Identification of Oyster Broodstock Conditioning Parameters that Minimize Inhibition of Gametogenesis by Dermo Disease

A Report to the

New Jersey Fisheries and Aquaculture Technology Extension Center

by

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INTRODUCTION

Hatchery operators providing eyed larvae or seed from MSX-resistant broodstock to the Maurice River Oyster Culture Foundation and other New Jersey growers must now contend with a second disease, Dermo, in the broodstock. Perkinsus marinus, the causative agent of Dermo, multiplies rapidly during the high temperature conditioning regime used to prepare oysters for spawning. Preliminary studies during 1991 suggested that the time of year when broodstock are collected from the field, and the temperature at which they are conditioned, may affect the speed with which Dermo proliferates relative to the speed with which gametes mature.

In this project, we assessed the effect of two collection dates (January and March 1992) and two conditioning-temperature regimes (22-25° C and 26-30° C), on Dermo disease development and gametogenesis in native Delaware Bay oysters that had been exposed to Dermo in 1991. Our results showed that Dermo intensity increased during the conditioning period after both collection dates, with no consistent differences between temperature regimes. In contrast, gonadal development was greater after the second collection date, especially in the warmer temperature tank, where a spontaneous spawning occurred after 50 days (740 degree days) of conditioning.

MATERIALS AND METHODS

Oysters were collected from Ground 455D on the New Jersey planting grounds on two dates: January 13 and March 21, 1992. We had been following the development of Dermo disease in these oysters since late summer 1990 and knew that by autumn 1991, prevalence had reached 100%. The majority of oysters on this ground were three and a half years old; we selected those with shell height of 60-80mm.

Two hundred and forty oysters were selected from those collected at each date (January collection - Experiment I; March collection - Experiment II). Of these, 100 were individually labelled, their shells were notched, and a hemolymph sample was cultured in fluid thioglycollate for an initial Dermo diagnosis (Gauthier & Fisher 1990). We rated the number of parasites in the initial sample from Experiment I in a semi-quantitative manner (stages 0-5 according to Mackin (1962)); thereafter, we counted parasites in hemolymph subsamples and extrapolated to cells per milliliter.

From the remaining 140 oysters, 20 were diagnosed for Dermo by two methods: hemolymph (quantitative as described above) and rectal/mantle tissue (Mackin's semi-quantitative ratings of 0-5). The Mackin ratings for individual oysters were summed and divided by the number of oysters in the sample to obtain a weighted prevalence for the sample. Subsequently, each individual was fixed in Davidson's fixative, sectioned, and examined for MSX (Haplosporidium nelsoni) disease intensity and gonadal development.

The oysters from each collection were maintained in the Maurice River Oyster Culture Foundation (MROCF) broodstock conditioning facility at Shellpile, NJ for 8-9 weeks. The facility is an unheated greenhouse with two 50-gallon tanks (used to hold the oysters) and two 100-gallon tanks (used to bloom algae) that were filled with water pumped from the Maurice River. Water was warmed with 300-watt submersible, thermostatically controlled heaters. During the second half of the study, we used shades to decrease solar heating in the greenhouse. Half the oysters (50 labelled and 60 unlabelled for each experiment) were held under a temperature regime of approximately 22-25° C and the other half, at approximately 26-30° C.

After the initial collection, oysters were sampled as follows during each week experiment:

1) At 4-week intervals, hemolymph screening was performed on all surviving labelled oysters.

2) At 1- to 2-week intervals, 10 unlabelled oysters from each tank were diagnosed for the presence of Dermo in hemolymph, then sacrificed for diagnosis by rectal and mantle culture, and finally fixed for determination of reproductive state (gonadal index) and MSX infection. Gonadal index was measured, using image analysis, as the ratio of the cross-sectional area containing gametes to the total visceral mass area (Barber et al. 1988).

3) Gapers (recently dead oysters still containing soft tissues) were collected at each water change and examined for Dermo by rectal/mantle culture. All were subsequently fixed in Davidsons for diagnosis of MSX if deemed necessary.

Water was changed daily six days a week and heated to the desired temperature before replacement. Algae was usually present in the incoming water and we added commercial nutrients (Fritz) to enhance blooming. When natural food was scarce, we seeded algal tanks with cultured algae. Temperature and salinity were recorded before each water change. We converted temperature into degree days by subtracting the measured temperature from 12° C. The latter temperature was described by Price and Maurer (1971) as the threshold below which they observed no gonadal development in Delaware Bay oysters and was their baseline for calculating degree days for reproductive conditioning.

The concentrations of Dermo cells in the hemolymph were $\log_{10}$ -transformed, and gonadal indices were arcsine-transformed, before statistical analysis, which was performed using a general linear model analysis of variance.

RESULTS

Experiment I - January Collection

Water temperature at the collection site on January 13 was 5.5^{o} C and salinity was 20 ppt. There was considerable variability in the temperature recorded in both conditioning tanks during the subsequent 8-

week study (Fig. 1). The mean in the low temperature tank was 22.5° C; that in the high temperature tank was 25.9° C. The number of degree days at the end of the experiment was 520 in the low temperature tank and 690 in the high temperature tank, a difference of 170 degree days (Fig. 2). Salinity ranged between 17 and 20 ppt.

The weighted prevalence of parasites in rectal and mantle tissues more than doubled, from 1.0 to about 2.5 during the first two weeks at elevated temperature, remained relatively stable over the next month, and finally increased again to about 3.5 during the last two weeks (Fig. 3). In contrast, there was a steady increase in the mean concentration of parasites in the hemolymph from 23,000 ml^{-1} in the 2-week sample to 600,000 ml^{-1} after eight weeks (Fig. 4). For the most part, there were few differences between the two temperature regimes. This was underscored by similarities in cumulative mortality, which increased steadily in both tanks to about 50% by the end of the conditioning period (Fig. 5). Nearly all of the gapers were infected with Dermo, with intensities usually from 2 to 5, especially after the first two weeks. The steady loss of the most heavily infected animals prevented the weighted prevalence in samples of live oysters from increasing during most of the conditioning period.

MSX was detected in 20-30% of oysters in the 2-week sample (February 3), but decreased during the course of the study. We have not yet been able to process slides of gapers to determine MSX prevalence, and it is possible that some of the early mortality was due to this disease rather than to Dermo

Gonadal maturation began almost immediately after the oysters were placed in the conditioning tanks, although it was very slow. After one month, half of the oysters had gonads that were measurable by image analysis of tissue sections, but there was no further development thereafter (Fig. 6). The best developed individuals had gonads that occupied 25% to 35% of the cross-sectional area of the visceral mass, but the mean of all individuals was only 4-5%. No spawning was detected.

Experiment II - March Collection

Water temperature at the collection site on March 21 was 4.0° C and salinity was 14 ppt. It was more difficult to control tank temperatures during this experiment, compared to the first, as outside air and water temperatures increased and it became more critical to cool than to heat the water. The means were 25.1° C and 27.1° C in low and high tanks, respectively, a differential of only 2° C with much fluctuation and overlap (Fig. 1). The number of degree days at the end of the experiment was 723 in the low temperature tank and 855 in the high temperature tank, a difference of 132 days. Salinity ranged between 15 and 20 ppt.

Weighted prevalence, which was nearly 2.0 in the initial sample, did not change during the first six weeks of conditioning (Fig. 2). As in the first experiment, there was an increase during the final two weeks, to between 3.5 and 4.0. In contrast to the first experiment, however, the concentration of parasites in hemolymph, which averaged 9,000 ml^{-1} when the oysters were placed in the system, appeared to decrease somewhat over the next month before increasing to a mean of about 200,000 ml^{-1} in the final sample.

There were no differences in Dermo levels between temperature regimes, but cumulative mortality was only about half as great (26%) in the low temperature as in the high temperature tank (45%) (Fig. 5). Most of the differential was established during the second week of the experiment. Although most of the oysters that died during this period had Dermo, the weighted prevalence (1.4) of all gapers combined was no greater than in live oysters sampled before and after (see Fig. 3) indicating that the disease was most probably not the primary cause of death. [Had it been, we would have found disease levels in dead oysters to be much higher than in the live ones]. During the remainder of the experiment, however, most of the gapers had intensities of 2-5. MSX was detected in only 3% of all live oysters examined and was not considered an important factor in Experiment II.

Gonadal maturation was more rapid in the second experiment than in the first. By the second week of conditioning, 80% of the oysters had measurable (by image analysis) gonads. Although maturation appeared to be more rapid at the high temperature (Fig. 6), there were no statistically significant ($p > 0.05$) differences between tanks. Nevertheless, a spontaneous spawning occurred in the high temperature tank on May 13, after 740 degree days, and most of the oysters were spawned out in the final sample. No spawning was recorded in the other tank.

Relationships between disease measures, survival, and gametogenesis

In both experiments, there was a statistically significant ($p < 0.001$) relationship between the two measures of disease intensity (i.e., quantitative in hemolymph and semi-quantitative in rectal and mantle tissues) (Fig. 7). In the first experiment, there was a regular (exponential) increase in hemolymph parasite concentration for every category increase in the Mackin scale. In the second experiment, hemolymph parasite concentrations showed a step-like increase relative to the Mackin rating: categories of 0.5 and 1 were similar, categories 2-4 were alike, and category 5 formed a third level (Fig. 7). These relationships were not affected by temperature regime or date of collection.

In the initial sample of the first experiment, hemolymph from 9 of 100 individually labelled oysters had Mackin ratings of 3 or greater. Seven died during the first two weeks of conditioning and the remaining 2 died within a month. The next screening, one month later, quantified the numbers of parasite in the hemolymph, as did all screening in the second experiment. In these screenings (both experiments), 18 individuals were found with parasite concentrations of 1at least 00,000 ml^{-1} (some had several million). Fourteen of these died within two weeks. There was no correlation between parasite loads and survival time for oysters with concentrations of less than 100,000 ml^{-1}. Most individuals that were negative for hemolymph parasites during the initial screenings eventually developed diagnosable infections. Only 2 of the 44 surviving oysters in the first experiment and 4 of 59 in the second were still without detectable hemolymph parasites in the final sample.

We found no consistent relationships between the level of Dermo, the conditioning temperature, or the date of collection - or any interactions among them - and the gonadal index. During the first experiment, there was a positive correlation ($p < 0.004$) between degree days and gonadal index, but the r^2 value was very low (0.08) indicating that degree days alone explained very little of the total variance among gonadal indices. No statistically significant relationship existed in the second trial; nevertheless, the degree days (740) at the time the high temperature oysters spawned in the second experiment was never reached by any other group (see Fig. 2).

DISCUSSION AND CONCLUSIONS

Of the two variables, collection time and conditioning temperature, that we tested during this study, the former appeared to be more critical for ensuring gamete maturation in oysters infected with Dermo disease. Not only did the gametes develop faster and to a greater degree in oysters collected in March compared to those collected in January, but oysters in the high temperature tank spawned naturally. Parasite loads, although higher to begin with in the second experiment, did not increase as rapidly in the second experiment and overall mortality was lower. We do not know what caused the disparity in mortality between tanks during the second experiment. Neither Dermo nor MSX was implicated.

Although there appeared to be some differences between high and low temperature tanks in the rate of gametogenesis and disease development, these were not consistent and could not be verified statistically. The fact that spawning occurred in the high temperature tank after a number of degree days that was never reached by other groups supported the indication of greater gonadal development in this tank, but this evidence should be viewed with caution as we did not verify it with replicated treatments.

Hemolymph diagnosis of potential broodstock previously exposed to Dermo will detect animals likely to die within a week or two, and these can be discarded immediately. Animals recorded as negatives during the winter or early spring are likely to have subpatent infections that will proliferate almost immediately under conditioning temperatures used in our study and will result in continuing mortality of oysters as they are conditioning. Nevertheless, oysters collected in March and conditioned at a mean temperature of 27° C spawned in 7 weeks, when half of the starting oysters were still alive, although fewer than half of these would have been ripe enough to spawn judging by the gonadal indices in the sample taken the previous week.

We are puzzled that we found no correlation between Dermo intensity and gonadal indices in the experimental oysters. It seems a reasonable hypothesis that Dermo disease, like MSX (Barber et al. 1988), would diminish the reproductive capacity of oysters. A possible explanation for our results is that overall Dermo pressure on all the oysters was so great that the effect of variations in intensity could not be measured. Also, food quantity and quality in the conditioning tanks may not have been sufficient to support the best gamete development, regardless of disease intensity. A third possibility is that Dermo infections harbored in autumn 1991 (which then became subpatent over the winter) reduced the quantity of stored glycogen available for gametogenesis during our conditioning experiments. Regardless of the reason, the "reproductive condition" of the oysters in our tests was relatively low. In samples of oysters with neither MSX nor Dermo diseases, which conditioned naturally on the Cape Shore flats, maximum gonadal indices may be nearly 50, compared to those in our experiment, which were less than 10 (see Fig. 6).

It would have been more desirable, in terms of experimental design, to have had better control of temperatures in the conditioning tanks, and it might have been more prudent biologically to raise temperature gradually rather than in a single step. On the other hand, this study was performed to answer a question of practical value to hatchery operators who might not have much better control than we did. In the case of the Maurice

River Oyster Culture Foundation, the facility used is the very one that has been and will be used to condition broodstock. Although we do not consider this a definitive study, its results suggest that a later collection period is more advantageous than an early one, and that a higher conditioning temperature may be better than a lower one.

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FIGURE LEGENDS

Figure 1. Water temperatures recorded daily (except Sunday) in the "high" and "low" temperature tanks at the Maurice River Oyster Culture Foundation conditioning facility at Shellpile, N.J. during the two experiments. Horizontal lines and numbers at right represent the mean temperatures in each tank. The occasional extreme low temperatures are dates when the heaters failed.

Figure 2. Cumulative degree days recorded during the study. The degree days for each day were obtained by subtracting 12° C from each daily temperature (see text). This value was then added to the sum of degree days up to that date.

Figure 3. Mean (+/- S.E.) weighted prevalence of Perkinsus marinus (Dermo) infections as determined by fluid thioglycollate culture of rectal and mantle tissues during conditioning experiments. Infection ratings are on a scale of 0 (no parasites seen) to 5 (heavy parasitism) according to Mackin (1962). N = 10 for each sample.

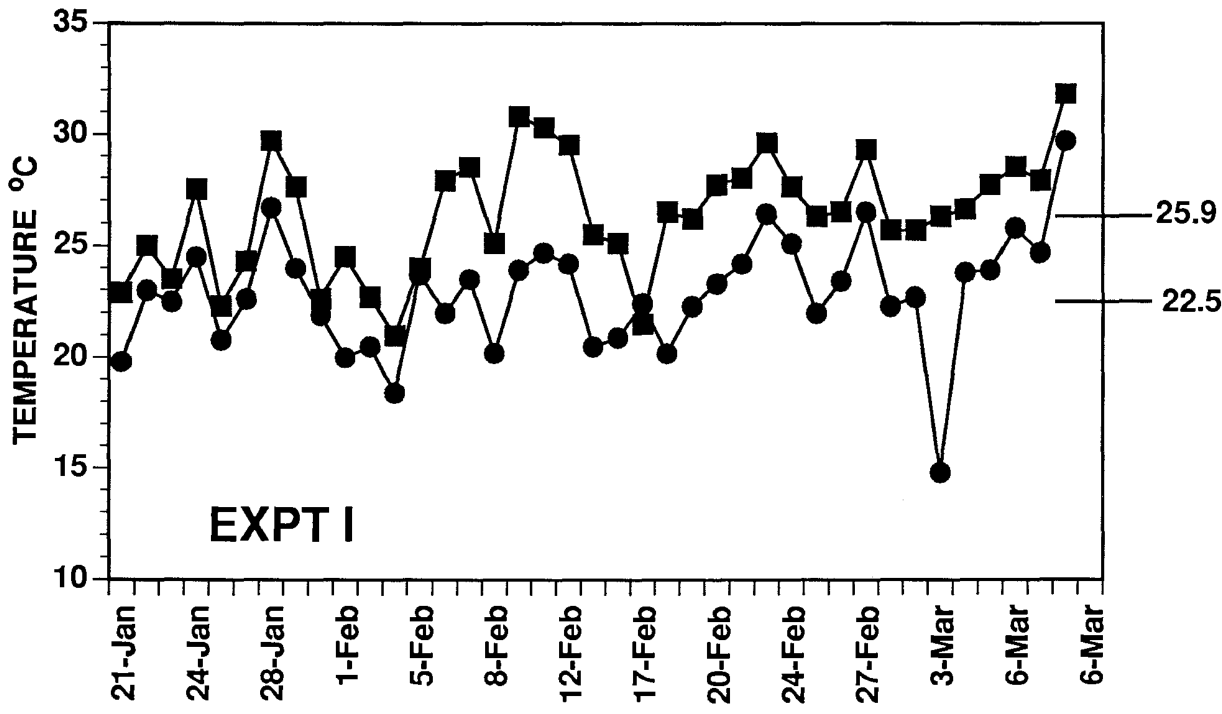
Figure 4. Mean (+/- S.E.) concentration of Perkinsus marinus (Dermo) cells in hemolymph of conditioning oysters. N = 10 for each sample.

Figure 5. Cumulative mortality of individually labelled oysters during conditioning experiments. N = 50 per temperature regime at the start of each experiment.

Figure 6. Mean (+/-S.E.) gonadal indices, measured as the ratio (x 100) of the cross-sectional area of developing gametes to the total visceral mass area in conditioning oysters. N = 10 for each sample.

Figure 7. Mean (+/-S.E.) of hemolymph parasite concentration grouped according to Mackin rating category of cultured rectal and mantle tissues.

FIGURE 1



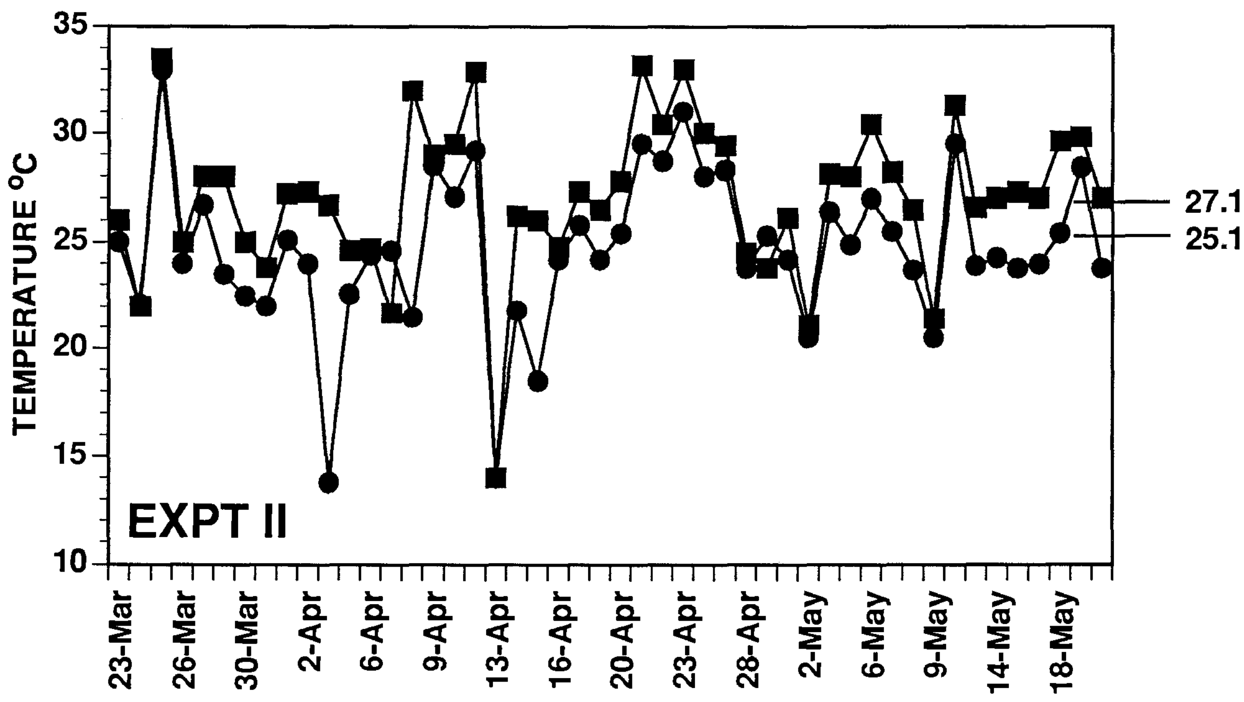
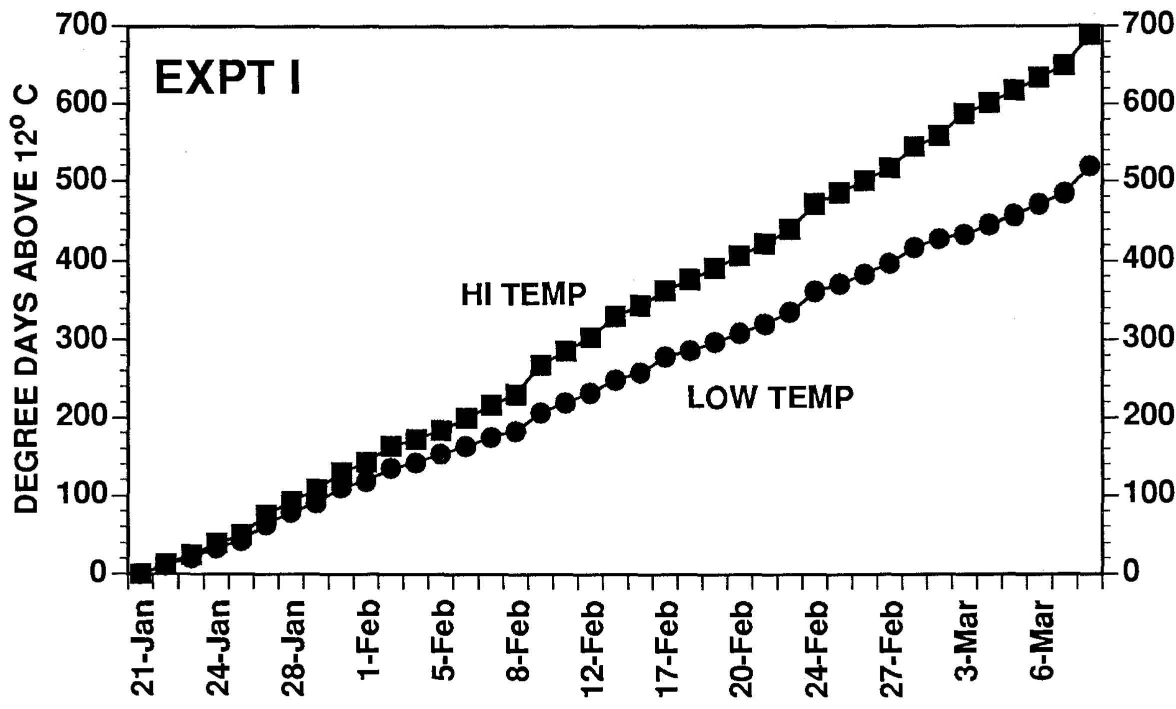


FIGURE 2



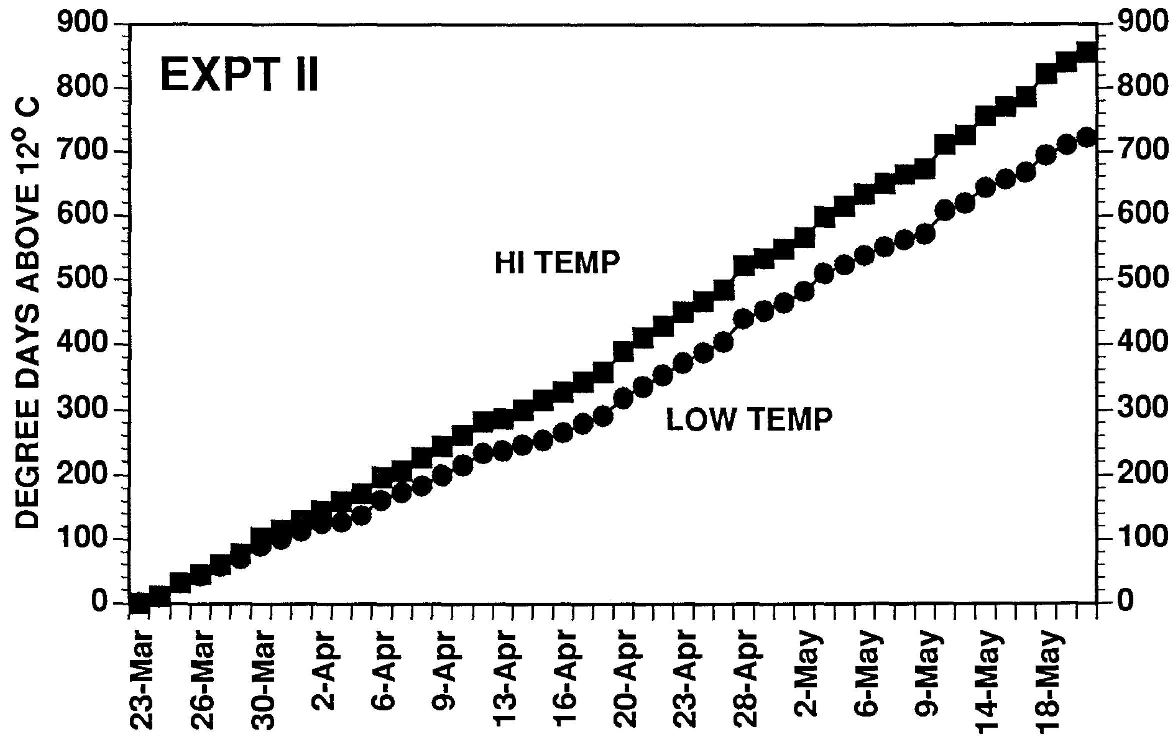
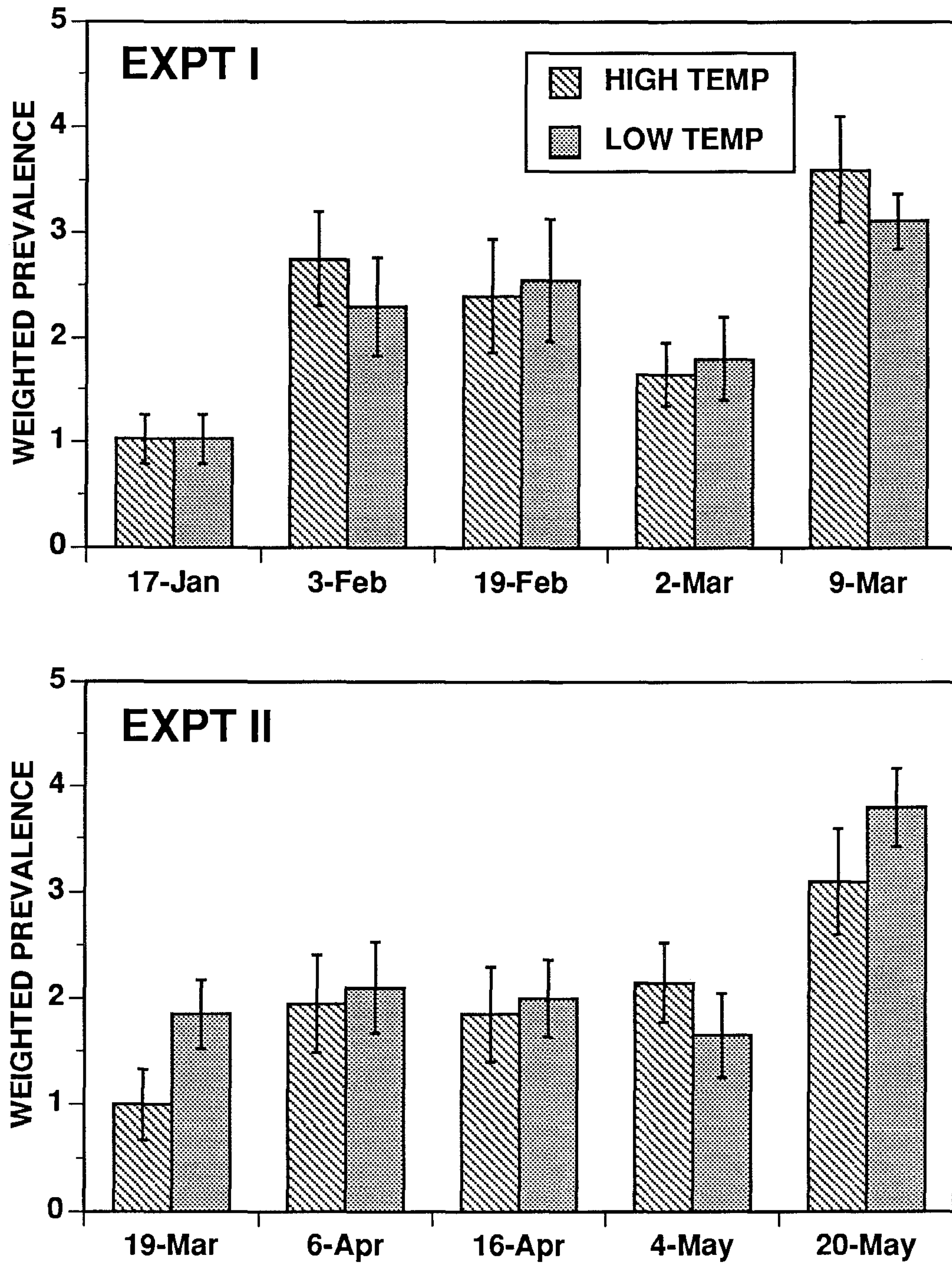


FIGURE 3



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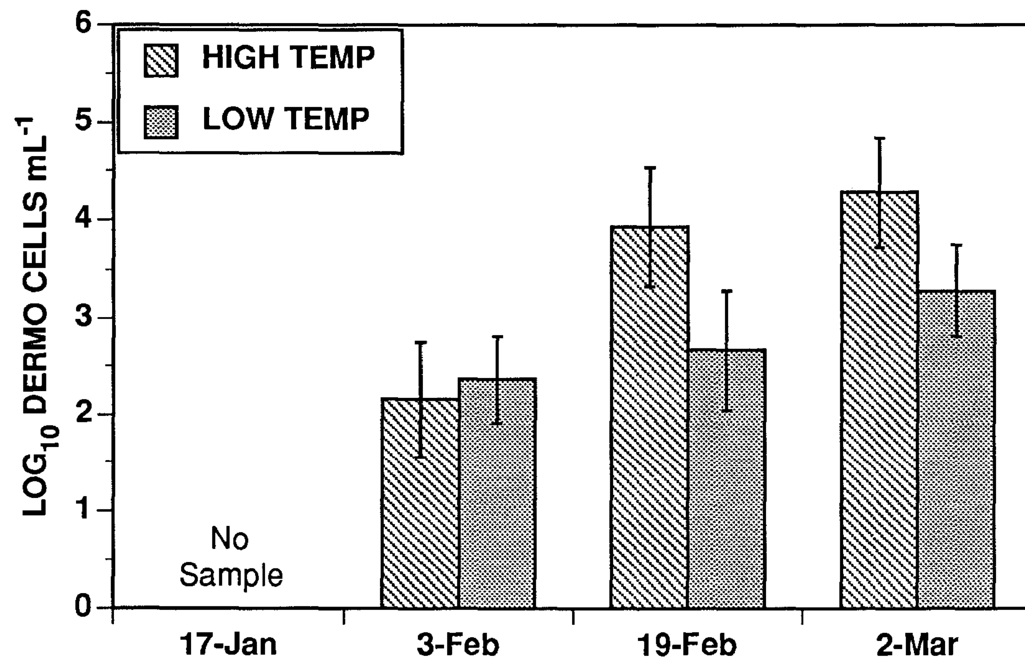


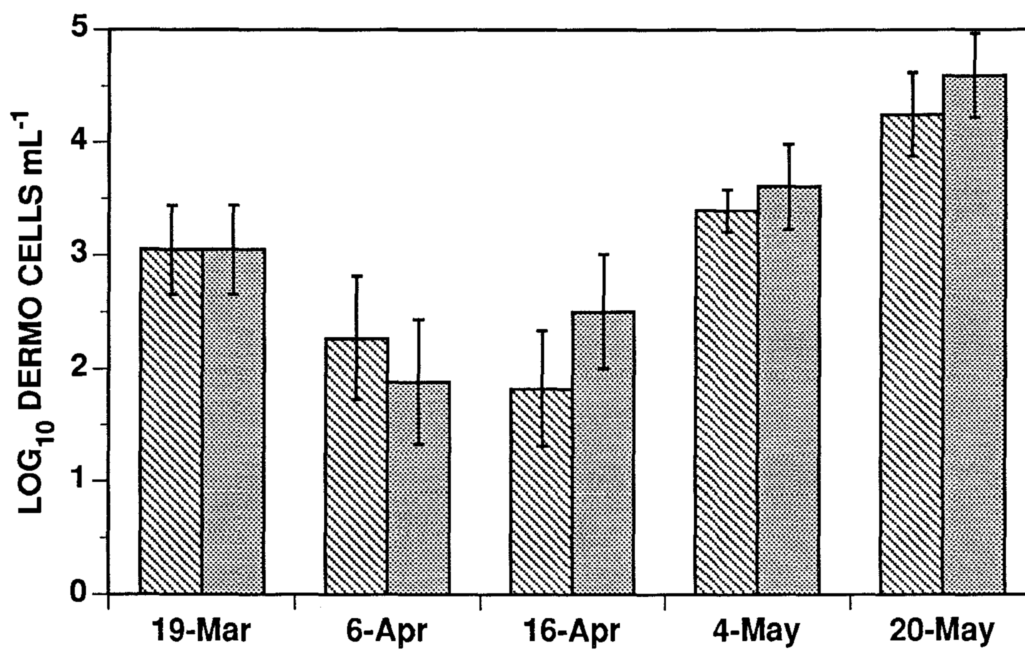
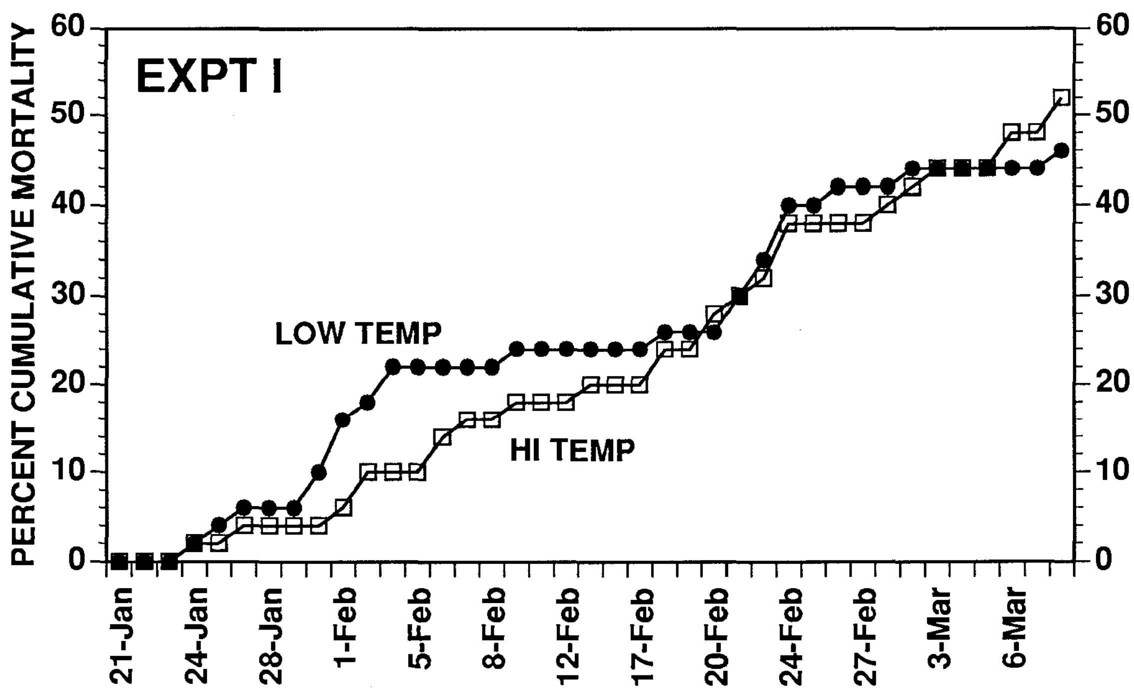


FIGURE 5



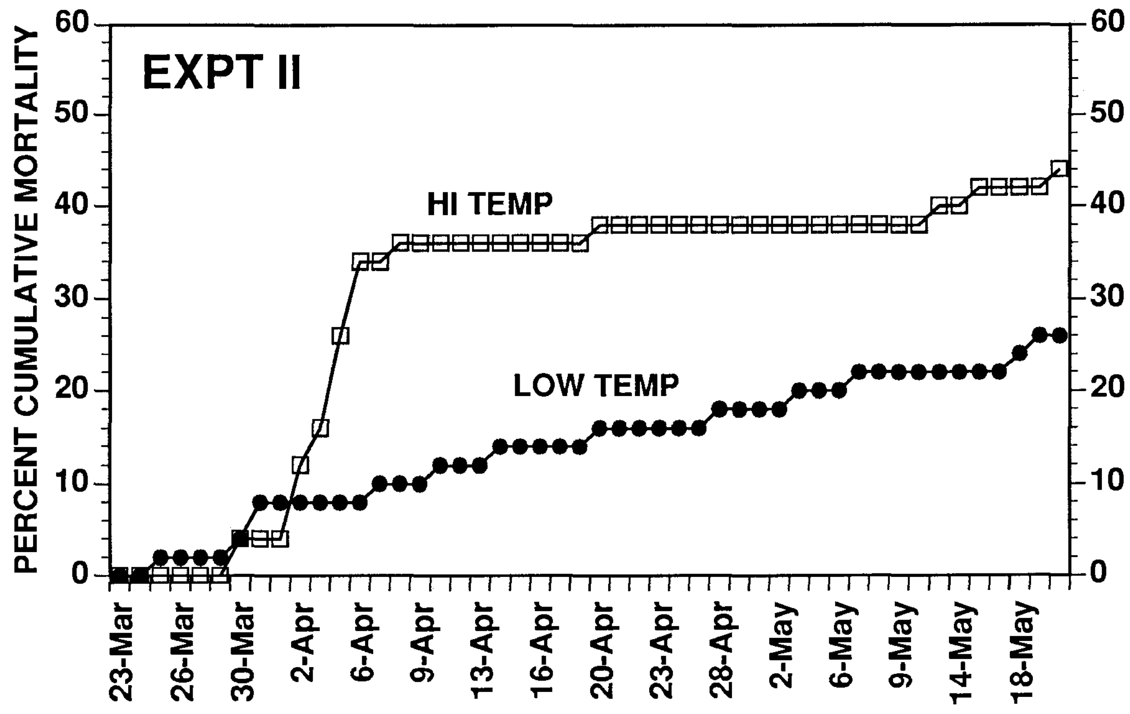
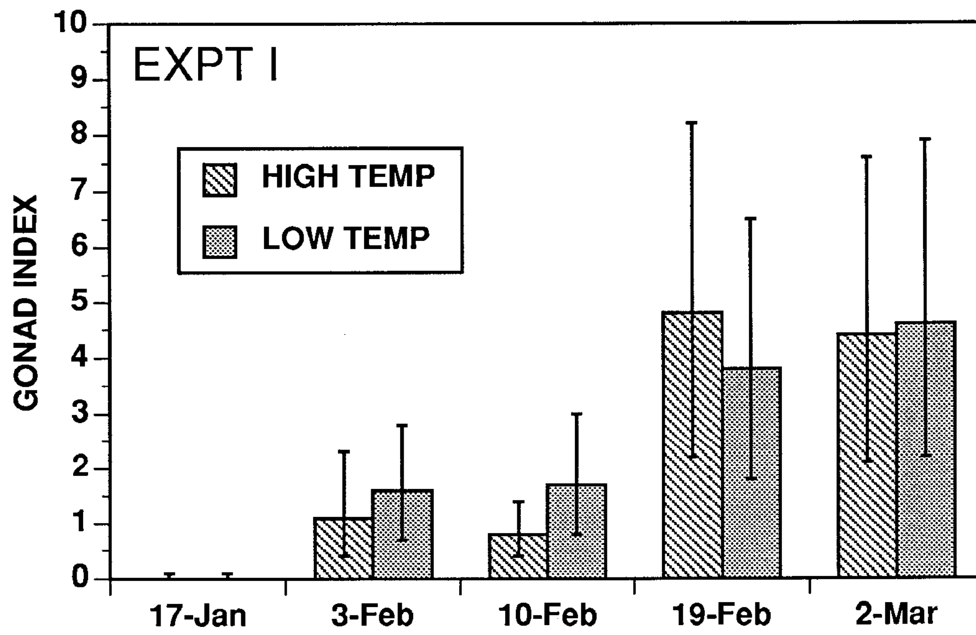


FIGURE 6



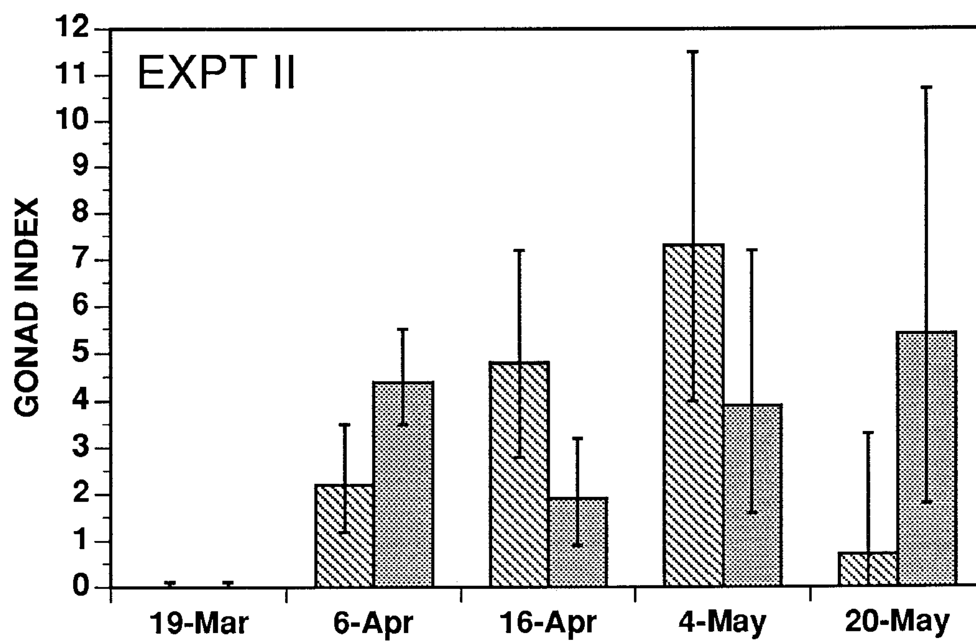


FIGURE 7