HATCHERY DEVELOPMENT OF MARINE BIVALVE MOLLUSCS

Special project/Internship

Report

by

E. Mark Solon

A Final Report Submitted to
Oregon State University
School of Oceanography

In partial fulfillment for the
Degree of Master of Science

Completed February 16, 1984

Commencement June, 1984

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PREFACE

The direction of my Marine Resource Management program has been to develop an expertise in a particular area of aquaculture, complemented by a general knowledge of oceanographic principles. Specifically, my area of interest is the culture of marine bivalve molluscs. Prior to entering the MRM program, I had worked with Systemculture Corporation in Hawaii on the research and development of large-scale phytoplankton cultures for the closed system production of the Pacific oyster, Crassostrea gigas. As part of my program at O.S.U., I interned with the Whiskey Creek Oyster Farm in Tillamook, Oregon. This hatchery is among the largest and most efficiently operated hatcheries in the world and has become the primary seed supplier to commercial oyster growers along the west coast of North America.

Aquaculture-oriented coursework in my program included Fish and Molluscan Culture, Fish Diseases, and Seafood Processing. In a special projects course under Wilbur Breese at the Marine Science Center, I learned the histological microtechinque for the preparation and interpretation of bivalve gonadal samples and worked with the Pacific weathervane scallop, Patinopecten caurinus, to develop an induced spawning method and investigate early larval development parameters. My courses in Agricultural Finance, Statistics and Microcomputers will be useful in developing and managing an aquacultural business.

Given my past experience and current education, I feel I am now qualified to work towards the development of hatchery production technology of aquaculturally desirable bivalve species.

This report presents an outline for the development of a bivalve

species to hatchery production. In addition, included are aspects of work with induced spawning and larval rearing of the Pacific weathervane scallop, Patinopecten caurinus, a species which shows potential for commercial hatchery production.

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INTRODUCTION

Culturing of bivalve molluscs has occurred since ancient times. Early Romans cleared growing areas and planted them with young oysters for later harvest. In Japan, records dating from 746 A.D. show clams being transported and planted in bays where the species did not formerly exist. In France, the blue mussel has been cultured for over 700 years (Magoon, 1981).

Although controlled rearing of young bivalves has long existed, hatchery production is a more recent advancement. Perhaps the first step toward hatchery development occurred in 1879 when eggs of the American oyster, Crassostrea virginica, were observed in early development at Johns Hopkins University (Borgese, 1980). In the 1920's and 1930's, development work concentrated on the rearing of larvae in outdoor tanks, supplemented by laboratory investigations of larval rearing requirements. During this time, in Conry, England, adult European oysters, Ostrea edulis, were placed in large concrete tanks which had been constructed for allowing harvested mussels to purge themselves of estuarine pollutants prior to marketing. Oysters were allowed to spawn naturally in these tanks, and the young oysters (spat) left to settle on lime and sandcoated tiles.

In the late 1930's. Galtsoff (1938) found that spawning of the American oyster could be induced by increasing water temperature. Induced spawning methods allowed great numbers of fertilized eggs to become available for larval rearing studies. However, it wasn't until the early 1950's that Loosanoff and Davis were able to cussessfully rear C. virginica from larvae to metamorphosis, fed on cultured phytoplankton,

thus completing the hatchery cycle. Since then, much of the hatchery development effort has been directed towards meeting the nutritional requirements of large numbers of bivalve larvae, with a general emphasis on monospecific high-density cultures of unicellular algae.

Perhaps the most recent development in bivalve hatcheries has been the marketing of eyed larvae to shellfish growers. Just prior to settling out of the water column onto a substrate, the larvae develop a darkened area, referred to as an eyespot. Eyed larvae can be bagged in moist towels and shipped in small, iced containers to oyster growers throughout the world. The eyed-larvae method reflects the state of the art in terms of shellfish supply and marketing and is rapidly replacing the former hatchery method in which spat already attached to shell or some other substrate (cultch) must be shipped, commonly requiring heavy duty machinery and many additional hours of labor.

Upon arrival at the oyster farm, eyed larvae are released into prepared tanks of warm, well-aerated brackish water where mesh bags of clean cultch, commonly oyster shell, have been placed. Within 24-48 hours, the larvae will settle onto the cultch and after a few additional days will be ready to transfer to growing areas.

HATCHERY CONSIDERATIONS

A major stumbling block for successful bivalve culture can be the sufficient availability of seed. A hatchery can meet these demand requirements and provide additional benefits including:

- Elimination of seasonal dependency on natural seed.
- Production of clean, disease-free monospecific seed.
- Elimination of labor costs associated with natural seed collection.
- Affords the potential capacity of genetic selection.
- Allows an option of producing individual (cultchless) vs. substrate-attached spat.

Before considering the aquacultural development of a bivalve species, a need for hatchery seed must be evident. It is assumed that the technology for rearing young to market size has been established and that a market for the product exists. If these criteria are met, investigations to produce hatchery seed may be initiated.

Hatchery-produced seed has several advantages but their production may be unnecessary if naturally occurring seed are abundantly available. In Japan, for example, several years were spent to develop a hatchery technology for the scallop Patinopecten yessoensis. However, populations of the bivalve were found sufficient to supply more than adequate amounts of seed and that only an efficient means of seed collection was needed to accelerate the industry. The problem was solved with the onion bag collector. Japan's scallops now account for more than half of the world's total harvest, largely due to this seed collection method (Magoon, 1981).

A scallop species under investigation for commercial development would

be the North American weathervane scallop. This species is similar in appearance to the Japanese scallop, but its populations are small and scattered, as in Puget Sound (Olson, 1982). Seed collection attempts of this species in the Canadian Puget Sound have, to date, been unsuccessful (Bourne, 1983). Other known scallop populations are found in deep offshore areas, as off the Oregon Coast. Because of the rigors of open ocean seed collection, collection attempts might prove to be impractical if at all fruitful. Results of continued investigations may indicate that the development of hatchery methods for this species would be the most suited means of achieving commercial production.

Other bivalve species with hatchery production potential include the tropical oysters. Various types of mangrove oysters have large, firm meats which could be desirable commodities for the international market. One such oyster species is Crassostrea belcheri, occurring in estuaries of Eastern Borneo. Although seed of C. belcheri can be obtained by setting collectors at the appropriate time of year, another small, undesirable oyster, C. cucullata, also spawns at the same time. The result is that collectors have 90% C. cucullata and only 10% C. belcheri (Lim, 1980). A hatchery may eliminate this problem, and perhaps produce an oyster species well suited to tropical aquaculture. An alternative approach to develop a desirable species for tropical aquaculture may be through genetic selection of the commonly cultured C. gigas, a temperate region oyster species. Along these lines the College of Oceanography and Fisheries Science of the University of Washington (Beatie, 1983) has been working with the Pacific Oyster to develop, through selective breeding, a strain resistant to summertime conditions which result in heavy oyster

mortalities in areas of Puget Sound.

Development of the necessary technology to produce bivalve hatchery seed may follow a sequence of steps, the first of which, identification of the need for hatchery seed, has already been discussed. Other steps are outlined in Figure 1.

DETERMINATION OF ANNUAL CYCLE OF GONAD MATURITY

The bivalve gonad is a large organ of ciliated tubules which surround the intestine (Barnes, 1974). In oyster species, it is composed of two gonads so close to one another that the paired condition is difficult to detect. The interconnected tubules have many sacs or follicles. From the walls of these follicles, sex products grow and during spawning move to the mantle cavity through one of two gonadopores. Alternatively, in some bivalves, the nephridia will provide exit for the sperm and eggs.

Although the majority of bivalves are dioecious, some (e.g. Pecten maximus) are true hermaphrodites and others are protrandric hermaphroditic (e.g. Ostrea edulis), alternating sex products after each spawning. Crassostrea gigas is sexually similar to O. edulis, but Walne (1979) believes that C. gigas first develop as described then the population divides into those which remain prodominantly male and those which are predominantly female.

Gonad condition is determined by microscopic examination of the stages of gametogenesis within the gonad follicles. However, once familiarity has been established for a given species, macroscopic or gonad weight indices may be used to determine gonad condition (see Appendix A for application of these methods on the scallop Patinopecten caurinus).

At Oregon State University's Marine Science Center, the method em-

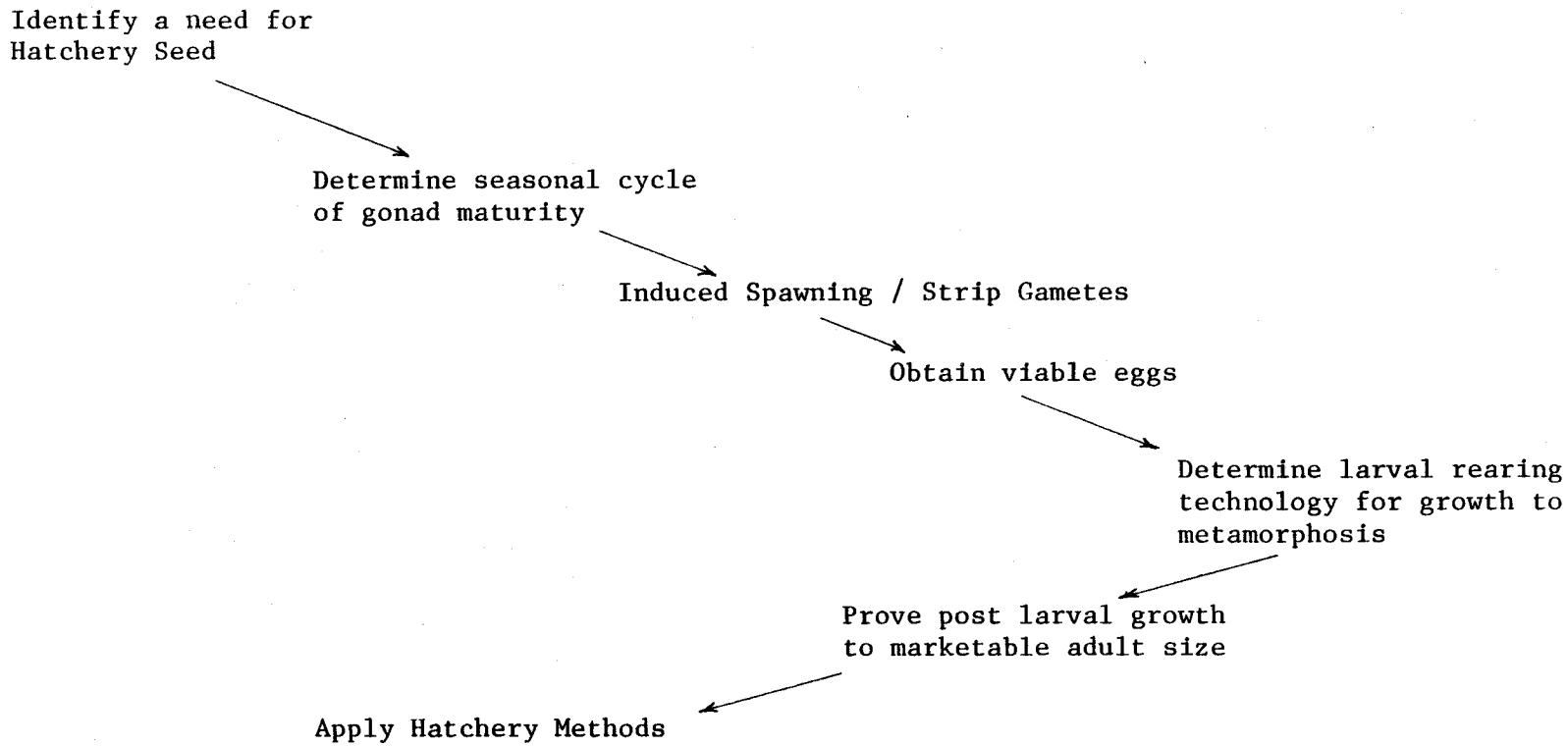

Figure 1. Development of a bivalve species to hatchery production.

ployed for determining seasonal sexual maturity in all bivalve molluscs is through microscopic examination of samples of at least twenty live specimens. From these samples, gonads are preserved and brought to the laboratory where approximately 1 cm^3 of tissue is taken from a designated area of each gonad. Tissue samples are dehydrated in alcohol, cleared in toluene, embedded in paraffin, and sectioned to a thickness of 6 microns with a microtome. The sections are then mounted and stained using the standard hemotoxylene and eosin method.

Female gonadal development is monitored by determining the percentage of oocytes in different growth stages present in follicle sections. Complete follicles are counted until a minimum of 100 oocytes have been recorded. The stages of oocyte maturation are identified as follows[1]:

I. SMALL OOCYTES: dark staining synaptic cells.

II. GROWING OOCYTES: light staining, often club-shaped cells extending from the wall of the follicle.

III. PRIMARY OOCYTES: fully developed oocytes in the follicle lumen which take on a polygonal shape due to pressure of adjacent oocytes.

Male histological samples are rated according to a predetermined index. The stages of development are:

I. EARLY DEVELOPING: Spermatogonia form a layer on the follicle walls. Inside this layer is another layer of spermatocytes. The lumen contains spermatozoa arranged radially.

II. LATE DEVELOPING: Few spermatogonia and spermatocytes remain near the follicle wall. Lumen becoming closely packed with spermatozoa.

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[1]Definitions are based on verbal descriptions by Robinson (1983) with reference to Mason's (1958) microscopic observations.

III. FULLY MATURE: Spermatozoa very dense and arranged radially from the lumen of the follicle. A few spermatogonia visible along follicle lining though little connective tissue remaining.

IV. MOSTLY SPAWNED: Center of follicle mostly empty. Few residual spermatozoa in periphery of the follicle but appear clumped.

V. SPENT - RECOVERING: Center of follicle empty. Spermatogonia around follicle wall, among them some spermatocytes.

After data have been recorded for a full year, monthly percentages of oocyte stages can be graphed to reveal seasonal trends (see Fig. 2). Because of the generally extended season of sexual maturity for male bivalves and the fact that few sexually ripe males are necessary for egg fertilization, emphasis on interpretation of seasonal gonad development trends is given to oocyte development. Also, prior to fertilization, sperm activity can and should be verified by stripping of sex products from an apparently ripe individual and examining the sperm suspension under high power of a light microscope. Pronounced sperm activity should be obvious.

In addition to seasonal patterns in gonad development, histological readings indicate the ratio of males to females in a population and the percentages of ripe individuals of each sex for a given month. This information will be essential for adequate sex representation when conducting induced spawning experiments.

STRIPPING AND INDUCED SPAWNING

The annual cycle of gonad development for a bivalve species is important because it indicates the time of year of peak sexual maturity. This is the time when stripping of eggs and sperm will most likely produce fertile eggs, and experiments on induced spawning methods should be undertaken.

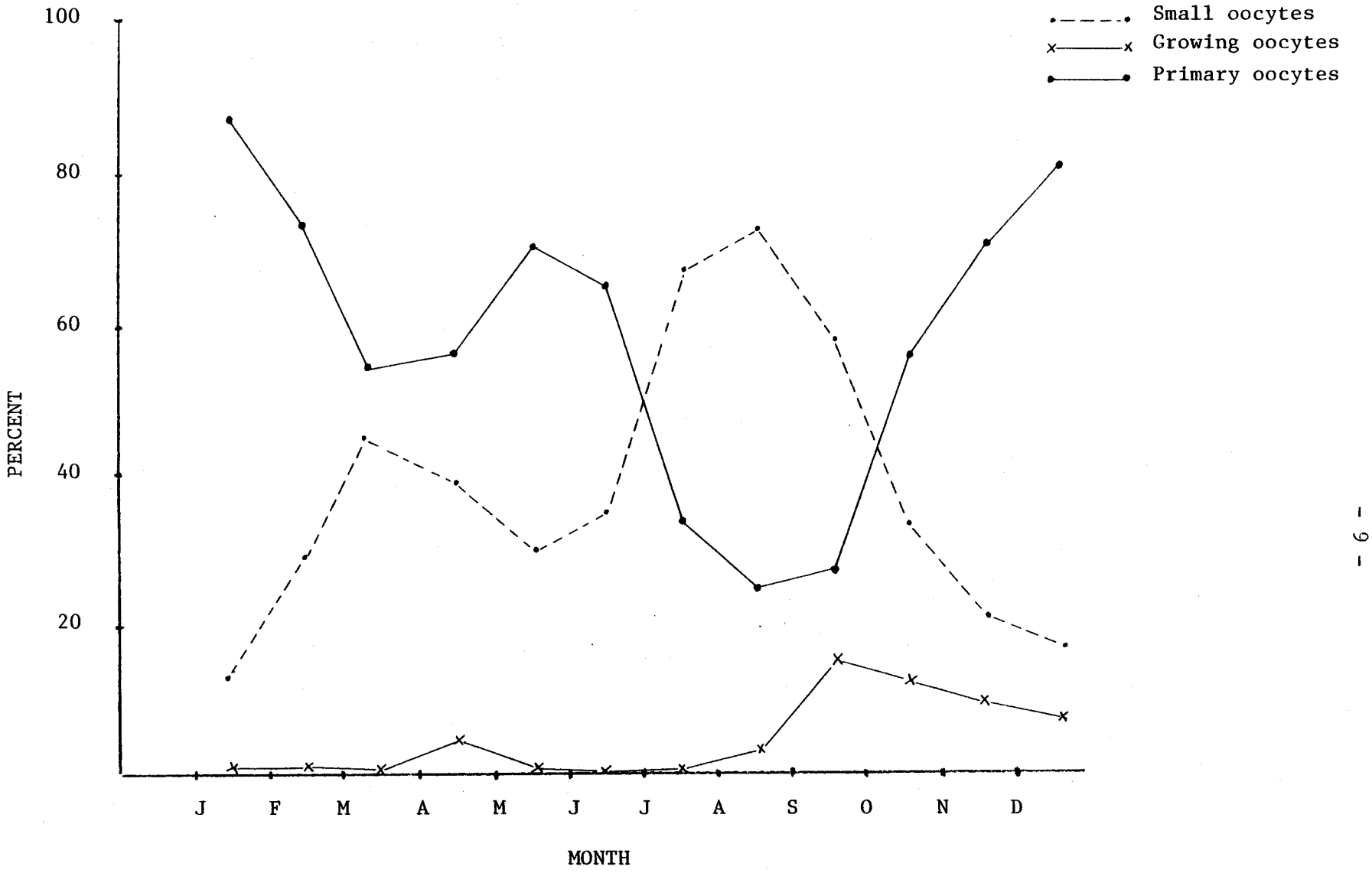


Figure 2. Annual reproductive cycle of Patinopecten caurinus off the Oregon Coast.

Before attempting induced spawning methods, the first step should be to strip eggs and sperm from mature specimens to obtain fertilized eggs. If adequate numbers of viable fertilized eggs can be collected with this method and if sufficient adult stocks are available, there may be no need to pursue induced spawning.

During the stripping process, sperm should be filtered through a small-meshed sieve, separating out ruptured tissue debris. Similarly, stripped eggs should be allowed to pass through a sieve of mesh size slightly larger than the egg diameter and held on a small mesh sieve to wash away debris. Adequate rinsing of stripped eggs is essential to minimize bacterial contamination. As a further aid to minimize bacteria, eggs may be held in rearing water slightly cooler than that for spawned eggs, until the straight hinge larval stage is attained (Hansen, 1983). Eggs should be held separately from sperm until stripping is complete. A sperm suspension is then added to the eggs and allowed to sit for 5 min to 1 hr, after which the fertilized eggs and water can be poured into the larval rearing tank. If the eggs are to be reared at high densities in a relatively small tank, rinsing of excess sperm is recommended prior to placing in the rearing tank. Similarly, if a high density sperm suspension is used, rinsing of excess sperm may be done sooner.

Typically, stripping of sex products yields much lower numbers of eggs than induced spawning. However, with species such as Crassostrea gigas in which a mature female may have greater than 100 million eggs (Walne, 1979) stripping may produce adequate amounts of viable eggs. Also, stripping of C. gigas is readily accomplished because primary oocytes of this species will complete the maturation process on exposure to seawater. This is not

the case for most bivalves (Yamamoto, 1964). With Patinopecten, Mytilus, and some clam species, stripping of eggs has limited or no success due to incompletion of oocyte maturation.

In nature, bivalve species usually exhibit seasonal patterns of gonad development. However, tropical species might not be so seasonally dependent. For these species, it is particularly important to be aware of other environmental parameters which may prove to be natural triggers for gonad ripening. It is by matching these environmental triggers or by substituting these with other spawning initiators that successful induced spawning may be accomplished.

Spawning begins with the onset of the final oocyte maturation process. In bivalves, meiosis is not completed until after fertilization. During oocyte maturation, the germinal vesicle breaks down and chromosomes which had been at the diakinesis stage of the first meiotic division move to metaphase, also of the first division (Gruffydd and Beaumont, 1970). Only then are spawned oocytes ready for fertilization (Fig. 3).

Spawning maturity, unfortunately, does not always correspond with the time when specimens exhibit peak microscopic ripeness. With Patinopecten yessoensis, natural spawning typically occurs 30-50 days past initial microscopic maturity (Yamamoto, 1964). Sperm stripped several weeks prior to the expected natural spawn may show little or no activity. According to Yamamoto, postponement of spawning may be due to some unfavorable environmental conditions or to the unreadiness of certain physiological mechanisms. Consequently, prior to attempting induced spawning methods, it is important to microscopically examine stripped sperm.

Determining an induced spawning method is a trial-and-error process of

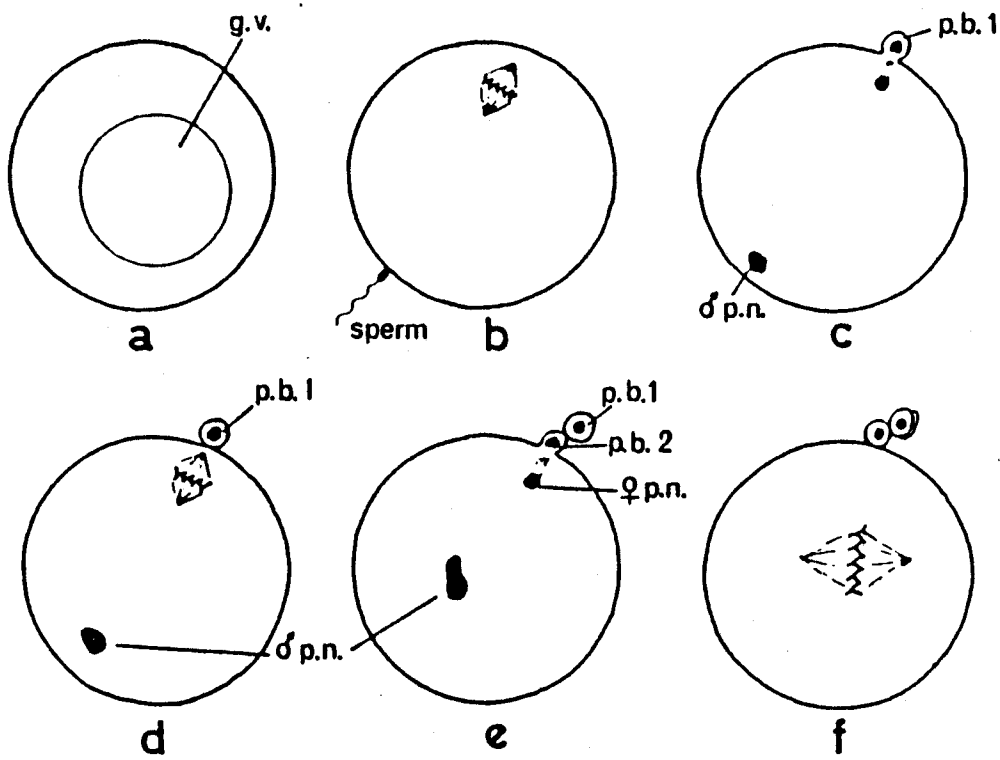


Figure 3 . Changes in the egg of *Pecten maximus* at fertilization. *a* Oocyte on release; g.v. = germinal vesicle. *b* Sperm penetration. *c*, *d* and *e* Formation of polar bodies (p.b.); male pronucleus (p.n.) migrates towards female pronucleus. *f* First cleavage metaphase (from Gruffydd and Beaumont, 1970).

tisting past proven, past modified, or new methods. It should be methodical, and a review of the literature, particularly for related species, may greatly facilitate the process. Some spawning methods applied to scallop species are listed in Appendix B.

To optimize success in spawning trials, performed when microscopic evidence suggests peak seasonal maturity, the following guidelines are recommended:

- Minimize stress to adult specimens. Maintain water at environmental parameters similar to those from which the specimens have been collected (e.g. temperature and salinity).
- Supplemental feeding of adult specimens is advised, particularly if duration of holding is prolonged.
- Determine stripped sperm activity.
- Numbers of specimens used in spawning trials should adequately represent ripe individuals of each sex based on microscopic evidence.
- If spawning occurs, employ techniques necessary to avoid excessive sperm (which could result in polyspermy).
- All spawning trials should be repeated with fresh specimens.

Helm et al. (1973) concluded that healthy adult oysters, supplementally fed with phytoplankton during conditioning, produced more viable larvae than those oysters whose diets had not been supplemented. Therefore, it is advisable to feed adult specimens.

If proven spawning methods fail, continued attempts should concentrate on physical methods rather than chemical methods since negative effects of the latter might not become apparent until later development.

One measure of induced spawning success can be taken as the percentage of fertilized eggs to develop to normal straight hinge stage. Until this stage of larval development it is generally accepted that supplemental

feeding is not necessary (Breese, 1981 and Hansen, 1983), and nutritional requirements should not influence this early growth.

LARVAL DEVELOPMENT THROUGH METAMORPHOSIS

Unless sufficiently high percentages of larvae can be reared through metamorphosis, development of hatchery technology should not be undertaken. For example, rearing success of Hinnites multirugosus, the purple hinged rock scallop, is currently limited by low percentages of larvae going through metamorphosis. Consequently, commercial application is not as yet practical (Leitan and Phlegler, 1981).

As previously mentioned, early hatchery technology advanced greatly when feeding with cultured phytoplankton was introduced. Meeting nutritional requirements of the developing larvae with unicellular algal cultures continues to play a major role in successful hatchery development. Hatchery production methods commonly utilize more than one algal species, emphasizing a smaller-sized plankton diet until larvae are 120μ (Dupuy et al , 1977) or 150μ (Hansen, 1983).

Pillsbury (1983) showed that growth rate and success of metamorphosis in Strombus gigas, the great conch, can be related to lipid content of the phytoplankton diet. Six unicellular algal species were analyzed for lipid content and artifically manipulated, through modification of growth regimes, to yield differences in lipid content within species. It was found that a lipid content of approximately 15% yields the greatest food value for the larvae of this conch.

Helm (1973) suggests that continuous monitoring of total lipid content of larvae in intensive culture systems can provide valuable information concerning their general condition. Gallager and Mann (1981) describe a

simple, inexpensive lipid staining techinque which can be used for rapidly assaying the condition of cultured larvae.

Using lipid staining techniques for bivalve larvae, in conjunction with biochemical lipid analysis of phytoplankton diet, may provide an efficient means of developing species-specific nutritional technology for larval rearing through metamorphosis.

HATCHERY APPLICATION

Included here is a general discussion of hatchery operational procedures, with some comparisons of preferred algal or larval rearing methods. The discussion presents the most current hatchery methods. For further details of bivalve hatchery operation or design specifications, the reader should refer to Hatchery Manual for the Pacific Oyster (Breese and Malouf, 1975) and Manual for Design and Operation of an Oyster Seed Hatchery (Dupuy et al., 1977).

Figure 4 illustrates the flow of operational processes within a shellfish hatchery. The type of hatchery products to be marketed will depend on the bivalve species and the market for the seed.

Although current applications are limited to oysters, many bivalve species could potentially be marketed as "eyed larvae". "Cultchless" seed is another alternative particularly popular among some oyster growers. In this process, larvae are allowed to settle on either a smooth substrate from which they will later be removed, or on small uniform particles of cultch (commonly ground shell) on which they will grow individually, as though without cultch. The advantage of cultchless seed is improved survival and more even growth to market size, resulting in a more attractive product. "Spat", in Figure 4, refers to young bivalve seed sold attached

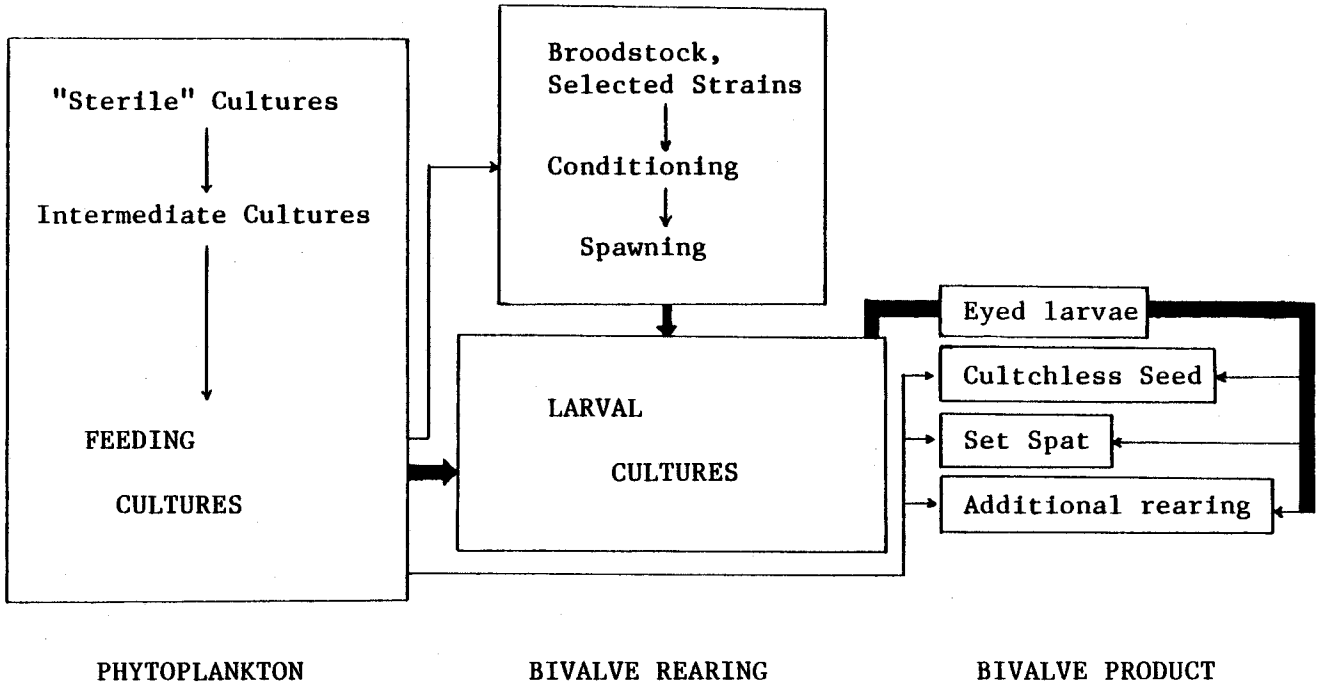


Figure 4. Flow of operational processes in a bivalve hatchery.

to cultch, commonly broken or half pieces of oyster shell. Although bivalve methods of substrate attachment vary (e.g. scallops have byssal threads), mussels, scallops and oysters might be marketed this way. "Additional rearing" is necessary for clam seed which does not set in a fixed place, but moves with foot and byssus tethers. Some scallop species which live attached to cultch for only a brief part of their lives might also require additional rearing before marketing. Manila clam, Tapes japonica, can be seeded on small-graveled, somewhat protected beaches. To survive, they must be planted at 1-3 mm size or greater, thus requiring additional hatchery rearing before planting. Rearing may be done in shallow flumes since larvae become benthic after metamorphosis.

If larval nutritional requirements have been determined for a particular species, and if preliminary culturing of phytoplankton and larvae has indicated success through metamorphosis, general hatchery methods can be applied to attempt commercial seed production.

Algal cultures:

Regardless of phytoplankton species or of culture methods to be used, nutrient solutions for feeding algae are roughly the same (Breese and Malouf, 1975). Table 1 lists the nutrients for four stock solutions. When prepared with distilled water and autoclaved, these solutions can be added at 1 ml/1 to culture media. For diatom species (e.g. Thalassiosira pseudonana, (3-H)), a stock solution of 2 gm/ 1 sodium metasilicate should also be prepared, to be added at 1 ml/ 1 (Hansen, 1983).

A hatchery facility should have a separate algal isolation room to allow for the maintenance of contaminant-free cultures. These "sterile" cultures, grown under fluorescent lamps in test tubes or small (e.g. 125 ml)

Nutrient	Formula	Stock Solution
1. sodium nitrate, granular, refined	$NaNO_3$	150 gm/l
2. sodium phosphate, monobasic, certified	$NaH_2PO_4 \cdot H_2O$	10 gm/l
3. trace metals[1]		
cupric sulfate	$CuSO_4 \cdot 5H_2O$	1.96 gm/l
zinc sulphate	$ZnSO_4 \cdot 7H_2O$	4.40 gm/l
cobalt (II) chloride	$CoCl_2 \cdot 6H_2O$	2.00 gm/l
manganese dichloride	$MnCl_2 \cdot 4H_2O$	36.00 gm/l
sodium molybdate	$Na_2MoO \cdot 2H_2O$	1.26 gm/l
4. vitamins		
biotin-crystalline		1 mg/l
vitamin B-12 crystalline		1 mg/l
thiamine hydrochloride		200 mg/l

[1] The concentrations of the trace metals given in the table are for the 5 primary solutions. The single secondary trace metal solution is made by mixing 1 ml of each primary solution plus 10 gm of ferric sequestrene and filling to 1 liter.

Table 1. Nutrient solutions in algal culture, after Matthiessen and Toner. Taken from "Possible methods of improving the shellfish industry of Martha's Vineyard, Duke's County, Massachusetts," by G. C. Matthiessen and R. C. Toner, 1966. Marine Research Foundation, Edgartown, MA.

stoppered flasks, maintain phytoplankton cell lines. Growth is retarded in these cultures by storing them under low light intensity and at a cool temperature. These cultures are used to inoculate working culture lines in flasks placed under brighter artificial light, also in the isolation room. These lines will be grown to greater densities for inoculating intermediate cultures. In the isolation room, all culture flasks and their contents should be autoclaved prior to inoculation.

Rapidly growing phytoplankton cultures, such as the intermediate and feeding cultures, increase the pH of their medium through utilization of CO_2. Certain algal species must remain within a pH range for optimal growth. Consequently, aeration of the cultures with CO_2-enriched air is advised to control pH and thus prolong growth of healthy cultures.

Another aspect of phytoplankton growth is that initial division enters an exponential growth phase, followed by a stationary phase and subsequent degeneration. To insure healthy cultures, when inoculating a newly prepared medium, the inoculant should always come from a culture still in exponential growth.

Traditionally, intermediate cultures have been grown in 20 l glass carboys. Although it is the practice of some hatcheries to autoclave these, it is sufficient to chlorinate the filtered seawater by the addition of sodium hypochlorite to 5 ppm. The chlorinated water is allowed to stand overnight and dechlorinated by addition of sodium thiosulphate. Nutrients are then added, followed by phytoplankton inoculation. An alternative to glass carboys are large (80-160 l), upright cylindrical fiber glass tanks, or plastic bags of similar volume contained in heavy mesh cylinders (Earnst, 1984). Both artificial and ambient light is recommended

for intermediate and large volume cultures. However, during seasons of greater insolation, ambient sunlight may be sufficient for all outdoor phytoplankton growth.

Pure intermediate cultures are used to inoculate the larger, open-air feeding cultures. Consequently, the desired volume of intermediate cultures is related to the size of the feeding culture. Based on the author's experience, when inoculating open-air feeding cultures an initial dilution no greater than 1/8 - 1/10 is advised. For example, if a 3,200 l feeding culture is to be inoculated, 80-100 l of intermediate culture would be pumped into the feeding culture and diluted to 800 l. The following day, if the culture has grown (darkened) significantly, dilution by filling to 3,200 l can ensue, with the culture expected for harvest on the third day. Ambient light, condition of inoculant, quality of filtered seawater, and frequency of air-borne contaminants are some of the many factors which may alter the expected growth schedule.

Three harvest methods are used independently by oyster hatcherymen in the Pacific Northwest. Batch culture method, which harvests a complete culture once it has attained its maximum density, is the most consistent harvest method in yielding clean, monospecific algal cells. Although this method is commonly used, semi-continuous harvesting can yield a greater number of algal cells with a minimum amount of maintenance over a fixed duration of time. With the semi-continuous method, once a culture has attained a harvestable density, rather than use it all at once, a fraction, commonly 1/2, is harvested and the remainder is filled with filtered seawater for algal purposes.[2] The culture will continue to be harvested,

[2]Algal water at Whiskey Creek Oyster Farm is double filtered through a fine-grained sand filter compared to larval water which has been once filtered, separating out particles > approximately 5μ .

once or twice daily, until it can no longer regain a harvestable density. At that time the culture should be drained, cleaned, and reinoculated. It is common for cultures to be harvestable for 7-10 days before draining and cleaning. Considering it usually takes a new culture 3 days to become harvestable, more cells can be grown and with less effort using semi-batch vs. batch harvesting method.

For open-air feed cultures, this investigator has found that unless the seawater source is fouled, there is little difference in the duration of semi-batch harvested cultures whether chlorinated and neutralized seawater or if untreated filtered seawater is used for refilling harvested cultures.

A third harvest method is continuous, using a chemostat. Although this would theoretically be an ideal situation, chemostats are very sensitive to changes and only relatively small, more manageable ones are in use (e.g. 200 1). Consequently, the numbers of cells is also small by comparison to batch or semi-batch harvested phytoplankton cultures grown in larger containers.

Broodstock and Conditioning:

An assumption made before attempting hatchery development of a bivalve species is that sexually mature adult specimens (broodstock) are to be available for spawning purposes. These adult specimens should be maintained where they can remain healthy without spawning. With the Pacific oyster, growers may bring their own specimens, usually selected for rapid growth, to the hatchery for spawning purposes.

Mature bivalve specimens are usually capable of spawning viable gametes for only a relatively brief portion of their annual reproductive

cycle. Consequently, the time of sexual ripeness, the "window", must be modified to extend or initiate sexual maturity. Conditioning is the artificial manipulation of the window for the purpose of obtaining viable sperm and eggs. This is usually accomplished by modification of environmental conditions such as food and temperature.

Although initial work with a bivalve species may require that stripping or induced spawning occur during the natural spawning cycle, continued work may allow year-round spawning through development of conditioning methods.

With C. gigas and C. virginica, seasonal sexual maturity can be manipulated by temperature. To initiate gonad ripening in recently spawned-out specimens of C. virginica, a period of cool water temperature should be provided before increasing the water temperature of the conditioning tank. This subsequent warm water will stimulate gamete formation. (For further descriptions of seasonal conditioning methods for C. virginica refer to Dupuy et al , 1977).

If conditioning methods are successful, it may be possible to supply year-round bivalve seed to interested shellfish growers.

Larval Maintenance:

From fertilization until metamorphosis, similar treatment is given to larvae of different bivalve species, with the exception of water parameters such as salinity and temperature. For Crassostrea gigas. although relatively wide salinity tolerances are allowed (20-30°/$_{oo}$), recommended temperatures have a narrowed range, 24-27°C, to insure optimal growth and development. Evidence from lipid staining tests (Gallager and Mann, 1981) suggests that fat reserves, necessary for healthy growth, are better stored

at lower temperatures, but that at higher temperatures (e.g. 30°C) less fat is stored presumably because of increased metabolic activity.

Frequent water changes are essential for bivalve larval management. Seawater contains dissolved organic nutrients which are essential to larval development (Courtright, 1967). Larval water changes serve several purposes: 1) to replenish organic nutrients which may not be sufficient in larval feed; 2) to avoid build up of metabolic wastes; and 3) to retain and sort the larvae, through use of sieves, allowing dead, bacteria-ridden shells to pass and to separate out larger contaminent zooplankton.

The first water change is recommended after the straight hinge stage has developed then once every third day until the larvae reach 150μ, followed by every second day until harvest (Hansen, 1983). At some hatcheries, twice weekly water changes are done for all larval tanks. Water flow capacities must allow for the required exchange volume to pass within a working day (unless nighttime filling is anticipated), in addition to water needed for algal culture.

Typically, larvae showing fast initial growth will continue to outcompete smaller larvae. By using different mesh sizes, larvae can be sorted at each water change, allowing larvae of similar size to be grown together. Larvae of different spawning dates, however, should not be kept together. In some hatcheries, larvae are refrigerated and held until tanks containing larvae of similar size become available. It is not known if this practice has a long-term effect on larval or postlarval growth.

Also during a water change, larvae can be reduced in density, by dividing one tank between two or three others. Breese and Malouf (1975) suggest a maximum egg or straight hinge stocking density of 200 eggs/ ml.

For growth of straight hinge to harvest, a concentration no greater than 5 larvae/ ml is recommended. At the Whiskey Creek Oyster Farm, approximately 10 ripe female C. gigas are induced to spawn with increasing water temperature. Loosanoff and Davis mention that 10 ripe C. virginica females can spawn well over 200 million eggs. If 200 million eggs are assumed spawned for C. gigas (and the 6,000 gal tanks are filled to 5,600 gal), then the resulting density at Whiskey Creek is 9-10 eggs/ ml. Because of subsequent splitting of larvae during water changes, a resultant harvest culture may have an estimated 30-50 million larvae per tank, roughly 1-2 larvae/ ml.

Feeding, which begins after straight hinge development, can follow the recommended daily schedule:

1st week - 30,000 algal cells/ ml
2nd week - 50,000 algal cells/ ml
3rd week - 80,000 algal cells/ ml

This schedule for C. gigas, is based on 21 day growth to 315 μ (eyed stage or metamorphosis) (Breese and Malouf, 1975). For faster growing or larger larvae, the feeding schedule should be modified as appropriate.

An alternative to cell counting and measured cell volume feeding, (described above) is to feed the larvae twice daily, visually estimating the algal density. Although both methods can be effective, taking regular cell counts and feeding designated water volumes is much more labor intensive than visual feeding. This additional labor, however, may be compensated for by faster and more uniform larval growth. Whiskey Creek, which uses visual feeding, produces eyed larvae in 14-15 days. Coast Hatchery in Quilcene, Washington, where larval feeding is with calculated water volumes of known density, claims an ability to grow Pacific oyster larvae to

the eyed stage in 11 days.

Cleaning:

Intermediate algal culture vessels are cleaned with acid and rinsed with hot water prior to filling with seawater and chlorinating. During semi-continuous harvesting, culture quality usually declines because of increasing concentrations of contaminant algal or zooplankton species. Also, the bottom of the culture accumulates dead cells and associated bacteria. Consequently, to maximize culture duration, care should be given to clean tanks thoroughly with hot water and bleach between use.

Similar to algal culture maintenance, larval cultures should be cleaned with every water change. Use of hot fresh water and bleach is advised, although extreme care should be taken to rinse adequately before refilling and adding larvae.

Handling of eyed larvae:

Larvae of a given species typically metamorphose upon reaching a certain size. Newly formed straight hinge larvae of C. gigas are roughly 90μ in length. At the eyed stage, just before metamorphosis, these larvae are approximately 315μ in length. Size, appearance of an eye spot, appearance of early gills and development of a foot (thus termed pedivelliger larvae) are criteria which can be used to indicate approaching metamorphosis.

When growing oysters, these clues indicate the product is ready for harvest. After draining the contents of a larval tank onto a sieve, the larvae are rinsed with cold water while being held on a sieve of appropriate size to retain only the largest eyed larvae. Smaller larvae

which rinse through the sieve are collected and placed into a fresh tank for additional growth.

The retained larvae are then passed through a larger screen to sort out debris and contaminant zooplankton. The filtered larvae are then rinsed (also with cold water) into a calibrated settling funnel and counted by volume, knowing the number of larvae per 100 ml. Alternatively, eyed larvae can be weighed given a predetermined weight per million count.

Oysters and presumably other bivalve larvae, can be refrigerated and stored for later setting. Henderson (1983) found that eyed larvae of Crassostrea gigas are able to retain their setting competancy while in refrigerated storage for up to 8 days at 5°C. The ability to store larvae ready for setting allows the product to be sold wrapped only in nylon cloth and moist paper towels and shipped to the oyster growers in small iced Styrofoam containers. Marketed this way, Whiskey Creek Oyster Farm guarentees a 20% set for the oyster growers, which generally yields 50% settlement or better. Marketing of shellfish by this method eliminates large shipping costs previously associated with the purchasing or collecting of shellfish already set on bulky cultch.

CONCLUSION

Several bivalve species could show potential as marketable products if grown commercially. In the Pacific Northwest, there is great interest in the aquacultural planting of the Manila clam, Tapes japonica, and the the culture of the weathervane scallop, Patinopecten caurinus (Bourne, 1983). Other bivalves such as the northern razor clam, Siliqua patula, and the geoduck, Panope generosa, have become depleted in some areas of their habitat, generating interest in their hatchery development. Reseeding pro-

jects for these two species are currently underway at the Washington State shellfish laboratories (Olson, 1983).

Through the successful development of spawning and larval rearing technology and through sound hatchery management, it will become increasingly possible to provide hatchery seed for many aquaculturally desirable bivalve species.

SCALLOPS

Investigations on Spawning and Larval Rearing of *Patinopecten caurinus*

ACQUIRING AND MAINTAINING SCALLOPS

Gonadal sampling of Patinopecten caurinus populations off the Oregon Coast has indicated that the highest percentages of sexually ripe individuals occur from late January through early June (Robinson, 1983). Consequently, investigations on induced spawning and larval rearing were undertaken during the winter academic term. At this time of year, however, river discharge into Yaquina Bay is at a maximum resulting in salinity problems for scallops at the Marine Science Center.

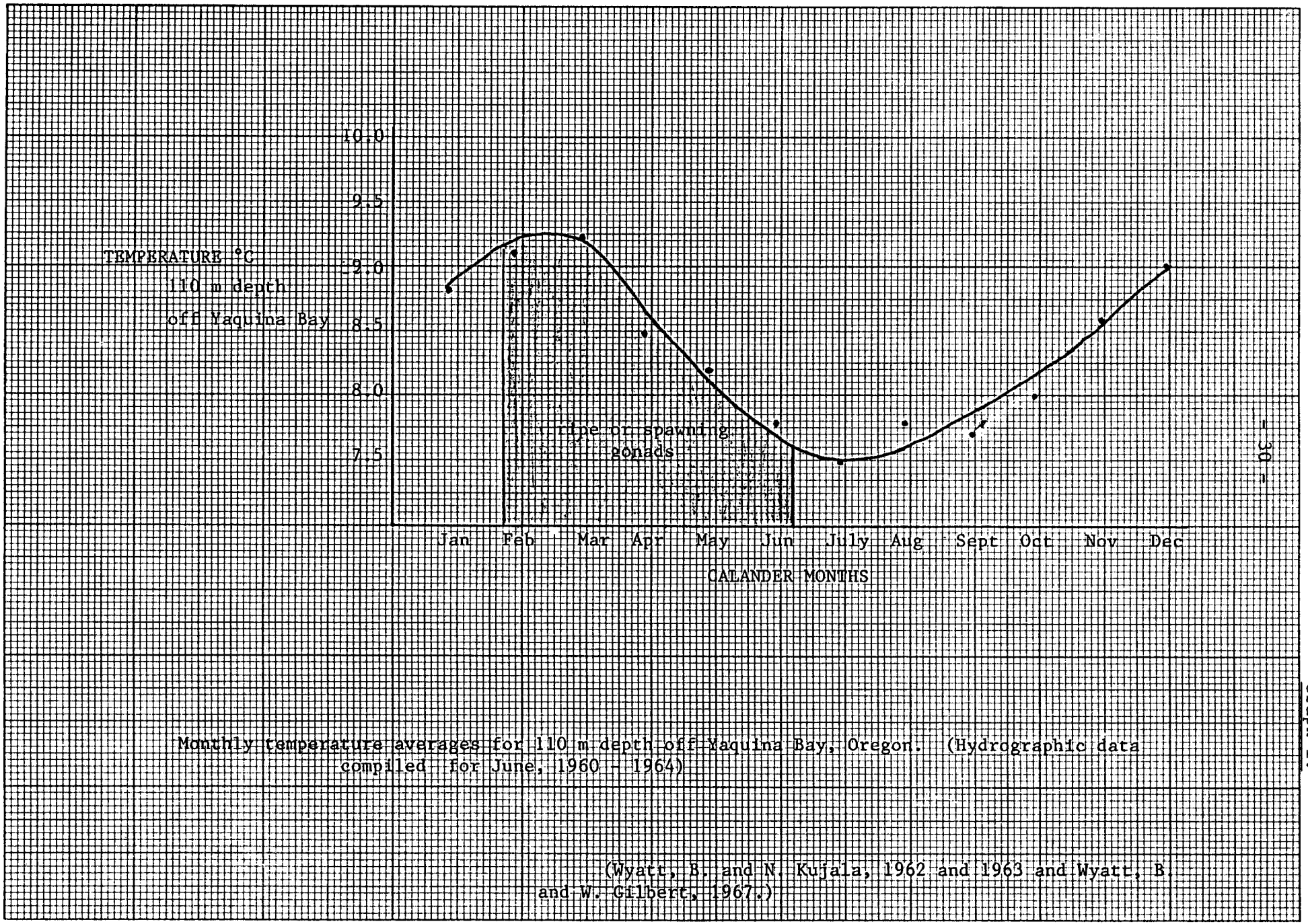
Scallops have little tolerance to salinity or temperature extremes and exhibit stress in reduced salinity environments (Yamamoto, 1964 and Itoh, 1982). Because of these potential problems, scallop specimens were kept in closed system aquaria fitted with aeration and maintained at salinities and temperatures similar to the areas from which they were harvested. Since there is no current hydrographic data on these locations, monthly salinity and temperature averages were calculated from previously existing data (Table 2 and Graph 1).

Table 2.

Month	Temperature °C	Salinity ppt	Month	Temperature °C	Salinity ppt
January	8.8	33.8	July	7.5	33.7
February	9.1	33.5	August	7.8	33.6
March	9.2	-	September	7.7	33.7
April	8.5	33.5	October	8.0	33.7
May	8.2	33.7	November	8.6	33.7
June	7.7	33.6	December	9.0	33.6

Hydrographic data off Yaquina Head, 1961-1964, 105 m depth (station NH-25). (Wyatt, B. and N. Kujala, 1962 and 1963, Wyatt, B. and W. Gilbert, 1967).

Newport fishermen primarily harvest off Yaquina Head north to Three Arches. Trawlers leave the bay during periods of calm seas and, if successful, return after 2-3 days with iced whole scallops. Unfortunately, there



Graph 1.

Monthly temperature averages for 110 m depth off Yaquina Bay, Oregon. (Hydrographic data compiled for June, 1960 - 1964)

(Wyatt, B. and N. Kujala, 1962 and 1963 and Wyatt, B. and W. Gilbert, 1967.)

were only 4 periods of calm seas during the 10 weeks of this investigation. Consequently, total numbers of adult scallops were less than anticipated.

The last scallops harvested are usually the first to be unloaded so it was desirable to be dockside when a trawler returns. Because the process of unloading to cold storage is hectic and potentially dangerous, when aquiring specimens, the only allowable sorting is to choose unbroken shells.

Scallops were brought by bucket to the Marine Science Center and placed in one of two 100 l fiber glass basins of desired temperature and salinity. The basins were in a freshwater bath in a large fiber glass circular tank. A refrigeration unit and submersible pump were used to maintain the waterbath temperature. Initial temperature and salinity were 8-9 °C and 33.5 ppt. This salinity was achieved by storing the highest salinity water available (28 ppt at spring tides) and boosting this by addition of a rock salt mixture (Courtright, 1967).

Newly arrived scallops contain sand and debris which will be purged within the first 24 hours. Some individuals, however, may be heavily laden with sand due to the rigors of dredging. These specimens can be assisted in purging by applying thumb and index finger gently to the sides of a gaping individual, forcing the valves to remain open. The specimen is then held vertically and dunked repeatedly but smoothly in seawater until voided of sand. Sample specimens with excessive amounts of sand would preferably be avoided since these individuals have the greatest amount of mantle damage, usually leading to death within the first 3 days of arrival.

Due to purging and death of some specimens, the holding basins may need to be changed several times in the first few days of the scallops' arrival. Specimens surviving to the third day generally remain healthy.

Once adjusted, adult scallops were fed approximately 4 l of a dense culture of Tahitian isochrysis daily. This amount could be cleared within 6 hrs by 25-30 specimens. Other than feeding, maintenance included a water change every other day by transfering scallops to a 100 l basin with prepared water of the same temperature and salinity.

SALINITY SUBSTITUTES

Salinity posed a problem at the Marine Science Center in that the available salinity of incoming water was less than desirable for scallops. Consequently, it was decided that a synthetic salt mixture should be used to boost available sea water to the desired salinity.

Commercially prepared salt water substitutes lack some organic constituents necessary for developing larvae (Courtright, 1967). To eliminate this problem, Courtright prepared his own salt water mixture, "Bio Sea", which proved suitable for developing Mytilus edulis larvae (Table 3).

Table 3. Preparation of "Bio Sea" (Courtright, 1967).

Compound	Concentration (g/l)
NaCl - "Leslie" coarse hide, solar evaporated rock salt.	33.0
$NaHCO_3$	.2
KCl	.5
$MgSO_4$	3.75
$CaCl_2$	1.0

(Resultant salinity approximately 35 ppt.)

The primary ingredient of "Bio Sea" is solar dried rock salt which was inexpensive and readily available at the time of Courtright's thesis. Unfortunately, the only rock salt currently available in Newport has been kiln dried, a process which denatures certain desirable organics. Consequently, various whole or partial salt water substitutes were compared

using available rock salts. Compared salt mixtures were:

(A) Control: 28 ppt collected seawater (MSC), air evaporated until 32.5 ppt.

(B) Courtright's formula, prepared in distilled water using "leslie" kiln dried rock salt.

(C) Courtright's formula, prepared in distilled water using "salt from the sea" salt (unknown whether solar evaporated).

(D) Fresh sea water (25 ppt) boosted to 32.5 ppt using appropriate "B" mixture.

(E) Fresh sea water (25 ppt) boosted to 32.5 ppt using appropriate "C" mixture.

(F) Fresh sea water (25 ppt) boosted to 32.5 ppt using 7.5 g "Leslie" kiln dried rock salt.

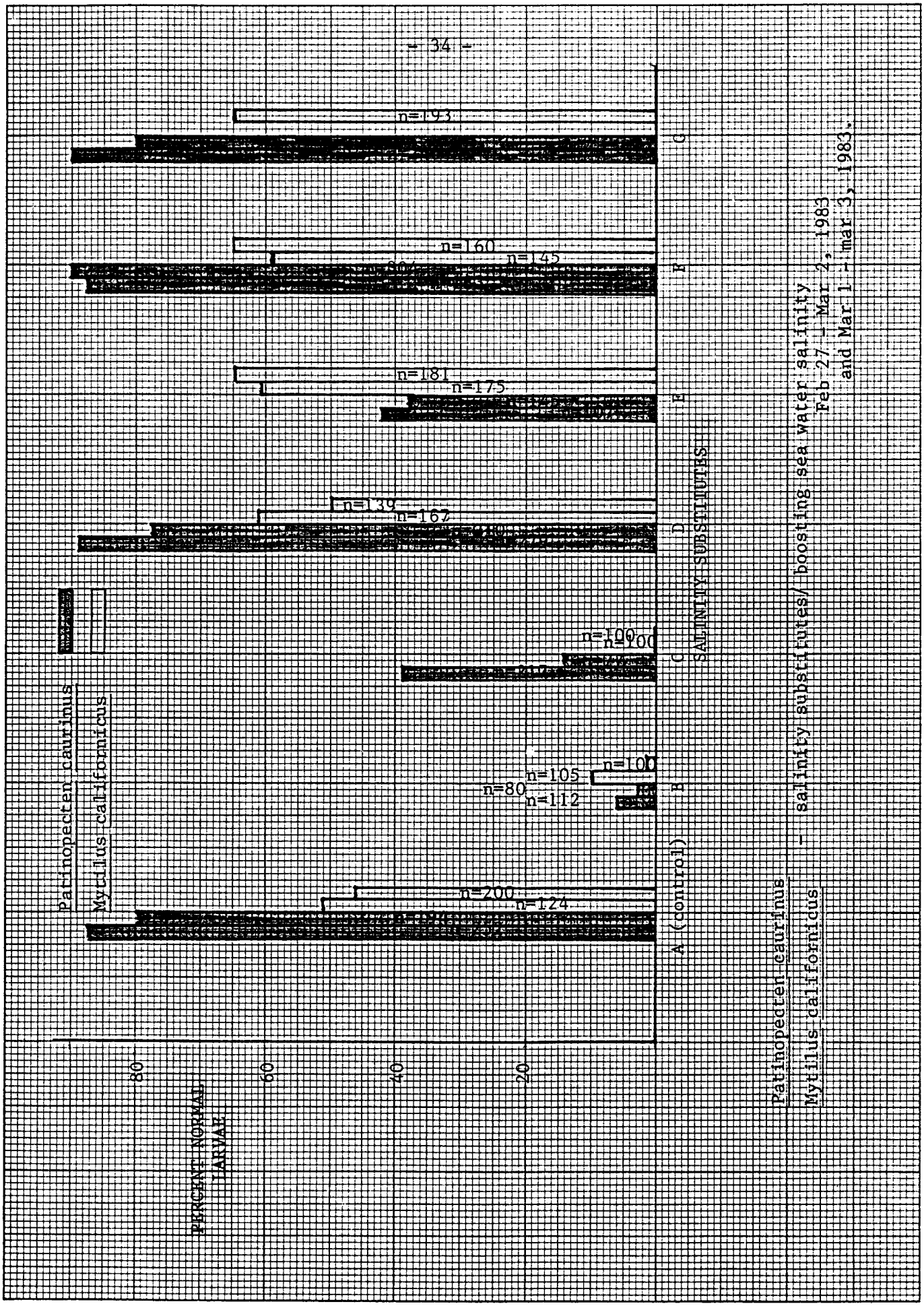
(G) Fresh sea water (25 ppt) boosted to 32.5 ppt using 7.5 g "salt from the sea" salt.

Standard bioassays were performed on larvae of both Patinopecten caurinus and Mytilus californianus. Procedures for bioassay are discussed in detail by Dimmick and Breese (1965). Criterion of effectiveness in all tests was taken as the percent normal shelled larvae at the straight hinge stage. Fertilized mussel eggs were obtained by induced spawning of adult specimens using the algal method (Appendix B). Scallop eggs were stripped, treated with ammonium hydroxide solution, and fertilized (see next section).

Bioassay results (Graph 2) indicate that substitution of kiln dried for solar evaporated rock salt in "Bio Sea" is unsuitable when preparing this mixture from distilled water. More importantly, however, the results indicate that fresh Marine Science Center water of 25 ppt salinity can effectively be boosted to 32.5 ppt by addition of rock salt alone, and that this new salinity water is safe for larval scallops or mussels. In fact, the performances of boosted salinity waters with rock salt (mixtures F and G) were slightly better than the control[3] which suggests that it is preferred

[3] A more appropriate control would have been sea water collected offshore at the necessary depth. However, difficulties involved made this impractical.

Graph 2.



to use freshly pumped, boosted salinity water than to use higher salinity water which has been stored for extended periods of time.

In view of these results, continued work with adult scallops and larvae utilized boosted salinity sea water.

TREATMENT OF STRIPPED EGGS

Although gonadal sampling indicated peak seasonal maturity at the time of this investigation, stripping of eggs and sperm from ripe individuals at this time yielded only low percentages of viable ova. In initial trials, only 6.7% of eggs obtained this way became fertilized (n=412). Yamamoto (1964) observed similar low fertility rates (10-15%) in stripped eggs of Patinopecten yessoensis, but noted that fertility rates could be improved greatly by treating eggs with ammonium hydroxide solution. However, although this compound can stimulate oocyte maturation, the incidence of abnormal embryos is increased.

Unsatisfied with the low fertilization rates of stripped eggs of P. caurinus, eggs were treated with various concentrations of ammonium hydroxide, yielding the following results:

Table 4. Percent fertilization of NH_4OH-treated eggs
fertilized / normal larvae

Concentration of NH_4OH	Duration of treatment			
	10 min	20 min	40 min	60 min
none (control)	(0.0/0.0)	(.22/.04)		
1×10^{-4} N	0.0/0.0	.06/.06	.17/.08	.14/.05
5×10^{-4} N	.52/.52	.85/.70	.87/.68	.72/.41
10×10^{-4} N	.83/.70	.96/.42	.76/.64	.71/.36

This data suggest that fertilization can be maximized and abnormalities minimized by treatment of stripped eggs with particular concentrations

of NH_4OH solutions for a particular duration of time. Based on this information, stripped eggs for future larval bioassays were treated with NH_4OH at 5×10^{-4} N for 20 min or 10×10^{-4} N for 10 min, rinsed with sea water then fertilized. Use of this method increased egg fertility to 87% in the next fertilization trial.

LARVAL SALINITY AND TEMPERATURE

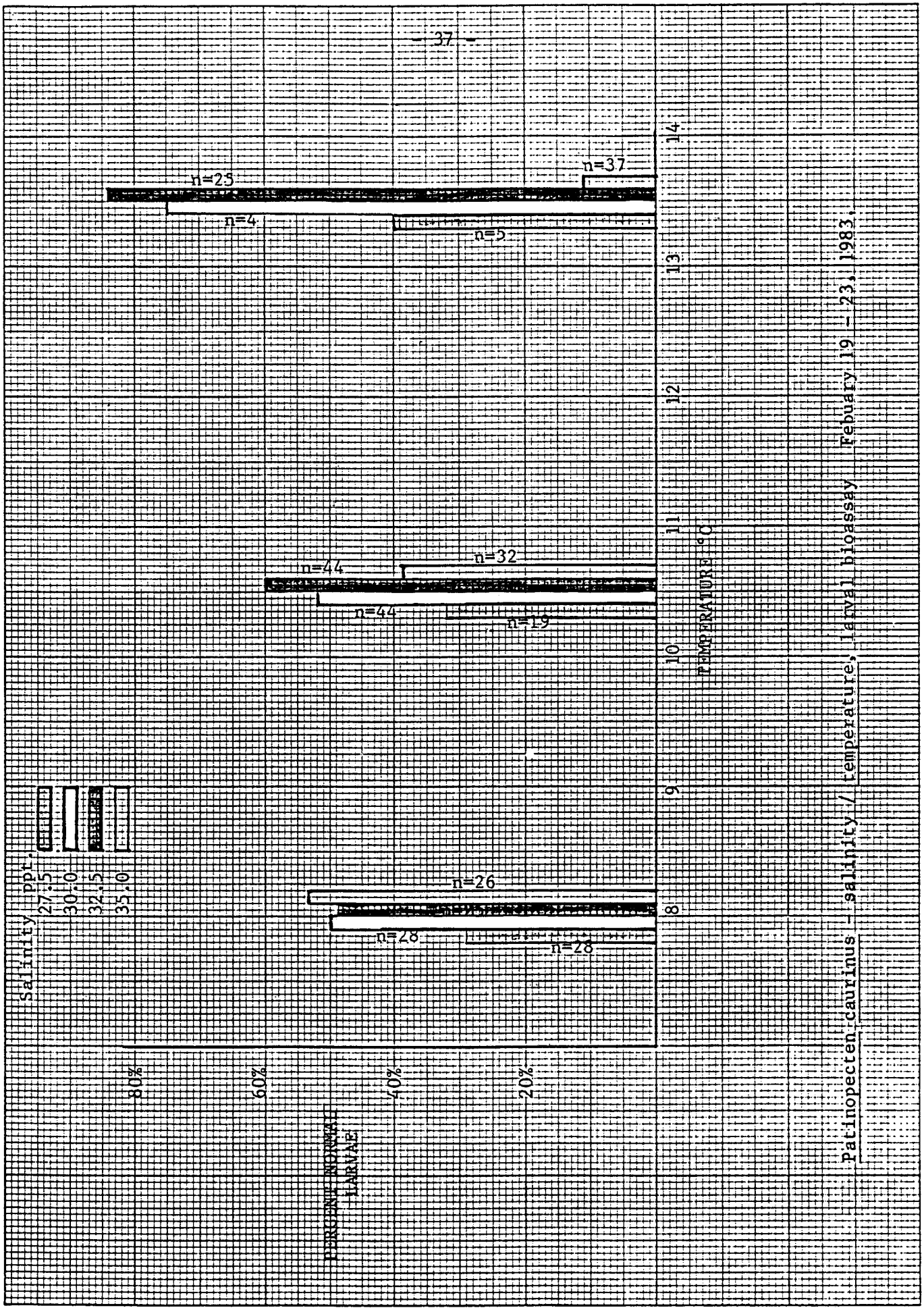
Once an adequate method of obtaining normal fertilized eggs was available, salinity and temperature bioassays were undertaken. For each bioassay, eggs of a visibly mature individual were stripped, filtered through a 120μ mesh nytex screen, and held on a 38μ mesh screen (egg diameter of *P. caurinus* is 72μ). This was followed by NH_4OH treatment, rinsing, and distribution among the bioassay beakers. To each beaker, .1-.3 ml sperm suspension was added. Sperm was obtained from at least 2 stripped males, passed through a 38μ mesh screen, and examined microscopically for activity.

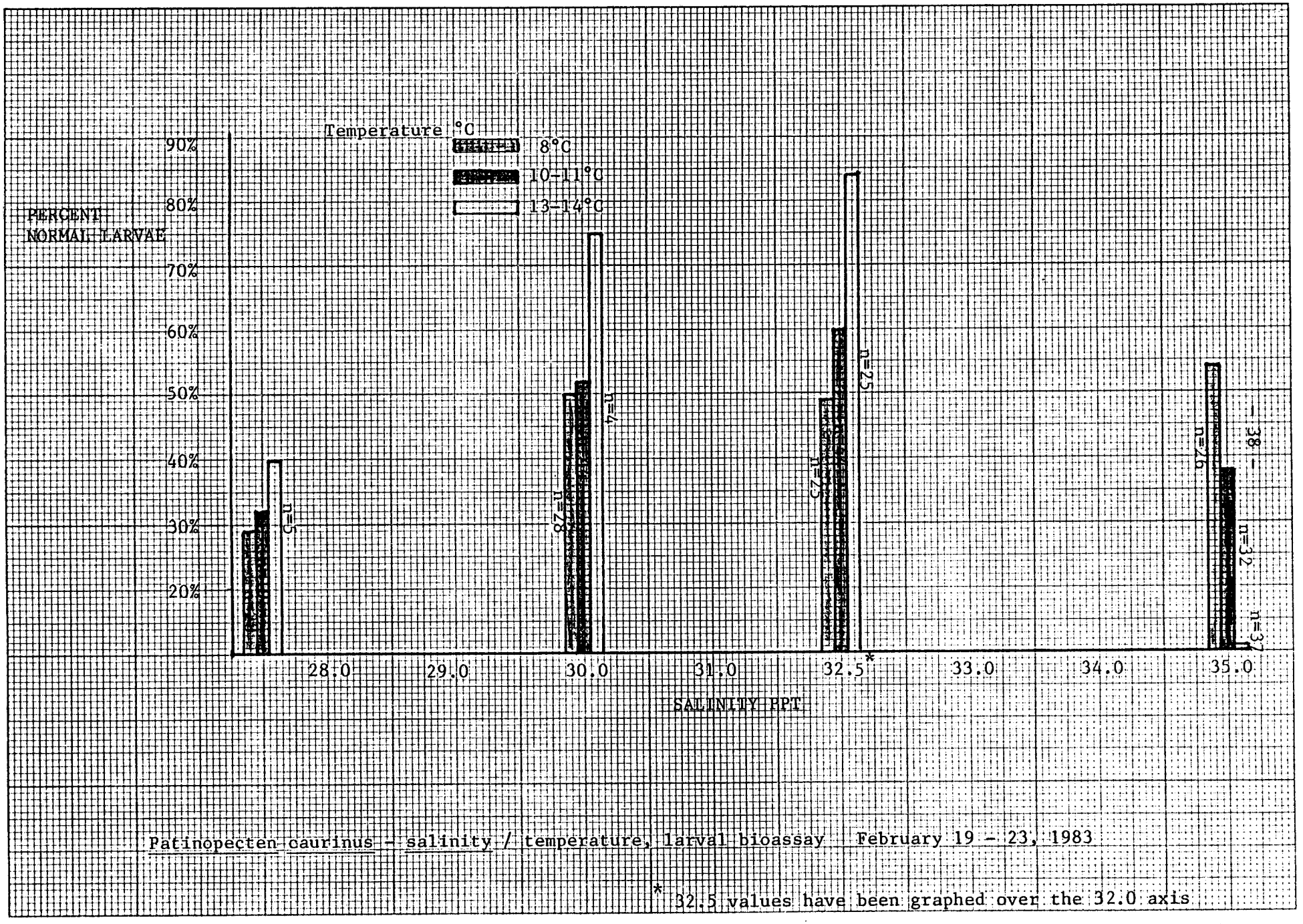
In the first bioassay, 24, 250 ml beakers were used. For each of 3 temperature ranges, 8°, 10-11° and 13-14°C, 4 different salinity levels were tested. Each parameter was done in duplicate. Results of the bioassay emphasizing temperature are presented in Graph 3. In Graph 4, salinity differences are emphasized.

Unfortunately, larval counts in this first salinity-temperature bioassay were very low. Consequently, results are inconclusive although perhaps indicative of trends. Considering all salinity data, a rough indication is that salinities of 30.0 and 32.5 were almost consistently better than the low or high salinities of 27.5 or 35.0 ppt.

Age to straight hinge development was probably the most relevant temperature information from this bioassay. At 8°C, larvae reach straight hinge

Graph 3.





Graph 4.

Patinopecten caurinus - salinity / temperature, larval bioassay February 19 - 23, 1983

* 32.5 values have been graphed over the 32.0 axis

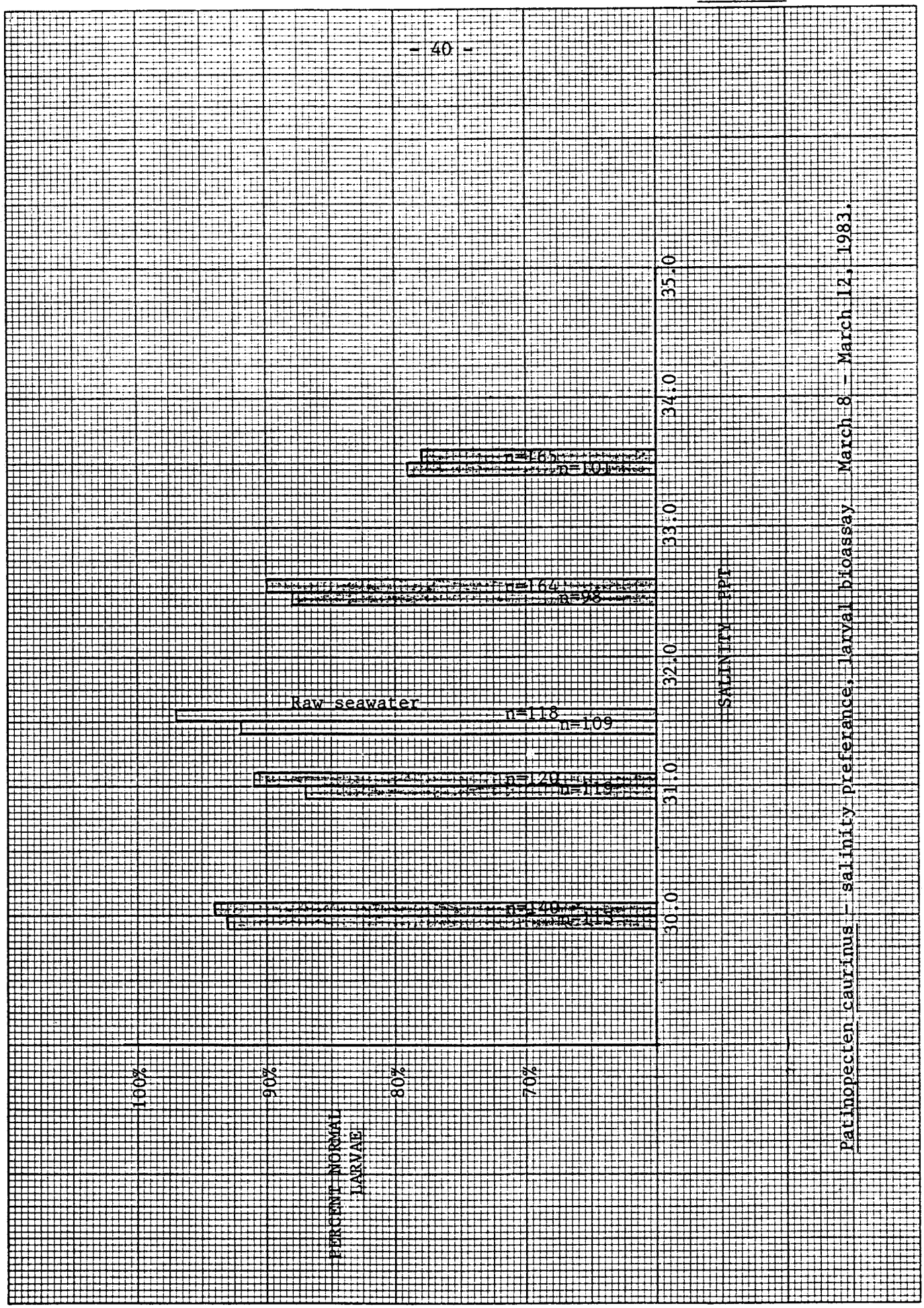
in 5-6 days, 3 and a half days at 10-11°C, and less than 3 days at 13-14°C. Straight hinge size was measured at 84μ x 100μ .

The second salinity bioassay was maintained at one temperature range, 10-11°C. This was set up such that the amount of rock salt added to achieve the desired salinity was the same percent amount for each salinity level. For example; for a salinity of 30 ppt, bay water at 24 ppt is collected to which 6 g/l rock salt was added, yielding a test water 4/5 bay salt and 1/5 rock salt. Similiarly, for 35 ppt, bay water at 28 ppt is collected to which 7 g/l rock salt is added, also yielding the 4/5 : 1/5 mixture. Salinities were checked by refractometer prior to placing in eggs. (In the previous bioassay it was learned temperature corrected hydrometer readings are not consistent with refractometer values.) Larger, 1 liter bioassay beakers were utilized, to which .3 ml sperm suspension was added. Salinities compared were; 30.0, 31.0, 32.5, and 33.5. One set of beakers was prepared using raw, MSC seawater which was bag-filtered. All other beakers were prepared with the standard, filtered and U.V. treated "P" water.

Results indicated that of the tested salinities, 30 ppt was preferred, with the exception of the raw sea water in which the percent normal larvae had their highest numbers, The raw sea water was at 31.5 ppt salinity (Graph 5).

Summarizing these results, the best available information indicates 30 ppt salinity and raw, bag-filtered sea water are preferred for rearing P. caurinus to the straight hinge stage. If held at 10-11 °C, this stage should be reached in 3½ days. It should be noted, to strengthen the statistical significance of this data, it is recommended that additional bioassay replicates be performed with scallop larvae.

Graph 5.



INDUCED SPAWNING

One of the primary goals in working with P. caurinus was to develop an induced spawning method. A dependable method would be a major step towards realizing hatchery potential of this species. At the time of this investigation no published information regarding induced spawning of weathervane scallops was located. However, Neil Bourne (1983) recently reported some success with the use of thermal shock and ultraviolet light treated water as spawning methods for this species.

It is important to acquire sexually ripe scallop specimens for spawning trials. Sex of individual scallops is determined easily and safely by visual inspection while gently forcing the valves to remain open after a specimen is lifted out of the holding tank. Size and color intensity of the gonad and a knowledge of microscopic examinations of gonadal tissue of similar animals are factors which allow the selection of the most gravid scallops. Hence, for each spawning trial, ripe individuals were selected and placed in round plastic spawning basins (15 cm diameter) and covered with 5 l of water. Two females and 1-2 males were placed in each basin without aeration, to avoid agitation. Since spawning attempts were done in duplicate, another treatment basin was prepared and two similar basins for controls. Although several spawning replicates would have been desired for each spawning method or concentration, replicates were subject to the availability of ripe scallops.

The established method for spawning P. yessoensis in Japan is to raise water temperature by about 5 °C. In fact, temperature alone is sufficient to induce the maturation process in oocytes of extracted gonadal tissue of this species (Yamamoto, 1964). Unfortunately, temperature in-

crease was not successful to induce P. caurinus to spawn.

Generally successful spawning methods attempted without success were:

(1) Temperature increase, from 9°C to 13°C and 9°C to 16°C.

(2) Temperature decrease, from 11-12°C to 8°C.

(3) Algae method. Algal culture grown without CO_2 (higher pH) is added to the spawning water to bring algal cell density to $2\text{-}5 \times 10^6$ cells per ml. Algal species: VA-12.

(4) Algae, with temperature increase of 5°C.

(5) H_2O_2, adjusted to pH 9.1 with 1×10^{-3} molar NaOH. Concentrations of $2\text{-}4 \times 10^{-3}$ were suggested for all bivalves. Concentrations tested were: 1.5×10^{-3} M
2.5×10^{-3} M
3.5×10^{-3} M
4.5×10^{-3} M.

(Morse, Hooker and Morse, 1978).

(6) KCl at 2 g/ 1. Scallop response to this method was the most adverse. Valves remained closed for the duration (3 hr) of treatment.

Considering the relative success of NH_4OH used for treating eggs, this compound was tried as a spawning stimulus. Yamamoto had mentioned NH_4OH as one of several induced spawning methods used for bivalve molluscs. It was used in 1936 to stimulate Crassostrea gigas. In the initial attempt, NH_4OH was added to achieve a 2×10^{-3} N concentration, adjusting pH with NaOH as described for the H_2O_2 treatment. The scallops appeared very relaxed with tenticles extended. Within an hour strands of mucous were visible in the water. After 3 more hours, scallops were inspected, and 3.4×10^5 eggs were found. Specimens were transferred to fresh water and allowed to stay overnight. By the next morning, 1.7×10^6 more eggs were spawned. Since 2 females were in the spawning basin, it was not known whether both or one spawned.

Subsequent investigations were directed to determine the minimum con-

centration of NH_4OH necessary to induce spawning and whether adjustment of pH was necessary.

It was determined that 1.5×10^{-3} N NH_4OH is effective to induce spawning in ripe female weathervane scallops. No pH adjustment is necessary and addition of NH_4OH at this concentration causes no measurable pH change.

Concentrations tested for induced spawning with NH_4OH were:

$.5 \times 10^{-3}$ N	(no spawning)
1.0×10^{-3} N	(no spawning)
1.2×10^{-3} N	
1.3×10^{-3} N	
1.5×10^{-3} N	
2.0×10^{-3} N	with and without pH 9.1.

With the 1.5×10^{-3} N treatment solution, spawning occurred in 2-3 hours. At lower concentrations, less eggs are spawned (although this may be related to ripeness of the individual) and the onset of spawning is later. In all cases where spawning was induced, only female scallops spawned. To obtain fertilized eggs, 1-2 males were stripped and a sperm suspension was added.

Due to the scarcity of ripe individuals, in some spawning trials, females used in earlier unsuccessful spawning attempts were used again. In these circumstances, however, there was always at least one fresh (untested) female. After success with NH_4OH was determined, most visually ripe female scallops were spawned regardless of previous unsuccessful attempts, implying that the earlier attempts had little effect on their spawnability.

DISCUSSION

Patinopecten caurinus shows potential for hatchery applications. It was found that spawning of mature female scallops can be induced with 1.5×10^{-3} N ammonium hydroxide solution to yield viable ova. Spawning was induced with

solutions as low as 1.2×10^{-3} N but numbers of eggs were less and the time to initiate spawning extended. Although male scallops have not been induced to spawn, this is not a problem since viable sperm are readily obtained from stripped male gonads.

Larval rearing studies indicated a preference for fresh (raw) sea water of 30 ppt salinity. Bioassays on larval temperature preference were not considered conclusive, although a temperature of 10-11°C yielding healthy straight hinge in 3½ days may be recommended.

Although icreasing water temperature was not successful as an induced spawning method in P. caurinus, water temperature may be related to gonadal development. Average temperature values for seasonal bottom water (Graph 1) reveal that gonad maturity in this species occurs after several months of increasing temperatures. Spawning occurs during months of decreasing temperatures. A knowledge of bottom temperature, where scallops are harvested, would contribute to understanding environmental factors affecting spawnings and populations. Kruse and Huyer (1982) suggest that hydrographic data, which include bottom temperature and salinity, can be determined from calculations of sea level and upwelling indices.

Manipulating temperature might prove to be a tool for conditioning scallops or for extending their spawning season. To test this, newly arrived scallops of of March 23, 1983 along with 12 tagged scallops of earlier collections were held at 10°C and fed 4 l of algal culture daily. On March 23, a description of the gonad condition of each tagged scallop was written for later comparison. On May 20, the tagged scallops were examined, and very little change was found in the color or texture of the gonads. Females that had been very ripe were still in the same condition. Spawning with

NH_4OH was successful with these specimens despite having been held for 2 months.

Physiological response to NH_4OH:

NH_4OH appears to induce primary oocytes in the ripe ovary of P. caurinus to mature as temperature does for oocytes of P. yessoensis. Changes that occur include a rounding of the cells, a breakdown of the germinal vesicle, and the assumed continuation of meiosis to metaphase of the first meiotic division. At this time the ova are ready to be fertilized.

Ova are enveloped by a vitelline membrane and a transparent jelly-like layer. In Mytilus edulis the vitelline membrane is about 1μ and the jelly layer 7-10μ (Reverbi, 1971). The membrane is thought to play an important role in maintaining the integrity of the developing embryo through early cleavage (Dan, 1964). Although blastomeres of early cleavage exhibit a considerable degree of mutual contact, they are held together primarily by restraint of the vitelline membrane. Dan isolated lysin derived from sperm acrosomes to dissolve away the vitelline membrane (Figure 5), which resulted in blastomeres that are only loosely connected. Embryos of the same appearance were often seen when eggs of P. caurinus were treated with strong solutions of ammonium hydroxide, or when spawning was induced using 2×10^{-3} N NH_4OH.

Stripped eggs treated with NH_4OH show varying degrees of vitelline membrane disruption. Where concentration and duration of treatment resulted in the highest percentages of abnormal larvae, the vitelline membrane was often greatly expanded around the ova or was ruptured. It is the belief of this investigator that minimizing the exposure of the egg to NH_4OH can allow optimal development of normal larvae due primarily to the ability to retain

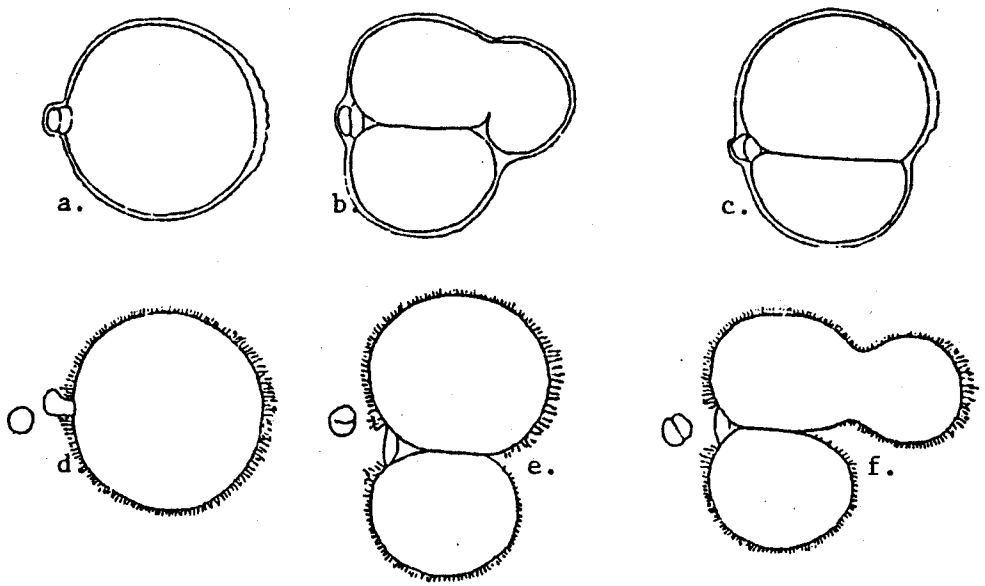


Figure 5. a. - c. Normal cleavage.
d. - f. Cleavage when hyaline material of the vitelline membrane has been dissolved. (adapted from J. Dan, 1964)

a healthy vitelline membrane. Also, visual inspection of the egg membrane may be useful to indicate success of induced spawning.

Although increased numbers of fertilized eggs have been made available through induced spawning of the weathervane scallop, larvae have not been reared beyond 150μ size. Larval rearing was not an objective in this investigation (other than larval parameters). Consequently, this would be one of the next major steps towards developing the hatchery potential for P. caurinus. It is hopeful that future studies will emphasize this problem area.

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APPENDICES

APPENDIX A. Gonad Indices for Patinopecten sps.

I. NUMERICAL

$$\text{gonad index} = \frac{\text{gonad weight x 100}}{\text{wt. of soft body parts}}$$

This method was developed in Japan for Patinopecten yessoensis but has been used in British Columbia for P. caurinus (Bourne, 1983).

For P. yessoensis, numerical indices are interpreted as:

<5%	- spent
5%	- resting
7-8%	- early developing
8-9%	- late developing
20%	- maturing
>20%	- breeding

II. MACROSCOPIC

Daniel Hennick (1970) developed the following gonad index for P. caurinus. This index was originally described by Mason (1958) for Pecten maximus but was adapted for use with the weathervane scallop.

(1) Empty or spawned out: Gonad reduced in size and collapsed, contains free water throughout. Transparent loop of alimentary canal clearly visible. Testes nearly colorless, ovaries dull amber to nearly colorless.

(2) Initial recovery: Gonad increasing in size, free water exists in portions. Loop of alimentary canal visible but fading. Portion of testes containing spermatogonia cloudy white, rest is transparent. Ovaries amber to dull pink or orange.

(3) Filling: Gonad near maximum size, contains 3/4 or more of the estimated capacity of sex products. Free water exists only as small canals or close to the surface. Testes pasty white; ovaries bright orange.

(4) Full or ripe: Gonad relatively large in relation to other body parts, completely full and rounded, contains no free water. Loop of alimentary canal not usually visible except for distal portion. Texture appears granular. Testes flat white; ovaries bright orange.

(5) Immature or juvenile: Gonad relatively small in relation to other body parts, angular and flattened, transparent and colorless. Loop of alimentary canal clearly visible.

III. MICROSCOPIC

The following gonad index was developed at the Marine Science Center in Newport, Oregon and is used for P. caurinus as well as other bivalve molluscs (Robinson, 1983).

In this method, preserved tissue samples taken from the distal portion of each gonad are preserved, stained, sectioned and slide-mounted for interpretation. Descriptions refer to observed stages of gametogenesis within gonad follicles. Definitions were based on verbal descriptions by Robinson with referance to Mason's (1958) microscopic observations.

Male bivalve molluscs:

(1) Early developing: Spermatogonia form a layer on walls of follicles. Inside them, a layer of spermatocytes. Lumen contains spermatozoa arranged radially.

(2) Late developing: A few spermatogonia and spermatocytes remain near the follicle wall. Lumen becomming closely packed with spermatozoa.

(3) Fully mature: Spermatozoa very dense and arranged radially from the lumen of the follicle. A few spermatogonia visible along lining. Little connective tissue.

(4) Mostly spawned out: Center of follicle mostly empty. Few residual spermatozoa in periphery of the follicle appear clumped.

(5) Spent-recovering: Center of follicle empty. Spermatogonia around follicle wall, among them some spermatocytes.

Female bivalve molluscs:

With female samples, numbers of oocytes at particular stages of oogenesis are recorded. Complete follicles are counted until a minimum of 100 oocytes have been recorded. Stages observed are:

(1) Young synaptic oocytes: Small, rounded cells, attached to follicle walls and darker staining than other oocytes.

(2) Growing oocytes: Elongated, often club-shaped cells, also attached to follicle wall.

(3) Primary oocytes: Fully developed oocytes in the lumen of follicles. Round when few in numbers, polygonal when packed with many of same.

Percentages of each of these stages is calculated and used to indicate gonad maturity (e.g. if % primary oocytes is > 50, spawning potential is indicated). If sampling is monthly, seasonal trends of gonad maturity can be determined.

APPENDIX B. Induced spawning method of some scallop species.

Species	Method	Referance
Argopecten irradians	Thermal stimulation	Sastry (1963) as cited in Yamamoto, 1964.
Hinnites multirugosus	H_2O_2	Morse, D.E., N. Hooker and A. Morse, 1978.
	U.V. treated water	Leiton and Phlagler, 1981.
Patinopecten caurinus	U.V. treated water	Bourne, 1983
	Thermal shock	Bourne, 1983
	NH_4OH	Solon, 1983 (also in this report).
Patinopecten yessoensis	Thermal stimulation (temperature increase)	Yamamoto, 1964 and Motada, 1973.
	Thermal stimulation (temperature decrease)	Motada, 1973
	U.V. treated water	Yamamoto, 1964
	Seretonin	Matsutani, T. and T. Nomura, 1982.
Pecten maximus	Desiccation and U.V. treated water.	Gruffydd, LL.D. and A.R. Beaumont, 1970.

Method descriptions:

Thermal stimulation - Usually accomplished by a sudden increase in water temperature of about 5°C. Spawning expected to begin 1-3 hours after temperature adjusted.

Thermal stimulation (temperature decrease) - Sudden decrease in water temperature af about 5°C. This method was used in conjunction with addition of a sperm suspension.

H_2O_2 - Scallops set in a bath of 7.5×10^{-3} molar H_2O_2 which has been pH adjusted to 9.1 by addition of 2 molar tris-hydroxymethyl aminomethane ("Tris"). Tris can be substituted with 1×10^{-6} molar NaOH.

U.V. treated water - Water which has been treated with ultraviolet light has been known to induce spawning in some species and is often used in conjunction with sperm suspension. U.V. light is known to form peroxide free radicles and it is believed that this may be the stimulus.

NH_4OH - Scallops set in a bath of 1.5×10^{-3} molar NH_4OH. Spawning expected to begin in 2-3 hours.

Desiccation and U.V. water - Scallops allowed to stand in air of same temperature as water. After 2 hours, place them in filtered, U.V. treated water. Spawning expected 2-4 hours after reimersion.

Seratonin - Single intragonadal injection of 0.4 ml Serotonin (5-hydroxy-tryptamine) creatinine sulfate.