spat per cm^2) x SA of sieve
   (Note: SA for 25 cm sieve = 532 cm^2; SA for 30 cm sieve = 696 cm^2)
9. To obtain biomass: Prepare several pieces of 15 cm^2 mesh (aperture size depends on age of spat – see Table 4.4).

10. Tare one piece using a balance (±0.1 gram).
11. Collect sieve from raceway, and using gentle jet of seawater, gather spat to corner of sieve.
12. Pass spat through two sieves for grading (see Table 4.3). Repeat with remaining sieves. Keep two size fractions separate and hold graded spat in two extra sieves sitting in tray with 5 cm of filtered seawater in bottom.
13. When all spat are graded, transfer aliquot of spat into tarred mesh piece using a spatula or small spoon. Wrap spat in mesh and blot dry on a piece of paper towel.
14. Weigh spat on same balance. Record.
15. Clean sieve thoroughly with chlorinated sponge for sides and strong jet of seawater through mesh to remove all debris. Suspend in raceway.
16. Weigh out aliquot of spat required for adequate coverage, dependent on age (see Table 4.4).
17. Transfer spat in cleaned sieve.
18. If require measurement of spat, use ocular micrometer on compound microscope at magnification of 4 until 2.5 mm in shell height. After which, use Vernier callipers (±0.1 mm).
19. Calculate total biomass in raceway and adjust flow based on 50 $ml.min^{-1}.g^{-1}$.
20. Feed according to Table 4.1 and Protocol–13 for indoor raceway and Appendix 21 for outdoor raceway.

4.5.4 Shell growth of calico and zigzag scallop spat

Post-larval shell growth is similar for calico and zigzag scallops. Figures 4.6 and 4.7 show shell height for both species when reared according to the protocols given above. In short, within 2 months of set, >90 percent of spat can be transferred to the field in 3 mm pearl nets. For additional assurance of post-transfer survival, spat can be maintained a further two weeks in the outdoor raceway; thus transfer of spat to pearl nets can be done 2.5 months after set with spat averaging 9 mm in shell height. The total time in hatchery and nursery prior to transfer to the natural environment does not therefore exceed 3 months. As for survival rate, mortality incurred following metamorphosis, setting of larvae and early post-larval growth, results in an average of 20 percent survival of larvae set to time of transfer (Figure 4.8). Growth rate given is associated with temperatures shown in Table 4.5.

In order to estimate total production of hatchery in terms of numbers, it is best to establish a baseline relating wet weight of spat to shell height and age. In this way, for subsequent batches, production can be estimated by extrapolating known biomass. This was done at the BBSR nursery for the calico scallop and is shown in Figure 4.9. In this way, if total biomass of Day-50 spat is known and growing at a known rate, the total number of spat produced can be calculated. This is evidently dependent on rearing conditions and growth and should be established for each nursery.

Table 4.5: Ambient temperature recorded in raceway systems in Bermuda during post-larval growth. Range represents monthly change.

Month	Temperature (°C)
March	18–2
April	20.5–22
May	22–24
June	24–26
July	26–27.5

Transfer of spat from the nursery to the natural environment for growout is described in Chapter 5.

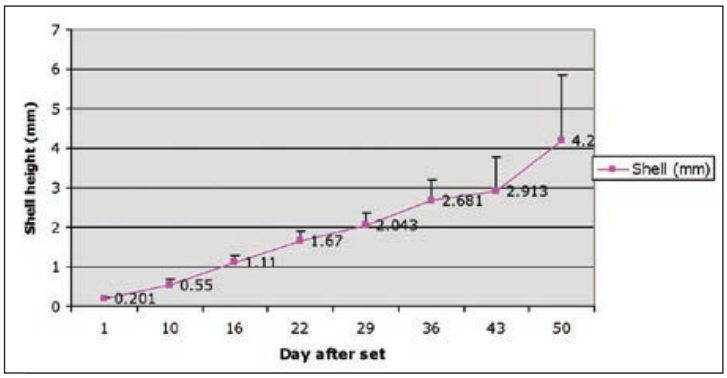


Figure 4.6: Shell height for calico scallop, *A. gibbus*, spat reared in raceway system.

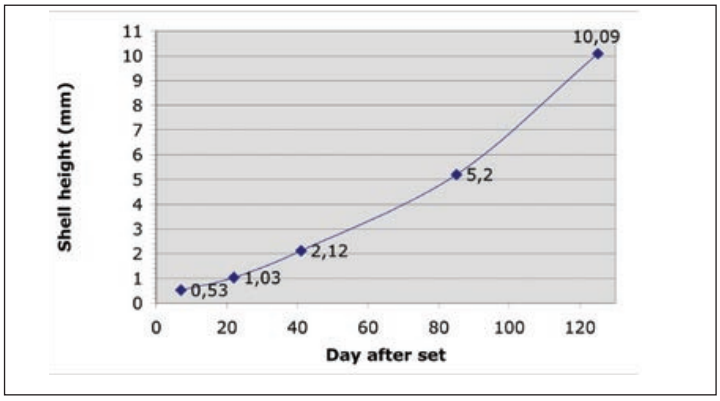


Figure 4.7: Shell growth (height) of the zigzag scallop, *E. ziczac*, following settlement.

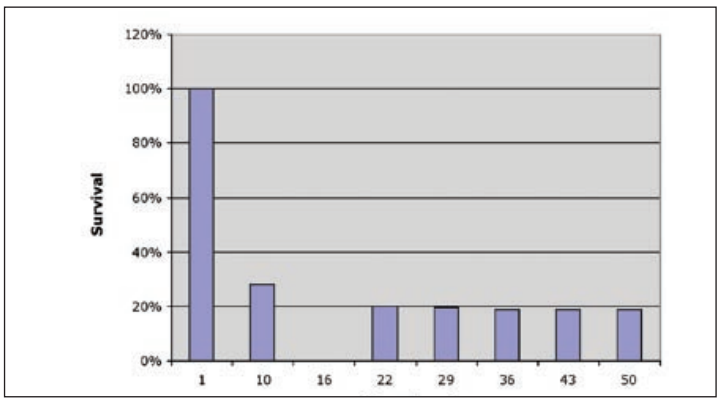


Figure 4.8: Survival rate of calico scallop, *A. gibbus*, post-larvae following settlement. Survival rate is calculated from number of larvae set.

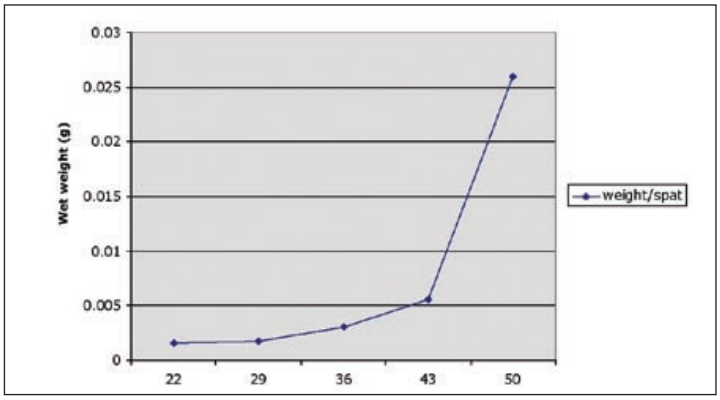


Figure 4.9: Wet weight of calico scallop, *A. gibbus*, spat (gram per spat) as determined during the nursery stage (n= 100).

Chapter 5

Growout of juveniles: transfer from nursery to field

5.1 HOLDING AND GROWOUT FACILITIES

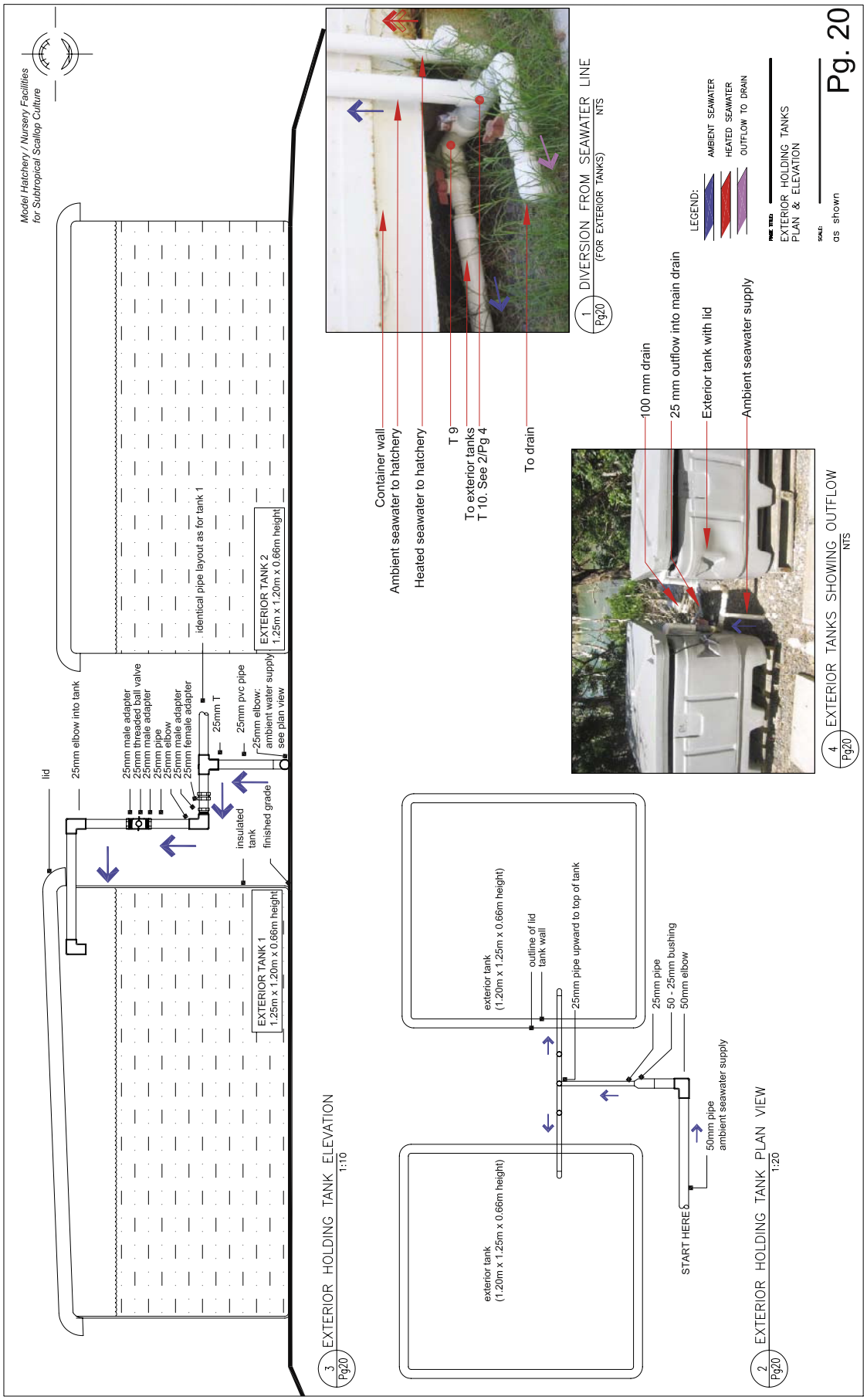
Facilities for holding scallop spat prior to transfer to the field for growout are described below. These exterior facilities can also be used for maintaining adult scallops, or for other purposes. These facilities are found extremely useful when bringing back young juveniles from the field for further sorting on land, if weather conditions do not allow sorting on the boat. The main requirements are cleanliness, as for the rest of the complex, and high water flow. Animals held in these tanks are usually not fed, and for this reason, cannot be kept for extended periods of time. Longline system used in Bermuda is described thereafter; this system is efficient for suspending pearl nets, scallop trays and/or collector bags.

5.1.1 Exterior holding tanks

Refer to Technical Drawing – page 20. Two square insulated "BONAR" tanks (400 litres volume) are installed outside the hatchery on a wooden base. They are supplied with seawater through an open system, with coarsely filtered seawater diverted from the main line, prior to entry into the hatchery complex. Technical drawing diagram – 1/Pg20 illustrates the main valve regulating the flow to the exterior tanks from the pump house. The relative position of the heated seawater line and ambient seawater line supplying the hatchery can also be seen. Finally, any excess flow, pumped in but not used, is diverted to a main drain pipe, buried sub-surface and not seen in this photo. *This photo is also represented in detail in technical drawing diagram – 2/Pg4.*

Technical drawing, Pg. 20

Exterior holding tanks: Plan & elevation



Plumbing for these tanks is very simple and done using a 50 mm pipe buried in the ground, running to the tanks. As seen in technical drawing diagram – 2/Pg20, a 50 mm elbow is fitted to the supply line, and points upwards to direct the flow of water to the tanks. From there, the pipe is reduced to 25 mm using a 50 mm to 25 mm bushing, and runs in the centre of the two tanks. A T-junction then divides the line into two. The tank elevation diagram (see technical drawing – 3/Pg20) shows the detailed connections for supply. As these tanks are cleaned, emptied and re-filled frequently, a flexible system, allowing complete drainage of the vertical pipe system, is required. The centre T-junction is fitted with a 25 mm female and male adapter threaded into one another, then glued to a clear elbow. This allows for the entire "arm" to swivel, and allows for draining of the supply pipe. The clear elbow allows to monitor any clogging of the coarsely filtered seawater supply. A 25 mm male adapter threads into a 25 mm threaded ball valve and regulates the flow of seawater into each tank. Once again this allows for turning of the pipe system to divert flow away from tank, required when cleaning. Finally a series of two 25 mm elbow direct the flow into the tank, and flow is supplied via a 25 mm inner diameter re-enforced tubing fitted to the pipe, running to the bottom of the tank.

Outflow is through a standpipe at the opposite end of the tank connected to a bottom drain. The drain is sealed with an O-ring and caulking, fitted with a thru-hull fitting, and connected to a 25 mm pipe draining into the main 100 mm drain pipe. This can be seen in technical drawing photo – 4/Pg20.

Despite high exposure to direct sunlight in Bermuda, the insulating capacity of these tanks, and high water flow generated, maintained an adequate environment for scallops throughout the year.

5.1.2 LONGLINES

Calico scallops have shown good shell growth and survival rate, as well as complete reproductive development when reared in suspended cultures. This method is used widely for various scallop species (Bourne, Hodgson and Whyte, 1989; Couturier, Dabinett and Lanteigne, 1989). Animals are reared in nets suspended from longlines in the water column. Longlines consist of two anchor lines and one transverse line with floats. There are various designs in use where transverse lines are maintained at the surface or submerged at various depths. The system in Bermuda consists mainly of a sub-surface transverse line, maintained at approximately 1.5 m depth with 30 cm diameter black floats (Figure 5.1). Each float has a floatation of 14.5 kg; they are spaced every meter and tied securely to the transverse line. The rope used is a floating type

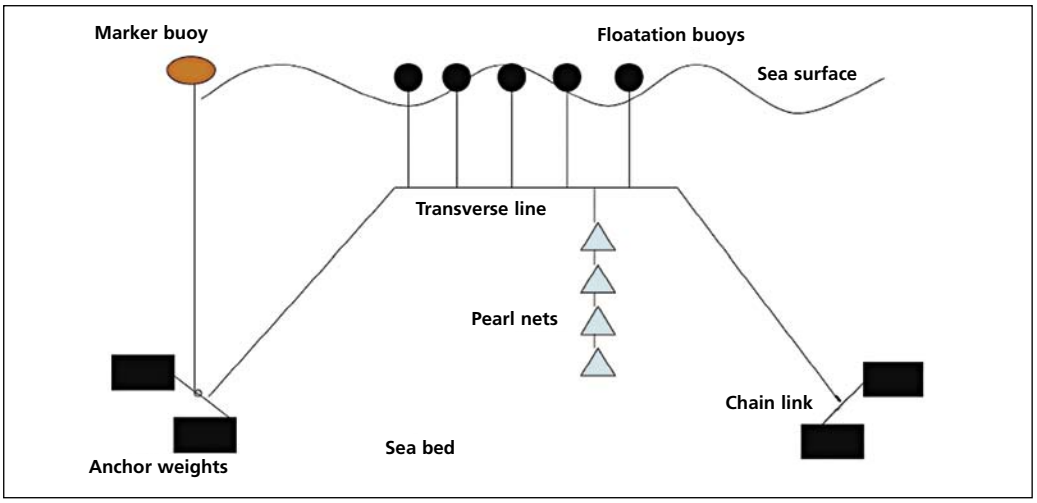


Figure 5.1: Sub-surface longline system used in Bermuda.

rather than sinking type. Maintenance of the line sub-surface serves two purposes: the first is related to the observed better performance of scallops reared below the surface, due most probably to the lack of inhibition from the UV rays. The second reason is related to minimized wave action due to wind, optimizing the longevity of the nets and of the whole system.

Anchor lines are, as a rule, twice as long as the depth of the water, so that in 10 m of water, each anchor line is 20 m long. They are attached to anchors made of any heavy material retrieved locally; in this case, plastic coated lead weights used for ballasts on boats were used, amounting to 68 kg per anchor. A 2 m chain length is connected to the anchors with shackles to facilitate replacing of the longline when needed. The anchor lines themselves are spliced into loops, protected by plastic tubing. Shackles connect the anchor lines to the chain and the plastic tubing avoids chafing of the rope.

In Bermuda, it is found, that a line needs replacing after two years. Square pearl nets are tied together to form a column of nets, with the last net being one metre off the bottom. As scallops grow, densities are reduced within the nets and nets of increasing mesh size are utilized; such that pearl nets of 3, 6 and 12 mm mesh size are used from juvenile to adult/market stage. Thus, in Bermuda, a 90 m longline set in 8 m of water, can support approximately 20 000–25 000 adult scallops. In the initial set-up, it is recommended to order more than the 12 mm nets needed, as some will tear due to bad weather or to predators.

Prior to transfer of spat to the field, longlines have to be prepared and set in selected sites. When selecting a site for scallop growout, the following considerations should be taken into account. Protection from storms, good water quality, high flow of water, water depth, protection from vandalism and other users, distance from nursery or ease of access.

5.1.3 BOTTOM CAGES

As mentioned in Chapter 4, growout of zigzag scallops to 25 mm juveniles is similar to that described for calico scallops. Unfortunately, this species does not fare well in suspended cultures for the rest of its life cycle, and exhibits optimal growth rates when cultured directly on the sandy bottom. Its natural behaviour, recessing in the sand, seems to play a role in limiting fouling on the shell, and enhancing growth. Growout cages used in Bermuda are shown in Figure 5.2. These cages protect scallops from predators as there is mesh on top and sides, but allow scallops to recess on the sandy bottom. Descriptions of the cages experimented with in Bermuda are not given in detail, as this system is not cost efficient. It is extremely labour intensive due to the continuous monitoring of the cages by SCUBA. Survival rate is very dependent on density, and loading density per cage is low (10 scallops per m^2). In order to make growout of zigzag scallops commercially feasible another growout method needs to be devised.

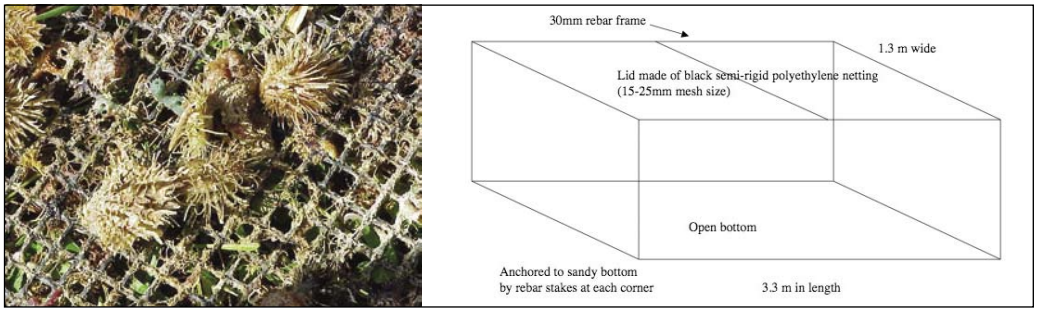


Figure 5.2: Schematic diagram of bottom cages made of rebar and plastic mesh used for protection of sand scallop (*Euvola ziczac*) juveniles and adults. Fouling on meshed lid shown close-up, necessitating retrieval of cages from field.

5.2 TECHNIQUES – TRANSFER OF SPAT FROM NURSERY TO FIELD

As two methods for set are used at the BBSR nursery (see Chapter 4), and resulting spat are transferred to the growout sites at different stages, procedures in transfer differ. Spat transferred, following the rapid approach set, are younger and smaller. They are more fragile than those transferred from the raceway system mainly due to their age. Although, success has been obtained in using the natural environment as growout site for young spat, survival rate has been enhanced when spat are reared to a larger size prior to transfer.

5.2.1 Transfer of 1.5 mm spat from 450 litres tank set

Spat set in 450 litres tanks on cultch are transferred to the field when a shell height of 1.5 mm is reached, or approximately 1 month after set (Figure 5.3). As these are very small and can be easily lost or damaged during handling, care is taken to always keep the spat submerged. Cultch is transferred to green collector bags and held in a container filled with ambient saltwater for transport to the growout sites (Figure 5.4).

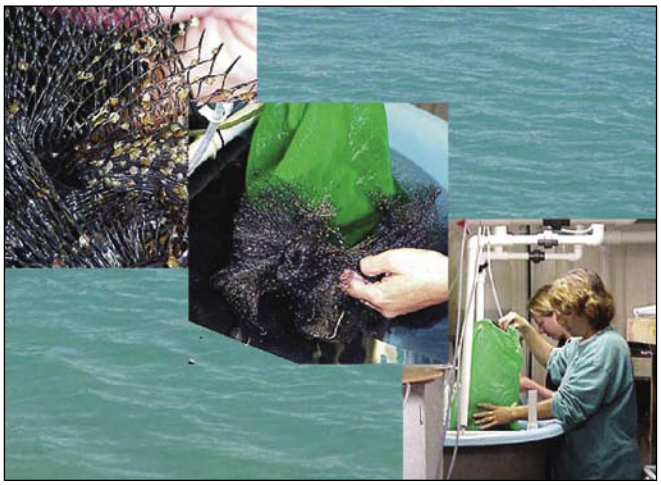


Figure 5.3: Transfer of one month old calico scallop spat in green collector bags for transfer to growout trays.

Figure 5.4: Spat pouches held in seawater prior to transfer to the field.

As outlined in detail in Protocol–16, green collector bags are suspended on longlines using scallop trays. Bags are inserted, one per tray, and trays are stacked one into the other in groups of six (Figure 5.5). The number of trays used in one stack is dependent on water depth, floatation of line, and equipment used for retrieving trays. In Bermuda, all growout handling is done by hand with a small (9 m) outboard motor boat; it is found that as scallops grow and fouling accumulates on trays, groups of six are easiest to handle.

On-site, trays are secured using a 15 mm rope, wrapped around the entire set, in a similar manner to a gift-wrap package. A bridle coming from each corner of the stack ties in the middle to a stainless steel clip. The clip attaches directly to the longline. Other methods can be used for securing a series of trays, dependent on preference of the grower. Transfer of green collector bags to trays, and securing the trays is done quickly on-site, to minimize the time period in which spat are exposed to the air. Trays are thereafter left for a period of 6 weeks in the field. For collecting of spat, trays are

Figure 5.5: Securing of pouches into trays for growout in the field on longlines.

dismantled in the field, collector bags with spat are transferred to containers filled with seawater and transported to the nursery. Upon arrival at the nursery, bags are placed in running seawater in exterior tanks or in 450 litres tanks. Spat are collected by removing mesh from cultch, and shaken into plastic trays filled with seawater. Spat at this time are passed onto a large mesh sieve (3 mm) and distributed by weight to pearl nets. For 3 mm pearl nets, a total of 150 spat (average of 7 mm shell height) are grown for a period of one month. This approximates 23 grams wet weight.

PROTOCOL–16

TRANSFER AND RETRIEVAL OF SPAT ON CULTCH TO FIELD

1. Prepare cooler or other container for transport on boat to growout site. Clean with fresh water and fill with ambient seawater.
2. Load boat with scallop trays, cut rope for securing, clips and tools. Prepare top trays with bridles and clips (see Figure 5.5).
3. Place green collector bag in 450 litres set tank and, with cultch remaining submerged in tank, transfer gently to collector bag. Fill collector bag to approximately 2/3. Close collector bag with drawstring.
4. Carefully transfer collector bag to transport container.
5. Repeat procedure until all cultch is removed from 450 litres tank.
6. Carry transport container to boat and fill to rim with ambient seawater once on board.
7. Immediately travel to growout site.
8. Upon arrival, prepare 7 trays; bottom tray should have a weight secured for stability (use twine or cable ties for securing 2.5 kg weight). Top tray has ready-made bridle. Begin transfer of green collector bag to tray: place one bag on tray, stack second tray on top, add one collector bag, and stack another tray, etc., until 6 trays have been filled with collector bags.
9. Add top tray with bridle.
10. Wrap rope around stack as for a gift-wrap.
11. Clip tray onto longline.
12. Leave for growth for a period of 6–8 weeks.
13. When ready for collecting and sorting, prepare outdoor tanks or 450 litres with clean seawater. Return to site with transport containers.
14. On-site, remove tray stack. Dismantle and transfer green collector bags to transport container filled with seawater.

15. Upon arrival to hatchery, transfer bags to prepared tanks.
16. For removal of spat, prepare trays with flowing seawater; place bags in tray and remove cultch, shaking off and picking spat.
17. All collected spat is left in trays.
18. Pour water and spat into 3 mm sieve to collect all spat.
19. Sample 150 spat on mesh, blot dry and weigh.
20. Transfer to pearl net.
21. Weigh out sub-samples of same weight for transfer to pearl nets.
22. Maintain pearl nets in flowing seawater (exterior tanks or 450 litres) until transfer to the field.
23. For transport of nets to growout sites, hold nets in containers filled with seawater. Pearl nets are attached in series of six, dependent on water depth, floatation and method of retrieval. Keep at least 1 m distance between last net and seabed.
24. Nets are hung on longline using clips or by a secure knot.

5.2.2 Transfer of 2–4 mm spat from raceway to longlines

Procedures for spat set in the raceway and transferred to the field at a size too small to be placed directly into 3 mm pearl nets (2-4 mm) is similar to that described in Section 5.2.1 above. Care is taken not to damage the spat and to maintain them continually submerged during transfer. As they are too small for 3mm pearl nets, insert pouches made of window fly screen material are used as inserts into scallop trays (Figure 5.6). This material is of larger mesh size than the collector bags, allowing for increased seawater flow for larger spat, and reduces the degree of clogging by fouling. Pouches are rectangular in shape with dimensions similar to that of the tray; pouches are sewn on three sides; the fourth side is closed using Velcro for ease of opening and closing. Spat are collected from the sieves as outlined previously. A sub-sample of 400 spat is weighed in a first instance; thereafter, aliquots of similar weight are taken and distributed in each pouch. Remainder of transfer is conducted as described from step 6 on in Protocol–16.

Figure 5.6: Transferring 2–4 mm spat into fly-screen pouches used as inserts for growout trays.

The older and larger the spat at transfer from the nursery to the field, the shorter the time period required for growth to 7 mm (and ready for growout in pearl nets). For raceway spat, transferred approximately two months after set, an intermediate growout period of one month is usually required in trays. Following this intermediate growout phase, spat are retrieved from the field and brought to the nursery for assessment of survival, and are re-distributed into 3 mm pearl nets.

5.2.3 Transfer of spat >4 mm

Spat reared in the raceway for a longer time period, whether it is in the indoor or outdoor system are ready to be transferred directly to 3 mm pearl nets. The larger the spat become, the easier they are to collect, and the more tolerant they become to handling. Nonetheless, care is still taken to minimize air exposure during handling. Spat from all sieves are pooled. An initial sub-sample of 150 spat is weighed; once the

Figure 5.7: Spat reared in raceway until 4–9 mm shell height and ready for direct transfer into pearl nets for growout. Pearl nets shown suspended from experimental system.

weight is known, spat are distributed into pearl nets by weight. This is more time efficient than counting spat per net. For calico and zigzag spat, sub-samples of 23 grams resulted in approximately 150 spat per pearl net. Once spat are distributed, pearl nets are closed and held in flowing seawater until transfer to the field. In Bermuda, pearl nets are usually prepared one day, prior to transfer to the field. On the day of transfer, nets are transported in containers filled with seawater. On site, nets are tied in series of six or seven and connected to the longline using a clip or simply tied. Spat are left in 3 mm pearl nets for a period of one month.

5.3 TECHNIQUES – GROWOUT OF JUVENILES

Calico and zigzag scallop juveniles grow quickly at a rate of approximately 5 mm per month. The first few months of growout are therefore labour intensive, in that biomass needs to be regularly controlled to maintain an optimal growth rate. A schedule is given below describing the time between transfers and pearl net size. Once scallops reach a size of approximately 40 mm, at a density of 30 scallops per net, maintenance becomes less labour intensive, involving monthly checks for rips and tears and control of fouling.

5.3.1 Calico scallop growout

Transfer of juvenile scallops to larger size pearl net is conducted on the boat. Large containers of seawater are prepared on board (minimum of two); series of pearl nets are brought on board and scallops removed. Dead scallops are discarded and live animals are transferred to holding containers. Collected scallops are re-distributed into a pearl

net of larger mesh size (6 mm) at a density of 75 scallops per net. Series of nets are then re-suspended on longlines. Scallops remain in these nets for a period of one month. Thereafter, they are transferred to 9 mm nets at a density of 40 scallops per net. They can be maintained up to two months at this density and in this net size; after which they are transferred to 12 mm nets at densities of 30 scallops per net.

Calico scallops average 5 mm per month in shell growth between 3 to 9 months of age; shell growth decreases thereafter allowing for energy utilization during gametogenesis in 10 months old individuals; as spawning period occurs during the winter months (December to May in Bermuda), shell growth remains minimal at this time, but increases once again during the second summer period. Calico scallops have a life span of approximately 2.5 years in Bermuda and can attain a maximal shell height of 65 mm during their second year of growth. The expected growth curve for calico scallops reared on longlines in Bermuda waters is given in Figure 5.8.

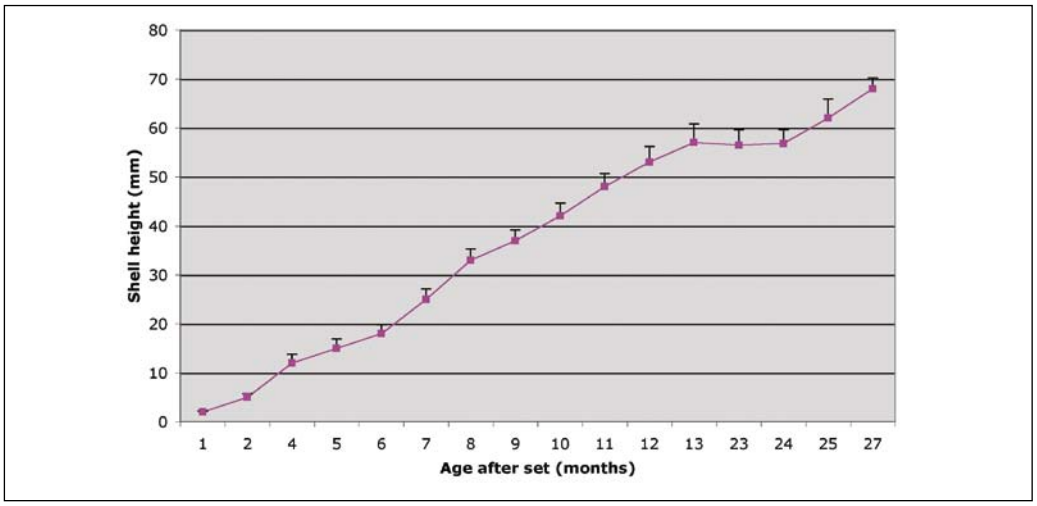


Figure 5.8: Shell growth of calico scallop, *A. gibbus*, juveniles reared on longlines in Bermuda.

For maintenance and control of fouling, monthly checks are made on the longlines. Wear and tear in the line is checked, along with rips in nets. Depending on season and water temperature, nets need to be cleaned monthly (during summer months), and every three months (during winter months). A saltwater power washer (electric motor driven on board) is used for cleaning of nets; it can be connected to the battery of an outboard engine, and nets can be cleaned with scallops remaining inside. This is a much quicker and efficient way of cleaning with no mortality ensued by scallops. Care must be taken to minimize the pressure in order to avoid damaging the scallops when using this method.

5.3.2 Zigzag scallop growout

Zigzag spat are treated in a similar manner to calico scallops until the size of 25 mm.The recessive nature of zigzag scallops seen in Figure 5.9 favours bottom rather than suspended culture (Figure 5.10). These results are in accordance with others reported in the literature, notably the investigation on zigzag scallops by Lodeiros and Himmelman (1994). Thus at 25 mm and above, zigzag

Figure 5.9: Adult zigzag scallops, *E. ziczac*, showing natural recessive behaviour in the sand.

juveniles are reared directly on the sandy bottom, enclosed by a cage, protecting scallops from predation. This is a much more labour intensive method of rearing, as all labour needs to be conducted by SCUBA diving. Cages are made of 20 mm rebar, and meshed on top and sides with plastic 15 mm to 25 mm mesh depending on size of scallops. The bottom is left open, allowing scallops to recess directly into the sand. Cages are of 2.5x1.5 m, yielding a surface area of 3.75 m^2.

The first transfer of 25 mm juveniles to bottom cages (15 mm mesh size) entails the collection of spat from pearl nets. Initial density is of 400 juveniles per cage. Juveniles only remain at such a high density for a period of one month. They then have to be collected by SCUBA, and thinned out to 200 juveniles per cage; again they remain as such for 1-month period, and are then transferred to a 25 mm cage at the final density of 100 per cage. This remains a high density, and periodically, scallops have to be collected and the cage moved. High density of scallops alters the composition of the substrate, leading to hypoxia or anoxia of the sediment, becoming an unfavourable environment for growth. Controlling fouling for cages has to be done monthly by SCUBA using a scrub brush for removal of epiphytes. Although this is intensive, it results in a noticeably faster growth rate than pearl net rearing. The difficulty in commercializing bottom culture lies in developing a time efficient and cost efficient system. To the author's knowledge, such a system for large scale rearing of the sand scallop has not been developed to date.

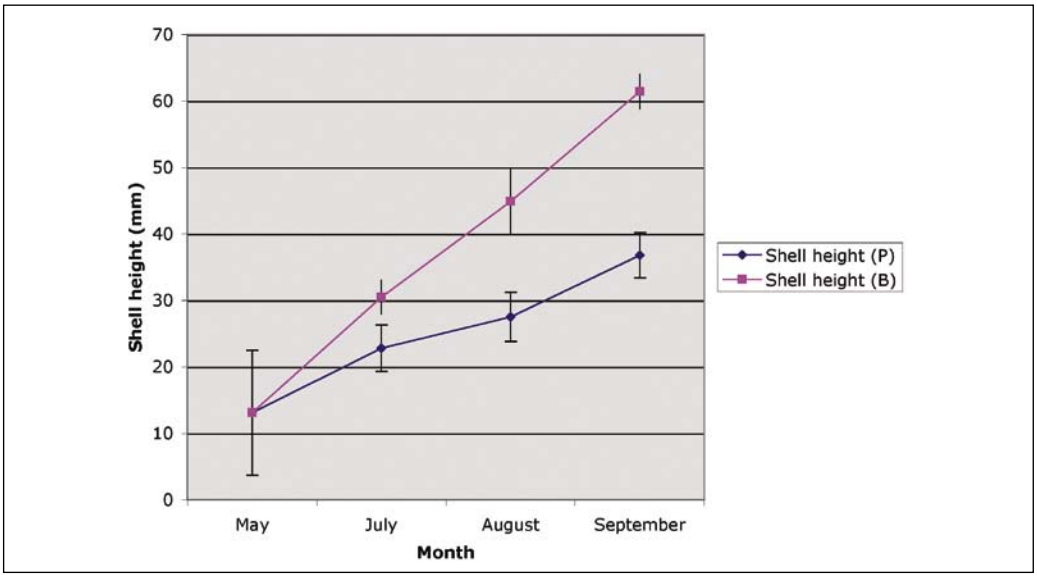


Figure 5.10: Comparative growth between zigzag scallops cultured directly on the sandy bottom (B) and suspended in pearl nets (P).

5.4 TECHNIQUES – TRANSPORT OF JUVENILES

Scallops produced in hatcheries or collected from natural settlements normally require transportation to other sites for continued culture. Scallops are particularly difficult to transport as they are usually sub-littoral organisms, unlike oysters or mussels, and seldom encounter aerial exposure. In part, for this reason, stress associated with transport often causes mortalities. Several methods of transport have been attempted; two of the most common are the use of insulated boxes with material soaked in seawater, referred to as dry transport, (Ventilla, 1982; Maguire, Cashmore and Burnell, 1999; Maeda-Martinez, Siscard and Reynoso-Granados, 2000), and vivier lorry, where animals are transported in seawater with a continuous oxygen flow (Maguire, Cashmore and Burnell, 1999). Although some success is obtained, survival

reported in the literature does not exceed 61 percent immediately following 19 hours of simulated dry transport (Maeda-Martinez, Siscard and Reynoso-Granados, 1999), and increased mortality (up to 51 percent) is often seen in subsequent months during growout (Maguire, Cashmore and Burnell, 1999). As for transporting in seawater, high densities cause a rapid deterioration in quality of holding seawater by reducing dissolved oxygen and increasing ammonia and bacterial levels, leading to gill tissue damage (Maguire, Cashmore and Burnell, 1999).

In Bermuda, due to the small size of the island, transport to growout site can be easily conducted by holding scallops in seawater-filled containers on-board a boat. However, because of interest expressed by other Caribbean islands to rear tropical scallop species and consequent need for transport of spat or adults, procedures for optimal transport were investigated. These procedures were tested for both the sand scallop and the calico scallop, aiming for maximal survival following 24 hours transport time and following transfer to the field.

Details of the experiments conducted are reported in Sarkis, *et al.*, 2005. The procedures are based on methods used for fish transport in the aquarium trade. The methodology developed has been used in two real life situations for the sand scallop. In both cases, scallop spat were taken by air to another site; the total duration of the trip – from packing of scallops to unloading of scallops in the receiving nursery – was of 16 hours. Survival rate upon arrival was >90 percent. It was found that for successful transport of scallop spat, care must be taken to minimize stress during transport, and enhance tolerance of spat to stress by acclimation prior and post transport. Starvation and gradual acclimation to lower temperature, prior to transport, allow for a reduction in metabolic rate, reducing oxygen consumption, amount of excretia and accumulation of acidity, carbon dioxide and ammonia during transport. Low temperatures during transport maintain a low metabolic rate, minimizing oxygen consumption throughout the duration. Following transport, gradual acclimation to ambient temperature minimizes recovery stress, possibly enhancing long term survival. Shell growth of scallops transferred to the natural environment was not affected by simulated transport. The protocol developed is given in detail below (Protocol–17). This procedure should become easily adaptable to various scallops species, with additional insight into some of the species-specific physiological requirements.

PROTOCOL–17

PROCEDURE FOR LONG TRANSPORT PERIODS OF JUVENILE SCALLOPS

1. Do not feed scallops to be transported for 24 hours prior.
2. Acclimatize juvenile scallops to cold water (14 °C) for a minimum period of 6 hours (without feeding).
3. Make a false bottom of polystyrene to fit the cooler, in order to avoid direct contact of scallops with gel packs. Drill several holes in false bottom to allow cooling of entire container.
4. Weigh 160 grams sub-samples of spat (in tarred mesh, blotted dry as in Protocol–14).
5. Fill 1 litre zip-lock bags with 600 ml of 1 µm filtered cold seawater (14 °C).
6. Add scallops to zip-lock bag. Partially close bag.
7. Fill zip-lock bag with oxygen using Pasteur pipette fitted to oxygen regulator and bottle. Close zip-lock bag.

8. Place zip-lock bag into second bag for additional security. Use duct tape to secure closure and avoid any potential tearing or opening.
9. Cool a polystyrene box (60x30x40 cm) to 14 °C using four gel packs. Install false bottom on top of the gel packs and place scallop bags on it.
10. Secure lid of polystyrene box with tape.
11. Following transport do a visual assessment of scallop behaviour and mortality. Record temperature in bag.
12. Transfer scallops into a cold water bath (14 °C) and gradually increase seawater temperature ambient over a 12-hour period.
13. Maintain spat in running seawater once ambient temperature is reached.
14. Following a minimum 3-day acclimation to ambient temperature spat can be transferred to the field.
15. If spat is kept for longer than 3 days, complimentary food supply should be added as described in Appendix 21.

Chapter 6

Economic considerations: costs of set-up and labour requirements

6.1 SET-UP COSTS OF A MODULAR HATCHERY

The equipment and materials required for the set-up of the hatchery/nursery complex and for the carrying out of the rearing protocols are given in detail in Appendix 22. This list is given as an aid to assessing the needs for setting-up and operating a modular hatchery and assist in costing out such a hatchery. In-depth economic studies have been conducted on other scallop species (Paquotte and Fleury, 1994; Dredge *et al.* 2002), which provide the capacity for modelling. The intent here is to provide baseline information which can thereafter be used in modelling, if required.

For ease of understanding, the list provided has been divided into several sections. Firstly, equipment and materials required for each section of the complex is given with price at the time of publication of this document. It must be noted that all prices are given in US Dollars (USD). Although prices will vary among sites, this can provide a general estimate of the funds required. Additionally, a list of suppliers used for the construction of the Bermuda modular hatchery is given, and may be useful for construction of hatcheries in North and South American regions. Secondly, the list provides small equipment and materials required for conducting the protocols described in this manual. Once again, associated costs (at the time of publication) and suppliers are provided.

Set-up costs and rearing costs involved for this "portable" model hatchery are summarized in Table 6.1 below. Rearing costs refer to requirements for farming scallops as outlined in the protocols of this manual. The price of PVC pipe and parts required for the whole complex is estimated at the price purchased in Bermuda. It has to be kept in mind that Bermuda has a high cost of living and prices given here need to be adjusted to a given region. The amount of PVC required can be estimated from the technical drawings. In total, in Bermuda, it is estimated that USD 16 000 is required for PVC parts. Set-up costs amount to USD 47 500 in Bermuda, and include all equipment outlined in Appendix 22 for set-up. Initial rearing costs amount to USD 25 100 and include all equipment outlined in Appendix 22 for rearing of scallops. The total cost for a turn-key modular hatchery, excluding labour costs, amounted to USD 88 600 in Bermuda. Yearly operating costs are not included here.

Table 6.1: Summary of the set-up costs for the hatchery/nursery complex. Set-up costs do not include shipping, PVC pipe/connections, construction materials (cement, concrete blocks, etc.) and electrical components. An estimated figure is given separately for PVC parts based on expenses made in Bermuda (***Note****: Table figures taken from Appendix 22 have been rounded off*).

Facility section	Set-up costs (USD)	Rearing costs (USD)	Estimated PVC costs (USD)
Seawater system	3 000		5 000
Heating seawater system	4 000		500
Housing containers (hatchery, nursery, algae)	12 000		
Broodstock	4 000	1 500	500
Algae	10 000	13 000	500
Hatchery	3 500	1 500	5 000
Nursery (indoor & outdoor)	6 000	600	4 000
Transfer	1 000	1 500	500
Growout (for 25 000 adult scallops – 90 m longline)	4 000	7 000	
Grand Total	47 500	25 100	16 000

Note:
- Rearing costs for broodstock include materials for spawning procedure (Appendix 22).
- Rearing costs for algae include materials for master and sub-culture, live & dry algae (Appendix 22).
- Rearing costs for hatchery include materials for construction of sieves and miscellaneous equipment (Appendix 22).
- Rearing costs for nursery include materials for construction of sieves for indoor and outdoor raceways (Appendix 22).
- Rearing costs for transfer include transfer materials (Appendix 22).
- Rearing costs for growout include growout materials (Appendix 22).

PVC costs for seawater include main seawater intake line, pump house, main supply to heating unit, algal unit and hatchery complex; as well as main drain pipe system. PVC parts for hatchery include main ambient and heated seawater line to all tanks and air line.

6.2 OPERATIONAL LABOUR REQUIREMENTS

Running costs for the entire operation are given in Table 6.2 below. These costs are shown as percentages, as prices and salaries vary widely among countries, and should provide a basis for assessing operational costs for a region. Running costs include electricity, replacement of equipment and consumables. The percent labour column results from an evaluation of the time involved in carrying out the tasks necessary for rearing of scallops at each phase of its life cycle. Both running and labour percentages are given for a 12-month period and are based on the hatchery operating at full capacity, yielding a production of 600 000 to 800 000 spat per year, of 2–9 mm shell height and ready for transfer to the field. It can be seen from Table 6.2 that highest running costs are associated with the larval rearing (hatchery) phase and the growout phase (juveniles to adults). The algal culture phase is least expensive in running costs due in part to the reliance on dry algae for the nursery phase (see Chapter 4). Running costs for the nursery phase are dependent on duration of spat rearing and the strategy followed for transfer to the field. Labour requirements follow a similar trend with the highest requirements during the larval rearing (hatchery) and growout. These requirements are based on rearing larvae in a conventional static system where water is changed every other day; it is anticipated that changing to a flow-through system would reduce the labour requirements for this phase (see Chapter 3). Growout is the most labour intensive part of the operation in Bermuda although needs are not distributed evenly throughout the twelve months and occur in bursts. For example, according to the cycle described in Bermuda, where larvae are reared in the winter months and spat are

transferred to the field in early summer, growout is most intensive during the summer months at the beginning of this phase. By late fall (October/November) growout requirements are much reduced.

Table 6.2: Operational costs as a percentage of time for full time aquaculture activities from spawning to growout.

Section	Running costs (in %)	Labour (in %)
Algae	13.7	14
Hatchery	28.3	33
Nursery	18.7	23
Growout	39.3	30

6.3 FINAL PRODUCT

This manual has strived to provide step-by-step guidelines for the production of scallop. Scallops are considered a seafood delicacy and the fresh product is, in general, very much appreciated by chefs. Several marketing studies have been conducted in Bermuda demonstrating the high demand for such fresh seafood on this island. Due to the difficulty in developing a cost-efficient growout methodology for sand scallops, large scale rearing is not commercially feasible for this species at this time. However, methods described for the calico scallop have proved successful on a commercial scale. Calico scallops are generally sold at 1.5–2 years old; the whole animal is used and often served in its shell. Scallops, with ripe gonads, are usually preferred by the restaurant trade. A second marketing scheme, selling 30 mm scallops to Italian restaurants, also became successful as young scallops are cooked whole in the shell and mixed in with pasta dishes. The colourful shell of calico scallops and the low price of younger individuals are found to attract this market (Figure 6.1).

Figure 6.1: Zigzag and calico scallops (adults and juveniles) ready for market and sold fresh to restaurants in Bermuda.

The rapid growth rate of these tropical species makes them a good candidate for culture, minimizing risks and costs associated with a long growout period.

This work provides an additional technology for the culture of tropical and sub-tropical scallop species. The techniques have been well tested over the past four years and scaling-up production for commercial purposes can be easily done following the Bermuda protocol by simply increasing the tank capacity (the number of tanks or tank volume) for larval and post-larval rearing. This technology can be adapted to other tropical and sub-tropical regions and to other species. Furthermore, the concept of the module hatchery housed in mobile containers provides a relatively inexpensive facility for the rearing of bivalves, minimizing initial capital costs for the set-up of a commercial operation. Due to the flexible nature of the facility, species-specific requirements can easily be accommodated with the current design. Finally, this type of modular or "portable" hatchery can be advantageous for those areas limited in space (like Bermuda), and/or in areas which are potentially prone to pollution issues. The only requirement for such a hatchery is access to clean seawater.

Appendixes

Appendix 1

Description of oocyte developmental stages

Stage 1: **Undifferentiated** – where follicular structure is indistinct and there is no sex cell primordial.

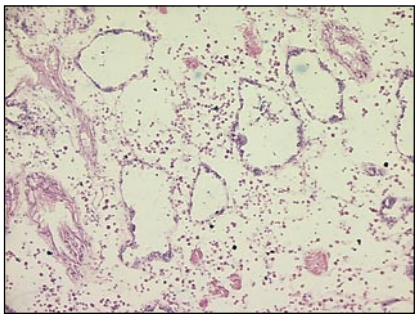
Stage 2: **Differentiated** – where oocyte development commences, primary oogonia are visible, sex cell range from 15–25 µm in diameter.

Stage 3: **Developing** – where there are mostly pre-vitellogenic and vitellogenic oocytes, follicle lumen is open or only partially occluded and oocyte diameter ranges from 30–70 µm.

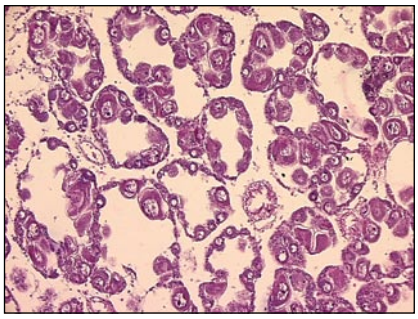
Stage 4: **Ripe** – where gonads show packed follicles, the follicular lumen is occluded, there is no inter-follicular space visible, and oocytes range 60–70 µm in diameter.

Stage 5: **Spawning** – where the follicle lumen is opening, follicle size diminishing and ripe oocytes range 60–70 µm.

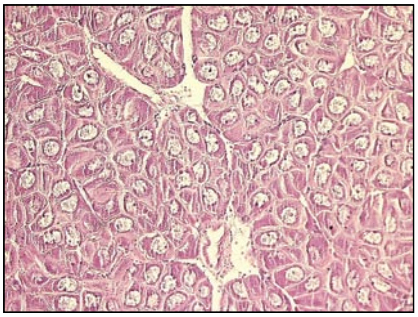
Stage 6: **Spent** – where follicles are empty and frequently filled with haemocyte aggregations on slide preparations and an occasionally atretic oocyte is present.



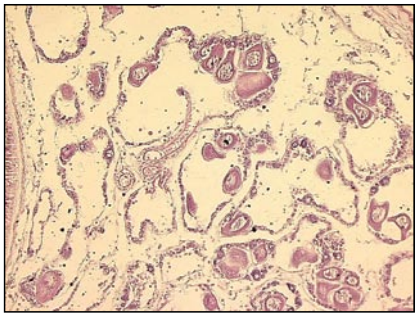
Stage 2: Differentiated



Stage 3: Developing



Stage 4: Ripe



Stage 6: Spent

Appendix 2

Sample data sheet for gonadal and muscle indices

SPECIES:

Scallop No.	Shell height (µm)	Shell lenght (µm)	Total wet weight (gram)	Empty shell weight (gram)	Gonad foil weight (gram)	Muscle foil weight (gram)	Gonad dry weight & foil (gram)	Muscle dry weight & foil (gram)	Gonad dry weight (gram)	Muscle dry weight (gram)	GI	MI

Appendix 3

Broodstock check sheet

YEAR:

MONTH:

Day	Time	B1 T (°C)	Algal food	B2 T(°C)	Algal food	B3 T (°C)/Flow	B4 T(°C)/Flow	COMMENTS
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								
26								
27								
28								
29								
30								
31								

Appendix 4

Heating unit set-up and take-down

Note: Refer to Technical Drawing – Pg 5A.

Set-up

1. Backwash entire seawater system first.
2. Rinse coarse filter, 10 µm and 1 µm filters with fresh water.
3. Turn off drain valve for coarse filter; open air vent slightly.
4. Install filters in cartridges.
5. Divert flow of water after 1 µm filter to drain for 15 minutes, clearing any chlorine residue in filters.
6. Open incoming valve to coarse filter halfway and valve to 10 µm filter halfway.
7. Rinse heating tank with fresh water.
8. After 15 minutes, divert 1 µm filtered seawater to fill tank.
9. Open Valves X and E all the way.
10. Make sure valves D, F and W are closed.
11. Once level of water in tank has reached the alarm switch and pump is turned on recycling the water into the tank, make sure plates of titanium heaters are covered with water.
12. Turn heaters and controllers on. Set temperature as desired.
13. A small red dot on some temperature controllers indicates that heaters are heating. Once set temperature is reached, red dot disappears.
14. Close incoming water valve from 1 µm filter to accelerate heating in the tank. So that water is only being heated and recycled.
15. Once desired temperature is reached, open incoming seawater valve from 1 µm filter halfway. You now have inflow of 1 µm filtered seawater and recycled water going into tank.
16. Open valve D halfway to supply hatchery with heated seawater and close valve E to halfway mark.
17. Depending on the supply and degree of heating required, balance may have to be altered by degree of valve closure.

Take-down

1. Turn off heaters and temperature controllers.
2. In hatchery unit: Open last red valve of heating line; Make sure all other red valves are closed.
3. In heating unit: Close incoming water from filters and Valve D so that water is only recycled within the tank.
4. Pour 100 ml of chlorine into tank and mix by letting water recycle for at least 15 minutes.
5. When hatchery has been cleaned, close valve E and open valve D completely. Chlorinated water will pass through the lines to hatchery.

6. Once water level drops and pump stops, close valve D and open drain valves F and W. Let tank empty completely.
7. Rinse empty tank and heaters with fresh water hose.
8. Remove filters from cartridges and rinse cartridges with fresh water.
9. Place filters in a bucket of fresh water with a capful of chlorine. Let sit for 24 hours.
10. The next day rinse filters with fresh water and let dry.

Appendix 5

Maintenance and cleaning of seawater system

Note: Refer to Technical Drawing – Pg 2.

1. Backwash first thing in the morning every day during months of hatchery operation. If needed (if water has high particulate matter), backwash several times a day.
2. For a continuous pumping system, cleaning of system should be done at the beginning of the season and at the end of the season.

End of season

1. Pump is turned off. Supply line is drained using drain valves located at lowest point.
2. Empty sand filter by opening drain valve. Remove sand and wash with fresh water. Let dry and store.
3. Clean sand filter and all parts with diluted muriatic acid and fresh water. Dry and store.
4. By SCUBA, remove mesh and drain of anchor. Block inlet with secured reinforced plastic bag. If possible, soak mesh and drain in a bucket of fresh water with a capful of muriatic acid to clean. Store until next season. If too fouled, replace with new mesh and a new drain.
5. Remove check valve, clean and place back in line.
6. Close all drain valves and pour gallons of chlorine into cleaner Y-junction after sand filter to clean supply line to hatchery. Let sit for a few days. Then drain line by opening all drain valves.
7. Once drained, close valves until next season.

Beginning of season

1. By SCUBA, remove mesh and drain of anchor. Block inlet with secured reinforced plastic bag. If possible, soak mesh and drain in a bucket of fresh water with a capful of muriatic acid to clean. If too fouled, replace with new mesh and a new drain.
2. Remove check valve, clean and place back in line.
3. Pour gallons of chlorine into cleaner T-junction in pump room to allow seepage into the inflow line. Let sit for a few days.
4. Set up sand filter with sand following dealer's instructions.
5. Chlorinate supply lines to hatchery as at the end of the season (see Point 6 above).
6. Remove bag from inlet hole, install cleaned or new drain and mesh.
7. Turn pump on so as to pump water through and send to last drain valve on supply line for 24 hours at least to remove all chlorine residue.

Appendix 6

Pump room log

YEAR:			
MONTH:			
Day	Time	PSI pressure	Comments
1			**Check**: Oil pump
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			**Check**: Oil pump
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			
31			

Appendix 7

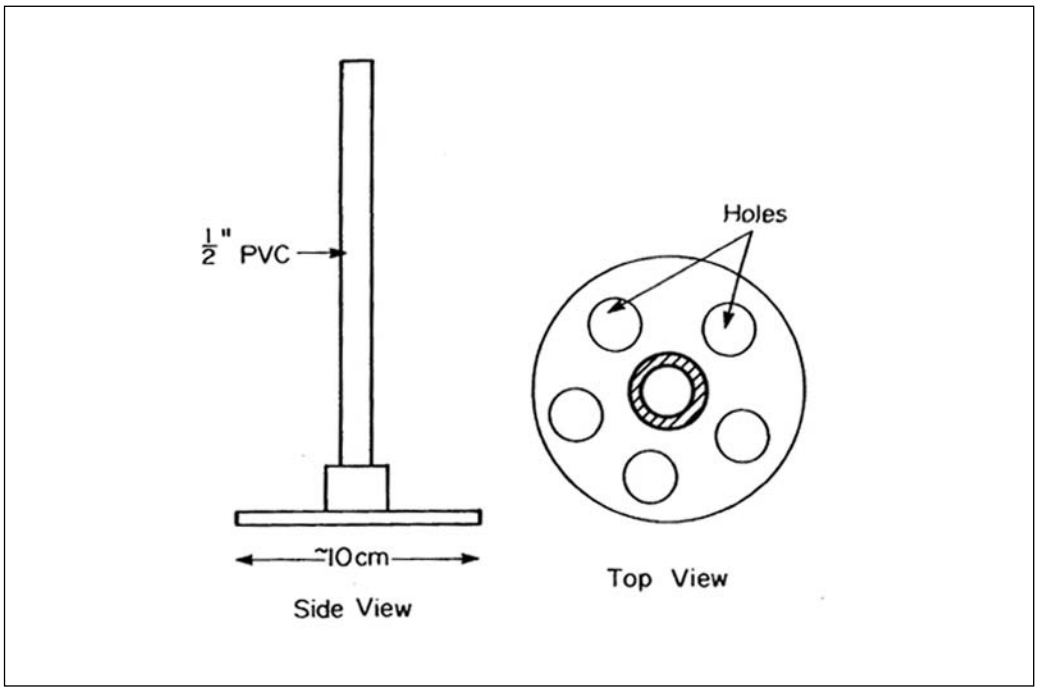
Cleaning hatchery after spawning

Note: Refer to Technical Drawings – 3/Pg 5A and Pg 7

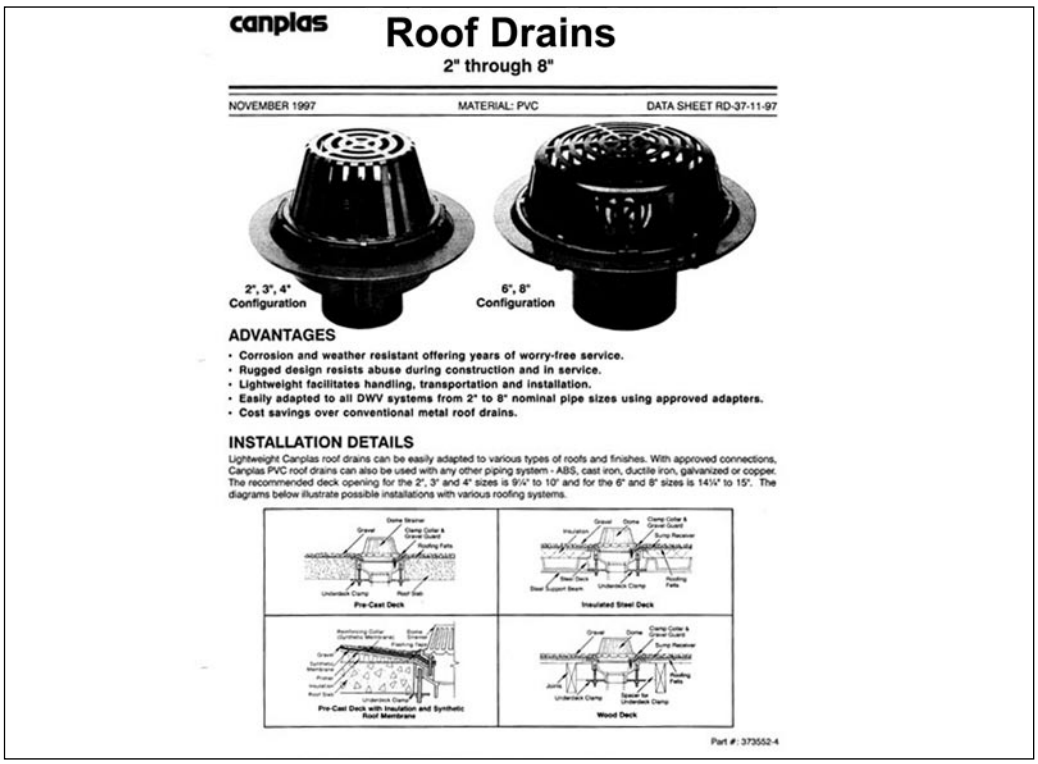
1. Remove all scallops to outdoor tank with high flow of water; allow to spawn completely.
2. Close inflow of water from heating unit.
3. Disconnect cartridge for double 1 µm filter.
4. Open last red valve (heating line) in hatchery; close other valves in heating line.
5. Once chlorinated water has passed, rinse heating line with fresh water hose.
6. Open all red valves for complete drainage of pipes. Once drained close valves.
7. Open valve W at exterior of hatchery to allow for complete draining of heating line.
8. Discard all extra spawn. If species is exotic, discard in a chlorinated tub first.
9. Wash all trays and beakers with chlorine and fresh water.
10. Pass a small piece of chlorinated sponge through flexible hoses with fresh water hose.
11. Dip flexible hose into tub filled with chlorinated fresh water and leave until next water change.
12. All filters are rinsed and placed into tub filled with chlorinated fresh water.
13. Filter housings and immersion heaters are washed with chlorine and fresh water.
13. Store trays, beakers, filter housings and all other materials when dry.

Appendix 8

Details of materials – Plunger and drain



PLUNGER – made of Plexiglas base and a plastic or PVC rod. Used for mixing of egg and larval solutions (taken from Bourne, Hodgson and Whyte, 1989).


canplas

Roof Drains

2" through 8"

NOVEMBER 1997 | MATERIAL: PVC | DATA SHEET RD-37-11-97

2", 3", 4" Configuration

6", 8" Configuration

ADVANTAGES

- Corrosion and weather resistant offering years of worry-free service.
- Rugged design resists abuse during construction and in service.
- Lightweight facilitates handling, transportation and installation.
- Easily adapted to all DWV systems from 2" to 8" nominal pipe sizes using approved adapters.
- Cost savings over conventional metal roof drains.

INSTALLATION DETAILS

Lightweight Canplas roof drains can be easily adapted to various types of roofs and finishes. With approved connections, Canplas PVC roof drains can also be used with any other piping system - ABS, cast iron, ductile iron, galvanized or copper. The recommended deck opening for the 2", 3" and 4" sizes is 9¼" to 10" and for the 6" and 8" sizes is 14¼" to 15". The diagrams below illustrate possible installations with various roofing systems.

Pre-Cast Deck

Insulated Steel Deck

Pre-Cast Deck with Insulation and Synthetic Roof Membrane

Wood Deck

Part #: 373552-4

DRAIN – plastic roof drain used on inlet of seawater pipe as a filter for larger marine organisms.

Appendix 9

Preparation of culture media

F/2 Nutrient solution

This solution was purchased from Aquatic Eco-systems. It is sold in two parts: Part A and Part B.

For 125 ml, 500 ml and 4 l cultures:

1. Mix equal volumes of each part into a 500 ml dark glass bottle (some of the chemicals are sensitive to light).
2. Cap the bottle with a cotton plug and autoclave according to manufacturer's instructions.
3. Wrap bottle top with foil and autoclave.
4. Once autoclaved let solution cool.
5. Add vitamin stock solution using microbiological sterile techniques.
6. Close bottle with autoclaved top and label with date.
7. Store at -4 °C.

For 100 l cultures:

1. Use solutions directly from purchased containers.
2. Add equal parts of solutions directly to cultures using 25 ml graduated cylinder or 10 ml graduated pipettes.
3. Add algae in accordance with volume of culture. Instructions on volume of F/2 required per volume of culture are given on the purchased bottles.

Vitamin stock solution

Materials:

- 50 ml volumetric flask and top.
- Glass funnel.
- Q-water bottle.
- Vitamin B12.
- Vitamin B1.
- Biotin.

Method:

1. Autoclave the volumetric flask and the glass funnel.
2. Weigh out vitamins:
   B12 = 0.005 grams
   B1 = 5 grams
   Biotin = 0.02 grams

3. Transfer vitamins into the flask.
4. Fill up the flask to total volume of 50 ml with Q-water.
5. Insert a magnetic stirrer and stir.
6. Keep the stock solution in the fridge in the algae container.

Vitamin solution is added to the autoclaved F/2 solution as follows:

1 ml vitamin solution per 1 liter F/2 solution

Sodium metasilicate solution (3 % w/v)

Materials:

- 500 ml dark glass bottle and top.
- Glass funnel.
- Q-water.
- Sodium metasilicate (Na_2SiO_3).

1. For a 300 ml volume, weigh 9 grams of sodium metasilicate.
2. Pour 300 ml of Q-water into a 500 ml graduated cylinder.
3. Fill a squeeze bottle with this water. In this way you'll keep track of the volume of water added for dissolution.
4. Gradually transfer weighed sodium metasilicate powder into bottle, diluting solution with Q-water from your squeeze bottle progressively. Make sure to add a small amount of powder at a time otherwise it is difficult to dissolve.
5. Once diluted, cap bottle with cotton plug and autoclave.
6. Wrap glass top in foil and autoclave.
7. Once cool, store solution at -4 °C.

Note: If there is no dark glass bottle, use clear glass bottle wrapped in foil. Sodium metasilicate is sensitive to light.

Sodium metasilicate is added as a supplement to F/2 and vitamin solution for diatom species. Volume added is 2 ml of sodium metasilicate for 1 l of culture.

Convay medium

Preparation of Solution C for a 25 l volume:

KNO_3	2 000 grams
K_2HPO_4	400 grams
EDTA	750 grams
H_3BO_3	50 grams
TMII	2 000 ml
SWII	125 ml
Vitamin Solution	25 ml

TMII Solution (25 l volume):

$Fe_2(SO_4)_3$	395 grams
$MnSO4.H_2O$	37.5 grams

$ZnSO_4.H_2O$	6.25 grams
$CuSO_4.5H_2O$	5.00 grams
$CoSO_4.7H_2O$	0.65 grams
$NaMoO_4.2H_2O$	0.35 grams

SWII Solution (10 l volume):

$SrCl_2:6H_2O$	13.00 grams
$AlCl.6H_2O$	0.50 grams
$RbCl.6H_2O$	0.20 grams
$LiCl.H_2O$	0.10 grams
KI	0.05 grams
KBr	0.65 grams

Vitamin Stock Solution (1 l volume):

B_{12}	0.10 grams
B_1	100.00 grams
Biotin	0.40 grams

F/2 media

1.	Nitrate	$NaNO_3$	75 g/l
2.	Phosphate	$NaH_2PO_4.H_2O$	5 g/l
3.	Silicate	$Na_2SiO_3.9H_2O$	30 g/l
4.	Trace Metals	$FeCl_3.6H_2O$	3.5 g
		Na_2EDTA	4.36 g

Dissolve in 900 ml distilled H_2O.

Add 1 ml of each of the following trace metal solutions.

- $CuSO4.5H_2O$ 0.98 g/100 ml
- $ZnSO4.7H_2O$ 2.2 g/100 ml
- $CoCl2.6H_2O$ 1.0 g/100 ml
- $MnCl2.4H_2O$ 18.0 g/100 ml
- $Na_2MoO_4.2H_2O$ 0.63 g/100 ml

Bring solution to 1 l with distilled H_2O (pH about 2.0).

Add 1 ml/l of filtered seawater of the above solution (#1-4).

5.	Vitamins:	Biotin	1.0 mg
		B_{12}	1.0 mg
		Thiamine HCl	20.0 mg

Dissolve in 1 l distilled H_2O. Store in refrigerator.

Add 0.5 ml of vitamin solution for every 1 l filtered seawater.

Appendix 10

Chemical sterilization procedure

Materials:

- Sodium Hypochlorite (12 %) solution or commercial grade chlorox.
- Sodium Thiosulfate (1 N) solution.
- Glass bottle with screw cap.

Procedure:

Prepare 1 N sodium thiosulfate by dissolving 158.1 grams sodium thiosulfate with de-ionised or distilled water. Store in glass bottle.

For sterilizing container with seawater:

1. Fill container or flask with filtered seawater.
2. Add 1 ml of chlorox (or sodium hypochlorite) per liter of seawater.
3. Stir or bubble and allow to sit for a few hours.
4. Close flask with lid or rubber stopper.
5. Neutralize chlorox with 1 ml of sodium thiosulfate per litre of seawater.
6. Stir or bubble.
7. Seawater and container are ready to be used for algal inoculum or other purpose.
8. You will probably need to pour out some of seawater for algal inoculum. But filling the entire container initially allows for complete sterilization.

Appendix 11

Set-up and take-down of seawater supply in algae container

Set-up of seawater supply in algae container:

Note: Refer to Technical Drawings – Pg 1 and Pg 9.

1. Remove filters and hose from chlorinated bin.
2. Rinse abundantly with fresh water all filters (coarse, 10 µm and 1 µm) and flexible water hose.
3. Set-up filters in respective cartridges fixed to outside of container, and connect flexible hose to UV sterilizer placing end in sink of algae container.
4. Open drain valve Z by Y-junction and allow chlorinated water to drain. Open union by inflow valve Q to coarse filter and rinse abundantly with fresh water.
5. Close drain valve Z and union.
6. Open inflowing valve Q to coarse filter all the way and close outflowing valve O of coarse filter.
7. Slightly open air vent of coarse filter.
8. Open main valve Y to algal container (from Y-junction) three quarters of the way.
9. Allow coarse filter to fill with water.
10. When water starts to come out of air vent (it takes a few minutes only), open outflow valve O halfway to allow water to pass through 10 and 1 µm filters.
11. Check that water is passing through UV sterilizer and flowing out of flexible hose into sink. Let run for 15 minutes to eliminate all residue of chlorine.
12. Plug UV sterilizer in when ready to use. Control seawater flow with main valve Y and outflow valve O.

Take down of seawater supply to algae container:

Note: Refer to Technical Drawings – pages 1 and 9.

1. Unplug UV sterilizer.
2. Close main valve K at Y-junction.
3. Close inflow valve I to coarse filter and open drain plug of coarse filter. Allow coarse filter to empty.
4. Remove filters from coarse 10 µm and 1 µm cartridges. Rinse salt off with fresh water and place in chlorinated bin until next use. Do the same with flexible hose.
5. Rinse coarse filter with fresh water allowing to empty via drain plug. Rinse lid as well and place back on coarse filter.
6. Undo union prior to inflow valve I and open drain valve H of pipe adjacent to Y-junction.
7. Blast fresh water through pipe for a few minutes. Close drain valve H. Fill pipe with fresh water and a capful of chlorox. Close union and let sit until next use.

8. Undo union after 10 and 1 µm cartridges and rinse with fresh water hose for a few minutes to rinse line through UV sterilizer with fresh water. Make sure to place a tray under UV sterilizer to collect fresh water and prevent spilling on algal container floor.
9. Allow pipe to drain. Rinse 10 and 1 µm cartridges with fresh water and replace on-line.

Appendix 12

Bactopeptone test

(from Bourne, Hodgson and Whyte, 1989)

Materials:

- Bactopeptone.
- Filtered seawater.
- 10 ml tubes with cap.

Procedure:

1. Dissolve 10 g bactopeptone in 1 l filtered seawater.
2. Fill test tubes with 10 ml of media and cap.
3. Autoclave tubes.
4. Inoculate tube containing bactopeptone media with 3–4 drops of algal culture.
5. Store in the dark for up to two weeks..

Assessement:

1. Some faster growing bacteria will become evident within 2–4 days.
2. If bacteria are present, contents of the tube will appear cloudy.
3. Degree of cloudiness reflects degree of bacterial contamination. The cloudier a tube the more contaminated.
4. Discard cultures with heavy bacterial contamination.

Appendix 13

Algal culture check list

YEAR:						
MONTH:						
Day	Time	Temperature	Lights/AC	Cultures bubbling	CO_2 level	Comments
1						
2						
3						
4						
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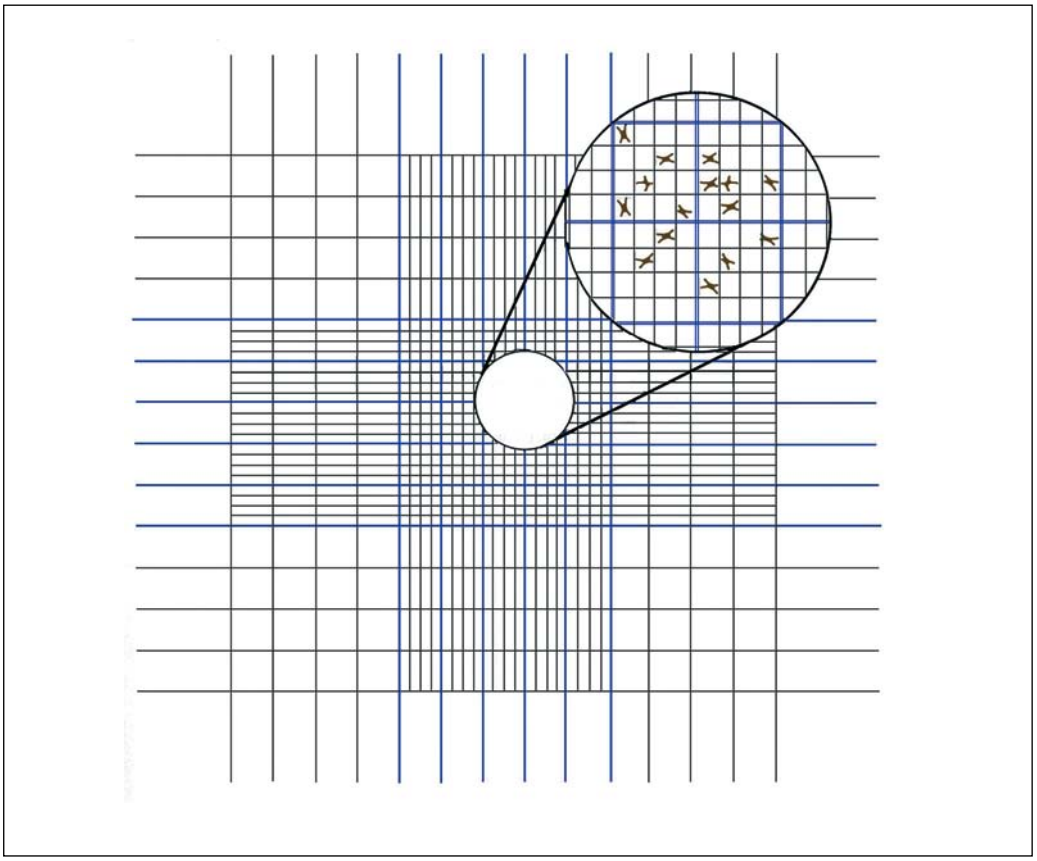
Appendix 14

Haemocytometer cell diagram

(from Bourne, Hodgson and Whyte, 1989)



Appendix 15

Larval check sheet

YEAR:

MONTH:

Day	Species	Day after set	T(°C)	Air	Density No./ml	Total No.	Volume	Rations and species	Comments
1									
2									
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Appendix 16

Determination of dry weight and ash-free dry weight

1. Number (with a pencil) and ash 4.7 cm GF/C filter in a muffle furnace at 450 °C until constant weight.
2. Weigh filters on an electronic microbalance (±1 µg). Record weights for each numbered filter.
3. Set-up a Millipore filtering apparatus and connect to a vacuum pump.
4. Place one filter on filtering apparatus.
5. Count larvae using a Sedgewick-Rafter cell.
6. Calculate number of larvae per ml.
7. Using a 7 mm Tygon tube, siphon required number of ml into a graduated cylinder to obtain total number of larvae. For Day 2 larvae collect minimum of 150 000 larvae. For pediveligers collect a minimum of 5 000 larvae.
8. Pour collected larvae through a 20 µm sieve.
9. Wash larvae with isotonic ammonium formate (3 percent) to remove mineral salts.
10. Wash down larvae with ammonium formate onto filter fitted to filtering apparatus.
11. Ensure that all larvae from sieve and sides of filtering apparatus are collected by thoroughly washing with isoosmotic ammonium formate.
12. Remove filter from filtering apparatus and place in a foil disc.
13. Record number of larvae collected and number of filter.
14. Dry filter in an over at 60 °C until constant weight (approximately 48 hours).
15. Transfer filters to a dessicator to avoid moisture affecting weight.
16. Record weight after drying using same electronic balance.
17. Place filters in a muffle furnace at 450 °C until constant weight.
18. Remove from furnace, transfer into a dessicator, and weigh with electronic balance.
19. Record weight and filter.
20. To calculate larval dry weight (DW):
    a) Dry weight (µg) of filter + Larvae - Initial filter weight
21. To calculate Ash-free dry weight (AFDW):
    a) Larval ashed weight (inorganic matter)= Ash weight of filter + Larvae - Initial filter weight
    b) Larval ash-free dry weight (organic matter)= Larval ash weight - Larval dry weight
22. To calculate Condition Index:
    a) Condition Index= Organic matter/Larval dry weight

Appendix 17

Sieve construction for larval and post-larval collection

Note: Refer to Technical Drawings – Pg 14 and Pg 17.

1. Cut with a circular saw a PVC ring to appropriate height (20 cm high for 20 cm and 25 cm larval sieves, 18 cm high for raceways).
2. Sand surface sieve on both ends to make it as smooth as possible.
3. For a 20 cm sieve for larval collection, drill two 15 mm hole on opposite sides, approximately 40 mm from top. For a 30 cm sieve for outdoor raceway, drill one 25 mm hole 50 mm from top.
4. Cut a square piece of mesh required so as to cover entire surface area of sieve.
5. Lay mesh on top.
6. Adjust hose clamp so that it can be placed on top of mesh and sieve; enough mesh should be available so that it sticks out below hose clamp.
7. Tighten hose clamp along top of sieve with a screwdriver, continuously pulling mesh.
8. Try to eliminate wrinkles in mesh by pulling uniformly around perimeter of sieve.
9. Label size of mesh on side of PVC with a permanent marker.
10. Once mesh is tight and hose clamp secured (approximately 1 cm from top of sieve) use PVC cleaner to prepare surface of sieve for gluing.
11. Glue mesh to PVC using PVC glue (or cement) along surface and side down to hose clamp.
12. Let dry for 24 hours.
13. Once dry, make sure mesh is uniformly glued so that larvae cannot accumulate in small unglued areas.
14. Remove hose clamp and cut unglued mesh with a razor blade.
15. For support rings used in larval collection, cut 25 cm diameter pipe in 2.5 cm height.
16. For larval collection and with 6 larval tanks, two sets of rings (20 cm and 25 cm diameter) of same mesh aperture are used, allowing take down of two larval tanks simultaneously. Approximately 10–15 mm pipes were cut to 30 cm length for suspension of 20 cm sieve onto 25 cm sieve.
17. A total of 16 sieves for post-larval settlement, suspended in indoor raceway are used in the present set-up.
18. A total of 12 sieves for outdoor raceway are used in the present set-up.
19. A range of sieves, with mesh size ranging from 1.2 mm in diagonal to 4.9 mm, are used in the present set-up for grading.

Appendix 18

Raceway check list

YEAR:						
MONTH:						
Day	Species	Day after set	Air	Density No./ml	Total No.	Comments
1						
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Appendix 19

Cleaning of raceway

Note: Refer to Technical Drawings – Pg 14 and Pg 17.

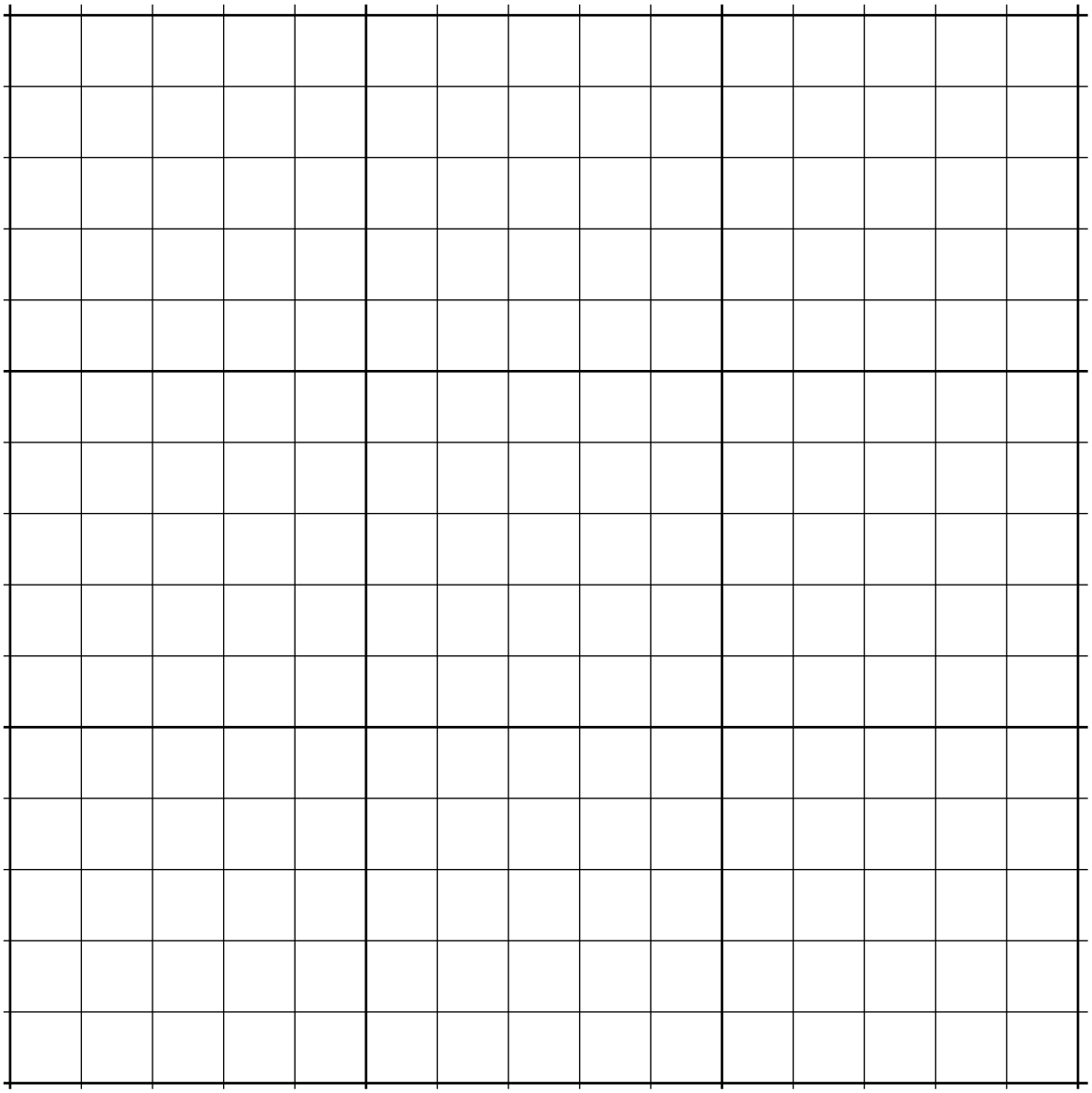
Raceway cleaning involves full chlorination of lines and troughs. The process is similar for both indoor and outdoor raceways. For the indoor raceway, care must be taken to chlorinate all recirculating pipes and sump tank. For the outdoor raceway, algal tank needs to be chlorinated.

1. Turn off pump for recycling system and/or algal supply.
2. Drain troughs, pipes and tanks.
3. Rinse with fresh water.
4. Plug troughs and fill with 50 cm of freshwater.
5. Add capful of chlorine and scrub to remove any detritus.
6. Fill sump tank/algal tank with fresh water to 50 cm and scrub.
7. Drain sump tank/algal tank. Fill with seawater and add capful of chlorine. Mix and let sit.
8. Drain troughs and rinsing with strong jet of fresh water.
9. Indoor raceway: start pump for recycling, open recycling valves and let chlorinated seawater pass through pipes. Watch water level in sump tank so that pump does not run dry.
10. When water level in sump tank/algal tank is low, close off pump.
11. Drain sump/algal tank.
12. Rinse with fresh water and saltwater.
13. Rinse troughs with saltwater.
14. Turn seawater supply on, fill sump tank, turn pump on, and let run through troughs and pipes for 15 minutes.
15. Place standup pipes in troughs, fill for use.
16. Reset recirculation system.
17. Outdoor raceway: Turn pump on to recycle chlorinated water through algal lines. Take care not to let pump run dry.
18. When water level low in algal tank, turn off pump. Open union on drain side of tank.
19. Drain tank and rinse with fresh water.
20. Close union, fill tank halfway with seawater. Turn pump on and let run through algal lines.
21. When low, fill tank with seawater and add algae as per usual.
22. Close trough with standup pipes and fill with seawater.

Appendix 20

Counting grid for spat

(Template to be copied and laminated for use)



Appendix 21

Preparation and ration for dry algae

Instant algae comes in 1 l ziplock bag and can be purchased from Reed Mariculture Inc.

- Packs of *Tetraselmis chuii* contain the equivalent of 1 800 l at 2 600 cells.µl^{-1}.
- Packs of others (*Pavlova lutheri, Isochrysis galbana* (Clone: T-Iso), *Thalassiosira pseudonana* (Clone: 3H)) contain the equivalent of 3 600 l at 410 cells.µl^{-1}.

They should be refrigerated and have a shelf life of 12 weeks.

To calculate food ration: F= (SxR)/7

Where:

F= dry weight of algae per day (mg)

R= ration as dry weight of algae per wet weight of spat (mg algae.mg spat^{-1})

S= total wet weight of spat (mg)

Example:

Total biomass= 75 g= 75 000 mg.

Ration= 0.4 mg dry weight of algae per mg wet weight of spat.

Diet: Mixture of *Tetraselmis chuii*, *Pavlova lutheri* (or T-Iso) and 3H

F= (75 000x0.4)/7= 4285.7 mg dry wt algae

Daily ration is 4.3 grams dry weight

Prepare aliquots of each algal species. Use at least three different species for daily food ration: In Bermuda, *Pavlova lutheri* and T-Iso is used alternatively. Ration is based on biomass as seen above. For ease of preparation, one week supply of aliquots can be made in advance, assuming an average 350 grams biomass in raceway.

Algal preparation and supply:

1. Transfer one 10 ml aliquot of one species to a screw cap 10 ml test tube.
2. Label the vial with species and date.
3. Keep in the refrigerator until use.
4. Daily: Mix 1 vial of 3H and *Pavlova lutheri* (or T-Iso)(10 ml) and 1/2 a vial of *Tetraselmis* (7.5 ml) into 1 l of seawater.
5. Pour into 150 l tank in outdoor raceway when full.
6. This provides an adequate food ration complimentary to any additional nutrition supplied by the raw seawater.

Appendix 22

List of equipment: template for costing out set-up of modular hatchery

Note: List is drawn by rearing stage (or manual chapter). Costs are based on North American and Bermuda prices, as of 2004, and are given as an indication of expenditures. Costs need to be calculated for each specific project and can be done using this template. PVC parts, shipping of equipment and labour are excluded.

Seawater supply (ambient and heated) and broodstock (Chapter 1)				
QTY	**Description**	**Unit price (USD)**	**Extended price (USD)**	**North American/other supplier**
Seawater inlet/pump house/heating/broodstock				
2	50mm PVC roof drain	43	86	Local hardware
6	50mm flange (2 flanges, gasket and bolts)	63	376	AES
2	Clear PVC check valve (2")	24	48	AES
1	Concrete anchor	500	500	Homemade
2	50mm flange	50	100	AES
1	Sweetwater centrifugal pump 2 hp	700	700	AES
1	Hayward self-priming super pump (1 1/2 hp)	365	365	AES
1	Jacuzzi sand filter	369	369	AES
2	Sand (22 kg bag)	50	100	AES
Sub-total			**2 644**	
Heated seawater unit				
1	1 000-litre insulated tank (43" x 48" x 50")	641	641	BONAR
3	Titanium immersion heaters (one-phase) 6 kw	199	597	AES
3	Digital temperature controllers (one-phase)	339	1 017	AES
1	Water level switch	44	44	AES
1	Hayward cartridge filter housing (C250)	240	240	Local pool supplier
2	C250 cartridges	50	100	Local pool supplier
2	25cm filter housings	22	44	AES
20	10µm and 1µm 25cm wound polypropylene filter cartridges	5	100	AES
1	Quiet one centrifugal pump (Vertical) 0.5 amps	117	117	AES
1	Shed (3m x 3.5m)	800	800	Local supplier
1	Shelves and brackets	20	20	Local hardware
Sub-total			**3 720**	
Containers for hatchery/broodstock complex				
2	6.5m x 3.5m fibreglass insulated containers	2 500	5 000	Local shipping company
	Connection between two containers (concrete and plywood)	1 500	1 500	Local hardware
8	Concrete pads for container corners	200	1 600	Local hardware
2	Doors (PVC with windows)	800	1 600	Local supplier
2	Air conditioners	600	1 200	Local supplier
10	Shelves for storage	20	200	Local hardware
Sub-total			**11 100**	

Broodstock area				
2	380-litre fiberglass aquarium tanks with viewing window	675	1 350	AES
4	Plexiglass for cover and filtration (8mm)	90	360	Local supplier
1	Sweetwater air pump 1.2 CFM@4 psi (9" x 8" x 9")	279	279	AES
20	Labcock ball valves for air control	5	100	AES
5	Nitex mesh 500µm per yard	12	60	Aquafauna Bio-marine
2	Tygon tubing (7mm ID) in a 18m coil	49	98	AES
1	Aqua Logic drop-in titanium water chiller 1/5 hp	910	910	Aqua Logic
8	250 W submersible heaters	20	160	Aquafauna Bio-marine
2	20-litre carboys	100	200	AES
2	Shelves and brackets	20	40	Local hardware
Sub-total			**3 557**	
Spawning procedure				
6	Trays (non-toxic)	30	180	Local hardware
5	Graduated 10-litre buckets	10	50	Local hardware
50	Graduated beakers (2 litre and/or 3 litres)	6	313	AES
2	Sedgewick-rafter cell	125	250	AES
1	Plunger			Homemade
1	Eppendorf pipette (0-1 000µl)	350	350	Sigma
1	Eppendorf tips (100µm and 1 000µm)(1box)	50	50	Sigma
1	Pasteur pipettes (1 box of 250)	14	14	AES
4	Bulbs	6	24	AES
10	Recording book (water resistant)	4	35	AES
6	Thermometers	5	30	AES
1	150µm PVC sieve (see Chapter 3)			Homemade
1	300µm sieve (see Chapter 3)			Homemade
Sub-total			**1 296**	

Algal culture (Chapter 2)				
QTY	**Description**	**Unit price (USD)**	**Extended price (USD)**	**North American/other supplier**
Algal seawater system				
1	4m x 3.5m fiberglass container	2 000	2 000	Local shipping company
1	Hayward cartridge filter (C250)	240	240	Local pool supplier
1	Replacement cartridge	50	50	Local pool supplier
2	10" Cartridge filter housing	22	44	AES
10	10µm filters (1box)	5	50	AES
10	1µm filters (1box)	5	50	AES
1	Lifeguard vertical UV sterilizer	172	172	AES
1	20mm ID re-inforced vinyl tubing (18m coil)	66	66	AES
1	Shelves and brackets	20	20	Local hardware
Sub-total			**2 692**	
Air and light requirements				
1	Air conditioning unit	600	600	Local hardware
1	Freshwater hose	13	13	Local hardware
1	Nozzle	5	5	Local hardware

1	Sweetwater air compressor	279	279	AES
1	Shelves and brackets	20	20	Local hardware
2	50lb CO_2 cylinder	75	150	Local beverage supplier
1	CO_2 regulator with flowmeter	188	188	VWR
1	Refrigerator	350	350	Local supplier
17	Double vertical ballasts	96	1 632	Local supplier
48	Coolwhite fluorescent lamps (30watts)	14	672	Local supplier
6	Single horizontal ballasts (1.2m)	70	420	Local supplier
2	Double horizontal ballasts (1.2m)	96	192	Local supplier
1	7mm ID Tygon tubing clear (36m coil)	11	11	AES
20	Male adapter (8mm NPT x 7mm BARB)	<1	6	AES
20	Labcock ball valve (7mm FNPT ports)	4	81	AES
20	Nipples (7mm NPT x 7mm NPT)	<1	7	AES
20	Bacteria filters 0.3µm (8mm hose barb connection)	7	135	AES
1	Soft tubing cutters	12	12	AES
1	Thread cutting tap (8mm NPT)	6	6	AES
1	Drill bit for 8mm NPT tap	4	4	AES
10	Y-connections	<1	4	Sigma
Sub-total			**4 787**	
Culture vessels and fittings				
6	100-litre vessels (transparent, cone bottom)	199	1 191	AES
6	Lids for 100-litre vessels	12	74	AES
1	Marine plywood for 100-litre vessel frame	96	96	Local supplier
1	West epoxy resin system	150	150	Local supplier
6	50mm male adapter for 100-litre drain	2	12	Local hardware
6	50mm-20mm reducers for 100-litre drain	3	15	Local hardware
6	20mm valves for 100-litre drain	3	20	Local hardware/ AES
8	4-litre Ernlemeyer flasks	45	360	Sigma
3	500ml Pyrex round flasks with flat bottom - 6 per case	69	207	Thomas Scientific
1	125ml Ernlemeyer flasks, screw cap (case of 24)	228	228	VWR
16	3-hole rubber stopper (no. 10)	2	31	AES
1	Glass rod (package)	30	30	Local supplier
3	Cheesecloth material (4 sq. yards)	6	18	Local hardware/ Biomarine Aquafauna
1	Cotton (1 roll)	10	10	Local supplier
1	Pasteur pipettes 1.5ml cap (pack of 400)	20	20	VWR
24	5 and 10ml sterile graduated pipettes (packs of 8)	10	240	AES
1	Incubator	500	500	AES
2	Bottle brush	5	10	AES
1	Thermometer	11	11	VWR
1	Utility brush	5	5	AES
Sub-total			**3 228**	
Master and sub-culture				
1	Deep plastic reservoir with lid (chemical resistant)	30	30	Local hardware
1	250ml graduated cylinder (glass)	12	12	AES
5	Foil	5	25	Local supplier
1	50ml volumetric flask with cap	8	8	AES

1	500ml glass bottles (dark amber) with cap (case of 12)	30	30	AES
1	Autoclavable funnel	2	2	AES
1	Autoclave	10 000	10 000	
4	Labelling tape	10	38	Sigma
4	Magic marker		0	Local supplier
2	Pipette latex rubber bulb	13	25	VWR
2	Haemocytometer cell	133	266	AES
1	25ml graduated cylinder	4	4	Sigma
1	100 ml graduated cylinder (polypropylene)	5	5	AES
1	F/2 Formula nutrient media (Solution A) (4 litres)	14	14	AES
1	F/2 Formula nutrient media (Solution B) (4 litres)	14	14	AES
1	D-Biotin (1g)	39	39	Sigma
1	Vitamin B12 (250mg)	17	17	Sigma
1	Thiamine hydrochloride (250g)	58	58	Sigma
1	Sodium metasilicate nonahydrate (250g)	25	25	Sigma
1	Scientific calculator	22	22	VWR
1	Propane torch	10	10	Local hardware
1	Propane bottle	7	7	Local hardware
2	10-litre carboy	83	166	AES
6	10-litre graduated buckets	10	60	Local hardware
12	2-litre graduated beaker with handle (polypropylene)	5	54	AES
1	Compound microscope	979	979	AES
5	Muriatic acid (4-litre container)	9	44	Local supplier
50	Chlorox (4-litre container)	4	213	Local supplier
1	Formaldehyde (1-litre)	30	30	VWR
Sub-total			**12 196**	
Live and dry algae				
8	stock cultures (15ml volume)	45	360	CCMP
2	Tetraselmis 3 600 (1 litre)	68	136	Reed Mariculture
2	Pavlova 1 800 (1 litre)	38	75	Reed Mariculture
2	Thalassiosira weisflogii 1 800 (1 litre)	30	60	Reed Mariculture
Sub-total			**631**	

Larval seawater system (Chapter 3)				
QTY	**Description**	**Unit price (USD)**	**Extended price (USD)**	**North American/other supplier**
Seawater and air supply (ceiling plan)				
20	50mm plastic pipe hangers	2	40	AES
20	25mm inner diameter clear flexible hose (per 30cm)	2	43	AES
20	Labcock ball valves	4	81	AES
15	8mm FNPT adapter for labcock ball valve	<1	6	AES
20	25mm plastic pipe hangers	1	24	AES
1	Sweetwater 100 V linear compressor	279	279	AES
1	Shelves and brackets	20	20	Local hardware
Sub-total			**493**	
Larval tanks				
4	1344-litre square insulated BONAR tanks with lids	536	2 144	BONAR Plastics
4	40mm-20mm reducer	3	12	Local hardware

4	20mm union	6	24	Local hardware
1	20mm elbow	1	1	Local hardware
1	20mm male adapter	1	1	Local hardware
1	Cartridge filter housing	22	22	AES
10	1µm filter	3	25	AES
10	10µm filter	3	25	AES
1	20mm threaded hose barb (20mm NPT x 8mm barb)	1	1	AES
10	20mm mm inner diameter clear flexible hose (per 30cm)	2	15	AES
1	8mm Tygon tubing (90m coil)	13	13	AES
4	25mm thru-hull bulkhead fitting	14	56	Local hardware
4	25mm female adapter	1	3	Local hardware
4	25mm elbow	1	5	Local hardware
4	25mm ball valve	5	18	Local hardware
20	25mm inner diameter clear flexible hose (per 30cm)	2	43	AES
1	Utility brush	6	6	AES
4	Silicon sealant	11	44	AES
Sub-total			**2 456**	
Larval sieves				
1	20µm nitex mesh (per metre)	60	60	Aquafauna
2	40µm nitex mesh (per metre)	56	112	Aquafauna
2	60µm nitex mesh (per metre)	42	84	Aquafauna
2	80µm nitex mesh (per metre)	34	68	Aquafauna
6	120µm nitex mesh (per metre)	24	144	Aquafauna
6	150µm nitex mesh (per metre)	20	120	Aquafauna
2	300µm nitex mesh (per metre)	16	32	Aquafauna
6	25cm (10”) diameter PVC (per 30cm)	16	93	Local hardware
6	20cm (8”) diameter PVC (per 30cm)	13	75	Local hardware
6	15mm PVC pipe (per 30cm)	1	3	Local hardware
2	15cm (6”) diameter PVC pipe (per 30cm)	8	15	Local hardware
8	Garden hose clamps (125mm)	2	14	Local hardware
Sub-total			**820**	
Miscellaneous equipment				
1	Freshwater garden hose (18m)	25	25	Local hardware
1	Hose nozzle	8	8	Local hardware
1	20-gallon bin for chlorination of small equipment	30	30	Local hardware
20	Chlorine (4-litre containers)	4	85	Local hardware
10	Recording notebook (waterproof)	4	35	AES
2	250ml graduated cylinders	12	25	AES
Sub-total			**208**	

450-L and raceway nursery systems (Chapter 4)				
QTY	**Description**	**Unit price (USD)**	**Extended price (USD)**	**North American/other supplier**
450-litre setting tanks				
4	450-litre tanks with skirt and standpipe assembly	600	2 400	Red-Ewald Inc.
4	Drain pipe assembly (50mm male adapter)	2	8	Local hardware
4	Recirculation assembly (25mm pipe and 25mm T)	120	120	Local hardware
4	20-litre carboys	100	400	AES
2	Shelves and brackets (20cm x 25cm)	20	40	Local hardware

1	3mm black polyethylene mesh (18m roll)	60	60	Atlantic Gulf & Fishing
2	Cable ties (100 per package) - 100mm long	3	5	AES
Sub-total			**3 033**	
Indoor raceway system				
1	220-litre tank	350	350	Bonar Plastics
2	Fiberglass troughs (120 litres)	200	400	Red-Ewald Inc.
1	Centrifugal pump (vertical)	120	120	AES
4	50cm x100cm wolmanized wood for frame	12	48	Local hardware
1	West system epoxy resin	150	150	Local hardware
2	Shelves and brackets	20	40	Local hardware
Sub-total			**1 108**	
Indoor sieves				
8	25cm (10”) pipe for sieves (per 30cm)	16	124	Local hardware
16	Tubing flow valve (for restricting flow)	<1	3	AES
12	Barbed fitting (8mm x 7mm barb)	<1	4	AES
2	20-litre carboys	100	200	AES
	150 and 120µm Nitex mesh - see larval sieves section			
Sub-total			**331**	
Outdoor raceway				
1	Cement pad	600	600	Local supplier
5	Untreated wood for frame (50mm x100mm)	12	60	Local hardware
1	West system epoxy resin	150	150	Local hardware
2	Corrugated plastic for roofing (per sheet)	35	70	Local supplier
1	Marine plywood for roof support	96	96	Local hardware
1	150-litre reservoir	60	60	AES
2	8mm stopcock valve	1	2	AES
30	Window screen (per 30cm, 1.2m wide)	2	60	Local hardware
30	1/2” pipe for screen (per 30cm)	1	23	Local hardware
10	Cup hooks for screen	1	7	Local hardware
1	Centrifugal pump (vertical)	120	120	AES
Sub-total			**1 248**	
Outdoor sieves				
8	30cm pipe for sieves (per 30cm)	18	140	Local hardware
20	750µm green collector bag	2	35	AES
5	1.5mm red collector bag	2	9	AES
12	25mm coupler and pipe for suspension	2	18	Local hardware
Sub-total			**202**	

Transfer phase: exterior tanks and field requirements (Chapter 5)				
QTY	**Description**	**Unit price (USD)**	**Extended price (USD)**	**North American/other supplier**
Holding tanks				
2	540-litre insulated tank with lid	450	900	BONAR Plastics
2	Drain fittings (bulkhead and female adapter)	17	34	AES
1	Silicone sealant	10	10	AES
10	Reinforced vinyl tubing (25mm inner diameter)(price per 30cm)	2	22	AES
Sub-total			**966**	

Growout longlines (for 90m line) in 10m depth				
1	15mm twisted polypropylene rope (200m per reel)	70	70	Atlantic Gulf & Fishing
50	30cm diameter black floats with mooring eye (14.8kg buoyancy)	15	750	Go-Deep
6	Marker buoys	9	54	Atlantic Gulf & Fishing
1	Salt water high pressure washer	2 500	2 500	Easy-Kleen
1	Rope cutting gun	40	40	Atlantic Gulf & Fishing
6	20mm shackles (stainless steel)	28	168	Atlantic Gulf & Fishing
Sub-total			**3 582**	
Transfer materials				
50	window screen and velcro for black insert pouches	3	150	Local hardware
100	750µm green collector bags	2	175	AES
100	Plastic shellfish trays	7	700	Aquaculture Supplier
15	15mm stainless steel snap hooks	10	150	Local hardware
4	Transport coolers	50	200	Local hardware
Sub-total			**1 375**	
Growout materials				
10	Snap-on connectors (12cm standard) (100 per package)	40	400	Atlantic Gulf & Fishing
220	3mm pearl nets	4	814	AES
440	6mm pearl nets	4	1 540	AES
1000	9mm or 12mm pearl nets	4	3 750	AES
20	Cable ties (150mm long - 100 per package)	4	75	AES
Sub-total			**6 579**	
TOTAL FOR EQUIPEMENT			**68 250**	
PVC Estimate	Pipes and parts (Bermuda prices)		16 000	
GRAND TOTAL			**84 250**	

Appendix 23

List of selected suppliers

This list is not comprehensive and provides only those suppliers used during the installation and operation of the Bermuda hatchery. The mention or omission of specific companies, their products or brand names does not imply any endorsement or judgement by the Food and Agriculture Organization of the United Nations.

Bonar Plastic Inc.
125 N. Christopher Court
Newnan, Georgia 30265
United States of America
Tel.: +1 770 2518264 / 800 7686246
Fax: +1 770 2518275

Aquafauna Bio-marine Inc.
PO Box 5
Hawthorne California 90250
United States of America
Tel.: +1 310 9735275
Fax: +1 310 6769387
Web: www.aquafauna.com

Aqua Logic Inc.
8280 Clairemont Mesa Blvd, Suite 127
San Diego, CA 92111
United States of America
Tel.: +1 858 2924773
E-mail: aqualogic@aol.com
Web: www.aqualogicinc.com

Aquatic Eco-systems Inc.
1767 Benbow Court
Apopka, FL 32703
United States of America
Tel.: +1 407 8863939 / 877 3474788
Fax: +1 407 886 4884
E-mail: aes@aquaticeco.com
Web: www.aquaticeco.com

Red Ewald Inc.
PO Box 519
Karnes City, Texas 78118
United States of America
Tel.: +1 800 2423524
Web: www.redewald.com

Easy-Kleen, Pressure System Ltd.
26 Eveleigh Street
Sussex, New Brunswick E4E 2N8
Canada
Tel.: +1 506 4333393
Fax: +1 506 4332443
Web: www.easy-kleen.com

Reed Mariculture Inc.
Instant Algae Products
511 Pamlar Ave, Suite #C
San Jose, CA 95128
United States of America
Tel.: +1 831 7683830
Fax: +1 831 4012474
Web: www.seafarm.com

Go Deep International Inc.
PO Box 493, Station "A"
Fredericton, New Brunswick E3B 4Z9
Canada
Tel.: +1 506 454 5341
Fax: +1 506 462 9883
E-mail: godeep@nbnet.nb.ca

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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 set-up of an inexpensive modular hatchery may be a simpler option to the start-up of a large commercial operation, or may be sufficient for 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, growout 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. Contains a CD-ROM of the complete publication.

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