

AN ABSTRACT OF THE THESIS OF

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Title: BROODSTOCK MANAGEMENT OF THE PACIFIC

OYSTER, CRASSOSTREA GIGAS (THUNBERG)

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Oyster culturists typically observe substantial variation in survival during hatchery rearing of larval oysters. A previous study has demonstrated that this variation is in part related to the degree of gonadal development of broodstock at the time of spawning. Larval survival was at a maximum when broodstocks were spawned in an optimum stage of gonadal development. Thus, culturists must manage broodstocks in order to spawn only oysters expressing the optimum gonadal condition. Consequently, hatchery productivity should be enhanced by increasing the number of broods that survive to metamorphosis while reducing operational costs associated with the rearing of ineffective broods. Because the rate of gonadal development is partially controlled by environmental factors, broodstocks can be accelerated or delayed to attain the optimum stage of development by manipulating the conditioning environment. Oysters in a ripe gonadal condition should then be available

for spawning during most times of the year.

The objective of this study was to observe the effects of temperature, salinity, and algal food supplementation during hatchery conditioning of broodstock on gonadal development and larval survival. Gonadal development was determined by direct observation of histological preparations of gonadal tissues and expressed in terms of the relative proportion of ova matured. With respect to temperature, the rate of development is a function of temperature intensity and time rather than accumulated thermal exposure. Salinity exerted a negative influence on the rate of development in oysters conditioned at salinity levels below 30 ppt. Furthermore, when oysters conditioned at 20 ppt relative to 30 ppt were spawned and the larvae were reared under standardized hatchery conditions, larval survival was markedly reduced in the former.

Proximate analysis of ova spawned by the two salinity conditioning regimes revealed significant differences in protein, carbohydrate and relative organic content. However, no correlation between proximate composition of ova and larval survival over the course of conditioning was evident. The apparent qualitative differences in the ova, inferred from broods exhibiting high or low survivals, does not appear to be detectable at the gross compositional level.

Fecundity of broodstock was 60% greater when fed an algal food supplement of the diatom Thalassiosira pseudonana relative to starved

controls although the rate of gonadal development and gamete viability was not significantly different.

A strategy to maintain broodstocks in an optimum gonadal condition for hatchery spawning is presented.

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Crassostrea gigas (Thunberg)

by

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TABLE OF CONTENTS

	<u>Page</u>
INTRODUCTION	1
MATERIALS AND METHODS	8
Experimental Animals	8
Conditioning Trials	8
Sea Water Supply	11
Oyster Conditioning System	11
Larval Culture	13
Histology	15
Index of Gonadal Development	15
Methods of Proximate Analysis	16
RESULTS	22
Effects of Temperature and Salinity on Gonadal Development	22
Effects of Salinity during Conditioning of Broodstock on Larval Survival	26
Proximate Composition of Ova	34
Effects of Feeding an Algal Food Supplement to Broodstock on its Reproductive Performance	39
DISCUSSION	44
CONCLUSION	49
BIBLIOGRAPHY	53

LIST OF FIGURES

<u>Figure</u>		<u>Page</u>
1	Schematic diagram of the oyster hatchery production system.	2
2	Oyster conditioning system designed for conditioning trials I, II, and III.	12
3	Oyster conditioning system designed for conditioning trial IV.	14
4	Primary oocytes in early stages of gonadal maturation.	18
5	Secondary oocytes growing into the follicular lumen.	18
6	Mature ova free in the follicular lumen.	19
7	A. Relationship between the rate of gonadal development of oysters exposed to four temperature levels and thermal exposure.	25
	B. Relationship between the rate of gonadal development of oysters exposed to three salinity levels and thermal exposure.	25
8	Relationship between the gonadal development after 1100 degree-days of conditioning and temperature at three salinity levels.	28
9	Relationship between gonadal development, survival to straight hinge larvae and larval survival and conditioning interval during conditioning trial II and III.	31
10	Relationship between the proximate composition of ova spawned during conditioning trial II and III and larval survival.	37
11	Relationship between gonadal development, fecundity and larval survival and conditioning interval during conditioning trial IV.	43

Figure

Page

- 12 Relationship between the conditioning requirements for broodstock to attain the optimum gonadal condition at four temperature levels with salinity held constant at 30 ppt. Conditioning commenced in January when oysters were sexually indifferent.

51

LIST OF TABLES

<u>Table</u>		<u>Page</u>
I	Analysis of variance of gonadal development.	29
II	Proximate composition of ova spawned during conditioning trial II.	35
III	Proximate composition of ova spawned during conditioning trial III.	40

BROODSTOCK MANAGEMENT OF THE PACIFIC OYSTER CRASSOSTREA GIGAS (THUNBERG)

INTRODUCTION

Crassostrea gigas (Miyagi variety) is native to the warmer semi-temperate areas of Japan and represents the primary species of oysters cultured in the Pacific Northwest. These transplanted populations seldom spawn and reproduce naturally at most locations in the northwest due to the low water temperatures. Nevertheless, the oysters do exhibit an annual reproductive cycle and attain spawning condition during the summer months although the gametes are subsequently resorbed in the fall. Therefore, oyster growers must depend on either imported or hatchery reared seed (Quayle, 1969) and the former is generally preferred over the cheaper hatchery counterpart because of the higher yields during growth to market size (Jones, 1979). However, the unstable supply and escalating costs of wild and imported seed has imposed greater reliance on hatchery production in recent years. Despite significant advances in the rearing of larval oysters, further improvement and refinement in culture methods must occur to establish hatcheries as reliable sources of quality seed. This warrants a brief discussion of the four major components comprising the hatchery production system to delineate areas of improvement that could potentially increase seed production (Figure 1).

OYSTER HATCHERY PRODUCTION SYSTEM

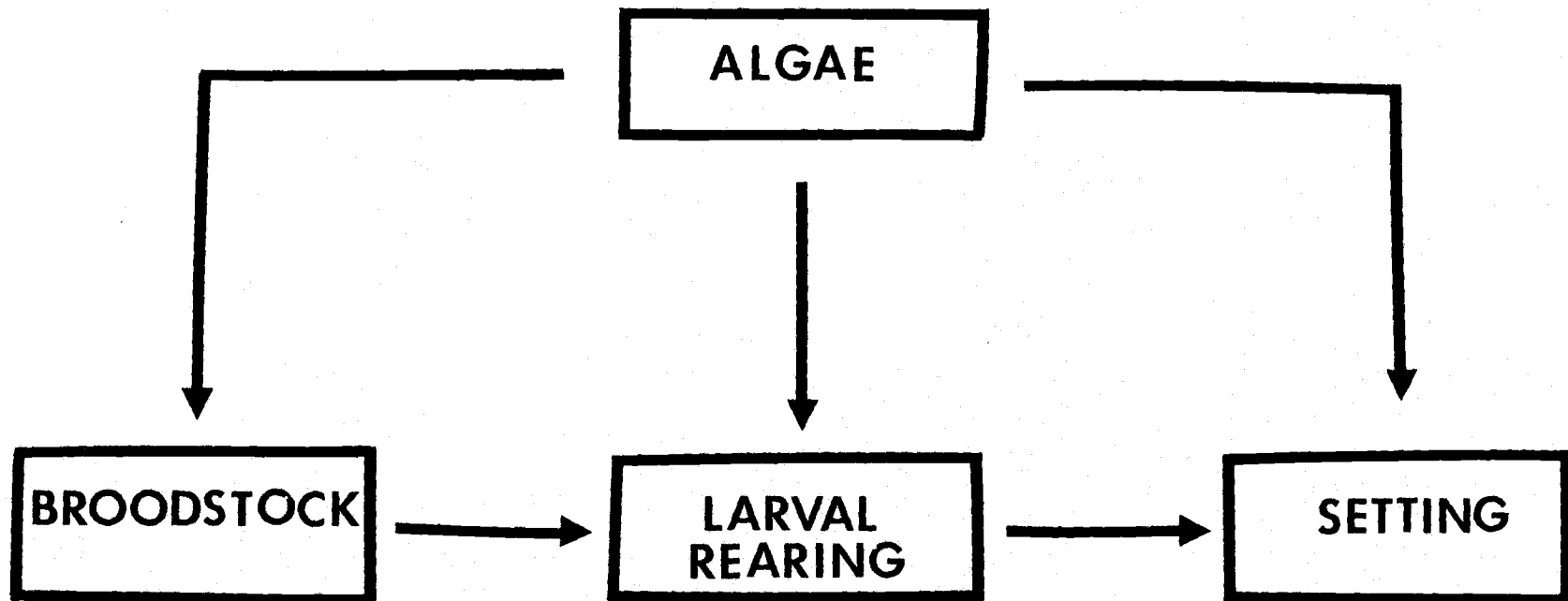


Figure 1. Schematic diagram of the oyster hatchery production system.

The algal culture component shown in Figure 1 must produce mass quantities of high quality algae for optimum larval and post-larval growth and survival, and maintenance of body condition of broodstock. Several algal species are recognized to be nutritionally adequate although current culture methods are relatively inefficient in producing algae of consistent quality and quantity (Pruder et al., 1976). Also, the development of artificial larval diets would represent a significant advancement in larval culture but past attempts have met with little success due to our limited understanding of the nutritional requirements of larvae coupled with technical problems in the food delivery system (Toner, 1977; Castell et al., 1972). Larval nutrition remains a major area of research in efforts to improve hatchery productivity.

Optimum environmental conditions promoting growth and survival in the larval rearing system have been established (Breese and Malouf, 1975). However, the incidence of disease is often implicated as a contributing factor to poor survival. Mortality due to bacterial and fungal diseases is usually a secondary effect of poor water quality although the exact cause is unclear and seldom determined. Antibiotics and disinfectants may be a short term and immediate solution but disease resistant strains are likely to develop eventually (Wood, 1974). Culturists should take a preventive course of action by filtering

the sea water and disinfecting hatchery equipment to prevent contamination and to maintain good water quality.

Inducing pre-setting stage larvae to undergo metamorphosis requires that an appropriate substrate be timely placed in the rearing tanks for maximum larval settlement. In addition, proper environmental conditions and adequate nutrition prior to metamorphosis are necessary for optimum setting success (Lund, 1976). Several techniques have been developed to minimize costs and handling during the critical transitional period from post-larvae to planting the seed on the growing grounds. The more notable are the advent of clutchless spat and the eyed larvae technique (Dupuy, 1971; Breese, 1979).

The broodstock must continuously provide viable gametes to the larval rearing system. Because the Pacific oyster populations of the northwest are in spawning condition only during the late spring and summer months, oysters must be artificially conditioned for spawning during other months of the year. The term "conditioning" as understood by culturists, refers to the acceleration of gametogenesis in the oysters usually by thermal enhancement. The prevailing method of conditioning broodstocks involves collecting oysters in the early stages of their reproductive cycle from the growing grounds and subjecting them to elevated water temperatures to accelerate gametogenesis. Spawning is usually attempted after two to four weeks of conditioning and if no spawning occurs because of insufficient

development of their gonads, the oysters are returned to the tanks for further conditioning. Furthermore, when spawning does occur and the larvae are reared, there is substantial variation in larval survival. Typically, certain broods exhibit excellent survival and set earlier than usual while others result in either complete mortality or poor setting success. This uncertainty in conditioning broodstocks and in setting success manifests in increased operational costs in terms of energy requirements to heat culture water, culturing of algae and the labor involved in spawning the oysters and rearing of the larvae. In the long run, hatchery productivity is reduced.

Lannan (1980) reports that this variation in larval survival is related in part to the degree of gonadal development of broodstocks. Larval survival is maximized when the broodstock is spawned in an optimum stage of gonadal development. Spawnings prior to and past this optimum resulted in gametes of reduced viability. This optimum stage occurs when the gonadal index is increasing but before full development is reached (Lannan et al., 1980). The gonadal index may thus serve as a predictive indicator of a ripe gonadal condition for spawning oysters.

Pacific oysters undergo an annual reproductive cycle adapted to the seasonal changes of the temperate climatic zone (Quayle, 1969). Because its rate is partially controlled by environmental factors (Loosanoff and Davis, 1952; Loosanoff, 1948), broodstocks can be

conditioned to reach the optimum stage of development at different times of the year by controlling the conditioning environment.

Biologists have long known that the rates of metabolic activities of oysters such as gonadal development were positively related to temperature (Nelson, 1928). Later studies determined temperature regimes required for oysters (Crassostrea virginica) to attain spawning condition outside their normal breeding season (Loosanoff and Davis, 1952; Price and Maurer, 1972). This represented an important advancement in oyster culture although the practical utility of these conditioning requirements for commercial hatcheries is severely limited for at least three reasons. First, these studies assumed that the criterion for an oyster in a ripe gonadal condition was its ability to spawn fertilizable gametes. This criterion may not be valid in terms of larval survival. Lannan et al. (1980) frequently observed spawned gametes exhibiting excellent fertilization success followed by extreme variation in survival to metamorphosis. Second, these studies neglected to mention how the conditioning requirements are altered when oysters are conditioned during different seasons of the year. Conceivably, less conditioning would be required as the oyster population advances through their reproductive cycle. Finally, the requirements do not account for the effects of other factors of the environment such as salinity and food supplementation on gonadal development.

These relationships must be established in order to regulate development of broodstocks. The culturist can then predict, with a reasonable degree of accuracy, when to spawn the oysters for gametes of maximum viability. In effect, hatchery productivity should be enhanced by increasing the number of broods that survive to metamorphosis while reducing operational costs associated with the rearing of ineffective broods.

This study investigates the relationships between several environmental factors and the reproductive performance of broodstocks during hatchery conditioning. A series of time course conditioning trials were conducted on Crassostrea gigas to observe the conditioning effects of temperature, salinity and an algal food supplement on gonadal development and larval survival. Gonadal development is defined as the sequential developmental stages of gamete formation (gametogenesis) in the oyster. Additionally, gross composition of ova spawned from parental oysters conditioned at two salinities was determined to gain insight on constituents in the ova that may be related to their subsequent survival and to observe possible differences in the ova due to the two salinity conditioning regimes. Based on these results, a strategy is proposed to maintain broodstocks in a ripe gonadal condition for spawning.

MATERIALS AND METHODS

Experimental Animals

Commercial grade two year old Miyagi strain C. gigas (mean wet body weight = 14.1 grams) were purchased from the Oregon Oyster Company, Newport, Oregon.

The oysters were scrubbed of fouling organisms and debris and placed in tanks with running sea water at approximately 10 to 12°C and ambient salinity for three days before acclimation to experimental conditions. Oysters were not fed unless otherwise stated. The water temperature was raised 2°C/day until the desired temperature level was attained. Acclimation to lower salinities was accomplished by adding dechlorinated fresh water over a period of several days so that the salinity was lowered at a rate of 5 ppt/day until the desired level was attained.

Conditioning Trials

Conditioning trial I was designed to test the effects on reproductive development of four temperature levels (16, 18, 20, 22°C) and three salinity levels (10, 20, 30 ppt) in a 4 x 3 factorial design. In January 1979, 1500 oysters were purchased and acclimated to experimental conditions. Histological examination of gonadal sections from 20 oysters revealed they were sexually indifferent containing no

gametes. Approximately 120 oysters were randomly selected and placed in each conditioning regime. Reproductive development of each treatment was monitored for 15 to 17 weeks.

In conditioning trial II, the effects of conditioning broodstock at 20 and 30 ppt on larval survival and composition of ova were observed. In April 1979, 480 oysters were purchased and acclimated to experimental conditions. Observation of gonadal samples from 17 oysters indicated they were reproductively undeveloped containing only primary oocytes. The remaining oysters were divided into two equal groups and conditioned at 20 and 30 ppt with temperature held constant at 20°C. Bimonthly gonadal samples were obtained from both groups during the 14 weeks of conditioning to monitor gonadal development. On each day of sampling, another 15 to 20 oysters from both conditioning regimes were artificially induced to spawn and the ova were fertilized, and the zygotes reared under standardized hatchery conditions. Samples of ova spawned from both groups were immediately stored for proximate analysis.

Conditioning trial III was of similar design to conditioning trial II except oysters already contained ripe gametes prior to treatment exposure. The objective of this trial was to test the effects of exposing such oysters to reduced salinities on larval survival and composition of ova. In June 1979, 100 oysters were purchased and a random sample of 22 oysters was spawned on the first day of conditioning.

The zygotes were reared to serve as a baseline with which to compare later spawnings. The remaining oysters were divided into two groups and conditioned at 20 and 30 ppt with temperature held constant at 18°C. Samples of 15 to 20 oysters were spawned bimonthly for 4 weeks and the zygotes reared. Samples of ova spawned from each group were stored for proximate analysis.

Conditioning trial IV tested the effects of an algal food supplement fed to the broodstock on gonadal development, fecundity and larval survival. Approximately 540 sexually immature oysters were purchased to April 1979. One group of oysters was batch fed a total of 4×10^8 cells/oyster-day of the diatom Thalassiosira pseudonana divided into two daily feedings. The amount of algae fed approximated levels suggested by Pruder et al. (1976) to meet requirements for feeding broodstock. To minimize pseudofeces production, algal concentrations in the tanks never exceeded 100,000 cells/ml. This type of discontinuous feeding has been shown to be an efficient method of feeding C. gigas (Langton and McKay, 1974). Temperature and salinity were kept constant at 20°C and 30 ppt. Gonadal samples were obtained bimonthly for 14 weeks to monitor gonadal development. On each day of sampling, 15 to 20 oysters from each group were induced to spawn and the ova were fertilized and zygotes reared.

Fecundity of female oysters was determined by integrating photographic enlargements of histological preparations. The ratio of the

weight of the area of the photographic print occupied by ova to the weight of the total area of the gonad was used as a measure of fecundity. The method assumes that this ratio is constant throughout the whole gonad and is deemed reasonable for the intended purpose.

Sea Water Supply

Sea water used in this study was drawn from the sea water supply of the Oregon State University Marine Science Center pilot oyster hatchery at Newport, Oregon. A sand bed water filter removed suspended particles greater than 5 μm and the sea water was subsequently sterilized by passage through an ultra-violet irradiation unit. Tap fresh water, used in diluting sea water to desired salinities, was previously dechlorinated by passage through a column of activated charcoal.

Oyster Conditioning System

The oyster conditioning system used during conditioning trials I, II, and III consisted of 18 liter polypropylene tubs each fitted with an undergravel biological filter utilizing silica grit as the filtering substrate (Figure 2). The tubs were partially submerged in a 500 liter fiber glass tank which served as a water bath adjusted to the desired treatment temperature. Temperatures were maintained within $\pm 1^\circ\text{C}$ of the desired level using Vycor immersion heaters connected to a



Figure 2. Oyster conditioning system designed for conditioning trials I, II, and III. Six 18 liter polypropylene tubs, each fitted with an undergravel biological filter, were partially submerged in a water bath set at the desired temperature. An airlift forced water through the biological filter and simultaneously oxygenated the water.

mercury "Red-top" thermoregulator via a mercury relay. Each fiber glass tank contained six polypropylene tubs.

Salinity fluctuations due to evaporative loss were restored daily to the desired level by addition of distilled water. Salinity was determined hydrometrically and did not fluctuate more than 1 ppt. Water in each tub was completely changed every three days to maintain water quality.

Experimental oysters in conditioning trial IV were placed directly in 500 liter fiber glass tanks filled with 280 liters of sea water and fitted with several air stones (Figure 3). Water was completely changed every three or four days to prevent toxic buildup of metabolites.

Larval Cultures

Techniques used in spawning oysters, enumerating gametes and the rearing of larvae followed methods outlined by Lannan (1980). Minor differences in culture methods existed during this study: 1) larvae were cultured 24 hours post-fertilization instead of 40 hours; 2) Sulmet (sulfamethazine) was administered prophylactically to larval cultures during conditioning trials II and IV from the tenth week and in all cultures during conditioning trial III; and 3) plastic bucket strips were used as cultch material instead of oyster shell during setting of larvae.

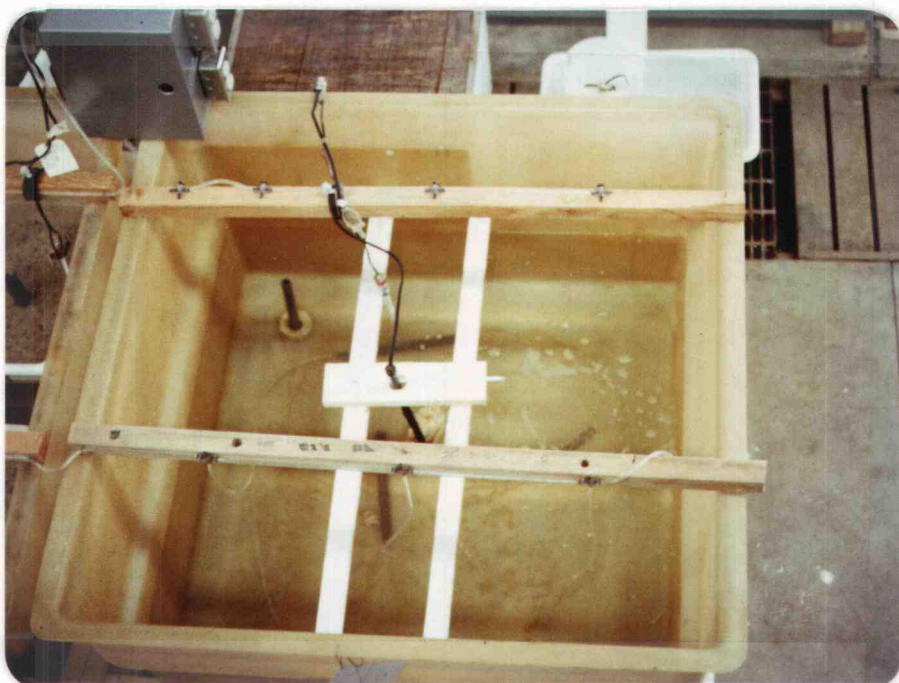


Figure 3. Oyster conditioning system designed for conditioning trial IV.

Pseudoisochrysis paradoxa and Thalassiosira pseudonana were cultured by methods adapted by Breese and Malouf (1975).

Larval survival, defined as the proportion of ova surviving to juvenile spat, was estimated in a two step process determining 1) the proportion of ova that survived to the straight hinge larval stage and 2) the proportion of straight hinge larvae that survived to juvenile spat. Multiplying (1) x (2) estimates larval survival from ova to spat.

Histology

Examination of histological sections of gonadal tissue allowed direct observation of gonadal development. The oysters were removed from their shells and a five to seven cm long cross section of gonad between the labial palps and the adductor muscle was excised and fixed in Bouin's solution. Samples were fixed for 72 hours and embedded in paraffin. Then 7 μ m sections were sliced on a microtome and stained with hematoxylin-eosin.

Index of Gonadal Development

Stages of gonadal development were determined in females by enumerating three distinct stages of egg development: 1) primary oocytes which are 10 to 15 μ m in diameter exhibiting a clear round nucleus and prominent nucleolus with little cytoplasm is present which is basophilic (Figure 4); 2) secondary oocytes undergoing

vitellogenesis exhibit an increase in cytoplasm and stains less basophilic, and are attached to the follicle walls by a stalk (Figure 5); and 3) mature ova appear free in the follicular lumen and are similar in appearance to secondary oocytes except slightly larger in size and having acidophilic cytoplasm (Figure 6). Direct counts of ova and oocytes were made in selected areas of the ovary. For the purposes of presentation, the index was calculated as the ratio of ova to ova plus oocytes.

Methods of Proximate Analysis

The composition of ova spawned during conditioning trials II and III were estimated by proximate analysis.

Wet weight of ova was estimated by pouring a suspension of ova over two 80 μ m Nitex screens to remove debris and the filtered ova suspension was left for one half hour in a beaker to concentrate the ova at the bottom. Then the supernatant was discarded and the remaining concentrated ova suspension was poured into a graduated cylinder to concentrate further. Ova concentration of the resultant suspension was estimated by dilution and sub-sampling. A 1.5 ml sample was drawn with a micro-pipette and placed in a 2 ml polypropylene centrifuge tube. The sample was centrifuged at 12,000 x G for two minutes and the supernatant decanted. The weight of the tube and the packed ova subtracted from the weight of the tube estimates

Figure 4. Primary oocytes developing along the walls of the follicle.

Figure 5. Follicles containing predominantly secondary oocytes. Note increase in size relative to primary oocytes and attachment of oocyte to follicle walls by a cytoplasmic stalk.

Figure 6. Mature ova free in the follicular lumen.

- p - primary oocytes
- s - secondary oocytes
- m - mature ova
- f - wall of follicle

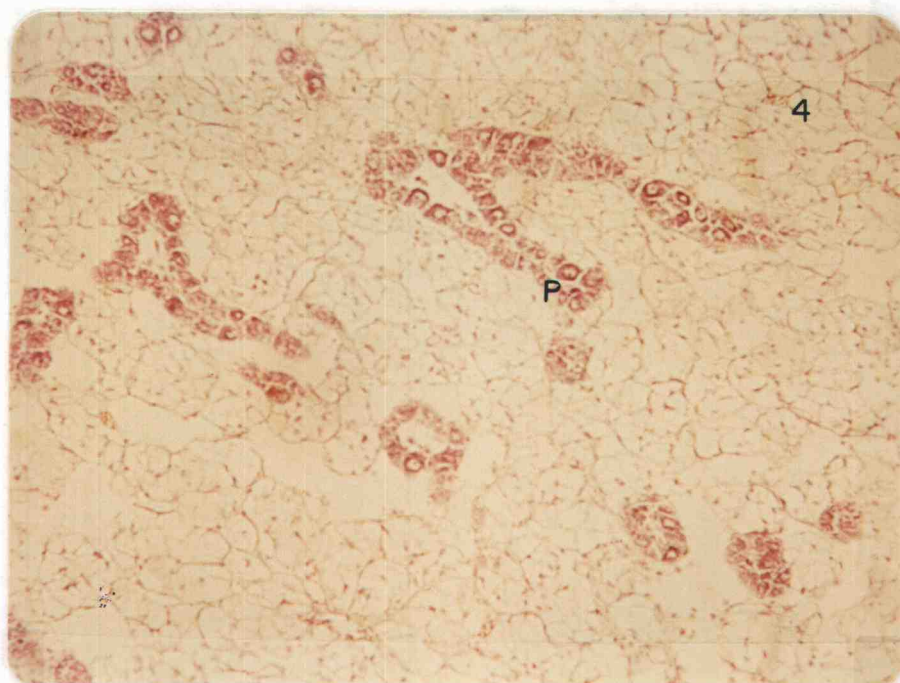


Figure 4 (100x magnification).

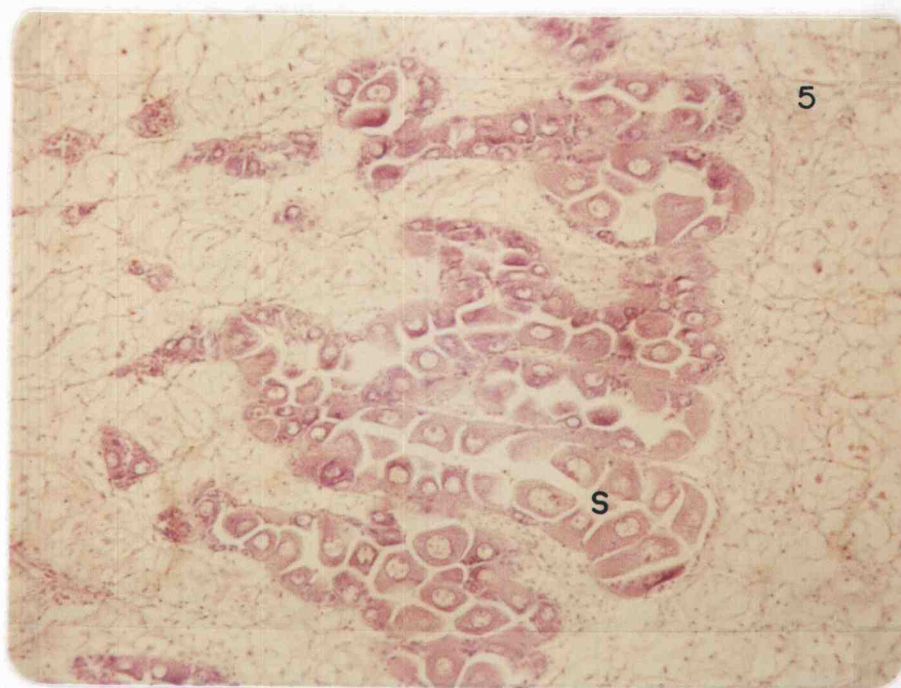


Figure 5 (100x magnification).

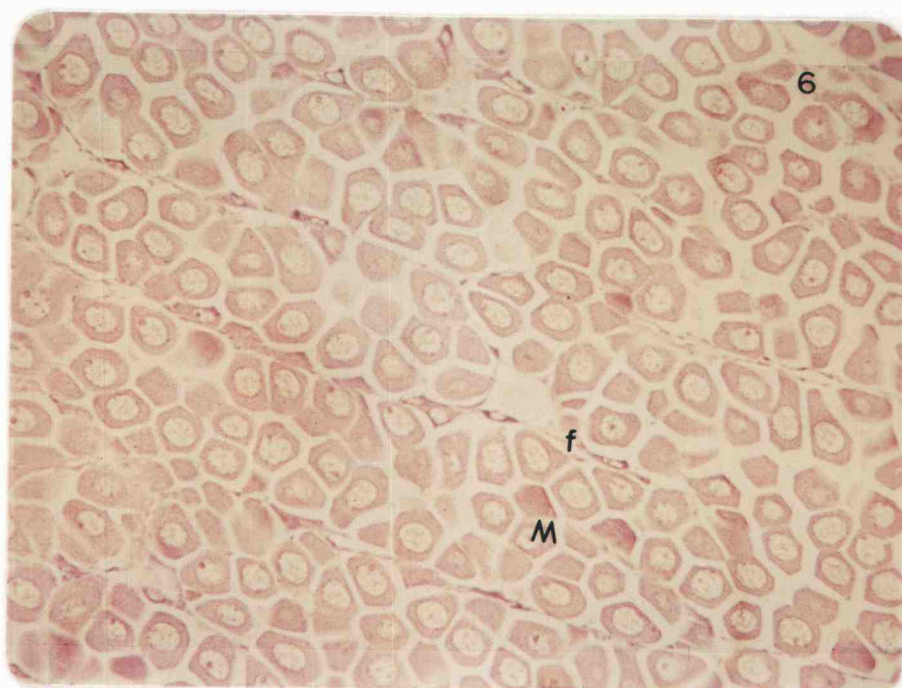


Figure 6 (100x magnification).

the wet weight of the ova. The centrifuge tube containing the wet packed ova was placed in a drying oven at 90°C for 24 hours. Clean, empty centrifuge tubes were also dried to correct for water loss of the tube. Subtracting the weight of the tube plus the dried ova from the weight of the tube alone estimates dry weight.

Ash content was estimated by placing dried ova of known weight in aluminum weighing pans and incinerated in a muffle furnace at 450°C for 48 hours. The weight of the pan was subtracted from the weight of the ash plus the pan.

Each determination of wet weight, dry weight and ash consisted of four to five replications.

Ova from each spawning were concentrated and stored in a 1.5 ml polypropylene centrifuge tube as previously described in estimating wet weight. The samples were immediately quickly frozen under liquid nitrogen and stored in a freezer at -15°C until analyzed.

Total protein was determined by a modified micro-Kjeldahl method (Dennis Gordon, personal communication). Dried ova samples of known weight were digested in concentrated sulfuric acid and commercial Kelpak catalyst. Bovine serum albumin was used as a standard. Nitrogen content of the sample was measured by a Model 95-10 Orion Ammonia Electrode with an Orion Model 701 pH meter and multiplied by 6.25 to give total protein. All determinations were performed in triplicate.

Carbohydrate was estimated indirectly by difference from 1000 μ g per mg dry weight of the summed contents of protein, lipid and ash which was determined empirically. Error in this estimate incorporates the sum of all errors inherent in the methods of proximate analysis for protein, lipid and ash and biological variation.

RESULTS

Effects of Temperature and Salinity on Gonadal Development

The effects of temperature or salinity on the rate of gonadal development will initially be described with the alternative factor held constant in order to clarify relationships not easily seen when the effects of both factors are presented simultaneously.

During conditioning trial I, oysters were conditioned at each of four temperatures and three salinities, and the rate of gonadal development was related to the amount of thermal exposure (degree-days) experienced during the course of conditioning. Arc sin $\sqrt{\text{percent ova maturation}}$ (Y) was regressed on the log degree-days (X) for each treatment and the slopes and y-intercepts of the regression lines were compared statistically (Snedecor and Cochran, 1976). The arc sin transformation of the data is necessary to stabilize the variances of binomially distributed data. Inasmuch as degree-days is the product of time and temperature, the amount of time the oysters are exposed to a given temperature is standardized, the gonadal development between temperature levels can be compared directly. For example, a group of oysters exposed to 20°C for five weeks and another group exposed to 10°C for 10 weeks can be compared directly because both groups received equivalent thermal exposure (700 degree-days).

If equivalent thermal exposure results in similar rates of gonadal development irrespective of temperature level, then gonadal development in broodstocks could be advanced or retarded simply by adjusting the amount of thermal exposure. The effect of temperature on gonadal development at 30 ppt salinity is presented in Figure 7-A. The rate of development increased from 16 °C to 20 °C and reached a maximum between 20 and 22 °C despite equivalent thermal exposure. Significant differences ($P < .01$) in y-intercepts between most temperatures was observed, suggesting that each temperature level has a differential effect on gonadal development. On the other hand, the rate of ova maturation, as indicated by the slopes, was constant over the range of temperatures. Therefore, the temperature effect must be related to oocyte development rather than ova maturation. These results demonstrate that thermal exposure (degree-days) in itself is insufficient to gauge accurately the rate of gonadal development in oysters.

Salinity is an important factor affecting the rate of gonadal development and follows a pattern similar to temperature. The rates of development particularly during oocyte development were evidently retarded at salinities below 30 ppt (Figure 7-B). This is inferred by the significant differences in y-intercepts between salinity levels ($P < .01$). The rate of ova maturation, as indicated by the slopes, was not affected by salinities as low as 20 ppt, but was significantly

Figure 7-A. The observed relationship between the rate of gonadal development of oysters exposed to four temperature levels and thermal exposure (degree-days). Salinity was held constant at 30 ppt.

Figure 7-B. The observed relationship between the rate of gonadal development of oysters exposed to three salinity levels and thermal exposure (degree-days). Temperature was held constant at 20°C.

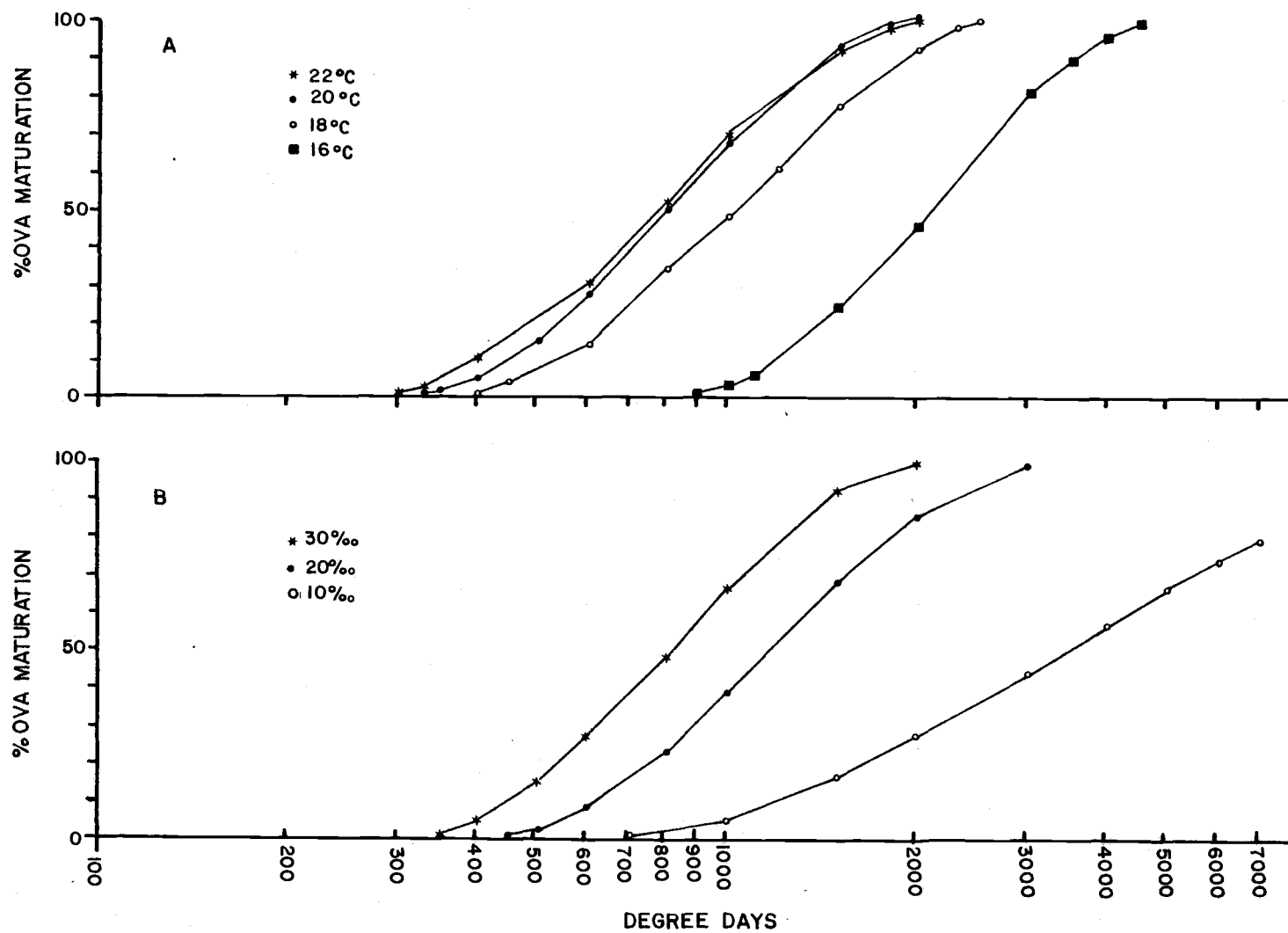


Figure 7.

depressed at 10 ppt ($P < .05$). The influence of salinity on gamete viability will be presented in the following section. The combined effects of temperature and salinity on gonadal development expressed in terms of percent ova maturation after 1100 degree-days of conditioning is illustrated in Figure 8. The rate of development consistently increased with temperature within each salinity level. Also the relative magnitude of response was augmented at higher salinity levels. Analysis of variance of this data, summarized in Table I shows a significant interaction however the variation explained by the main effects were relatively large in comparison. This implies that there is only minor variation in the response to temperature when salinity is at a high or low level and vice-versa.

At 10 ppt, gonadal development was sufficiently depressed so that no gametogenic activity was observed at 16° and 18°C. The physiological stress imposed by this low salinity was manifest in high mortality, which ranged from 38 to 57%. These oysters appeared "watery" suggesting that stored body reserves were depleted.

Effects of Salinity during Conditioning of Broodstock on Larval Survival

The rate of gonadal development of broodstock was retarded by salinities below 30 ppt as observed in Figure 7-B. Despite the stress imposed by low salinity, the gametes appeared morphologically

Figure 8. The observed relationship between the gonadal development after 1100 degree-days of conditioning and temperature at three salinity levels.

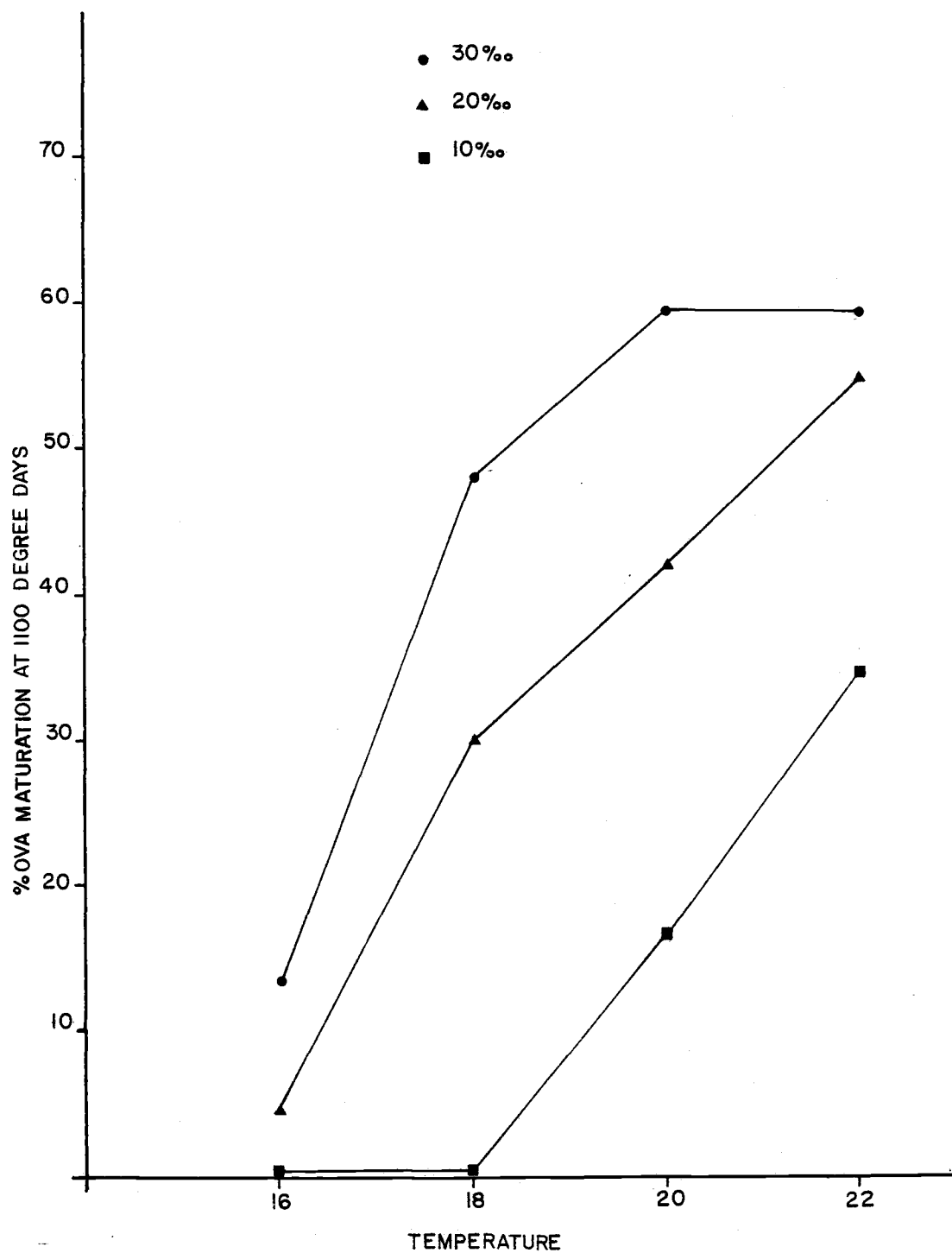


Figure 8.

Table I. Analysis of variance of gonadal development of oysters after 1100 degree-days of conditioning at four temperatures and three salinities.

Source of Variation	df	Sums of Squares	Mean Squares
Treatments	11	28500.4	2590.9**
Temperature	3	15981.4	5327.0**
Salinity	2	10433.7	5216.9**
T x S	6	2085.6	347.6**
Error	84	257.0	3.1
Total	95	28757.4	

Figure 9. The relationship between gonadal development (A), survival to straight hinge larvae (B), and larval survival (C) and conditioning interval during conditioning trial II.

The relationship between gonadal development (D), survival to straight hinge larvae (E), and larval survival (F) and conditioning interval during conditioning trial III.

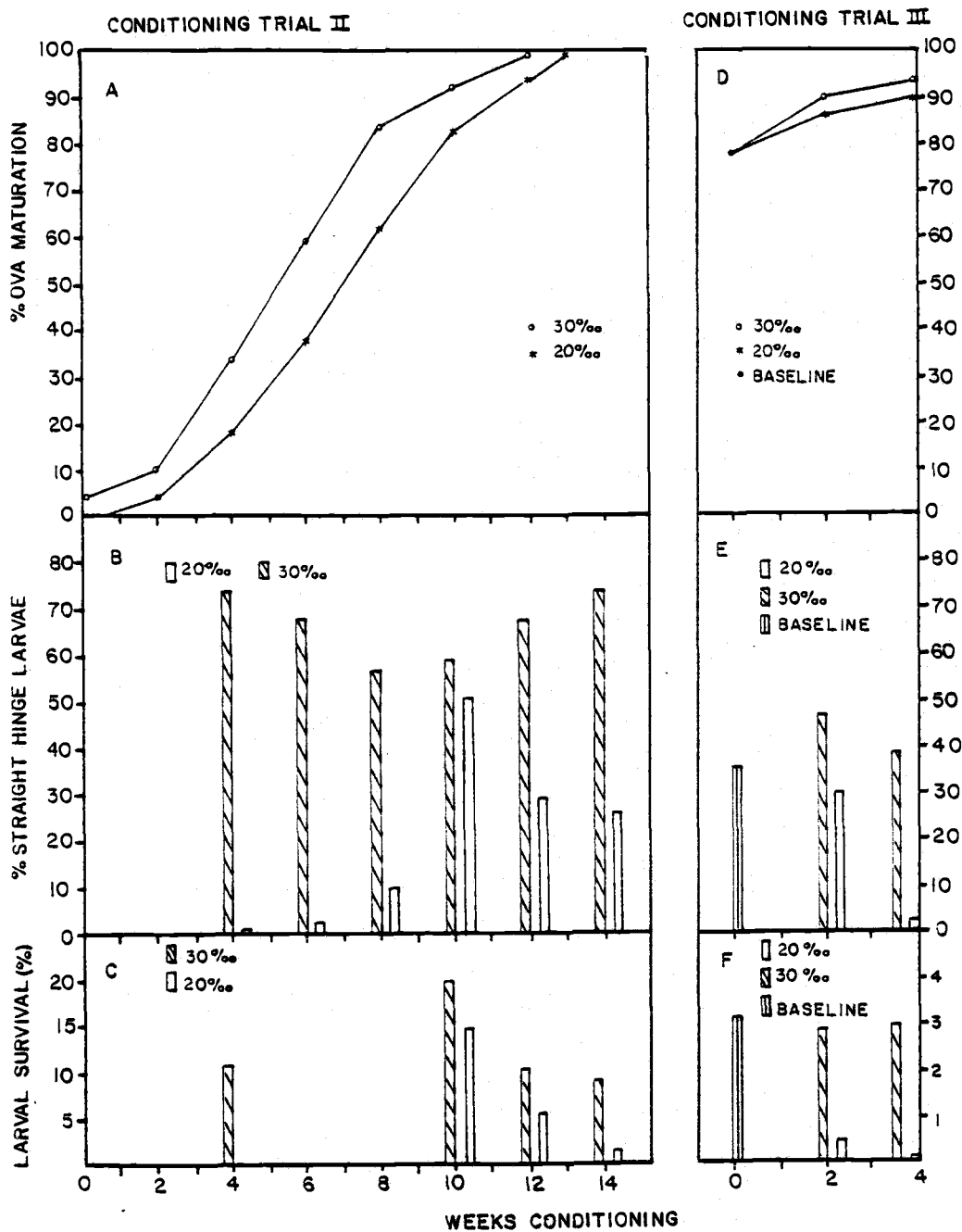


Figure 9.

normal. However, it does not necessarily follow that the gametes were viable. In conditioning trial II, larval survival was compared from spawnings between oysters conditioned at 20 and 30 ppt as shown in Figure 9-B, C. Larval survival ranged from 11.5 to 19.8% ($\bar{X}=13.0\%$) during the 14 weeks of conditioning at 30 ppt. No spat was observed during the sixth and eighth week cultures although zygotes developed normally to the straight hinge larval stage. The failure of larvae to survive further rearing was presumably due to contaminated water. These failures preclude assessing relative condition of parental oysters at six and eight weeks of conditioning. Apparently, the optimum conditioning interval extended to 10 weeks. The gonadal index of the broodstock was increasing during this interval and reached maximum development on the twelfth week (Figure 9-A). Lannan et al. (1980) also observed maximum larval survival when the gonadal index was increasing but before full development was reached.

Larval survival from parents conditioned at 20 ppt ranged from zero to 14.6% ($\bar{X}=3.7\%$). This represents a substantial reduction relative to the 30 ppt treatment. No spat was observed during the first eight weeks due to the extremely poor survival to the straight hinge larval stage at 34 hours post-fertilization. That larvae were abnormal could be easily identified by the characteristic spiral swimming behaviour and irregular body and shell shape.

Gonadal development progressed normally at 20 ppt although the rate was depressed relative to the 30 ppt treatment confirming the results of conditioning trial I (Figure 9-A). The fact that spat was observed in subsequent spawnings exemplifies the resiliency of the oyster's reproductive system and can be regarded as an adaptation to reduced salinities which ensures recruitment to the next generation.

Conditioning trials I and II demonstrated that the rate of gonadal development was retarded and gamete viability was reduced in oysters conditioned at 20 ppt relative to 30 ppt. Conditioning trial III tested whether or not exposing broodstock already containing ripe gametes to 20 ppt had a similar effect on larval survival. A sample of oysters was spawned on the first day of conditioning to provide a baseline with which to compare later spawnings. Figure 9-E and 9-F illustrates that survival to the straight hinge larval stage was consistently lower in the 20 ppt treatment. Also, larval survival was substantially reduced ($\bar{X}=0.33\%$) relative to the 30 ppt ($\bar{X}=2.92\%$) and baseline (3.24%) treatments during the course of conditioning. Apparently, the exposure of sexually mature oysters to 20 ppt affected the viability of their gametes further emphasizing the critical importance of controlling the salinity during conditioning of broodstocks.

Proximate Analysis of Ova

An intimate relationship exists between the stage of gonadal development at the time of spawning and larval survival (Lannan et al., loc. cit.). There are apparent qualitative differences in the gametes as evidenced by the different survivals observed in the 30 ppt treatment in conditioning trial II. For example, Figure 9-C shows that the gametes spawned on the tenth week of conditioning exhibited much higher survivals relative to other spawnings. Also, the markedly different survivals observed between salinities in conditioning trials II and III implies that there must be qualitative differences in gametes beyond random variation.

Proximate analysis of ova spawned from parents conditioned at 20 and 30 ppt during conditioning trial II was determined to identify constituents that may be related to subsequent survival. The data are summarized in Table II and Figure 10. The differences between means of each constituent in ova spawned from the two conditioning regimes were tested statistically using a t-test for two independent samples (Snedecor and Cochran, 1976).

There were no significant differences between conditioning regimes with respect to the relative dry weights of the ova. Ova spawned from the 30 ppt treatment contained a significantly higher proportion ($P < .05$) of total organics than the 20 ppt treatment, the ash

Table II. Proximate composition of ova spawned from oysters conditioned at 20 ppt and 30 ppt salinity during conditioning trial II.

Conditioning Regime	Weeks of Conditioning	Larval Survival	Lipid ($\mu\text{g}/\text{mg}$)	Protein ($\mu\text{g}/\text{mg}$)	Carbohydrate ($\mu\text{g}/\text{mg}$)	% Organic Dry Weight	% Dry Weight
30 ppt	4	11.5%	--	--	--	--	--
	6	*	141.4	463.3	350.0	95.5	17.2
	8	*	147.5	522.8	241.6	91.2	24.5
	10	19.8	155.2	503.3	283.6	94.2	26.2
	12	11.4	159.5	493.0	270.5	92.3	27.3
	14	9.3	171.6	519.9	247.9	93.9	26.4
20 ppt	4	0	--	--	--	--	--
	6	0	--	--	--	--	--
	8	0	170.8	561.2	139.6	87.2	21.6
	10	14.6	145.8	595.3	135.4	87.6	27.9
	12	5.9	164.3	530.2	199.4	89.4	26.5
	14	1.6	163.2	526.5	245.1	93.5	27.4

(--) not determined

(*) contaminated cultures

Figure 10-A, 10-C. The relationship between proximate composition of ova spawned during conditioning trial II(A) and larval survival (C).

Figure 10-B, 10-D. The relationship between proximate composition of ova spawned during conditioning trial III (B) and larval survival (D).

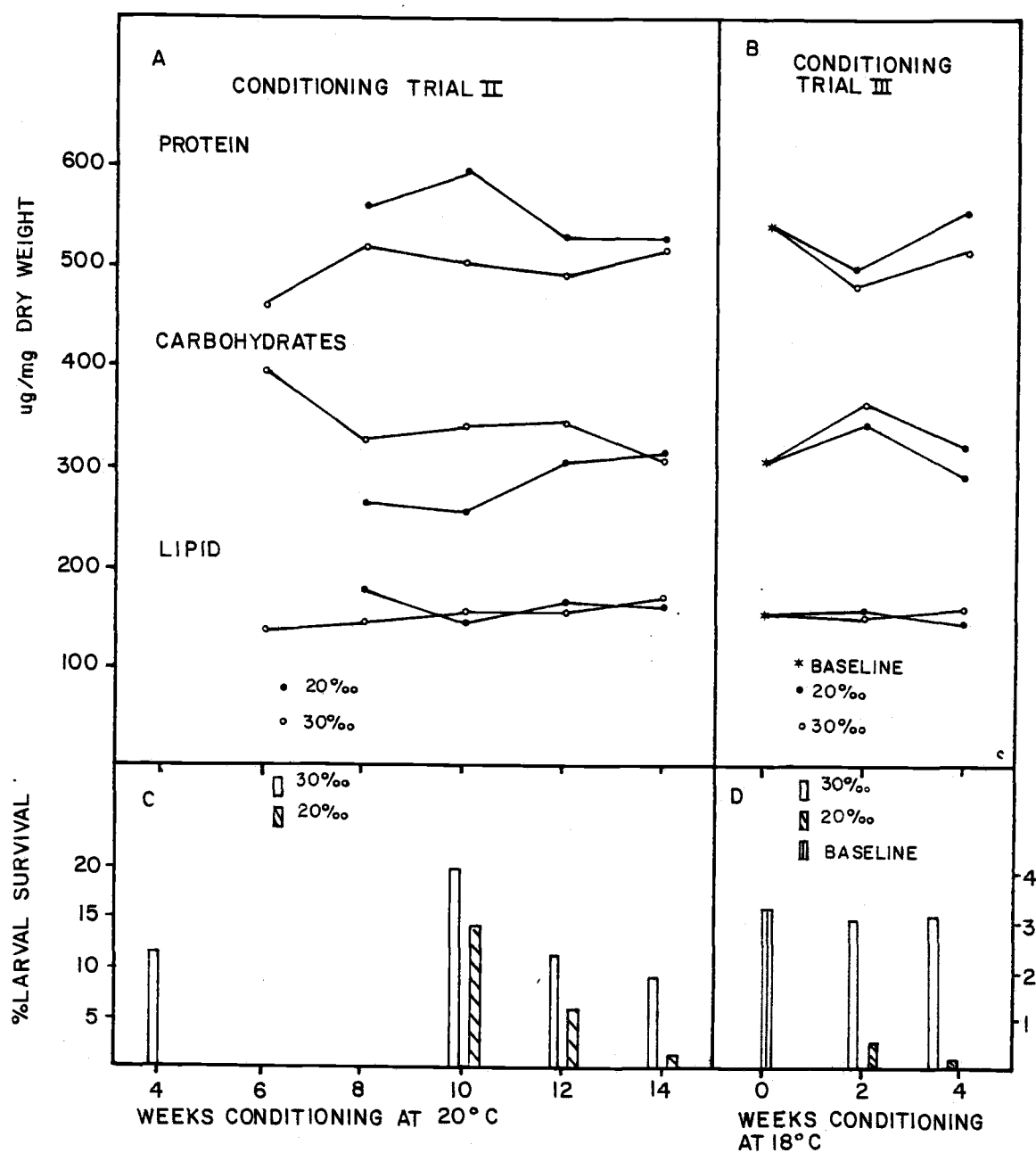


Figure 10.

content of ova from the latter being on the average 70% greater than the former. Total protein ranged from 463 to 520 $\mu\text{g}/\text{mg}$ dry weight and 526 to 595 $\mu\text{g}/\text{mg}$ dry weight in the 30 and 20 ppt treatments respectively. The difference is significant ($P < .10$). Total lipid in ova from the 30 ppt treatment increased steadily from 141 to 171 $\mu\text{g}/\text{mg}$ dry weight as conditioning progressed while no meaningful trend was apparent in the 20 ppt treatment. The difference is not significant. Carbohydrate in ova from the 30 ppt treatment decreased from 350 to 248 $\mu\text{g}/\text{mg}$ dry weight as conditioning progressed whereas an increasing trend from 139 to 245 $\mu\text{g}/\text{mg}$ dry weight was observed in the 20 ppt treatment. Carbohydrate was significantly higher in ova in the former case ($P < .10$).

Irrespective of the conditioning regime, the results indicate that no apparent relationship exists between proximate composition of the ova and its subsequent survival. None of the major constituents in the ova could be correlated with the observed variation in larval survival, although significant differences were observed in certain components of ova from the 20 ppt treatment which may account for the reduced viability.

Proximate composition of ova spawned from sexually mature oysters that were subsequently exposed to 20 ppt and 30 ppt during conditioning trial III are summarized in Table II and Figure 10.

Although gamete viability was reduced in oysters exposed to 20 ppt relative to 30 ppt, no significant differences between any constituent were observed. This is not surprising because the ova were already developed prior to the start of conditioning. More likely, the reduced salinities affected regulatory processes during embryonic and larval development.

Effects of Feeding Broodstock an Algal Food
Supplement on its Reproductive Performance

Conditioning trial IV tested the effects of an algal food supplement during conditioning on gonadal development, fecundity and larval survival. The rate of gonadal development was not significantly different between the fed and starved broodstock as shown in Figure 11-A.

The fed and unfed broodstocks were spawned bimonthly for 14 weeks and survival of the larvae was observed. Contamination of the hatchery water supply decimated all larval cultures for the first eight weeks of conditioning. Nevertheless, in subsequent spawnings no significant differences in larval survival were observed (Figure 11-C). Larval survival from spawnings from the fed group ranged from 4.72% to 14.92% ($\bar{X}=8.42\%$) compared to the starved group for which the survival ranged from 2.68% to 17.68% ($\bar{X}=8.32\%$).

The added food supplement increased fecundity in the broodstock. Fecundity, as measured by the ratio of the area of the gonadal section

Table III. Proximate composition of ova spawned from oysters conditioned at 20 ppt and 30 ppt salinity during conditioning trial III.

Conditioning Regime	Weeks of Conditioning	Larval Survival	Lipid ($\mu\text{g}/\text{mg}$)	Protein ($\mu\text{g}/\text{mg}$)	Carbohydrate ($\mu\text{g}/\text{mg}$)	% Organic Dry Weight	% Dry Weight
Baseline	0	3.2%	152.7	543.1	241.0	93.7	28.2
30 ppt	2	2.9%	152.2	482.0	293.6	92.8	27.0
	4	2.9%	159.8	517.9	247.4	92.5	26.5
20 ppt	2	0.5%	153.4	498.6	278.4	93.0	26.4
	4	0.15%	147.2	557.6	221.4	92.6	29.1

occupied by ova to the total area of the gonad, was 60% greater in the fed group (Figure 11-B). Supplemental feeding thus increases fecundity but has a negligible effect on gamete viability.

Figure 11. The relationship between gonadal development (A), fecundity (B), and larval survival (C) and conditioning interval during conditioning trial IV.

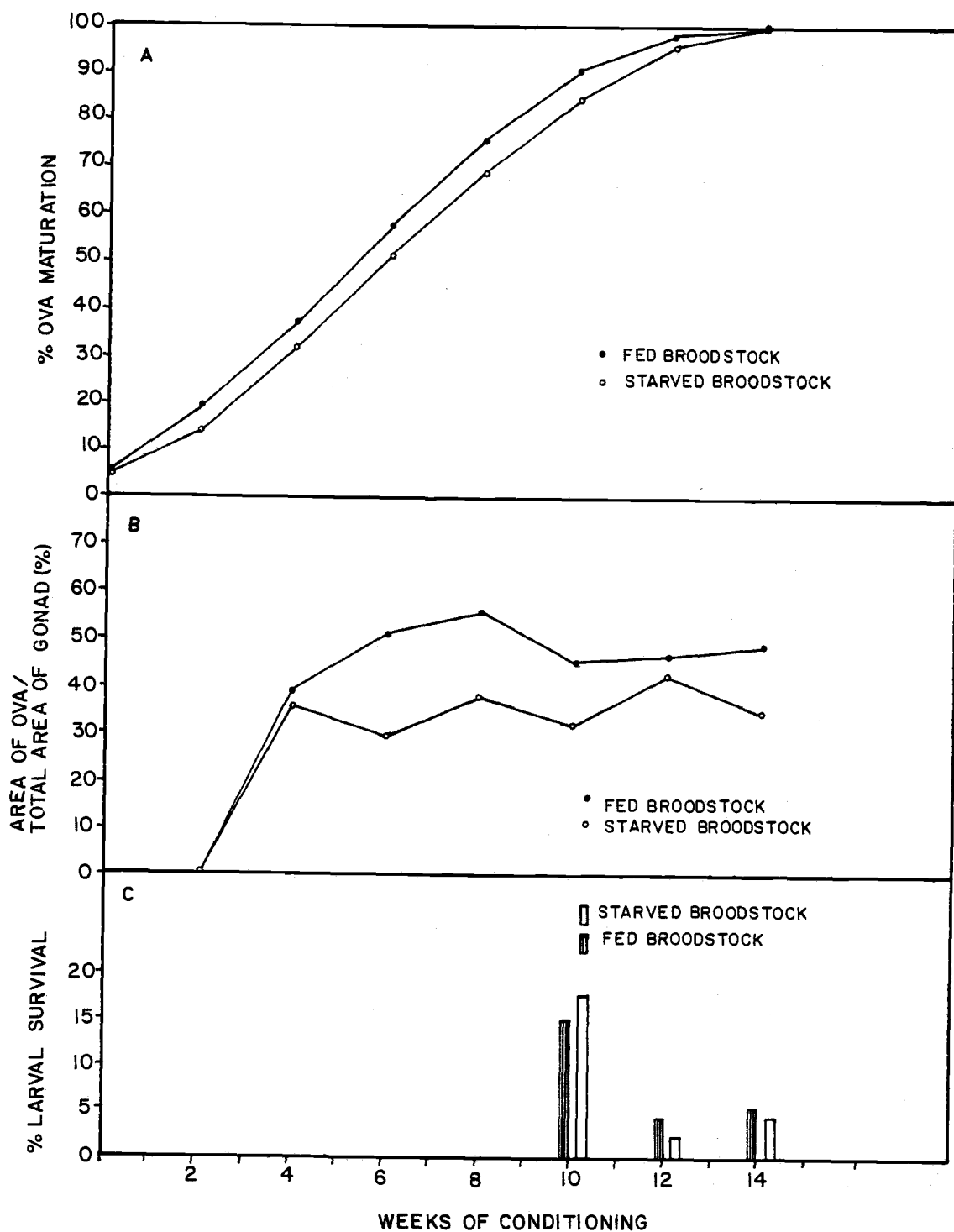


Figure 11.

DISCUSSION

This study demonstrates the importance of controlling temperature, salinity and algal food supplementation during hatchery conditioning of broodstock. These factors were observed to have a profound influence on the oyster's reproductive performance.

Temperature is generally regarded as the dominant factor controlling metabolism and activity of aquatic poikilotherms. Rates of physiological processes generally increase with temperature and reach a maximum near the upper thermal tolerance limit (Kinne, 1963). Similarly gonadal development in C. gigas exposed to higher temperature levels progressed at increasing rates although each temperature group experienced equivalent thermal exposure. With respect to temperature, the rate of gametogenesis is a function of temperature intensity and time of exposure rather than accumulated thermal exposure. This relationship is consistent with a basic physico-chemical law with which chemical reactions in biological systems must concur. The rate effects on chemical reactions due to temperature changes are dependent on the proportion of molecules participating in the chemical reaction that possess the minimum kinetic energy (energy of activation) to be reactive (Newell, 1976). A relatively small increase in temperature recruits a proportionally larger number of reactive molecules to drive the chemical reaction. Manifestations of

this relationship between temperature and rates of chemical reactions are quite pronounced in most organisms. For example the Q_{10} concept is a basic illustration of how metabolic rates in the organism doubles or triples in response to a 10°C change in temperature. At the cellular level, a series of intense biochemical synthetic events are occurring during the development of the oocyte (Wolfe, 1972). The rates of the biochemical reactions are determined by temperature intensity which over time results in a temperature dependent rate of reproductive development observed in Figure 7-A.

Salinity is another dominant factor characterizing the environment of aquatic organisms. Because estuarine bivalve molluscs such as C. gigas are euryhaline, culturists tend to minimize efforts in controlling salinities experienced by broodstock during conditioning. Schleiper (1957) demonstrated that low salinity (15 ppt) altered the metabolic activity of Mytilus edulis in terms of lowered ciliary activity, heart rate and elevated oxygen consumption. Their metabolic capacity appeared to be reduced under low salinity conditions and may account for the progressive decrease in the rate of gonadal development in C. gigas shown in Figure 7-B. Temperature and salinity have similar effects on the rate of gonadal development and behave as two forces constantly modifying the effects of each other yet maintaining a constant pattern of response at each level.

The viability of gametes spawned from oysters exposed to

reduced salinities were markedly reduced when the zygotes were reared under standardized hatchery conditions. Larval mortality was the highest when the intense morphogenic activities involving cleavage, gastrulation and development to the first veliger larval stage were occurring. Several possible explanations are advanced to account for this reduction in gamete viability. First of all, the gametes may have been adapted to the salinity at which gonads developed or at which their parents were exposed prior to spawning. Thus the salinity tolerance range of gametes spawned by oysters exposed to 20 ppt was below the salinity at which the larvae were reared (27-29 ppt). Davis (1958) demonstrated that the optimum salinity and salinity range for normal development to straight hinge in Crassostrea virginica were dependent on the salinity at which the parents developed gametes or were held prior to spawning. Secondly, proximate analysis of the ova indicated that the composition was altered and this was attributed to the 20 ppt salinity at which the gonads of the parental oysters were developed. These differences in composition may be critical to larval survival because embryonic development in planktonic species as C. gigas are energetically dependent on stored reserves of the egg cortex until the larval velum is developed for feeding. Thirdly, agents such as specific ions and salinity of the medium that affect the egg cortex noticeably influences the normal course of embryonic development and may result in characteristic malformations of

the embryo. The egg cortex regulates interactions between the cell and its environment by selective permeability. Also the cortex appears to coordinate the movement of various substances within the egg such as yolk granules and organelles during early development. It thus functions as a vital regulatory organ the integrity of which must be maintained for normal embryonic development (Raven, 1958). Therefore, when sexually mature oysters containing ripe ova were subsequently exposed to 20 ppt salinity, the inherent properties of the cortex of the egg such as permeability and electrical conductivity may have been altered resulting in reduced larval viability. Conclusions based on these observations recommend that salinity must be controlled during hatchery conditioning of broodstock, especially at locations where the water supply fluctuates more than 10 ppt to ensure proper development of gametes and maximum survival of zygotes.

Larval survival was not enhanced by supplemental feeding of the diatom Thalassiosira pseudonana, which has been observed to be an excellent food organism for supporting growth of oysters (Pruder et al., 1976). This demonstrates the oyster's capacity to produce viable gametes from stored body reserves despite starvation and a decline in body condition. The algal food supplement positively influenced gamete production as fecundity was 60% greater than starved controls. This is contrary to other bivalves in that supplemental feeding was observed to be necessary for normal gonadal development

and increased larval growth and survival (Helm et al., 1973; Bayne et al., 1975; Sastry, 1975). Nevertheless, the observed increase in fecundity is sufficient justification to feed broodstocks especially if they are to be reused.

The conclusive measure of gamete quality in the hatchery is its survivability to juvenile spat. Recent studies have implicated the lipid content of ova or early larvae as a predictive indicator of growth and survival of larval oysters (Helm et al., 1973; Creekman, 1977).

Lipid has been identified as the principal energy source for early development of various planktonic species (Millar and Scott, 1967; Sargent, 1976; Bartlett, 1978). Based on the results shown in Figure 10, no correlation exists between concentration of major constituents in the ova and larval survival. The apparent qualitative differences in ova, inferred from broods exhibiting high or low survivals, are very subtle and not detectable at the gross compositional level. Factors that distinguish ova produced by oysters in an optimum relative to sub-optimum gonadal condition are yet unclear. Identification of these factors would be a major step in developing an assay for gametes of maximum viability or a ripe gonadal condition and would function as a valuable hatchery tool for managing broodstocks. Until then, culturists must conduct conditioning trials to determine the conditioning requirements for spawning broodstocks in an optimum gonadal condition.

CONCLUSION

A strategy is proposed to maintain C. gigas broodstock in an optimum gonadal condition by controlling temperature, salinity and algal food supplementation during hatchery conditioning. Reproductive performance of broodstock is critically dependent upon control of these factors because each factor has a specific effect on gametogenesis.

Figure 12 illustrates the relationship between the number of weeks of conditioning required for oysters to attain the optimum gonadal condition and conditioning temperature with salinity held constant at 30 ppt. Based on previous conditioning trials, broodstocks are in an optimum gonadal condition when they contain gametes of which 40 to 85% are mature ova. When Miyagi strain C. gigas from Yaquina Bay are brought into the hatchery in January, four and one half weeks of conditioning at 22 °C are necessary to attain the optimum gonadal condition and will maintain this condition through the eighth week. By then, the next lower temperature group will already be in an optimum condition for spawning. Each group is thus spawned in series as they attain the optimum condition resulting in a greater proportion of viable gametes entering the larval rearing system. It must be emphasized that these conditioning requirements will change for oysters conditioned during other seasons of the year.

Figure 12. The relationship between the conditioning requirements for broodstock to attain the optimum gonadal condition (40%-85%) and temperature when conditioning started on January 15.

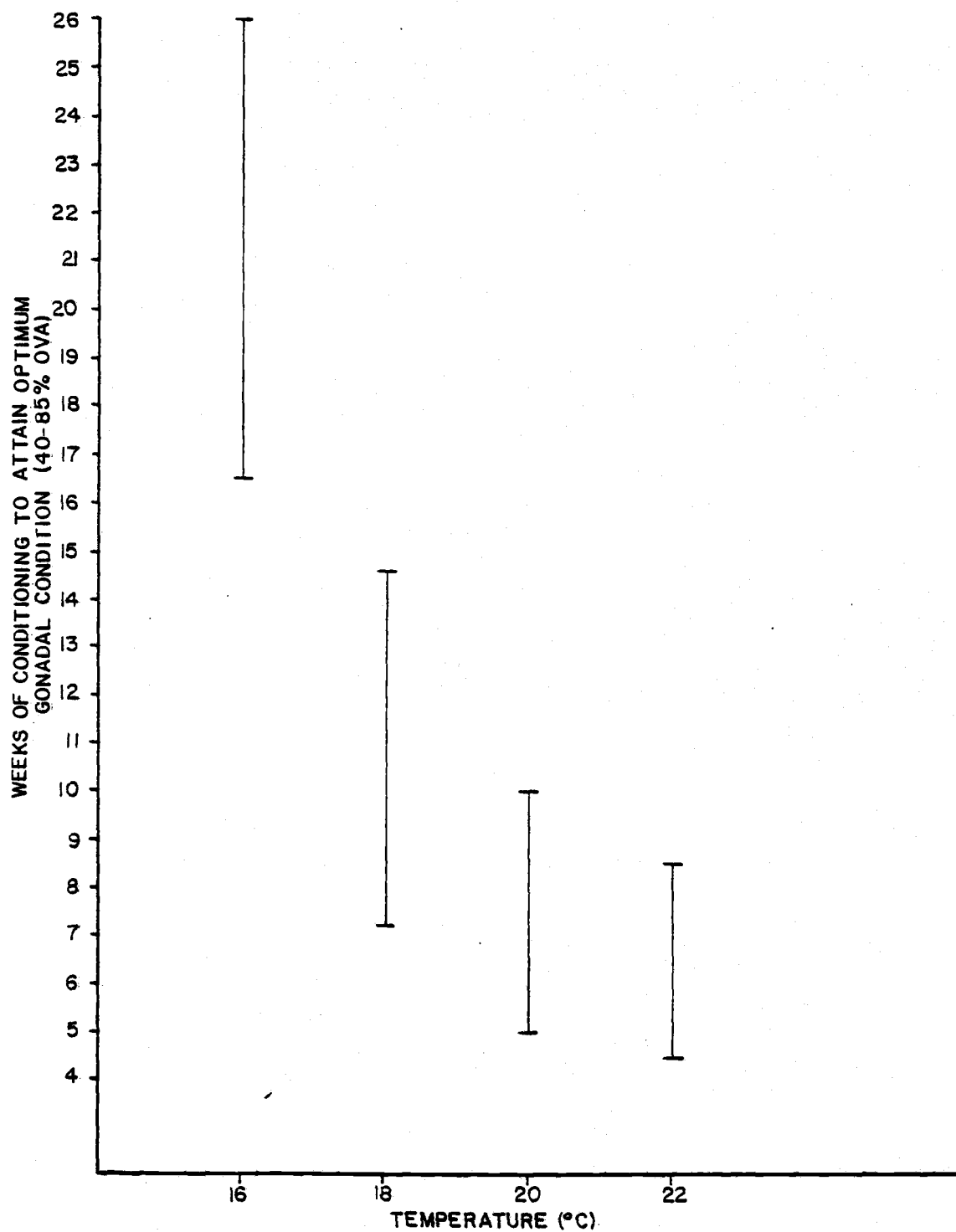


Figure 12.

Also, these requirements do not necessarily apply to other populations of oysters. The reproductive cycle of a particular population of oysters is adapted to their specific environment and progress at a rate determined by the unique environmental conditions prevailing at each locale. Therefore, culturists operating at other locations must empirically determine the specific conditioning requirements relative to the annual reproductive cycle of the local oyster population. This may be accomplished by collecting an adequate number of oysters from the field noting the time of year including observations on their gonadal condition. The oysters are divided into equal groups and each conditioned at a specific temperature over a range of temperatures thus assuring that different groups of oysters are in various stages of development and progressing at different rates. The optimum conditioning interval for each temperature group may then be determined empirically through spawning and larval rearing trials. It is my contention that management of oyster broodstocks will increase hatchery seed production substantially.

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