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Mictic patterns of the rotifer *Brachionus plicatilis* Müller in small ponds

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Abstract

Populations of the rotifer *Brachionus plicatilis* were monitored in three small ponds in a marsh on the Mediterranean coast. Samples were taken approximately every three weeks from July 1992 to November 1993. Salinity, temperature, conductivity, pH and oxygen concentration were measured in the field. Population density was determined from preserved quantitative samples. Individuals were classified as mictic females, amictic females, non-ovigerous females, and males, differentiating between two morphotypes ('S' and 'L'). From these counts, a level of mixis was calculated. We also determined the proportion of mictic females in natural populations by culturing females isolated from fresh samples. From these data, mictic patterns over time and correlation between levels of mixis and environmental and population parameters were analyzed. From a previous study 'S' and 'L' morphotypes were known to correspond to genetically different clonal groups. Our data showed that reproduction was predominantly parthenogenetic in these clonal groups, but mictic females were found in most samples, the proportion of mictic females ranging from 0 to 29%. The clonal groups showed different patterns of mixis. L clonal group presented a continuous sexual reproductive pattern. In contrast, S clones showed a rather punctuated mictic pattern. A positive correlation between levels of sexual reproduction and population density was found for S and L groups. However, they differed in their density threshold for mictic reproduction. The adaptive meaning of these patterns and their implications in maintaining genetic diversity within and between populations are discussed.

Introduction

In recent years an increasing number of laboratory studies has addressed mixis in rotifers, stressing the role of environmental conditions and internal factors on mixis induction (for reviews see Gilbert, 1977; Pourriot & Clément, 1981; Pourriot & Snell, 1983). In contrast, field studies about occurrence of sexual reproduction in rotifer natural populations are scarce, and only a few have provided quantitative data on the observed degree of sexual reproduction. Most of the older literature has been reviewed by Gilbert (1974) and King (1980). However, while mixis studies in the field have received little recent attention, insights into mixis can be obtained from studies of natural populations, particularly by analyzing mixis patterns over time, correlating mixis level with environmental and population parameters and studying the implications of mixis in maintaining genetic diversity within and between populations.

In this study we have monitored populations of *Brachionus plicatilis* O. F. Müller 1786 in three small ponds within a marsh of the Mediterranean coast. We focussed on quantification of the level of sexual reproduction.

Materials and methods

The study was conducted in three shallow ponds (Poza Sur, Poza Norte and Canal Central) located in the southern part of Torreblanca Marsh (Castellón, Spain; 40 ° 10′ 04″ N, 0 ° 10′ 57″ E). Torreblanca Marsh is a brackish area near the seashore and composed of small ponds and canals that may flood in winter. The three sampling sites are man made drainage ponds, for which maximum area ranged from 88 to 210 m^2, and maximum depth between 0.8 and 1.5 m. All three ponds can dry up in summer.

Samples were taken, at 10–11 a.m., approximately every three weeks from July 1992 to November 1993. Salinity, temperature, conductivity, pH and oxygen concentration were measured in each sampling site. Since ponds were shallow, plankton samples were obtained by sweeping a 1-liter plastic container several times through the water. The water was sieved through a 30 μm Nytal mesh, which according to our observations retains effectively female and male rotifers. Live rotifer samples were carried in pond water to the laboratory. For quantitative samples, 2–4 liters of pond water were filtered and the sample fixed with Lugol's iodine.

The incidence of sexual reproduction in natural populations was determined by performing the following procedure. If possible, up to 96 females of *Brachionus plicatilis* were randomly picked from each live sample. Within 5 hours after water collection, females were placed individually in wells (24-well plates, Costar, USA) containing 1 ml fresh culture medium. Culture medium was *Tetraselmis suecica* algae growing in 9 or 12 g l^{-1} Instant Ocean artificial sea water enriched with f/2 medium (Guillard & Ryther, 1962). Females were kept at 25 °C and constant illumination (PAR: approx. 35 μEm^{-2} s^{-1}) and they were monitored daily until their first offspring appeared. Each female was classified as mictic, if she gave rise to resting eggs or male progeny, or amictic, if she gave rise to female offspring. The proportion of mictic females in the cultures was used as an estimate of mictic ratio in the field. Isolated amictic females produced clones through parthenogenesis. In a parallel study (Gómez *et al.*, in press), these clones were screened for allozyme variation, and, thus, information about the presence of groups of related genotypes (clonal groups) is also available.

From preserved quantitative samples population density estimates of *B. plicatilis* were obtained by counting with a CK2 Olympus inverted microscope at 100 × magnification using sedimentation chambers. When population densities were high, samples were sub-sampled at random before counting. Individuals were classified as mictic females, amictic females, non-ovigerous females and males, differentiating for two morphotypes on the basis of body size and shape, particularly the shape of anterior spines (the so-called 'S' and 'L' types; Ito *et al.*, 1981; Hirayama, 1985). Amictic and mictic females were scored by their egg type: amictic parthenogenetic (large and light brown), mictic parthenogenetic (small and light brown) and mictic resting eggs (large, dark brown and thick-walled). From these counts, sex-ratio (ratio of males to females), and mictic ratio (proportion of ovigerous females that were mictic) were calculated. In this way we obtained a second estimate of the proportion of mictic females in rotifer natural populations.

To compare mixis estimates from live and fixed samples a multiple comparison using G-tests (Sokal & Rohlf, 1981) was carried out, the significance level being modified according to Bonferroni (see Weir, 1990). Relationships between levels of mixis and environmental and population parameters were analyzed by a correlation analysis using SPSS for Windows release 5.0.2. Pearson's correlation coefficient was calculated to evaluate correlation between (1) mictic ratio and (2) values of limnological parameters and variation rate of these parameters. Parameter variation rate was computed as $(P_{i+1} - P_i)\ /\ (P_i \,.\, d_i)$, where P_i is the value of the parameter P in the sampling i, and d_i is the number of days from i to $i+1$. Population density values were log-transformed before use in the analysis. A correlation analysis was also performed to explore the relationship between sex-ratio and mictic ratios for 'S' and 'L' morphotypes. An ANOVA analysis on the ratio between males and mictic females for both groups ('L' and 'S') was performed using SPSS for Windows release 5.0.2. This routine was also used to perform an ANCOVA on mictic ratio for both groups using population density as a covariable. Integration of the number of total females and mictic females with respect to time was calculated as

$$\sum_i d_i(N_i + N_{i+1})/2,$$

where N_i is the population density of sample i and d_i the number of days from i to $i+1$. Density of mictic females in the population was estimated from the total number of females by using mictic ratios.

Results

The studied ponds, Poza Sur, Poza Norte and Canal Central, offer to rotifer populations an environment with a high level of temporal heterogeneity. First, all three have an unpredictable character: although the ponds remained filled with water throughout summer 1992, they dried up in summer 1993. Second, they show a wide variation in limnological parameters stressed by dry periods in summer and fall-winter floods. Poza Sur is the most saline pond and salinity values varied from 5 to 64 g l^{-1}. The pond was warm during the study period, with winter lows of 8 °C in 1992 and 13 °C in 1993, and summer highs of 25 and 24 °C. The pond is slightly alkaline with pH values between 6.9 and 8.5. Oxygen values ranged between 0.2 and 15.1 mg l^{-1}. Poza Norte and Canal Central had a lower cyclic variation in water salinity, variation being around 5 and 3 g l^{-1} of salinity respectively. Temperature values ranged between 8 and 28 °C in Poza Norte and between 8 and 25 °C in Canal Central. As in Poza Sur, water in these two ponds was slightly alkaline. Oxygen concentration in Poza Norte ranged between 0.3 and 18.1 mg l^{-1} and Canal Central between 3.2 and 19.6 mg l^{-1}.

In Poza Sur, *B. plicatilis* was found on most sampling dates, population densities being highly variable throughout the annual cycle and ranging from 3 to 16 700 ind l^{-1}. Population densities peaked in late summer and fall and crashed in January and May 1993. In Poza Norte, this species was present from the end of summer to the middle of fall 1992, and in fall 1993 with population densities never exceeding 4 individuals per liter. Finally, in Canal Central, *B. plicatilis* was detected in some preliminary surveys, but during the study it was only found on a single sampling date, September 1992, density being 1200 ind l^{-1}.

Individuals from samples taken in Poza Sur were identified on a morphological basis as belonging to either 'S' or 'L' morphotype. There are remarkable shape and size differences between morphotypes. Measurements performed on laboratory cultures indicated that average lorica length of females ranged from 117 to 163 μm for 'S' individuals and from 220 to 237 μm for 'L' individuals. Both morphotypes were also found in Poza Norte, but only individuals of 'S' morphotype were found in Canal Central. The parallel allozyme study performed with clones isolated from live samples revealed that clones can be grouped into three clonal groups according to their genetic similarities (Gómez *et al.*, in press). Results from this study established a clear correspondence between clonal group named L and strains with 'L' morphotype, while strains with 'S' morphotype were found to be genetically heterogeneous, the morphotype clustering in two clonal groups, named by these authors SS and SM clonal groups. No evidence of gene flow between clonal groups was found. In this paper we refer to SS and SM clonal groups as S clonal groups, because when the population is a mixture of SS and SM clones it is not possible to discriminate readily between the two genetic groups using morphological characters.

During the study, L clonal group was present in Poza Sur from October 1992 to May 1993 (Fig. 1A). This group reappeared in October 1993 after pond refilling. Density peaked in fall (October 1992: 1600 ind l^{-1}; November 1993: 420 ind l^{-1}). L clonal group was found at water temperatures lower than 20 °C and high oxygen concentrations, according to its fall-winter presence in Poza Sur. Nevertheless, this group occurred over a wide range of salinities.

S clonal groups were present in Poza Sur from the beginning of the study in July 1992 to November 1992, when it was replaced by group L (Fig. 1B). During this period density of the S groups reached a maximum (15 100 ind l^{-1}) coinciding with group L first density peak. S clones reappeared in May 1993, then having a high density (847 ind l^{-1}) and replacing L group. S clones remained until population extinction caused by the Poza Sur summer drought in 1993, density peaking again (785 ind l^{-1}) just before extinction. After the pond refilling, S individuals were not detected in Poza Sur. S groups were found over a wide range of salinities but at higher temperatures and lower oxygen concentrations than L group as would be expected in spring-summer groups.

Lower panels in Fig. 1 (A and B) show the change of mictic ratio over time for L and S groups respectively. Since estimated frequencies of mictic and amictic females were found to be independent of the estimation procedure (two way independence G-test, $P>0.05$), mictic ratios on Fig. 1 were calculated by pooling mictic and amictic female counts from live and preserved samples.

Reproduction is predominantly parthenogenetic in both groups, mictic females were found in most samples but the proportion of mictic females was never more than 29% of the reproductively classified females. With only three exceptions for each group of clones, all the samples contained males, sex-ratio being lower than 0.14 in the L group and lower than 0.07 in the S groups. No correlation was found between sex-ratio and mictic ratio in either group. ANOVA on the ratio of males to mictic females showed no significant differences between L and S groups ($F=1.77$; df$=1$; $P=0.31$).

L clonal group presented a relatively high incidence of sexual reproduction throughout its presence in Poza Sur (see lower panel in Fig. 1A). Thus, in 67% of cases mictic ratio was higher than 0.05, values ranging between 0.05 and 0.23 if considering only samples in

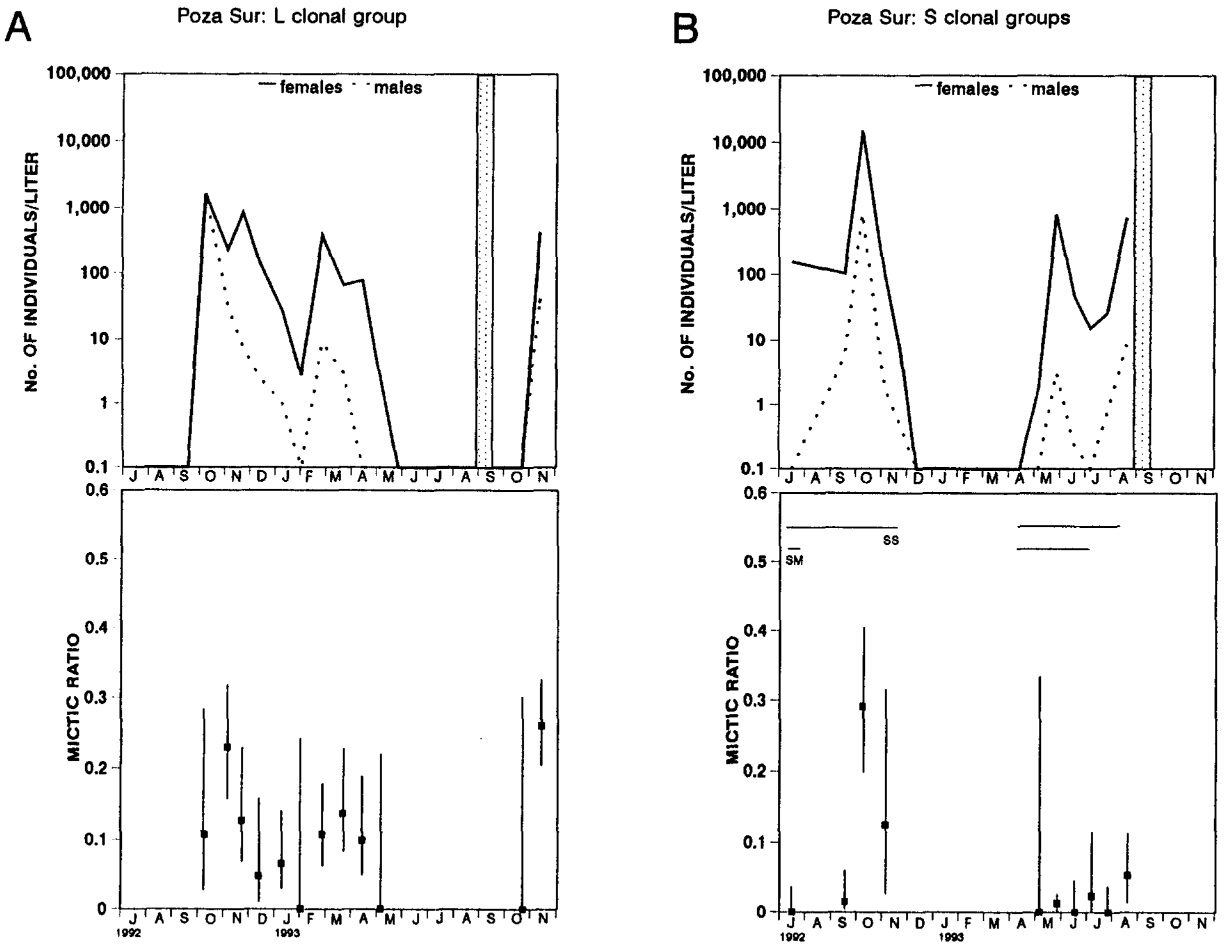

Fig. 1. A: Upper panel shows temporal changes in density of *Brachionus plicatilis* L clonal group in Poza Sur. Variation of mictic ratio over time for L clonal group is showed in lower panel. B: Upper panel shows temporal changes in density of *Brachionus plicatilis* S clonal groups in Poza Sur. Variation of mictic ratio over time for S clonal groups is shown in lower panel. Horizontal bars indicate presence of SS and SM clonal groups in the pond (see explanation in the text). Stippled areas indicate periods when the pond was dry. Vertical bars are 95% confidence intervals.

which number of females classified as mictic or amictic was higher than 20. In contrast, S clonal groups showed low mictic ratios most of the time that they were present in the pond (see lower panel in Fig. 1A). Only in 20% of cases was mictic ratio higher than 0.05, values ranging between 0 and 0.29 in samples in which the number of females classified as mictic or amictic was higher than 20. Periods with high levels of sexual reproduction were always close before the crash of S clonal groups in Poza Sur. These mictic ratios can be attributed to SS clones belonging to S clonal groups, since during those periods, only SS clones were present in Poza Sur.

Integrating over time the total number of females and mictic females from the whole study period in Poza Sur allows one to obtain an estimate of each group's investment in sexual reproduction. The ratio of integrated number of mictic females to integrated number of total females was 0.260 for S clonal groups and 0.124 for L clonal group.

No mictic females were detected in the single sample from Canal Central where *Brachionus plicatilis* was present. All individuals belonged to S clonal groups and were characterized by Gómez *et al.* (in press) as SM clones. In Poza Norte, where S clonal groups consisted only of SM clones, mictic females were found in three out of four samples where *B. plicatilis* was present, mictic ratios ranging between 0.07 and 0.21. L clonal group only appeared in one of these four samples with a mictic ratio of 0.04.

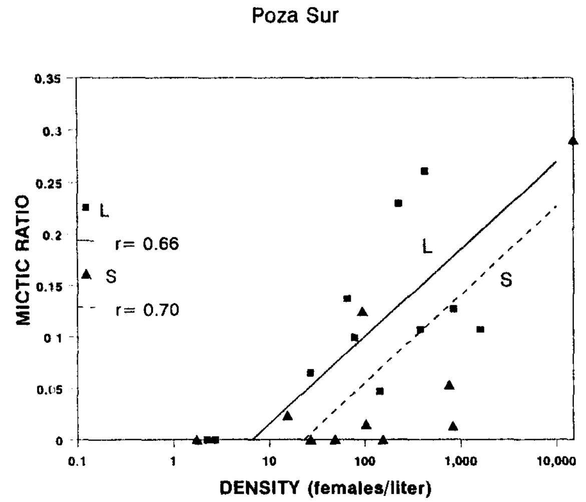

Fig. 2. Relationship between population density and mictic ratio for L and S clonal groups of *Brachionus plicatilis*. Teissier's regression lines are plotted and coefficients of determination indicated.

When the relationships between limnological parameters or their variation, and mictic ratios were explored, only temperature and temperature variation were negatively correlated with mictic ratio of S clonal groups ($r=-0.71$, $P=0.022$; and $r=-0.88$, $P=0.002$ respectively). Despite the scattering of data, a positive correlation between mictic ratio and log-transformed population density was detected in both groups (L clonal group: $r=0.66$, $P=0.026$; S clonal groups: $r=0.70$, $P=0.023$). The relationship between mictic ratios and clonal group densities in Poza Sur is graphed in Fig. 2. Teissier's linear regression of mictic ratio and log-density for L clonal group has a slope of 0.086. Linear regression of mictic ratio and log-density for S clonal groups has the same slope. Although the two regression lines do not differ in slope, their levels do; ANCOVA indicates significant effects of log-density ($F=15.93$; df $=1$; $P=0.001$) as well as group ($F=4.51$; df $=1$; $P=0.048$). The regression analysis allows us to estimate that, in order to get a similar mictic ratio, the density of S groups should be approximately 3.5 times higher than that of the L group. Assuming that the function is sufficiently accurate, the density threshold for mixis would be 6.6 ind l^{-1} for L group and 22.9 ind l^{-1} for S group.

Discussion

Results from the present study, give for the first time, the quantitative temporal patterns of sexual reproduction in natural populations of the species *Brachionus plicatilis*. There are few studies about the occurrence of sexual reproduction in natural populations of the genus *Brachionus* (Wesenberg-Lund, 1930; Buchner, 1941; Ito & Iwai, 1958; Bogoslavsky, 1963; Pourriot, 1965; Halbach & Halbach-Keup, 1972; Miracle & Guiset, 1977), and only two of them have provided data on *B. plicatilis*. Ito & Iwai (1958) noted that mixis in this species was associated with high levels of population density. Miracle & Guiset (1977) evaluated effects of enclosure on mixis by counting the number of males produced, as a whole, by populations of three species of *Brachionus*. However, a higher amount of information comes from laboratory studies. Data from different authors about levels of sexuality in laboratory populations of *B. plicatilis* have been compiled by Snell (1987). In these studies, the proportion of mictic females ranged from 0 to 0.50, which is consistent with the values found in Torreblanca Marsh in our study, which ranged from 0 to 0.29.

From our results, two methodological considerations arise. First, the two estimates of mictic ratio – from live and fixed samples – correspond to two different definitions of mictic ratio because the former involves both pre-reproductive and reproductive age classes, and the latter only the egg-bearing females, which belong to the reproductive age classes. However, the production of mictic females seems to be constant enough so that these mictic ratio definitions are equivalent in practice, since the estimates were not statistically different. Second, the number of males seems to be a very sensitive indicator of mixis but a poor index of mictic ratio in the population, since males were detected in samples in which mictic females were not, but no correlation was found between sex- ratio and mictic ratio.

One of the problems in determining the environmental factors or population parameters which control induction and degree of sexuality in natural populations is the difficulty in isolating effects of individual controlling factors. Thus, our results suggest that sexual reproduction in S and L groups is related to population density, but they do not provide a direct test for causal relationships. However, a positive causal link between mixis and density in *B. plicatilis* has been supported by several authors working with laboratory cultures (Ito, 1960; Pozuelo, 1977; Hino & Hirano, 1976; Lubzens *et al.*, 1985; Snell & Boyer, 1988; Carmona *et al.*, 1993). In contrast to this association, limnological parameters did not show a correlation with the ratios of mixis of the two groups with the exception of temperature and variation rate of temperature, which were negatively correlated with mixis in S groups. This correlation is very probably spurious due to the coincidence between a decrease of temperature and a population peak in fall 1992.

A remarkable finding in this study is that different mictic patterns can be clearly attributed to populations of genetically differentiated clones. L group showed a sexual reproductive pattern that can be considered continuous, the sexual period of this group spanning its presence in Poza Sur. In contrast, S groups presented a rather punctuated mictic pattern. In spite of differences in mictic pattern, a partial overlapping of sexual reproductive periods between groups of clones occurred. Thus, the observed temporal patterns of mixis, i.e. seasonal reproductive isolation, do not account for reproductive isolation between S and L clonal groups. Cross-mating experiments performed in our laboratory (Gómez & Serra, this volume) revealed the occurrence of behavioral reproductive isolation between clonal groups.

Both L and S groups presented a correlation between levels of sexual reproduction and population density. However, they differ in their density threshold for mictic reproduction and this difference generates a remarkable divergence of sexual reproductive patterns. S clonal groups, with a higher threshold, showed periods of very low levels of mixis. Preliminary studies performed in our laboratory support these differences in the density threshold for mictic reproduction between S and L clonal groups (E. Aparici, *personal communication*).

Our findings indicate a population density threshold for *B. plicatilis* of 6.6 ind l^{-1} for L group and 22.9 ind l^{-1} for S group. These values are higher but in the same order of magnitude than the density threshold for mictic reproduction of 2.3 females per liter recorded by King & Snell (1980) in a natural population of the rotifer *Asplanchna girodi*, and lower than the density threshold of 147 females per liter for mictic female production in a *B. plicatilis* L strain estimated by Snell and Boyer (1988) from an experimental study. According to all these data, mixis in natural populations could occur at population densities far lower than experimental densities used in lab studies on sexual reproduction.

The proportion of sexual females produced in the population during the sexual phase of the rotifer life cycle involves a trade-off between population growth and resting egg production, and it is probably optimized (Snell, 1987; Serra & Cammona, 1993). A pattern of continuous sexuality has the effect of depressing population growth rate but, in contrast, guarantees a regular resting egg production; while a punctuated sexual pattern, producing a very low proportion of mictic females most of the time, allows higher population growth rates, so that a switch to high mictic ratios just before population crash, when density is still high, would result in an enhanced production of mictic females. Probably, as a result of the two different mictic patterns, L clonal group showed lower maximum population densities and had a lower sexual reproductive allocation than S groups. Therefore, the pattern showed by S clonal groups seems to be more efficient at producing mictic females, and presumably to produce resting eggs, an important component of fitness. However, the fitness of this pattern would be dramatically dependent on the long-term habitat suitability, so that high population densities could be achieved and mixis would start. In contrast, the sexual reproductive pattern showed by L clonal group would produce fewer resting eggs in favorable conditions but it is not so critically dependent on long-term habitat suitability.

We hypothesize that punctuated pattern would be optimal in predictable habitats, mixis occurring at the end of the suitable period. On the other hand, continuous pattern, as a 'bet-hedging' strategy, would be optimal if the habitat is unpredictable, so that resting egg production would be guaranteed by starting mixis as soon as possible, e.g., when population density allows male-female encounter, because mictic ratios are rather low, the population would maintain itself by parthenogenetic growth till habitat deterioration or competitive exclusion. Features of the habitat of each clonal group seem to be consistent with our hypothesis. Therefore, L clonal group, which showed a continuous mixis with low mictic ratios, is a fall-winter ecotype. During this period, Torreblanca Marsh may suddenly flood, with a subsequent sharp decrease in salinity, which would cause population extinction. In contrast, two putative hazards for spring–summer populations are either pond desiccation during summer, with a previous smooth increase in salinity and population density, or graded temperature decrease during early fall, both phenomena being more predictable than marsh floods.

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