

BIVALVE MOLLUSC HATCHERIES: A CRITICAL APPRAISAL OF THEIR DEVELOPMENT AND A REVIEW OF THEIR POTENTIAL VALUE IN ENHANCING THE FISHERIES OF DEVELOPING NATIONS.

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INTRODUCTION

In the century since Brooks succeeded in culturing the larvae of the Eastern oyster Crassostrea virginica the dream of producing shellfish seed from controlled hatcheries and subsequently growing these seed to a size where they could be sold for human consumption has become a reality. This realization has, however, not been without difficulty and much work remains to be done before we can successfully and reliably apply shellfish hatchery technology throughout the world, especially in the tropical regions. This report reviews the historical development of marine bivalve mollusc culture and emphasizes hatchery development. A discussion is made of hatchery development and the applicability of this technology to present problems in bivalve aquaculture throughout the world.

HISTORICAL PERSPECTIVE

Although the modern era of hatchery development began with Brooks' (1879) work on Crassostrea virginica the history of bivalve culture reaches back over 2000 years. The first accounts of active enhancement of bivalve production are seen in the Roman literature of Plinius. Where he describes the work of Sergius Orata who, while working in Lake Avermis, noted that in nature the European flat oyster, Ostrea edulis, occurred predominantly on the submerged brush wood of the mastic tree (Pistacea leutiscus) and the evergreen oak (Quercus ilex). By providing supplementary substrate of the same type Orata noted an increase in settlement of young bivalves and a subsequent increase in production. Although Orata was unaware of the planktonic develop-

ment and larval substrate specificity in O. edulis he set a precedent for techniques that are still in use in Norway and Yugoslavia for culturing Ostrea edulis (see Korringa, 1976a), in Cuba and throughout Latin America for Crassostrea rhizophorae (see Nikolic et al., 1976), and in Sierra Leone, West Africa for Crassostrea tulipa (Kamara, 1982).

The development of oyster culture on the rim of the Pacific ocean probably began in earnest in the seventeenth century (see Korringa, 1976b) and was again the result of observations of preferred settlement, this time of Crassostrea gigas on bamboo fish weirs. Subsequently culturists implanted bamboo specifically for collecting juvenile oysters. Today, shell strings are used for the same purpose (see Korringa, 1976b). Work on an artificial substrate for collecting settling oysters was first undertaken in France by Coste and DeBon in the early nineteenth century. Their work was continued by Kimmerer (see Orton 1937, p. 123) and resulted in the lime coated pottery tile collector that is still used extensively today in Northern Europe.

During the late nineteenth century work on the larval development of fishes had taken great strides forward (see review in Shelbourne, 1964) and in the early 1870's remarkable success was recorded in rejuvenating the Atlantic stocks of the shad, Alosa sapidissima, through hatchery programs. From the same source shad were also introduced to the Pacific Coast of the United States where they became well established as a commercial crop. This, and the work of Sars (1866) in Norway on cod, inspired work on Gadus callaris L. at Gloucester, Massachusetts. In 1878 over 1.5

million cod fry were released from this hatchery program. W.K. Brooks, then working at Crisfield, Maryland, U.S.A. had carefully observed the early work on fish larvae and, applying these techniques to the eastern oyster Crassostrea virginica, succeeded in 1879 in being the first individual to culture a bivalve completely through larval development. Brooks' methods were observed by Winslow who, in 1880, subsequently used them to culture Crassostrea angulata in Cadiz Spain. Although Brooks went on to examine the relationship of settling of larval oysters to the presence of shell substrate he was not the first worker to combine larval culture with provision of an artificial substrate. This distinction goes to M. Bouchon-Brandelely who in 1882, combined Winslow's work on C. angulata with the previous work of Kimmerer and succeeded in spawning and setting oysters in a closed system: that is the first production of oysters suitable for planting in the natural environment.

EARLY DEVELOPMENT OF BIVALVE HATCHERIES

The work of Kimmerer, Brooks, Winslow and Bouchon-Brandelely provided a basis for subsequent development of the highly intensive bivalve hatchery. Several major subject areas were identified as requiring investigation. These included induction of spawning, the provision of food for larvae, a means of separating and maintaining larvae throughout their lengthy free swimming development, the preparation of suitable substrate for settlement and metamorphosis, and the subsequent growth of metamorphosed juveniles in the natural environment. Before continuing with the chronology of work to address these problems it is relevant to summarize the rationale for developing hatcheries.

Mortality rate in marine organisms is not constant throughout the life span. It is common to record very high mortalities during the first days to weeks of larval development. In the natural environment these losses may be due to predation, starvation, disease or other causes. Consequently only a very small per

centage of the spawned individuals reach maturity and harvestable size. This mortality is counterbalanced by huge fecundity. In commercially valuable bivalves production of millions or even tens of millions of eggs per spawning is not uncommon. Hatcheries seek to maximize survivorship to harvestable size by minimizing mortality during the early development. A small decrease in early mortality rate can significantly influence numbers surviving to harvestable size. Hatcheries are, therefore, a conservative mechanism.

Early developments in hatchery work were as often accidental as premeditated. Wells and Glancy (see Wells, 1920) while working at the New York Conservation Commission and investigating the use of commercial cream separators (continuous centrifuges) for separating larvae, accidentally discovered a means of removing a large number of grazers from the plankton community and in doing so provided the inoculum for subsequent forced blooming of plankton or "green water"; a technique that is now commonly used throughout the world and, indeed, is an integral part of such development hatcheries as that described in Castagna and Kraeuter (1981). Using forced plankton blooms and "centrifuge-separated" larvae Wells and Glancy successfully reared oyster (C. virginica) larvae and metamorphosed them on oyster cultch (substrate). Meanwhile in Europe work with Ostrea edulis was also progressing. At Conwy Cole (1937) developed a technique to spawn O. edulis in large concrete tanks and culture the larvae through to metamorphosis in that same tank by maintaining a continuous phytoplankton bloom. This he did by regular small additions of nutrients in the form of minced crab. The oysters metamorphosed on limed, half-round tile collectors and were subsequently transported, on these collectors, to a grow out site on local intertidal mudflats.

HATCHERY DEVELOPMENT: 1940-1980

In the mid-1940's Loosanoff and Davis, working at Milford, Connecticut succeeded in incorporating induced spawning of

Crassostrea virginica into the procedure developed by Wells and Glancy thus facilitating long term planning of hatchery production through the provision of regular spawning.

Following the early work of Cole (1937) and Bruce, Knight and Parke (1939) in the identification of phytoplankton as food organisms for developing larvae a considerable effort was made to isolate and identify and culture potential food species. Much of this work was effected at Conwy, in the United Kingdom by Walne (see Walne, 1963) and at the Milford Laboratory in the United States by Loosanoff and his co-workers (see Davis and Guillard, 1958). Work was tedious and repetitive but good diets emerged. Following these studies the use of such food species as Tetraselmis suecica, Isochrysis galbana, Monochrysis lutheri and Thalassiosira pseudonana became common and, indeed they are still in extensive use today (see review by Epifanio, 1976). The advances made in the years 1945-1960 are summarized in several important contributions including Loosanoff and Davis (1963) and Walne (1956, 1965, 1966).

The early 1960's can be characterized as a period when significant advances in microbiology, phytoplankton and larval culture were made. Materials used at that time were, however, generally limited to glass, stainless steel, concrete and other expensive commodities. With the advent of plastics technology the economics of building and maintaining culture facilities were to change markedly and allow both cost reduction and improved systems design. The period following the publication of the major works of Loosanoff and Walne could be viewed as a period of refining techniques rather than pioneering development. This refinement was, in turn, based upon increased understanding of the biology of the species in culture at the organism level and resulted from critical experimental work rather than mimicry of field observations. Techniques in biochemistry and physiology were employed to aid in interpretation of experimental data and better refine culture methods. Significant contributions relating to larval culture during this period include those of Helm, Holland and Ste-

venson (1973) on conditioning regimes for broodstock in relation to subsequent larval survival, Holland and Spencer (1973) on larval energetics; Parsons, Stephens and Strickland (1961) on composition of algal food in relation to culture conditions, and Helm and Millican (1977) on definition of growth optima for larval culture. This is only a small selection from a large volume of pertinent literature.

As larval culture technique improved a stimulus was provided to examine problems in post larval culture. The use of shell bags as a settlement substrate or cultch was commonplace but problematic in that it required prior cleansing of the shell, resulted in significant handling and maintenance problems when used in growth of juveniles in closed systems, and often produced irregular shaped adults that were unsuitable for the premium, halfshell market. Several options for the production of "free" or "cultchless" juveniles were investigated. These included settlement on and subsequent removal from plastic or metal screens or sheets, or settlement on small particles of calcium carbonate which would be of little influence in subsequent growth. All of these techniques proved valuable in providing juveniles that could be grown in high densities in controlled hatchery systems. Investigations of the food and culture requirements of the juvenile life stages followed methods of earlier work with larvae (see for examples Walne 1970, Walne and Spencer, 1974). Even though "cultchless" seed was to prove valuable in hatchery production of bivalves their small size resulted in significant losses to predation or smothering and high cost of containment in mesh trays on transfer to the natural environment.

Major comprehensive syntheses of work describing hatchery culture of bivalves have been slow to materialize despite the wealth of relevant scientific literature. Perhaps the first major attempt at production of a complete hatchery manual was that of Dupuy et al. (1977). This manual focusses on a high technology, energy intensive, year-round operation to produce Crassostrea virginica. Extensive pretreatment of culture water and the culture of phytoplankton

food are a prerequisite to hatchery success. Also, the capital investment required is considerable— a minimum of \$ 500,000 at 1976 value to build and operate for a period of 18 months. This cost estimate does not include initial land purchase or any portion of subsequent grow out to adult size. Obviously, such a formidable investment of capital is beyond most individuals or even cooperatives. Underestimates of capital expenditure in such high technology hatcheries have and will continue to result in their economic failure.

By comparison a less complex hatchery manual, focussed predominantly on the production of Mercenaria mercenaria using the Wells-Glancy technique, was produced by Castagna and Kraeuter (1981). This manual also addresses the problems of growth of post settlement stages in areas protected by stone aggregate and mesh covers (see also Walne and Davies, 1977 for application of mesh covers to the grow out of oysters). It describes a seasonally operated hatchery, thus eliminating the need for broodstock conditioning programs, and is aimed at a much lower capital investment level and, perhaps more important, at the nonprofessional. The advantages of simplicity and low cost are counteracted by the fact that such a hatchery is more constricted in operation by climate and geographical location than an intensive, self contained hatchery of the type described in Dupuy et al. (1977).

HATCHERY DEVELOPMENT: 1980-PRESENT DAY

Recent changes in hatchery design, operation and success have been strongly influenced by advances in materials, engineering and, above all, application of new understanding of the spawning, growth and metamorphic processes of commercially valuable bivalves at the molecular level. Also, hatcheries now orient methods to the production of specific seed, for example, production methods for Crassostrea gigas seed differs markedly from those for Mercenaria mercenaria seed.

Plastics, nylon and fiberglass laminates have essentially replaced concrete

stainless steel and glass in modern hatcheries. All algal food cultures that exceed 20 L in volume are now usually effected in tanks made of translucent fiberglass, these often being cylindrical in shape to optimize surface area:volume ratios and photosynthesis. Polyvinyl chloride or polyethylene plumbing is standard, this being easily installed and non-toxic. Fine meshes for use with larval culture are available in a range of sizes in nylon or polypropylene and are easily cleaned or sterilized. These advances have eased hatchery operation and maintenance problems considerably.

Efforts have also been made to make the processes of spawning, larval rearing and metamorphosis more predictable. The stimulation of spawning by thermal shock has been successfully employed for many species of bivalves since the early work of Loosanoff. By contrast the biochemical basis of the spawning mechanism received comparatively little attention until the recent work of Morse et al. (1977) on the abalone, Haliotis rufescens, which demonstrated the central role of prostaglandin endoperoxide synthesis in the spawning response. The authors suggest the controlled, synchronous induction of spawning by stimulation with chemicals such as hydrogen peroxide as a valuable tool for mariculture operations with a number of molluscan species. Subsequent work by the same authors Morse et al., (1979) demonstrated the existence of specific chemical inducers of the settlement and metamorphic process in Haliotis. These inducers, analogs of gamma-amino butyric acid, can be used simply, safely and inexpensively to induce rapid settlement and metamorphosis in many commercially valuable mollusc species.

In the manner that the work of Morse and his collaborators has increased the predictability of events in spawning and metamorphosis, recent work by Gallagher and Mann (1981) has focussed on development of a simple, rapid, inexpensive technique to assess larval health. This technique uses the stain Oil-Red-O to emphasize stored lipid reserves in cultured larvae. Healthy larvae have been shown to contain extensive lipid reserves whereas unhealthy larvae have low lipid reserves. From standardized culture

techniques Gallagher and Mann have prepared a series of color photographs of healthy and unhealthy individual larvae of several commercially valuable species. This photographic series is presently being evaluated in commercial hatcheries throughout the United States as an on site tool for use in daily management decision relating to whether or not larval cultures should be retained or discarded. This staining technique has the added feature that it can also be used as a diagnostic tool for the early detection of Vibrio sp. bacterial contaminants in larval cultures.

As mentioned earlier techniques for culture of post metamorphic (seed) forms are species specific. On the west coast of North America a large industry exists for the culture of the Japanese oyster Crassostrea gigas. The market product of this industry is predominantly a shucked processed or canned item rather than a premium half shell oyster. Despite an increasing dependence upon hatchery produced seed to sustain this industry very little post larval culture is effected in the hatchery. Larvae are cultured in very large volumes, 10,000 l or more is not uncommon, and, when metamorphic competency is indicated by the presence of pediveliger stage larvae, shell bags containing approximately 20-40 kg of clean, washed, whole oyster shells are suspended in the culture tank. Larvae settle and metamorphose on these shells and the shell bags are subsequently transferred to the natural environment where they are suspended from floats or lines tied between large stakes. The shell bags and attached juveniles remain suspended until the latter have attained a length of approximately one centimeter at which time the bags are reclaimed and their contents spread on the bottom. The final product is harvested by dredging. By contrast the present state of the art of culture of cultchless seed of Crassostrea gigas, Mercenaria mercenaria, and to a lesser extent Crassostrea virginica and Ostrea edulis during early post settlement and metamorphosis uses upwelling culture vessels. These differ from the previously common mesh bottomed trays, on which the juveniles were maintained as a monolayer in a downward flowing water current, in that an up-

welling unit is usually cylindrical, varying from 20 cm to 1 m in diameter depending upon need, and have a mesh bottom of appropriate size on which the juvenile bivalves are placed. The upwelling units do not use monolayers but are filled with many individuals to a depth of 10 cm or more, that is a very high density of individual animals. The system offers the advantage of much smaller volume requirement than the mesh-bottom tray arrangement and easier maintenance in that a majority of the fecal material is carried away with the high flow of water. Upwelling systems have proved particularly useful in the culture of Mercenaria mercenaria where continuous, active movement of individuals within the culture container results in uniform growth of the cultured population.

Irrespective of the culture method used, food requirements of post-metamorphosis individuals are considerable and efforts to transfer them to running natural seawater at the earliest time is advised.

APPLICATION OF HATCHERY TECHNOLOGY IN NEW FISHERY DEVELOPMENT PROGRAMS

A large volume of information of the culture of bivalve molluscs in hatchery systems is now available. It is relevant to ask how much, if any, of this is of direct application to the enhancement of fishery production efforts in countries which have little or no previous experience in mollusc culture? As a general comment it is notable that many such development efforts have similarities with problems in industrialized nations where hatcheries form a small component in a larger plan to counteract overfishing and environmental degradation resulting from urbanization and industrialization. In both situations it must be emphatically stated that the potential contribution of hatcheries is and probably will remain to be very small. There is no substitute for a clean, natural environment.

The first major impediment to hatchery development is inevitably capital. Costs quoted earlier will generally preclude high-intensity, year-around opera-

tions unless substantial industry (possibly with investment from first world nations) or governmental funding is available. Even under these conditions large initial investment is unwise without prior demonstration of suitability of siting and proven capability to culture the target organism. I am assuming at this time that the organism to be cultured is native and of present economic value as a fishery. I will comment on the use of non-native species later.

Given that capital is available probably the most important decision relating to hatchery construction is site selection: an adequate and dependable supply of unpolluted water is absolutely essential. Geographical location should be such that the natural water supply has salinity and temperature characteristics appropriate for the species that are to be cultured. Appropriate siting will allow seasonal operation with minimal expenditure on water heating - a significant cost saving. In planning a site access to services such as roads, electricity and fresh water should also be considered of prime importance. Hatchery buildings should be constructed with cleanliness in mind and consideration given to greenhouse type structures if any algal culture or forced blooming of phytoplankton is considered. Seawater systems should be constructed as simply as possible with no buried pipelines and, wherever possible, dual pipelines so that one line can be used while the other is being cleaned. Open gutter drains are preferable to enclosed drains in that they are much easier to clean.

Assuming that the physical structure is available the selection of broodstock can proceed. If asynchrony in gametogenic cycles in widespread populations of the cultured species exists it can be exploited to lengthen the hatchery operational season through shipping of ripe broodstock. A survey of potential broodstock sources is advised as an integral part of site selection in that the presence of the target species in a site does not necessarily mean that those individuals will provide highest fecundity or highest quality eggs. An integral part of successful hatchery operation is exploiting traits in the biology of

the species to be cultured that will make them more amenable to culture. Notable examples include using larviparous species, notably Ostrea species and especially Ostrea chilensis, which brood the larvae for part or all of the larval development period and thereby relieve the culturist of many larval culture problems. Mytilids form strong byssal attachments at the time of settlement. Setting these species on rope directly in the culture container as is effected for Mytilus edulis in China (Zhang Fusui, 1983) or using ropes as collectors in the natural environment (as used extensively in France, see also Tortell, 1976) eases subsequent handling problems considerably. Use of simple rope settling collectors might also be appropriate for early, byssus forming stages of various scallops. In designing methods to exploit amenable traits of the culture species it is important to avoid complexity and to utilize inexpensive and locally available materials. This will allow both the culture techniques to be taught to others who have no prior training in aquaculture, and the industry to expand without excessive reliance on distant material sources.

Assuming that these guidelines are followed a realistic first appraisal of a relatively inexpensive, uncomplicated hatchery can be made. Only then can the need for greater size, complexity or even multiplicity of small efforts be evaluated on both a biological and economic basis. It is important to emphasize that this "first stage" in hatchery development will take considerable time perhaps several years to choose a site, construct a small hatchery and produce several crops of juvenile animals for subsequent grow out to commercial size in local sites. Hatchery construction and operation will not provide rapid solutions to juvenile or "seed" production problems. During a development or transitional period seed supply should be satisfied from elsewhere using the fast shipping service provided by modern land and air transport systems.

A word of caution is needed when considering the movement of shellfish over considerable distances, even if movements are to be sustained for only

short periods. Movements of marine mollusc species around the world for culture purposes have contributed significantly to the associated spread of pest and even disease organisms (Elton, 1958; Mann, 1978, 1983). Although modern hatchery production offers a generally cleaner shellfish seed than wild collections it is still impossible to certify molluscs as free of diseases, parasites or potential pest organisms. Consequently, great care is required in long distance movement of seed shellfish stock. Recent demonstrations of the feasibility of shipping pediveliger larvae of cultured species rather than post metamorphic juveniles offers the possibility of both decreasing the cost of shipping (many millions of larvae can be shipped in a container of less than one litre volume) and reducing the shipment of associated species. This new shipping method places the responsibility of settlement and metamorphosis procedures on the purchaser; however, it also allows the purchaser to use whatever substrate is appropriate for subsequent local grow out. A less appealing facet of this much improved shipping ability is the temptation to import non-native species in the hope that they will exhibit superior growth and survival. This has been the major stimulus to the rapid spread of the Japanese oyster Crassostrea gigas from its native oriental range to North America, Europe, the Mediterranean Sea, Australia and now even South America. In a few locations, the United Kingdom for example, these new fisheries are completely dependent upon hatchery produced juveniles because water temperatures are never high enough to stimulate spawning; however, where natural spawning can occur (at greater than 18-20°C) the result may be uncontrollable proliferation and spread of the species, possible competition with and displacement of native species and considerable economic and ecological problems. Examples of such uncontrolled and unpredictable biological problems already exist in both terrestrial and freshwater systems (see Elton, 1958) and emphasize the need for care in further introductions. A series of guidelines for consideration of possible introductions and methods for effecting introduction has

been prepared by the International Council for Exploration of the Seas (I.C.E.S.) and I urge individuals who are contemplating culture programs with non-native species to study this document carefully before proceeding with introductions.*

CONCLUSIONS

Techniques for bivalve hatchery development are moving toward but have not yet reached the point in time where a hatchery can be built and operated following pre-set instructions and be absolutely assured of both economic and biological success. In most countries; however, shellfisheries operate on either the family or small cooperative level, and rarely have financial or technical backing to support extensive hatchery facilities. The problems of seed supply still remain and require attention if economic prosperity is to be attained. Even though the importance of preserving natural set cannot be over emphasized it is relevant to ask how can hatchery technology be applied to the problems of fishery enhancement? The answer to this question must, to a certain extent, be both site and species specific. The importance of knowing the basic biology of the species in question is crucial to good hatchery management. Certain guidelines can be followed: (1) Initially, work should de-emphasize complexity, encourage simplicity and be effected in the natural breeding season with native species and in as optimal a site as possible. The improved Wells-Glancy larval culture technique recently described by Castagna and Kraueter (1981) for seasonally operated, low technology hatcheries provides a good foundation on which to build. (2) It should be constantly emphasized that there is no substitute for good water quality. This should be considered in the selection of a site for culture activities. (3)

* Copies of this document can be obtained from: Dr. Carl J. Sindermann, Chairman, Working Group on Introductions and Transfers of Marine Organisms, I.C.E.S. National Marine Fisheries Service, Sandy Hook Laboratory, Highlands, NJ 07732, U.S.A.

Cleanliness and simplicity are valuable assets in hatchery operation and design. (4) Test possible broodstock from a number of localities. Avoid broodstock conditioning programs. They are expensive, complex and unnecessary in a seasonal operation. (5) In seeking a suitable substrate for metamorphosis consider what the species naturally sets upon. Use methods which will relieve potential handling problems and be compatible with grow out in most hatchery operation. (6) If manpower is abundant and relatively inexpensive then use it to keep energy costs to a minimum. (7) Throughout the whole development process remember that the final methods should be simple enough to be taught to, and mastered by all potential users and should use inexpensive readily available natural resources whenever possible. (8) The participation of the local user groups should be actively encouraged. Such efforts take time and results are not instantaneous; however, a cautious, gradual approach can, I believe, show the value of hatcheries in fishery development efforts throughout the world.

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LITERATURE CITED

- Brooks, W.K. 1890. The Oyster. Johns Hopkins Press Baltimore 225 p.
- Bruce, J.R., Knight, M. and Parke, M.W. 1939. The rearing of oyster larvae on an algal diet. J. Mar. Biol. Ass. U.K., 24:337-374.
- Castagna, M. and Kraeuter, J.N., 1981. Manual for growing the hard clam Mer-
cenaria. Special Report in Applied Marine Science and Ocean Engineering N° 249. Virginia Institute of Marine Science.
- Cole, H.A., 1937. Experiments in the breeding of oysters (Ostrea edulis) in tanks, with special reference to the food of the larva and spat. Fish. Invest. London, (Ser. 2) 15:1-28.
- Davis, H.C. and Guillard, R.R.L., 1958. Relative value of ten genera of micro organisms as food for oyster and clam larvae. U.S. Fish Wildl. Ser. Fish Bull., 136, 58:293-304.
- Dupuy, J.L., Windson, N.T. and Sutton, C.E., 1977. Manual for Design and operation of an Oyster Seed Hatchery for the American Oyster Crassostrea virginica. Special Report in Applied Marine Science and Ocean Engineering, N° 142. Virginia Institut of Marine Science.
- Elton, C.S., 1958. The ecology of invasions by animals and plants. Methuen and Co. Ltd., London, 181 pp.
- Epifanio, C.E., 1976. Culture of Bivalve Mollusks in Recirculating Systems: Nutritional Requirements. pp. 173-194 in First International Conference on Aquaculture Nutrition. ed. by K.S. Price, Jr., W.N. Shaw and K.S. Danberg. U. Delaware Press. 323 pp.
- Gallager, S.M. and Mann, R., 1981. Use of Lipid Specific Staining Techniques for Assaying Condition in Cultured Bivalve Larvae. J. Shellfish Res. 1(1):69-74.
- Helm, M.M., Holland, D.H. and Stephenson R.R. 1973. The effect of supplementary algal feeding of a hatchery breeding stock of Ostrea edulis L. on larval vigour. J. mar. biol. Ass. U.K., 53:673-684.
- Helm, M.M. and Millican, P.F., 1977. Experiments in the hatchery rearing of Pacific Oyster Larvae (Crassostrea gigas Thunberg). Aquaculture, 11:1-12
- Holland, D.H. and Spencer, B.E., 1973. Biochemical changes in fed and starved oysters, Ostrea edulis L., during larval development, metamorphosis and early spat growth. J. mar. biol. Ass. U.K., 53:287-298.
- Kamara, A.B., 1982. Oyster Culture in Sierra Leone. pp. 91-108. In: "Aquaculture Development in Less Developed Countries." L.J. Smith and S.Peterson

- eds., Westview Press, Boulder, Colorado, 152 pp.
- Korringa, P., 1976a. Farming the flat oyster of the genus Ostrea. Elsevier, 238 pp.
- Korringa, P., 1976b. Farming the cupped oysters of the genus Crassostrea. Elsevier, 224 pp.
- Loosanoff, V.L. and Davies, H.C., 1963. Rearing of bivalve mollusks. *Advances in Mar. Biol.*, 1:1-136.
- Mann, R., 1978. Ed. Exotic Species in Mariculture. MIT Press, 363 pp.
- Mann, R., 1983. The Role of Introduced Bivalve Mollusc Species in Mariculture. *Jour. World Maric. Soc.* (in Press).
- Morse, D.E.; Duncan, H.; Hooker, N. and Morse, A.; 1977. Hydrogen Peroxide Induces Spawning in Mollusks, with Activation of Prostaglandin Endoperoxide Synthetase. *Science*, 196: 298-300.
- Morse, D.E.; Hooker, N.; Duncan, H. and Jensen, L., 1979. Gamma Aminobutyric acid, a Neurotransmitter, Induces Planktonic Abalone Larvae to Settle and Begin Metamorphosis. *Science*, 204: 407-410.
- Nikolic, M.; Bosch, A. and Alfonso, S., 1976. A System for Farming the Mangrove Oyster (Crassostrea rhizophorae Guilding, 1828). *Aquaculture*, 9:1-18.
- Orton, J.H., 1937. Oyster Biology and Oyster Culture. Arnold and Co. London 211 p.
- Parsons, T.R.; Stephens, K. and Strickland, J.D.H., 1961. On the chemical composition of eleven species of marine phytoplankters. *J. Fish. Res. Bd. Canada*, 18:1000-1016.
- Sars, G.O., 1866. "Om Vintertorskens (Gadus morrhua) Forplanting og Udvikling." *Forhandl. Vid. Selsk. Christiana*, 1866. (Translation in Rep. U.S. Comm. Fish, 1873-5, Appendix A 213-22)
- Shelbourne, J.E., 1964. The artificial Propagation of Marine Fish. *Adv. Mar. Biol.*, 2:1-83.
- Tortell, P., 1976. A new rope for mussel farming. *Aquaculture*, 8:383-388.
- Walne, P.R., 1956. Experimental rearing of the larvae of Ostrea edulis L. in the laboratory. *Fish. Invest. London*, (Ser. 2) 20(9): 23 pp.
- Walne, P.R., 1963. Observations on the food value of seven species of algae to the larvae of Ostrea edulis. 1. Feeding experiments. *J. Mar. biol. Ass. U.K.*, 43:767-784.
- Walne, P.R., 1965. Observations on the influence of food supply and temperature on the feeding and growth of the larvae of Ostrea edulis L. *Fish. Invest. London*, (Ser. 2). 24(1):45 pp.
- Walne, P.R., 1966. Experiments in the large-scale culture of the larvae of Ostrea edulis L. *Fish. Invest. London* (Ser. 2) 25(4):53 pp.
- Walne, P.R. 1970. Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera Ostrea, Crassostrea, Mercenaria and Mytilus. *Fish. Invest. London*, (Ser. 2) 26(5): 62 pp.
- Walne, P.R. and Davies, G., 1977. The Effect of Mesh Covers on the Survival and Growth of Crassostrea gigas Thunberg Grown in the Sea Bed. *Aquaculture*, 11:313-321.
- Walne, P.R. and Spencer, B.E., 1974. Experiments on growth and food conversion efficiency of the spat of Ostrea edulis in a recirculating system. *Cons. Int. Explor. Mer.*, 35:303-318.
- Wells, W.F., 1920. Growing oysters artificially. *Conservationist* 3, 151, New York Conservation Commission.
- Zhang, Fusui, 1983. Mussel culture in China. In: *Recent Innovations in Cultivation of Pacific Molluscs*. Ed. by D.E. Morse, K.K. Chew and R. Mann. Elsevier (in press).