

# Hatchery culture of bivalves

A practical manual



***Cover photographs:***

*Clockwise from top left* – fibreglass cylinders used for microalgae culture; interior of a small bivalve hatchery; raft nursery for bivalve spat; photomicrograph of *Crassostrea gigas* D-larvae (courtesy Michael M. Helm); a spawning female Manila clam (courtesy Brian Edwards).

# Hatchery culture of bivalves

A practical manual

FAO  
FISHERIES  
TECHNICAL  
PAPER

471

Prepared by  
**Michael M. Helm**  
FAO Consultant  
Nova Scotia, Canada

and

**Neil Bourne**  
FAO Consultant  
British Columbia, Canada

Compiled and edited by  
**Alessandro Lovatelli**  
Inland Water Resources and Aquaculture Department  
FAO Fisheries Department  
Rome, Italy

The designations employed and the presentation of material in this information product do not imply the expression of any opinion whatsoever on the part of the Food and Agriculture Organization of the United Nations concerning the legal or development status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

ISBN 92-5-105224-7

All rights reserved. Reproduction and dissemination of material in this information product for educational or other non-commercial purposes are authorized without any prior written permission from the copyright holders provided the source is fully acknowledged. Reproduction of material in this information product for resale or other commercial purposes is prohibited without written permission of the copyright holders. Applications for such permission should be addressed to:

Chief

Publishing Management Service

Information Division

FAO

Viale delle Terme di Caracalla, 00100 Rome, Italy

or by e-mail to:

[copyright@fao.org](mailto:copyright@fao.org)

© FAO 2004



# Preparation of this document

This manual is part of the publications programme of the Fisheries Department Inland Water Resources and Aquaculture Service of the Food and Agriculture Organization of the United Nations. It is a synthesis of the current methodologies applicable to the intensive hatchery culture of bivalve molluscs covering similarities and differences in approach in rearing clams, oysters and scallops in different climatic regions. All aspects of the culture process are described, together with considerations in choosing a site for hatchery development and in the design of suitable facilities. The manual also includes the post-hatchery handling of “seed” bivalves in land- and sea-based nursery culture preparatory to on-growing. This publication is intended to assist both technicians entering this field as well as investors interested in evaluating the complexity of intensive hatchery production.

The authors bring together a combined 80 years of experience in the biology, management and operation of hatcheries encompassing a range of the more commonly cultured bivalve species in different parts of the world. Preparation of the manual has been under the overall coordination of Alessandro Lovatelli, Fishery Resources Officer (Aquaculture).

The authors wish to acknowledge the contributions of their many colleagues past and present and industry leaders, without which this publication would not have been possible.

Unless otherwise acknowledged, all photographs were taken by the authors.

**Distribution:**

Directors of Fisheries

FAO Fisheries Department

FAO Fisheries Officers in FAO Regional and Subregional Offices

FI Marine Aquaculture Mailing List

# Abstract

Bivalve mollusc culture is an important and rapidly expanding area of world aquaculture production, representing approximately 20 percent of the sector's output at 14 million tonnes in 2000. The majority of production is from natural populations although increasingly stocks are approaching or have exceeded maximum sustainable yields. Stock enhancement through the capture and relaying of natural seed in both extensive and intensive forms of culture is common practice worldwide but the reliability of natural recruitment can never be guaranteed, and conflicts over the use of the coastal zone are becoming ever more pressing. A solution to meeting the seed requirements of the bivalve industry, applicable to the production of high unit value species such as clams, oysters and scallops, is hatchery culture. The production of seed through hatchery propagation accounts at the present time for only a small percentage of the total seed requirement but it is likely to become increasingly important as work continues to produce genetically-selected strains with desirable characteristics suited to particular conditions.

The advent of bivalve hatcheries was in Europe and the United States in the 1960s. Since those early pioneering days, knowledge of the biological requirements of the various species that predominate in worldwide aquaculture production and the technology used to produce them has grown and continues to improve. This manual brings together the current state of knowledge in describing the various aspects of hatchery culture and production from acquisition of broodstock to the stage at which the seed are of sufficient size to transfer to sea-based growout. Focus is on intensive methodology in purpose-built hatchery facilities rather than on more extensive methods of seed production in land-based pond systems. For a complete view, the intermediate nursery phase of production, which is the interface between the hatchery and sea-based growout, and the concept of remote setting are also described and discussed in some depth.

This manual is not intended as a scientific treatise on the subject. Rather, it provides the reader with a practical insight as to what is required in the way of resources and details of how to handle and manage the various life history stages of bivalves in the hatchery production cycle. Examples are largely drawn from the more commonly cultured temperate climate species including the Pacific oyster, *Crassostrea gigas*, the American (Eastern) oyster, *Crassostrea virginica*, the European flat oyster, *Ostrea edulis*, the Manila clam, *Tapes philippinarum* and a range of scallop species. Consideration is also given to the culture of tropical bivalves. Methods described are equally as applicable to bivalves of lesser significance in terms of worldwide production.

The authors recognize that bivalve hatchery production is as much an art founded on science as it is a science *per se*. There are as many ways of operating and managing a hatchery as there are hatcheries in terms of the sophistication of the facility and the precision with which each part of production is approached. In this respect, many experienced hatchery managers will consider much of the detailed information as "overkill." However, the authors have considered the need for a thorough grounding for new entrants in this field, not just how the various procedures are done but the biological basis of why they are done in that way. Thus, the content is equally as appropriate to the operation of a closely controlled experimental hatchery as it is to a commercial-scale hatchery.

In addition to explanations of culture technology and methodology, the manual includes a brief discussion of the processes of identifying a suitable site for locating a hatchery

and considerations in planning and designing the hatchery. It also includes advances that are likely to improve the reliability and economic viability of the hatchery industry in the near future, featuring topics such as polyploidy, the development of selected strains, cryopreservation of gametes and the need for novel, non-living foods.

**Keywords:** marine aquaculture, bivalve culture, bivalve hatcheries, bivalve nurseries, bivalve seed production, oysters, clams, scallops

**Helm, M.M.; Bourne, N.; Lovatelli, A.** (comp./ed.)

Hatchery culture of bivalves. A practical manual.

*FAO Fisheries Technical Paper*. No. 471. Rome, FAO. 2004. 177p.





# Contents

Preparation of this document .....	iii
Abstract .....	iv
List of figures .....	xi
List of tables .....	xv
Glossary .....	xvi
Abbreviations, acronyms and conversions .....	xix

<b>Introduction .....</b>	<b>1</b>
---------------------------	----------

## **Part 1 - Site selection, hatchery design and economic considerations**

<b>1.1 SITE SELECTION .....</b>	<b>5</b>
1.1.1 Introduction .....	5
1.1.2 Considerations .....	6
1.1.2.1 <i>Government regulations</i> .....	6
1.1.2.2 <i>Seawater quality</i> .....	6
1.1.2.3 <i>Siting the hatchery</i> .....	7
<b>1.2 HATCHERY DESIGN CONSIDERATIONS .....</b>	<b>8</b>
1.2.1 Introduction .....	8
1.2.2 Seawater system .....	9
1.2.3 The physical plant .....	12
1.2.3.1 <i>Algal culture facility</i> .....	13
1.2.3.2 <i>Broodstock holding and spawning area</i> .....	14
1.2.3.3 <i>Larval culture area</i> .....	14
1.2.3.4 <i>Juvenile culture area</i> .....	14
1.2.3.5 <i>Other space requirements</i> .....	15
<b>1.3 ECONOMIC CONSIDERATIONS .....</b>	<b>15</b>
<b>1.4 SUGGESTED READING .....</b>	<b>16</b>

## **Part 2 - Basic bivalve biology: taxonomy, anatomy and life history**

<b>2.1 TAXONOMY AND ANATOMY .....</b>	<b>19</b>
2.1.1 Introduction .....	19
2.1.2 External anatomy .....	20
2.1.3 Internal anatomy .....	21
<b>2.2 LIFE HISTORY .....</b>	<b>23</b>
2.2.1 Gonadal development and spawning .....	23
2.2.2 Embryonic and larval development .....	25
2.2.3 Metamorphosis .....	26
2.2.4 Feeding .....	26
2.2.5 Growth .....	27
2.2.6 Mortalities .....	27

<b>2.3 SUGGESTED READING</b> .....	29
------------------------------------	----

## **Part 3 - Hatchery operation: culture of algae**

<b>3.1 INTRODUCTION</b> .....	31
<b>3.2 MAINTENANCE OF STOCK AND STARTER CULTURES</b> .....	34
3.2.1 Procedures for the management of stock cultures .....	34
3.2.2 Starter culture management .....	38
<b>3.3 INTERMEDIATE-SCALE CULTURE</b> .....	39
3.3.1 Growth phases of cultures .....	40
3.3.2 Details of intermediate-scale culture operation .....	41
3.3.3 Estimating algal density .....	43
<b>3.4 LARGE-SCALE CULTURE</b> .....	45
3.4.1 Bag and cylinder cultures .....	46
3.4.2 Internally illuminated cultures .....	48
3.4.3 Principles of large-scale culture management .....	49
3.4.4 Automated large-scale culture .....	52
3.4.5 Troubleshooting .....	53
3.4.6 Extensive outdoor culture .....	54
<b>3.5 SUGGESTED READING</b> .....	56

## **Part 4 - Hatchery operation: broodstock conditioning, spawning and fertilization**

<b>4.1 BROODSTOCK CONDITIONING</b> .....	59
4.1.1 Introduction .....	59
4.1.2 Conditioning methods .....	62
4.1.2.1 <i>Tank systems and water treatment</i> .....	62
4.1.2.2 <i>Feeding broodstock</i> .....	65
4.1.2.3 <i>Calculating food ration for conditioning</i> .....	66
4.1.2.4 <i>Adjusting ration for flow-through systems</i> .....	67
4.1.2.5 <i>Two-stage early season conditioning</i> .....	67
4.1.3 Conditioning bivalves in the tropics .....	68
<b>4.2 SPAWNING AND FERTILIZATION</b> .....	68
4.2.1 Introduction .....	68
4.2.2 Gamete stripping .....	70
4.2.3 The special case of flat oysters .....	71
4.2.4 Induced spawning of oviparous bivalves .....	74
4.2.4.1 <i>Thermal cycling procedure</i> .....	74
4.2.4.2 <i>Spawning dioecious bivalves</i> .....	76
4.2.4.3 <i>Spawning monoecious bivalves</i> .....	77
4.2.5 Fertilization procedures .....	79
<b>4.3 SUGGESTED READING</b> .....	80

## Part 5 - Hatchery operation: culture of larvae basic methodology, feeding and nutrition, factors influencing growth and survival, and settlement and metamorphosis

<b>5.1 BASIC METHODOLOGY</b>	84
5.1.1 Introduction	84
5.1.2 Methods for embryo development	84
5.1.2.1 Tanks for embryos and larvae	84
5.1.2.2 Water treatment	85
5.1.2.3 Culture of embryos	86
5.1.3 Methods for rearing larvae	92
5.1.3.1 Starting a new culture	93
5.1.3.2 Husbandry of larval cultures	94
5.1.4 Growing larvae more efficiently	97
5.1.4.1 High density culture	97
5.1.4.2 Flow-through culture	98
5.1.5 Growth and survival of larvae	101
<b>5.2 FEEDING AND NUTRITION</b>	102
5.2.1 Introduction	102
5.2.2 Dietary considerations	103
5.2.3 Diet composition and ration	105
5.2.3.1 Feeding strategies	108
5.2.3.2 Calculating food rations	109
<b>5.3 FACTORS INFLUENCING GROWTH AND SURVIVAL</b>	111
5.3.1 Introduction	111
5.3.2 Effects of temperature and salinity	111
5.3.3 Seawater quality	113
5.3.4 Egg and larval quality	117
5.3.5 Disease	121
<b>5.4 SETTLEMENT AND METAMORPHOSIS</b>	121
5.4.1 Introduction	121
5.4.2 Maturation of larvae	122
5.4.3 Setting larvae	123
5.4.3.1 Settlement stimuli	123
5.4.3.2 Suitable settlement substrates	124
<b>5.5 SUGGESTED READING</b>	129

## Part 6 - Hatchery operation: culture of spat in remote setting site, in the hatchery and in nurseries

<b>6.1 INTRODUCTION</b>	135
<b>6.2 REMOTE SETTING</b>	137
6.2.1 Background	137
6.2.2 Preparing larvae for shipment	138
6.2.3 Preparations at the remote site	139
6.2.4 Receiving the eyed larvae	140
6.2.5 Setting the larvae and growing the spat	140

<b>6.3 METHODS FOR GROWING SMALL SPAT</b> .....	142
6.3.1 Introduction .....	142
6.3.2 Growing systems for spat set on cultch .....	142
6.3.3 Growing systems for unattached spat .....	143
6.3.4 Operation of closed upwelling systems .....	146
6.3.5 Operation of closed downwelling systems .....	147
6.3.6 Grading and estimating spat .....	148
6.3.7 Operating systems on flow-through .....	150
<b>6.4 DIETS AND FOOD RATIONS FOR SMALL SPAT</b> .....	151
6.4.1 Species composition of diets .....	151
6.4.2 Calculating food ration .....	152
<b>6.5 GROWTH AND SURVIVAL</b> .....	153
6.5.1 Variability in spat growth between species .....	154
6.5.2 Effect of ration on growth .....	155
6.5.3 Combined effects of ration and temperature .....	157
6.5.4 Survival .....	157
6.5.5 Hatchery production .....	158
<b>6.6 NURSERY CULTURE</b> .....	159
6.6.1 Land-based nurseries .....	161
6.6.2 Barge-type nurseries .....	164
<b>6.7 SUGGESTED READING</b> .....	166
 <b>Part 7 - The future of hatcheries: developing technologies</b>	
<b>7.1 GENETICS</b> .....	169
7.1.1 Polyploidy .....	170
7.1.2 Quantitative and molecular genetics .....	171
<b>7.2 THE FUTURE</b> .....	173
<b>7.3 SUGGESTED READING</b> .....	175

# List of figures

<b>Figure 1:</b> Production of bivalves from capture fisheries and aquaculture during the decade from 1991 to 2000 .....	1
<b>Figure 2:</b> A comparison of production from capture fisheries and aquaculture broken down into the relative contributions of the major groups of bivalves in 1991 and 2000 .....	2
<b>Figure 3:</b> A selection of photographs of hatcheries depicting the variability in size and sophistication of construction that exists around the world .....	8
<b>Figure 4:</b> A diagram of the various stages of seawater treatment for hatchery usage from the intake pipes to the points at which water is used in the different aspects of the operation .....	10
<b>Figure 5:</b> A generalized floor plan for a purpose-built bivalve hatchery .....	12
<b>Figure 6:</b> External and internal features of the shell valves of the hard shell clam, <i>Mercenaria mercenaria</i> .....	20
<b>Figure 7:</b> The internal, soft tissue anatomy of a clam of the genus <i>Tapes</i> .....	20
<b>Figure 8:</b> The soft tissue anatomy of the European flat oyster, <i>Ostrea edulis</i> , and the calico scallop, <i>Argopecten gibbus</i> , visible following removal of one of the shell valves .....	21
<b>Figure 9:</b> The internal, soft tissue anatomy of a hermaphroditic scallop .....	21
<b>Figure 10:</b> Photomicrographs of histological sections through the ovary of the scallop, <i>Argopecten gibbus</i> , during gametogenesis .....	24
<b>Figure 11:</b> Representation of the developmental stages of the calico scallop, <i>Argopecten gibbus</i> , which take place within a hatchery .....	26
<b>Figure 12:</b> Photomicrographs of two algal species commonly cultured in hatcheries, <i>Isochrysis</i> sp. (A) and <i>Tetraselmis</i> sp. (B) showing the relative difference in cell size .....	31
<b>Figure 13:</b> Steps in the production of algae .....	33
<b>Figure 14:</b> The process of algal culture showing the various required inputs .....	33
<b>Figure 15:</b> Illuminated, temperature controlled incubators for the maintenance of small algal cultures .....	34
<b>Figure 16:</b> A – schematic diagram of a culture transfer chamber. B – an autoclave suitable for the sterilization of small volumes of culture medium .....	35
<b>Figure 17:</b> Photographs showing typical facilities for maintenance of starter cultures .....	38
<b>Figure 18:</b> Two different approaches to the intermediate-scale culture of algae .....	39
<b>Figure 19:</b> Phases in the growth of algal cultures illustrated by a typical growth curve for the large, green flagellate, <i>Tetraselmis suecica</i> .....	40
<b>Figure 20:</b> Diagram of the grid marked on a haemocytometer slide .....	43
<b>Figure 21:</b> Electronic particle counters used in hatcheries for counting the density of cells in algal cultures .....	45
<b>Figure 22:</b> Large-scale culture was often in large, circular or rectangular tanks with overhead illumination .....	45
<b>Figure 23:</b> Efficient 200 l volume, water cooled, internally illuminated algal culture tanks .....	46
<b>Figure 24:</b> Examples of polyethylene bag and solar grade, fibreglass cylinder algal culture systems .....	47
<b>Figure 25:</b> The relationship between the productivity of a culture system (yield) and light energy input .....	49
<b>Figure 26:</b> The effect of light intensity on yield of <i>Tetraselmis</i> in 200 l internally illuminated culture vessels .....	50
<b>Figure 27:</b> Effects of A - post-harvest cell density (PHCD) and B – pH on cell division rate, and the influence of salinity on the productivity of cultures of <i>Tetraselmis suecica</i> – C. ....	51

<b>Figure 28:</b> Relationships between post-harvest cell density (PHCD) and the size of cells in terms of weight and the productivity of semi-continuous culture of <i>Tetraselmis suecica</i> .....	52
<b>Figure 29:</b> Relationships between post-harvest cell density and yield at standard cell density of <i>Skeletonema costatum</i> cultures operated semi-continuously at two light intensities and silicate concentrations .....	52
<b>Figure 30:</b> Schematic of a continuous ("turbidostat") culture system .....	53
<b>Figure 31:</b> Examples of large-scale, outdoor algal production .....	55
<b>Figure 32:</b> A typical broodstock conditioning system .....	60
<b>Figure 33:</b> The anatomy of a fully mature calico scallop ( <i>Argopecten gibbus</i> ) .....	60
<b>Figure 34:</b> A selection of clams commonly cultured in hatcheries .....	61
<b>Figure 35:</b> Diagrammatic representation of A – a flow-through broodstock tank in which adults are suspended off the bottom in a mesh tray with large apertures in the base so as not to retain faeces and detritus; B – a similar tank fitted for sub-gravel filtration .....	62
<b>Figure 36:</b> Examples of various types of flow-through tanks used for broodstock conditioning .....	63
<b>Figure 37:</b> A 120 l broodstock tank stocked with 55 oysters averaging 80 g live weight .....	64
<b>Figure 38:</b> A spawning female Manila clam .....	69
<b>Figure 39:</b> Stripping and transferring gametes from Pacific oysters to a beaker of filtered seawater using a Pasteur pipette .....	70
<b>Figure 40:</b> Anatomy of a developing flat oyster, <i>Ostrea edulis</i> .....	71
<b>Figure 41:</b> Brooding stages of the European flat oyster, <i>Ostrea edulis</i> .....	72
<b>Figure 42:</b> The appearance of <i>Ostrea edulis</i> veliger larvae (175 µm mean shell length) at release from the adult .....	72
<b>Figure 43:</b> Experimental broodstock conditioning of <i>Ostrea edulis</i> .....	73
<b>Figure 44:</b> Stripping <i>Ostrea edulis</i> larvae from a brooding adult .....	74
<b>Figure 45:</b> Diagram of a tray arrangement widely used for the spawning of oviparous bivalves .....	75
<b>Figure 46:</b> <i>Pecten ziczac</i> adults undergoing thermal cycling in a spawning tray .....	76
<b>Figure 47:</b> This sequence of photographs illustrates the spawning of the dioecious calico scallop, <i>Argopecten gibbus</i> , at the Bermuda Biological Station for Research, Inc .....	78
<b>Figure 48:</b> Dividing <i>Crassostrea gigas</i> eggs about 50 minutes after fertilization .....	79
<b>Figure 49:</b> Stages in the early development of eggs .....	79
<b>Figure 50:</b> Fertilized eggs can be incubated in various types of tanks in filtered seawater for a period of 2 to 3 days, depending on species and temperature .....	84
<b>Figure 51:</b> Photomicrograph of <i>Crassostrea gigas</i> D-larvae .....	85
<b>Figure 52:</b> Suitable rearing vessels for embryo (and larval) development .....	85
<b>Figure 53:</b> Examples of suitable equipment for water treatment .....	86
<b>Figure 54:</b> Development of embryos from the early trochophore to the fully shelled D-larva stage .....	87
<b>Figure 55:</b> Measuring larvae: each larva is oriented and lined-up with the calibrated eye-piece graticule .....	88
<b>Figure 56:</b> The arrangement of sieves to capture D-larvae .....	88
<b>Figure 57:</b> The appearance of almost 5 million calico scallop, <i>Argopecten gibbus</i> , larvae concentrated in a 20 cm diameter sieve (A) and after transferring to a 4 l graduated jug, preparatory to estimation (B) .....	89
<b>Figure 58:</b> Equipment used in estimating numbers of larvae .....	90
<b>Figure 59:</b> The steps in taking sub-samples of larvae for counting in the estimation of total numbers .....	91

<b>Figure 60:</b> An example of a daily record sheet and the type of information that is useful to record in order to follow the progress of a batch or a tank of larvae .....	95
<b>Figure 61:</b> Draining static larval tanks on water change days .....	96
<b>Figure 62:</b> Experimental automatic control of food cell density in high-density cultures of bivalve larvae .....	98
<b>Figure 63:</b> A typical arrangement for flow-through larval culture .....	99
<b>Figure 64:</b> Detail of the top of an experimental flow-through tank showing the “banjo” filter attached to the out-flow pipe .....	100
<b>Figure 65:</b> Photomicrographs of the growth and development of Pacific oyster, <i>Crassostrea gigas</i> , (A) and sand scallop, <i>Pecten ziczac</i> , (B) larvae .....	101
<b>Figure 66:</b> Comparative growth of larvae of some warmer water bivalve species .....	102
<b>Figure 67:</b> Larvae feed as they swim .....	103
<b>Figure 68:</b> Growth, development and settlement of <i>Ostrea edulis</i> larvae fed various single and mixed diets .....	103
<b>Figure 69:</b> Comparison of total lipid as a percentage of ash-free dry weight and the relative abundance of various highly unsaturated fatty acids (HUFAs) in a number of algal species ...	104
<b>Figure 70:</b> The growth of (A) <i>Crassostrea gigas</i> , (B) <i>Crassostrea rhizophorae</i> , (C) <i>Mercenaria mercenaria</i> and (D) <i>Tapes philippinarum</i> larvae fed T-Iso, <i>Chaetoceros calcitrans</i> and a two species mixture of these two algae .....	107
<b>Figure 71:</b> Effects of temperature and salinity on the growth of larvae of the Japanese scallop, <i>Patinopecten yessoensis</i> .....	112
<b>Figure 72:</b> The growth of Mangrove oyster, <i>Crassostrea rhizophorae</i> and Pacific oyster, <i>Crassostrea gigas</i> , larvae at various temperatures and salinities .....	112
<b>Figure 73:</b> The growth of Manila clam, <i>Tapes philippinarum</i> , larvae from D-stage to metamorphosis at 3 temperatures .....	113
<b>Figure 74:</b> The relative survival in bioassays comparing development to D-stage larvae of fertilized Pacific oyster eggs in artificial and in normally treated hatchery seawater .....	115
<b>Figure 75:</b> The comparative growth of Pacific oyster larvae over a 6-day period at 25°C in normal hatchery seawater and artificial seawater calculated as a growth index .....	116
<b>Figure 76:</b> Growth indices of samples of broods of European flat oyster, <i>Ostrea edulis</i> , larvae grown at the beaker scale in hatchery .....	116
<b>Figure 77:</b> The highly unsaturated fatty acid composition of Manila clam, <i>Tapes philippinarum</i> , eggs from a hatchery-held broodstock provided with different diets during conditioning ....	117
<b>Figure 78:</b> A comparison of the highly unsaturated fatty acid composition of hatchery conditioned and wild stock European flat oyster, <i>Ostrea edulis</i> , larvae .....	117
<b>Figure 79:</b> Relationship between total lipid as a percentage of dry weight and the percentage of Pacific oyster, <i>Crassostrea gigas</i> , eggs that develop to the D-larva stage .....	118
<b>Figure 80:</b> Relationships between the total lipid content of freshly spawned Pacific oyster eggs and, (A) months of the year in two different years and (B), the chlorophyll – $\alpha$ content of unfiltered seawater supplied to broodstock in a hatchery when employing a standard conditioning protocol .....	119
<b>Figure 81:</b> The relationship between the growth increment of <i>Ostrea edulis</i> larvae in a 4-day period from liberation and total lipid content at liberation from hatchery conditioned broodstocks .....	120
<b>Figure 82:</b> A comparison of increases in both (A) ash-free dry (organic) weight and (B) lipid content per larva relative to mean shell length in larvae of four bivalve species .....	120
<b>Figure 83:</b> Photomicrographs of (A) swimming <i>Argopecten gibbus</i> larvae showing the ciliated swimming/feeding organ, the velum, and (B) eyed pediveligers of the same species .....	122
<b>Figure 84:</b> “Stringing” (or “funneling”) behaviour of mature larvae prior to settlement .....	123
<b>Figure 85:</b> A remote, oyster setting system located on Vancouver Island, British Columbia, Canada .....	124



<b>Figure 86:</b> In this example, matt surfaced PVC sheets used as settlement substrate for oyster spat are placed on the base of larval culture tanks .....	125
<b>Figure 87:</b> Scallop pediveligers can be set at a density of up to 2 000 per l in cultch filled tanks equipped for static water, recirculation or flow-through .....	127
<b>Figure 88:</b> Cylindrical, nylon mesh based trays used to set scallop pediveligers at the Bermuda Biological Station for Research, Inc. ....	128
<b>Figure 89:</b> Receiving a consignment of eyed Pacific oyster larvae wrapped in nylon mesh at a remote setting site in British Columbia, Canada .....	138
<b>Figure 90:</b> Setting tanks at a site in British Columbia, Canada .....	139
<b>Figure 91:</b> Simple tank systems are used for growing spat set on cultch .....	143
<b>Figure 92:</b> A closed tank system designed for holding scallop spat in cylinders with a downwelling flow of water .....	144
<b>Figure 93:</b> Diagram illustrating the difference in flow circulation in upwelling and downwelling spat systems .....	145
<b>Figure 94:</b> Closed upwelling systems in use for the growing of small oyster spat .....	146
<b>Figure 95:</b> Grading of spat with hand-held sieves (screens) in shallow tanks .....	148
<b>Figure 96:</b> Upwelling tank units for larger size spat operating on flow-through .....	150
<b>Figure 97:</b> An example of a proprietary algal paste product suitable as a partial or complete replacement for hatchery grown live algae in the culture of bivalve spat .....	152
<b>Figure 98:</b> Comparison of the growth of Pacific oyster, Manila clam and calico scallop spat in similar conditions .....	154
<b>Figure 99:</b> The relationship between food ration and growth for Pacific oyster spat .....	155
<b>Figure 100:</b> Comparison of the growth of European flat oyster and Pacific oyster spat at 24°C when fed various rations of a mixed diet of <i>Isochrysis</i> and <i>Tetraselmis</i> .....	156
<b>Figure 101:</b> The survival and growth of calico scallop, <i>Argopecten gibbus</i> , spat during a 6-week period post-settlement .....	158
<b>Figure 102:</b> A summary flow diagram of the various aspects of hatchery production showing the temperature range and the daily food requirement per unit number of animals at each stage .....	159
<b>Figure 103:</b> A land-based nursery and a floating barge or raft nursery .....	160
<b>Figure 104:</b> Examples of land-based nurseries .....	161
<b>Figure 105:</b> Data from a land-based nursery pond system in Nova Scotia, Canada, operated from early May to the end of October .....	162
<b>Figure 106:</b> Examples of raft or barge-type nurseries .....	163
<b>Figure 107:</b> A small, commercially manufactured upwelling nursery powered by an axial flow pump in use at Harwen Oyster Farm, Port Medway, Nova Scotia, Canada .....	165
<b>Figure 108:</b> Tidal powered, floating upwelling systems – “FLUPSYS” .....	165
<b>Figure 109:</b> Representation of the process of triploidy induction .....	171
<b>Figure 110:</b> A – a device for exerting pressure on eggs to prevent chromosomal reduction through the suppression of meiosis. B – experiments in the cryopreservation of bivalve gametes and larvae .....	174

# List of tables

<b>Table 1:</b> The cell volume, organic weight and gross lipid content of some of the more commonly cultured algal species used as foods for bivalve larvae and spat .....	32
<b>Table 2:</b> The composition and preparation of Erdschreiber culture maintenance medium .....	35
<b>Table 3:</b> Guillard's F/2 media used for culturing algae in bivalve hatcheries from Guillard (1975) .....	36
<b>Table 4:</b> HESAW media used for culturing algae in bivalve hatcheries. From Harrison <i>et al.</i> (1980) .....	37
<b>Table 5:</b> Nutrient salt stock solutions for the enrichment of diatom cultures in treated seawater .....	42
<b>Table 6:</b> Cell densities at harvest (cells $\mu\text{L}^{-1}$ ) achieved in small-scale batch (B) and semi-continuous (SC) 2 l or 20 l cultures for a selection of nutritionally valuable species .....	43
<b>Table 7:</b> Comparison of yields of <i>Tetraselmis</i> and <i>Phaeodactylum</i> from various large-scale culture systems .....	48
<b>Table 8:</b> Effect of diet on the production of <i>Ostrea edulis</i> larvae .....	65
<b>Table 9:</b> Summary of information relevant to the conditioning and egg (or larval) production for a number of commonly cultured bivalves .....	69
<b>Table 10:</b> Summary data of typical embryo densities, initial D-larva size, densities of D-larvae and culture conditions in terms of suitable temperature and salinity for the culture of embryos and early larvae of a number of bivalves .....	87
<b>Table 11:</b> The relationship between the mesh aperture of sieves (screens) and the minimum size of larvae they will retain .....	90
<b>Table 12:</b> The average number of larvae stocked initially ( $N_0$ ) and surviving immediately prior to settlement ( $N_p$ ) in 5 comparisons of high and normal density rearing with the European flat oyster, <i>O. edulis</i> , and 3 comparisons with the Pacific oyster, <i>C. gigas</i> .....	98
<b>Table 13:</b> The number of algal cells ingested per larva per day by three commonly cultured bivalves relative to the mean shell length of the larvae .....	110
<b>Table 14:</b> Tank water volume and daily food requirements for bivalve spat of different sizes when grown at a biomass of 200 g live weight per 1 000 l .....	137
<b>Table 15:</b> Mean live weight of <i>Ostrea edulis</i> and <i>Crassostrea gigas</i> spat at the end of a 7-day period .....	156
<b>Table 16:</b> The combined effects of temperature and food ration on <i>Ostrea edulis</i> spat beginning a weekly growth period at 2 mg mean live weight .....	157

# Glossary

<b>Adductor muscle</b>	large muscle (or muscles) that pull the two shell valves together
<b>Algae</b>	aquatic plants that reproduce by spores
<b>Anterior</b>	front or head
<b>Auricle</b>	with respect to scallops, the ear or wing-like projections at the hinge of a scallop (can also refer to the chamber of heart that receives blood from the body)
<b>Axenic</b>	culture of a single species in bacteria-free conditions
<b>Biting</b>	condition where shell margins of two scallops become interlocked, and subsequently damage the inner soft parts
<b>Bivalve</b>	mollusc of the Class Pelecypoda, having a shell of two valves that are joined by a hinge
<b>Byssus</b>	thread-like filaments used by bivalves to attach themselves to a substrate
<b>Cilia</b>	hair-like structures whose rhythmic beat induces a water current in bivalves
<b>Ctenidia</b>	leaf-like appendages that function in respiration and filtration of food from water (used interchangeably with the term gills)
<b>Cultch</b>	material used to collect bivalve spat
<b>Demibranch</b>	single plate or leaf of a bivalve gill
<b>Detritus</b>	fragmented or decomposing organic material from plant and animal remains
<b>Diatom</b>	a single-celled alga of the Class Bacillariophyceae; cells are enclosed in a siliceous shell called a frustule, cells can form chains
<b>Dimyarian</b>	bivalves with two adductor muscles, e.g. clams and mussels
<b>Dioecious</b>	organisms in which male and females reproductive organs occur in different individuals
<b>Diploid</b>	the normal number of chromosomes (2n) in cells
<b>Dorsal</b>	the back or part of an organism away from the ground
<b>Downwelling</b>	in hatchery terminology, a growing system in which the flow of water enters at the top of a spat holding container (compare with upwelling)
<b>D-larva</b>	the early veliger larval stage of bivalves, also known as straight-hinge larva
<b>Embryo</b>	organism in early stages of development; in bivalves, prior to larval stage
<b>Exhalant</b>	area of bivalve where water currents have an outward direction
<b>Exotic</b>	introduced from foreign country or geographic area
<b>Eyespot</b>	simple organ that develops near centre of mature larvae of some bivalves and is sensitive to light

<b>Fertilization</b>	union of egg and sperm
<b>Flagellate</b>	group of single-celled algae characterized by having a locomotory organ called a flagellum
<b>Frustule</b>	siliceous shell-like covering of a diatom
<b>Gamete</b>	mature, haploid, functional sex cell capable of uniting with the alternate sex cell to form a zygote
<b>Gametogenesis</b>	process by which eggs and sperm are produced
<b>Gill</b>	a leaf-like appendage that functions in respiration and filtration of food from water (see ctenidia)
<b>Growout</b>	the process of growing seed produced in hatcheries to market size
<b>Halocline</b>	a zone of sharp vertical salinity change
<b>Hinge</b>	dorsal area of bivalve shell where two valves are joined together
<b>HUFA</b>	a highly unsaturated fatty acid, referred to also as polyunsaturated fatty acid (PUFA)
<b>Indigenous</b>	native, not imported
<b>Inhalant</b>	area of bivalve where water current have an inward direction
<b>Larva</b>	a stage of bivalves from the embryo to metamorphosis
<b>Ligament</b>	fibrous spring-like material joining two valves of a bivalve at the hinge
<b>Mantle</b>	the soft fold enclosing the body of a bivalve which secretes the shell
<b>Mean</b>	average
<b>Meiotic Division</b>	process in which normal number of chromosomes (2n) is reduced to the haploid (n) number
<b>Metamorphosis</b>	in bivalves, the period of transformation from the larval to the juvenile stage
<b>Microalgae</b>	small cell-size algae, either single celled or chain forming diatoms, cultured as foods for larvae and spat in a hatchery
<b>Microlitre (μl)</b>	one millionth of a litre or one thousandth of a ml
<b>Micrometer (μm)</b>	one millionth of a metre or one thousandth of a mm
<b>Monoecious</b>	organisms in which both male and female reproductive organs occur in the same individual
<b>Monomyarian</b>	bivalves with one adductor muscle, e.g. oysters and scallops
<b>Natural Set</b>	in bivalves, obtaining spat from spawning of natural populations
<b>Pallial Line</b>	faint circular line on inner surface of shell of bivalves showing location of attachment of mantle to shell
<b>Palp</b>	a sensory appendage near the mouth used to assist in moving food into the mouth
<b>Pedal</b>	pertaining to the foot
<b>pH</b>	a measure of acidity
<b>Plankton</b>	floating or weakly swimming aquatic organisms, can be phytoplankton (plants) or zooplankton (animals)
<b>Planktotrophic</b>	organisms that feed on phytoplankton

<b>Polar Body</b>	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
<b>Polyloid</b>	animals having more than the usual number of diploid (2n) chromosomes
<b>Posterior</b>	the rear, away from the head
<b>Pronuclei</b>	in the egg, the haploid nucleus after completion of meiosis but before infusion with the sperm nucleus
<b>Pseudofaeces</b>	false faeces, waste material not taken into the digestive tract
<b>PSU</b>	a measure of salinity, equivalent to parts per thousand
<b>Resilium</b>	internal portion of the ligament located centrally along the hinge of a bivalve; causes the valves to open when the adductor relaxes
<b>Salinity</b>	the salt content of seawater usually measured in parts per thousand (ppt) or practical salinity units (PSU)
<b>Seed</b>	a hatchery term for spat of a size ready for sale
<b>Settlement</b>	behavioural process when mature bivalve larvae seek a suitable substrate for attachment
<b>Shell Height</b>	the straight line distance measured perpendicularly from the umbo to the ventral margin of the shell
<b>Shell Length</b>	the straight line distance from the anterior to the posterior margins of the shell
<b>Spat</b>	a newly settled or attached bivalve (also termed post larval or juvenile in bivalves)
<b>Straight-hinge larva</b>	early part of larval stage, sometimes termed D-stage
<b>Tentacle</b>	long, unsegmented threadlike protuberance from edge of mantle that has specialized sensory function
<b>Tetraploid</b>	polyploid animal with twice the normal complement of chromosomes (4n)
<b>Thermocline</b>	a zone of sharp vertical temperature change
<b>Triploid</b>	a polyploid animal with an extra set of chromosomes (3n)
<b>Trochophore</b>	planktonic stage of bivalve embryo
<b>Umbo</b>	beak-like projections at the dorsal part of the shell; it is the oldest part of a bivalve shell (also called the umbone)
<b>Upwelling</b>	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).
<b>Urogenital System</b>	system with organs concerned with excretion (kidney) and reproduction (gonad)
<b>Valve</b>	one of the two parts of a bivalve shell, two valves make up one shell
<b>Veliger Larva</b>	the larval stage of most molluscs, characterized by the presence of a velum
<b>Velum</b>	ciliated locomotory organ of the larva
<b>Ventral</b>	pertaining to the under or lower side of an animal
<b>Zygote</b>	diploid (2n) cell resulting from union of male and female gametes

# Abbreviations, acronyms and conversions

<b>BBSR</b>	Bermuda Biological Station for Research
<b>DHA</b>	Docosahexaenoic Acid
<b>DOPA</b>	Dihydroxyphenylalanine
<b>EDTA</b>	Ethylene Diamine Tetraacetic Acid
<b>EPA</b>	Eicosapentaenoic Acid
<b>FAO</b>	Food and Agriculture Organization of the United Nations
<b>FLUPSY</b>	Floating Upwelling System
<b>FSW</b>	Filtered Seawater
<b>GI</b>	Growth Index
<b>GRP</b>	Glass-Reinforced Plastic
<b>HUFA</b>	Highly Unsaturated Fatty Acid
<b>LDR</b>	Light Dependent Resistor
<b>MAFF</b>	Ministry of Agriculture Food and Fisheries
<b>NTM</b>	Net Treatment Mortality
<b>PHCD</b>	Post-Harvest Cell Density
<b>PUFA</b>	Polyunsaturated Fatty Acid
<b>PVC</b>	Polyvinyl Chloride
<b>RSR</b>	Resistance Sensing Relay
<b>SI</b>	Système International
<b>TBT</b>	Tributyltin
<b>TCBS</b>	Thiosulfate Citrate Bile Sucrose
<b>UV</b>	Ultra-Violet

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

<b>&lt;</b>	less than
<b>&gt;</b>	greater than
<b>n.a.</b>	not analysed or not available (also written as N/A)
<b>µm</b>	micron
<b>mm</b>	millimetre
<b>cm</b>	centimetre
<b>m</b>	metre
<b>km</b>	kilometre
<b>inch</b>	inch
<b>ft</b>	foot
<b>yd</b>	yard
<b>mi</b>	mile
<b>ft<sup>2</sup></b>	square foot
<b>yd<sup>2</sup></b>	square yard
<b>mi<sup>2</sup></b>	square mile
<b>m<sup>2</sup></b>	square metre
<b>ha</b>	hectare
<b>km<sup>2</sup></b>	square kilometre
<b>cc</b>	cubic centimetre (= ml)
<b>m<sup>3</sup></b>	cubic metre

ft <sup>3</sup>	cubic foot
yd <sup>3</sup>	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 ft³ = 1.308 yd³ = 219.97 UK gallons = 264.16 US gallons
1 ft³	0.02832 m³ = 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 m³/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 ft² = 1.196 yd²
1 ha	10 000 m² = 100 ares = 2.471 acres
1 km²	100 ha = 0.386 mi²
1 ft²	0.0929 m²
1 yd²	9 ft² = 0.836 m²
1 acre	4 840 yd² = 0.405 ha
1 mi²	640 acres = 2.59 km²

**Temperature:**

°F	$(9 \div 5 \times ^\circ\text{C}) + 32$
°C	$(^\circ\text{F} - 32) \times 5 \div 9$

**Pressure:**

1 psi	70.307 g/cm²
-------	--------------

**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<sup>-1</sup> in scientific journals. 1 g/kg is written as 1 g kg<sup>-1</sup>. 12 mg/kg would be written as 12 mg kg<sup>-1</sup>. 95 µg/kg would be written as 95 µg kg<sup>-1</sup>. A stocking density of 11 kg/m³ would be written as 11 kg m<sup>-3</sup>. This system of standardization is not normally used in commercial aquaculture hatcheries and growout units and has therefore not been used in this manual. More information about this topic can be found on the internet by searching for SI Units.



