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CONDITIONING OF EASTERN OYSTERS IN A CLOSED, RECIRCULATING SYSTEM

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ABSTRACT Techniques were developed for holding and conditioning of eastern oysters, *Crassostrea virginica* (Gmelin), in a recirculating system. Oysters collected in February from public oyster grounds off the coast of Louisiana were maintained in a recirculating system for 8 wks. For conditioning, water temperature in the system was gradually raised with a heat pump from 14°C, and held at 25°C for 6 wks. Oysters were fed a diet of algal paste (*Isochrysis galbana* for the first 6 wks and *Chaetocerus calcitrans* for the last 2 wks). Water quality, mortality, *Perkinsus marinus* infection, gonad development, and physiologic condition (dry tissue-to-dry shell ratio, dry tissue-to-wet tissue ratio, digestive diverticula tubule ratio) were monitored. At weeks 7 and 8, the laboratory-held oysters were compared with field controls held at Grand Isle, Louisiana. Water quality in the system remained within target ranges. Mortality was low (18 of 300 oysters stocked) and not associated with *P. marinus* infection. In the laboratory at week 1, the gonads of all oysters sampled were classified as immature or in early development. By week 5, the gonads of 73% of oysters sampled were classified as mature. Physiologic condition decreased in the laboratory. Field controls reached a higher mean gametic stage and were in better physiologic condition at the end of the 8-wk study. These differences were attributed to differences in nutrition available between the field and laboratory. This study demonstrated that conditioning of *Crassostrea virginica* is possible in a closed, recirculating system, although improvements in nutrition would be useful.

KEY WORDS: *Crassostrea virginica*, conditioning, gametogenesis, histology, aquaculture, recirculation

INTRODUCTION

The eastern oyster, *Crassostrea virginica* (Gmelin 1791), supports a valuable commercial fishery. The annual harvest value is measured in millions of dollars in the United States (MacKenzie 1996). However, the industry has been plagued with multiple problems in recent years, resulting in decreased oyster production (MacKenzie 1996, Andrews 1991) and creating a need for research in oyster genetics.

This has made necessary the development and improvement of culture techniques, including design of recirculating systems for holding and conditioning of broodstock. Typically, work is limited to the natural spawning period (April to October), and even then oysters require repeated monitoring for gonadal maturation. Additionally, research away from the coast requires continual monitoring and transport of oysters for use in the laboratory. Further problems include the costs and labor associated with obtaining suitable water sources. Thus, development of a recirculating system for holding and ripening of oysters in the laboratory would extend the oyster spawning season and expand research opportunities. In addition, such systems would allow containment of genetically modified organisms, including those produced by gene transfer (Kapuscinski and Hallerman 1991). The ability to condition oysters in an artificial system is a first step toward containment of the complete life cycle in the laboratory.

The reproductive ecology of *C. virginica* is well described (Shumway 1996, Thompson et al. 1996). The primary cue for gamete development seems to be temperature, and research has addressed the effects of temperature on gametogenesis (Price and Maurer 1971, Loosanoff and Davis 1953). Gametogenesis and spawning begin with increasing temperatures in the spring and summer, and the existence of oysters acclimatized to local environments, with specific temperature requirements in reproduction, have been reported (Loosanoff 1969). Oysters are routinely conditioned through temperature manipulation in research hatcheries using natural seawater and foods (Dupuy et al. 1977). Laboratory studies on temperature and gametogenesis have utilized natural seawater for water exchange in holding systems (Robinson 1992, Price and Maurer 1971). Some research has been conducted on the maintenance of oysters in a laboratory environment. Tolerance levels of oysters to various water quality conditions have been reported (Epifanio and Srna 1975, Epifanio et al. 1975), as have guidelines for feeding regimes and rations (Epifanio and Ewart 1977). Several studies have also addressed design and maintenance of recirculating systems (MacMillian et al. 1994, Thiekler 1981).

The goal of this project was to develop techniques for holding and conditioning of *C. virginica* in a recirculating system. To our knowledge, this is the first study on the conditioning of oysters in such a system. Oysters collected during winter in Louisiana coastal waters were brought into the laboratory, and conditioning was attempted over an 8-wk period by incremental raising of temperature from 14°C to 25°C. Laboratory-held oysters were compared with oysters from natural waters before and after the holding period. Our objectives during the study were to monitor (1) water quality, (2) mortality and disease, (3) changes in gonad condition, and (4) changes in physiological condition.

MATERIALS AND METHODS

Eastern oysters were collected from public oyster grounds in Hackberry Bay Louisiana (29°40′00″N, 90°02′30″W) on February 19, 1997. Hackberry Bay oysters were selected because of reportedly low levels of *Perkinsus marinus* infection in this population (Supan, unpublished data). The oysters were kept on ice, and transferred to the Louisiana State University Aquaculture Research Station (ARS) on February 21. Of these, 50 were processed upon arrival at the ARS to establish a baseline reference. Another 600 oysters were cleaned of external debris and split into two groups of 300 each.

One group was transported to the Louisiana State University oyster hatchery at Grand Isle, Louisiana (29°12′30″N, 90°02′30″W) to serve as a field reference. These were suspended from a pier in 1.25-cm mesh ADPI® bags, and were exposed to ambient conditions over the course of the experiment.

The second group was placed in a closed, recirculating system in the laboratory for conditioning (Fig. 1). The system was composed of two 500-L rectangular, fiberglass tanks (259 cm × 91 cm × 20 cm) connected to a 1500-L sump (244 cm × 122 cm × 65 cm). Standard PVC pipe (5.08-cm, schedule 40 pipe, unless otherwise indicated) was used for all plumbing. Each tank had a ball valve to control water delivery, a Venturi orifice on the water inlet for aeration, and an internal standpipe to control water level. Temperature was maintained within 1°C by an in-line heat pump (1-hp, 13,300 BTU/hr; ACRY-TEC, San Diego, CA) with an electric remote control (model T775B, Honeywell Inc., Golden Valley, MN) between the sump and holding tanks. A separate loop from the sump provided filtration and sterilization. In this loop, water flowed through a 0.30-m^3 upwelling bead filter (Malone et al. 1993) and a 25-W ultraviolet light (Rainbow Plastics, El Monte, CA). Nitrifying bacteria in the effluent of a functioning bead filter were used to inoculate this filter. Bacterial growth in the filter was promoted with the addition of sodium nitrite and ammonium chloride for 4 wks before the study began (Malone and Manthe 1985). Two 5.2-cm diameter PVC foam fractionators were constructed (Wheaton 1977) and used in an additional sump loop to remove dissolved organics when excessive foaming was noticed. A ¾-hp centrifugal pump (Maxim, Moorpark, CA) was attached to the sump and drove water through the system. The system was filled with artificial salt water (Fritz Super Salt, Fritz Industries Inc., Dallas, TX) at a salinity of 15 ppt.

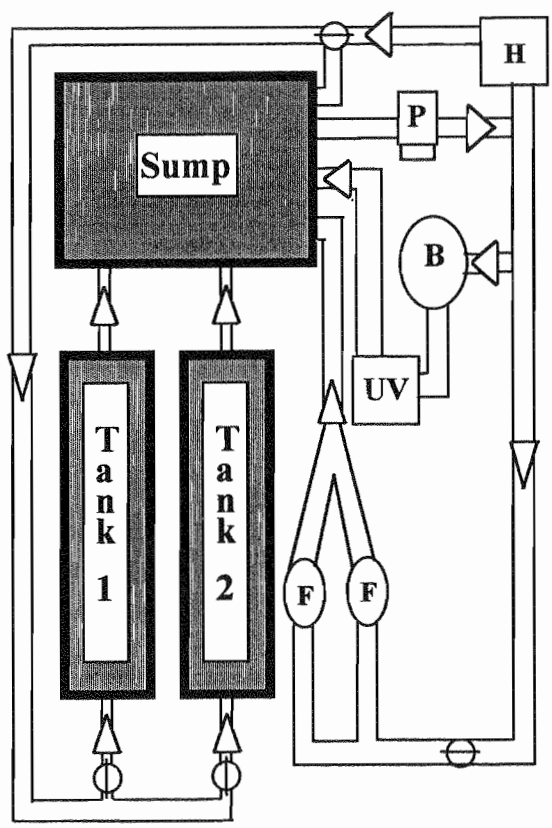

Figure 1. Design of recirculating system used to condition *Crassostrea virginica*. B = biological filter, H = heat pump, P = pump, F = foam fractionators, UV = ultraviolet light.

The laboratory oysters were fed a diet of algal paste produced from a continuous turbidostat culture maintained at the ARS (Theegala 1997). Paste was stored refrigerated until use (within 2 months). *Isochrysis galbana* (clone T-Iso) was fed for the first 6 wks and *Chaetocerus calcitrans* for the final 2 wks. The amount of paste fed was calculated based on dry weight (Epifanio and Ewart 1977), and prepared daily in the following manner: 140 g of paste was removed from storage at 4°C, weighed, dissolved in system water, and passed through 75-μm and 15-μm nylon mesh to disperse the algal cells. This mixture was added to the oyster tanks and allowed to circulate through the system. The backwash effluent from the beadfilter was returned to the sump daily, allowing total conservation of salt water, and providing a potential nutritional supplement of bacteria and dissolved organics to the oysters.

The study lasted 8 wks, from February 21 to April 15, 1997. Oysters were acclimated during week 1 in the system at 15°C. At day 10, temperature was increased 2°C every 2 days until reaching 25°C, which was maintained for the remainder of the study. Laboratory samples of 50 oysters were removed from the tanks at weeks 1, 3, 5, 7, and 8 for analysis of disease, gonad development, and physiologic condition. Of each sample, 30 oysters were assayed for *P. marinus* infection and sectioned for histology. The remaining 20 were processed for determination of physiologic condition. On weeks 7 and 8, 50 reference oysters were brought from Grand Isle as field samples and processed as described above. The whole wet weight of every oyster processed was recorded.

Water Quality

Water quality was monitored weekly (although daily measurements were made during weeks 2 and 3). A Hach® test kit (Model FF-3, Loveland, CO, USA) was used to measure ammonia con-

TABLE 1.

Comparison of observed and target water quality values in the recirculating system during an 8-wk study on conditioning of *Crassostrea virginica*

Parameter	Observed Values	Target Values
Salinity (ppt)	14.0–17.0	15.0
Temperature (°C)	13.0–26.0	15.0–25.0
Ammonia (mg/L)	0.10–4.2	0.0–5.5
Nitrite (mg/L)	0.05–1.90	0.0–460.0
pH	7.8–8.5	8.0–8.5
Dissolved oxygen (mg/L)	5.6–7.8	>3.6

TABLE 2.

Gametic stages assigned to histologic sections, and used to classify gonad development in *Crassostrea virginica*

Stage	Description
1 (immature)	Follicles small and contracted. Some early sex cells (such as pro-oogonia) may be poorly visible.
2 (developing)	<5% of all gametes mature.
3 (early maturity)	6–50% of all gametes mature.
4 (maturity)	51–75% of all gametes mature.
5 (late maturity)	76–100% of all gametes mature.
6 (regression)	Proliferation of hemocytes and cytolysis evident, some mature cells may remain.

centration (mg/L NH_3-N), nitrite concentration (mg/L NO_2-N), pH, hardness (mg/L $CaCO_3$), and alkalinity (mg/L $CaCO_3$). Dissolved oxygen was measured with a YSI oxygen meter (Yellow Spring, CO), and salinity with a hand-held refractometer. Tolerance levels from the literature (Galstoff 1964, Epifanio and Srna 1975, Epifanio et al. 1975) were used as guidelines for acceptable water quality conditions (Table 1). Temperature was measured daily with a submersible thermometer.

Mortality and Disease

Levels of *P. marinus* infection were measured using the fluid thioglycolate method (Ray 1966). A sample of rectal tissue was obtained aseptically and incubated in thioglycolate media for at least 7 days. The tissue sample was smeared on a glass microscope slide, stained with Lugol's iodine, and examined at 100× with brightfield microscopy for presence of hypnospores. Infection levels were assigned a value ranging from 0 (no detectable infection) to 6 (heavily infected, more than 1,000 hypnospores in a 5-mm field) (Quick and Makin 1971). From these values, infection prevalence (percent infected oysters per sample), intensity (average intensity of infection among infected oysters), and weighted incidence (average intensity of infection for the entire sample) were calculated (Quick and Makin 1971).

Dead oysters (individuals with gaping shells) were identified and removed from the tanks daily. If possible, a small rectal tissue sample was collected and inspected for *P. marinus* infection (12 of 18).

Gonad Development

Gonad development was assayed histologically. Standardization of sectioning is necessary for histologic comparisons among oysters (Morales-Alamo and Mann 1989). Accordingly, processing involved removing a 4–5 mm cross-section of each oyster just posterior to the junction of the labial palp and gill, and preserving this tissue in Davidson's fixative (Howard and Smith 1983). A 4-μm section was obtained ~1 mm from the junction, mounted and stained with Gill's hematoxylin and eosin (Howard and Smith 1983). Sections were characterized using features identified by Morales-Alamo and Mann (1989). Gonad development was classified as one of six stages (Table 2). For each oyster sectioned, gametic stage and sex (male, female, hermaphrodite, or unidentifiable) were recorded. Observations were made with brightfield microscopy at 100×. The mean gametic stage for each sample was calculated.

Physiological Condition

Physiological condition was closely monitored in this study for several reasons. A primary concern was the usefulness of the algal paste as a complete food source for oysters. Other concerns were the effect of temperature stress, water quality, and *P. marinus* infection. Therefore, physiologic condition was characterized with three assays, two traditional condition indices and an assay based on the morphology of the digestive diverticula.

The two traditional condition indices used were the ratio of dry tissue-to-dry shell (Rainer and Mann 1992), and the ratio of dry tissue-to-wet tissue (Lucas and Benniger 1985). Oysters were cleaned of external organisms and debris, opened, and the tissues and shell were separated and weighed (wet weight). Tissues and

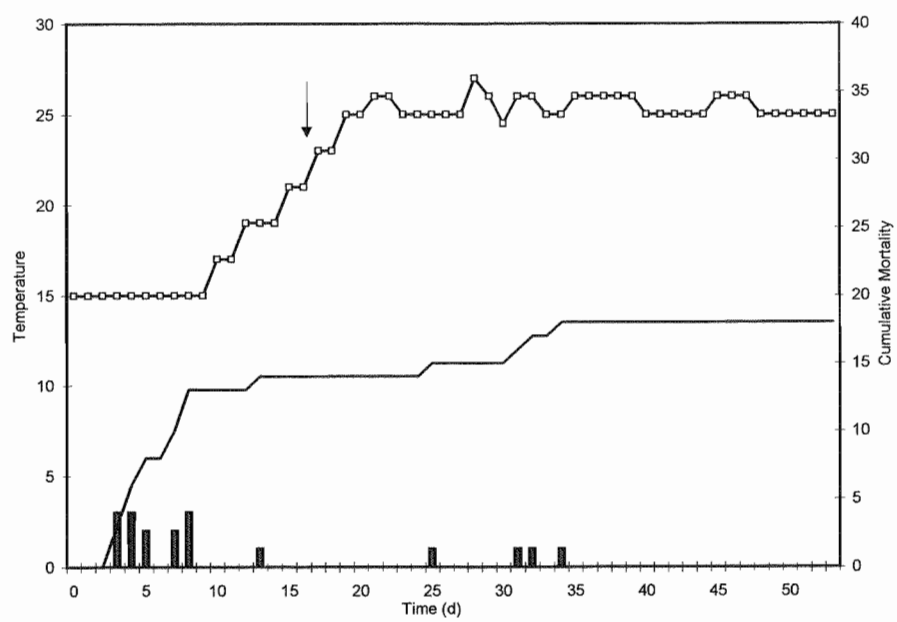

Figure 2. Water temperature (open squares) in recirculating system over the 8-wk experiment. Daily (shaded columns) and cumulative mortality (line) are indicated. The date (day 16) of highest ammonia level (4.20 mg/L) is indicated by an arrow.

shells were dried at 100°C for 48 h, dry weights were recorded, and the ratios were calculated.

Histologic slides were used for evaluation of digestive diverticula atrophy based on the methods of Winstead (1995). The area of the slide containing the digestive gland was divided into four quadrants, and five tubules were measured from each quadrant, for a total of 20 measurements from each oyster. Evaluations were done with 100× phase contrast microscopy using image analysis software (Optimas® 5.1a, Bioscan, Inc., Edmunds, WA). The total tubule area and the tubule lumen area were outlined by hand with a computer pointer (mouse), and the ratio of lumen area to total area was calculated and recorded in spreadsheet software (Microsoft Excel®, Microsoft Corp., Roselle, IL). A diverticula score was calculated for each oyster as the average of these 20 ratios, and a mean diverticula score was calculated for each sample.

Statistical Methods

Data were analyzed with the General Linear Models procedure (SAS Inc., Cary, NC). One-factor analysis of variance (ANOVA) was used to compare among mean values of weighted incidence of *P. marinus* infection, gametic stages, dry tissue-to-dry shell ratios, dry tissue-to-wet tissue ratios, and diverticula scores. A Duncan's Multiple Range Test was used to separate sample means. A significance level of $p < .05$ was used in all statistical analyses.

RESULTS

Water Quality

Water quality remained within the desired ranges throughout the study (Table 1). The system maintained temperatures within 1°C of the target temperatures (Fig. 2). However, transient increases in ammonia concentration were observed in weeks 2 and 3 as the temperature was increased (Fig. 2). The highest ammonia concentration was recorded on day 16 (4.2 mg/L). By day 21, concentrations had declined (0.2 mg/L) and remained negligible throughout the rest of the experiment.

Mortality and Disease

Mortality and *P. marinus* infection were low throughout the experiment. Of the 300 oysters stocked in the system, 13 deaths occurred during the acclimation period (week 1), followed by 5 deaths over the next 7 wks (Fig. 2). The weighted incidence of *P. marinus* infection did not exceed 1.00 (lowest level of infection) during the study. However, significant differences in weighted incidence of infection were noted among samples ($p < .017$) (Fig. 3). The highest observed weighted incidence of infection (0.70) was found in the week 7 field sample, followed by the week 7 laboratory sample (0.65). The lowest observed level of infection (0.10) was from the week 1 sample. During the study, 12 of the 18 oysters that died were examined for *P. marinus* infection. Of these, 2 were found to be infected, and each had an infection level of 1 (1 to 10 hypnospores per sample) (Table 3).

Gonad Development

Gametogenesis proceeded in the oysters during the experiment. At week 1, all of the gamete scores in the oysters sampled (29 of 29) were Stage 2 or lower (immature or developing). By week 8, 73% (22 of 30) of oysters sampled in the laboratory were at Stages 4 or 5 (mature) (Table 4). However, 93% (28 of 30) of field oysters sampled in week 8 were at Stages 4 or 5. The mean gametic stage increased through time, and significant differences were found ($p < .0001$) (Fig. 3). For all samples, the lowest mean value

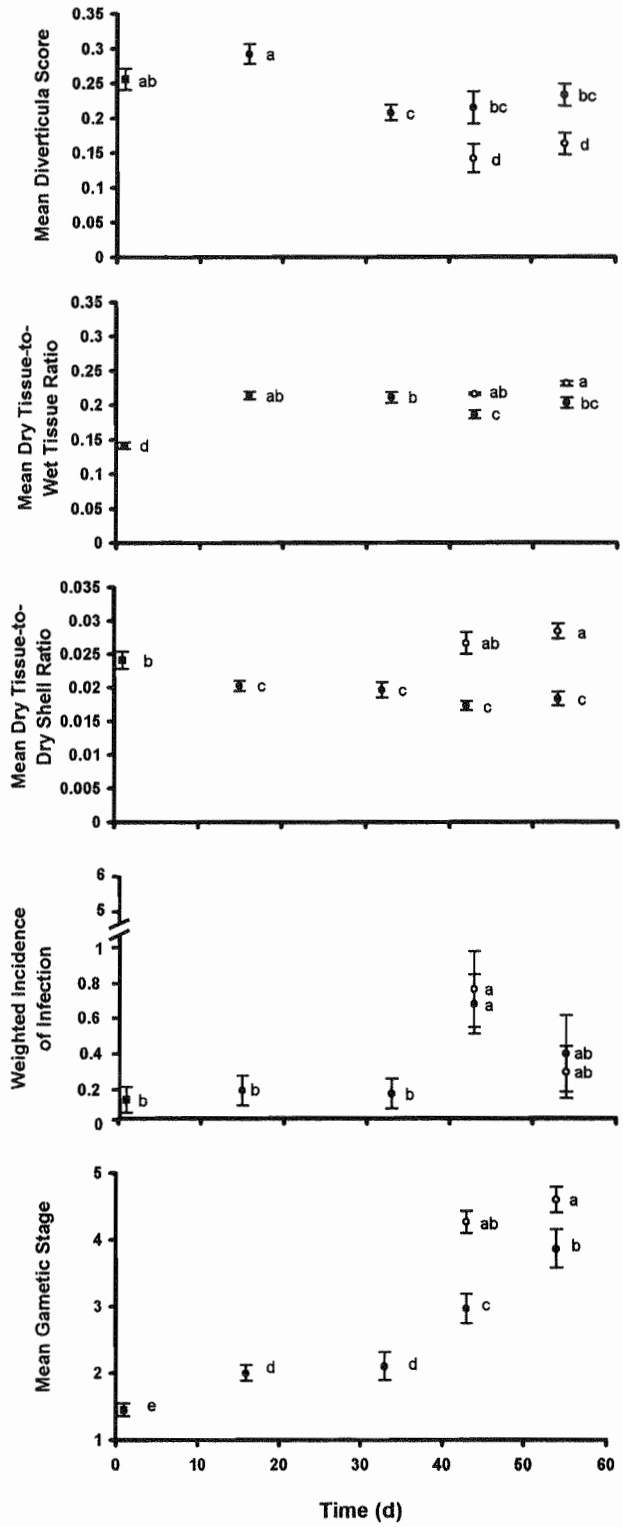

Figure 3. Weighted incidence *of Perkinsus marinus* infection, mean gametic stages, mean ratios of dry tissue weight-to-dry shell weight, mean ratios of dry tissue weight-to-wet tissue weight, and mean diverticula scores, over the 8-wk experiment. Initial samples are indicated with a square (these oysters were separated into laboratory and field samples). Laboratory samples are indicated with a closed circle. Field controls are indicated with an open circle. Bars indicate ± 1 SE. Within each panel, points sharing letters were not significantly different ($p > .05$).

TABLE 3.

***Perkinsus marinus* infection in *Crassostrea virginica* samples during an 8-wk study[a]**

Week	Source[b]	Individual Level of Infection								Prevalence	Intensity	Mean
		n	0	1	2	3	4	5	6			
1	F	20	18	2	0	0	0	0	0	10%	1.0	0.10
3	L	20	17	3	0	0	0	0	0	15%	1.0	0.15
5	L	20	18	1	1	0	0	0	0	10%	1.5	0.15
7	L	20	10	7	3	0	0	0	0	50%	1.3	0.65
7	F	20	11	5	3	1	0	0	0	45%	1.6	0.70
8	L	20	16	3	0	0	1	0	0	20%	1.8	0.35
8	F	20	17	1	2	0	0	0	0	15%	1.7	0.25
Mortalities	L	12	10	2	0	0	0	0	0	17%	1.0	0.167

[a] Included is individual level of infection, prevalence (% infected oysters), infection intensity (mean level of indection among infected oysters), and weighted incidence of infection (mean level of infection for the entire sample) for each sample. At the foot of the table, all mortalites tested during the study are combined in one sample.

[b] F, field sample; L, laboratory sample.

(1.4) was observed in the initial sampling (week 1), and the highest mean value (4.6) in the field sample at week 8. In the laboratory, a high value of 3.9 was observed in week 8. Gametic stage was significantly higher in each of the field samples than in the corresponding laboratory samples (Fig. 3).

Physiological Condition

Mean dry tissue-to-dry shell scores were significantly different among samples ($p < .0001$), with field samples higher than laboratory samples, and laboratory samples decreased after the first week (Fig. 3). The highest ratio (0.0284) was from the week 8 field sample. The week 1 initial ratio was 0.0241. Laboratory values after week 1 were not significantly different (Table 4).

Mean dry tissue-to-wet tissue ratios were significantly different among samples ($p < .0001$) (Fig. 3). The field samples from weeks 7 (0.216) and 8 (0.231) were highest. The laboratory values from weeks 7 and 8 were significantly lower than the corresponding field values (Fig. 3). The lowest laboratory value (0.141) was from the initial sampling at week 1.

The mean diverticula scores from each sample were significantly different ($p < .0001$). Scores of the field samples on weeks 7 and 8 were significantly lower than any score obtained for laboratory samples (Fig. 3). The lowest observed score (0.142) was from the week 7 field sample, and the highest (0.292) from the week 3 laboratory sample.

DISCUSSION

The goal of this project was to develop techniques for holding and conditioning of *C. virginica* in a recirculating system. To achieve this, water quality, levels of disease and mortality, gametogenesis, and physiologic condition were monitored over an 8-wk period. Although oysters have been held for extended periods in recirculating systems (Epifanio and Mootz 1976, Macmillan et al. 1994), to our knowledge, there are no reports of manipulation of gonadal maturation in such systems.

Water quality remained within desired ranges throughout the study. The required control of temperature for broodstock conditioning was achieved. However, ammonia concentrations approached stressful levels during weeks 2 and 3, as the temperature was increased to 25°C. It is likely that bacterial recolonization of the biologic filter was unable to keep pace with increased metabolic activity of the oysters, allowing nitrogenous wastes to accumulate. Five days after peaking on day 16, ammonia levels decreased to almost undetectable levels. With a higher stocking density (greater than 8.3 oysters/L), ammonia concentration could have reached a stressful level.

Although infections by *P. marinus* can cause extensive mortalities in *C. virginica,* and can reduce reproduction and physiologic condition (Hoffman et al. 1995, Kennedy et al. 1995), levels of this parasite were low throughout the experiment. The occur-

TABLE 4.

Gonadal development of *Crassostrea virginica* during the 8-wk study. Included is sample source and sample size, sex, number of individuals at each stage of development (1–6), and mean gametic stage for the entire sample

Week	Source[a]	n	Sex[b]				Gametic Stage						Mean
			M	F	H	U	1	2	3	4	5	6	
1	F	29	2	11	0	16	16	13	0	0	0	0	1.45
3	L	30	3	22	0	5	5	21	3	1	0	0	2.00
5	L	29	1	17	1	11	11	11	3	4	1	0	2.10
7	L	30	2	22	0	5	5	2	6	12	1	0	2.97
7	F	30	7	22	0	1	1	2	0	15	13	0	4.47
8	L	30	3	21	0	6	6	0	1	9	13	1	3.87
8	F	30	9	18	1	2	2	0	0	4	24	0	4.60

[a] F, field sample; L, laboratory sample.

[b] M, male; F, female; H, hermaphrodite; U, unidentifiable.

rence of infected individuals in Gulf Coast populations of *C. virginica* can be 100% (Craig et al. 1989). However, the initial infection incidence of *P. marinus* in the oysters in this experiment was 10% (Table 3). Although the technique used here to diagnose infection was less sensitive than other techniques (e.g., full body burden), especially with light levels of infection, high levels of infection would be obvious (Bushek et al. 1994). The mean level of infection for all samples remained less than 1.0 (lowest level of infection) for the entire 8 wks of the study. No mortalities during the experiment appeared to be due to *P. marinus* infection. Most mortalities (13) occurred during week 1 before the temperature was raised, and were presumably due to stress from harvesting, storage, transport, and stocking. The low level of mortalities (18 of 300 oysters stocked) and *P. marinus* infection suggest that other disease problems and severe stress due to poor nutrition or water quality were not present.

In healthy eastern oysters, gametogenesis would be expected to proceed at a water temperature of 25°C, and during the study, the majority of the oysters in the system reached maturity within 8 wks. Because gametogenesis in oysters in coastal waters begins during the time covered in this study (Shumway 1996), development was expected in the field controls. However, at the end of the study, oysters from the field control had a higher mean level of development. Nutrition is important in broodstock conditioning (Robinson 1992, Munanaka and Lannan 1984), and it is probable that oysters in natural waters obtained superior nutrition. Moreover, because artificial salt water was not replaced in the laboratory system, the oysters in the natural environment may have benefited from nutrients lacking or depleted in the laboratory. It should be noted that a mixture of algal species fed to oysters enhanced long-term growth in a recirculating system (Epifanio and Mootz 1976), whereas the oysters in our study were fed only a single algal species (resuspended from paste) at any time.

Factors causing oysters to utilize energy reserves, such as starvation, disease, spawning, or elevated temperatures, can cause a decrease in tissue weights and lower dry tissue-to-dry shell ratios (Mann 1978, Gabbot and Walker 1971). In this study, this condition index in the laboratory was reduced after week 1. The oysters came into the laboratory in good condition, remained in good condition in the field, but declined in the laboratory. Several explanations are possible. Spawning (Lucas and Beninger 1985) and *P. marinus* infection (Paynter and Burreson 1991) can cause decreases in dry tissue-to-dry shell ratios. Because little (if any) spawning occurred in the laboratory and field populations, and *P. marinus* infection was low in each, the likely causes for the poorer condition in the laboratory were temperature and nutrition. After week 2, oysters in the laboratory were exposed to an average temperature of ~25°C. A temperature-related increase in metabolic demand coupled with potentially poorer nutrition in the laboratory could have reduced condition. Previous studies with *Ostrea edulis* maintained for 4 wks in flow-through systems showed decreased condition compared with field oysters, attributable to higher temperature and less available food (Gabbot and Walker 1971).

Differences were observed when the ratio of dry tissue-to-wet tissue was considered as an indicator of physiologic condition. A high proportion of water in tissues (and the associated low ratio of dry tissue:wet tissue) indicates a state of depleted energy reserves, possibly from starvation, disease, or winter conditions (Lucas and Benniger 1985). The oysters apparently suffered stress before stocking, resulting in a decreased ratio in the initial sample (week 1). Oysters recovered in the system and ratios increased in the following wks (although ratios decreased slightly in weeks 7 and 8, they remained significantly higher than in the week 1 sample). The ratios from the field samples of weeks 7 and 8 were higher than in the laboratory samples from those wks. A decrease in this ratio has been reported for *C. virginica* in a food-limited environment (Rheault and Rice 1996), and nutritional problems in the laboratory could have caused the differences observed between the field and laboratory samples. The decrease in weeks 7 and 8 in the laboratory was associated with a shift in the species of algae being fed to the oysters. For the last 2 wks of the study, *Chaetocerus calcitrans* replaced *Isochrysis galbana* as the food source, possibly causing the observed decreases.

The final measurement of physiologic condition was based on morphology of digestive diverticula. This technique has been correlated with starvation or salinity stress, and provides a general indicator of stress in oysters (Winstead 1995). The tubule ratios from weeks 1 and 3 were high as the oysters became acclimated to the increased temperature and artificial environment. Scores then became significantly lower, indicating reduced stress on the oysters in the laboratory, and possibly acclimation to the laboratory environment. However, scores obtained from the field samples were significantly lower than the laboratory samples in weeks 7 and 8. These scores agreed with the other measures of condition and suggested that the oysters in the laboratory were more stressed than those in the field. Again, a probable explanation is that field oysters received a superior diet.

In this study, gamete development was obvious in oysters held in the recirculating system with temperature manipulation. Water quality was maintained despite temperature changes, and mortality and disease were not problems. Oysters from field controls achieved a higher level of gametic development in 8 wks, and had a better physiologic condition, perhaps due to a more complete diet. This indicates a need for nutrition improvement, but demonstrates that broodstock conditioning of *C. virginica* is possible in a closed, recirculating system.

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