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CHANGES IN THE RATE OF BEATING OF CTENIDIA CILIA IN RESPONSE TO SALINITY AND CATION VARIATION.

by
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ABSTRACT

The sensitivity of cilia and cirri on the ctenidia of the American oyster *Crassostrea virginica* to rapid salinity change was tested *in vitro* with excised, innervated ctenidium preparations. Quantitative measurements of metachronal wave activity and particle transport rates showed that on ctenidia from oysters acclimated to 15 o/oo sea water, ciliary activity was immediately inhibited by exposure to salinities below 15 o/oo, but was unaffected by higher salinities. Inhibition was followed by partial or complete recovery within the 120-minute experimental period. Exposure of oysters to 15 o/oo sea water for 24 hours shifted the maximum activity to 5 o/oo and established sensitivity to higher salinities; this shift was largely reversed if oysters were re-exposed to 15 o/oo sea water for an additional 24 hours. When total salinity was held constant at 35 o/oo but the Ca2+ concentration was varied equivalently to the salinity variations, the effect of rapid salinity changes was duplicated; holding Ca2+ concentration constant at the level expected for 35 o/oo seawater and varying the total salinity greatly diminished salinity sensitivity. These results indicate that the responses of ctenidial cilia and cirri to rapid salinity change is mediated principally by the Ca2+ concentration.

Under field conditions, the compensatory mechanisms will maintain ciliary activity at high levels even under varying salinity regimes.

INTRODUCTION

The American oyster *Crassostrea virginica* (Gmelin) is a euryhaline marine bivalve mollusk forming extensive subtidal reefs in estuaries on the Atlantic and Gulf Coasts (Galtsoff, 1964). The species is abundant in lower Mobile Bay, Alabama, a habitat characterized by a complex fluctuating salinity regime (Schroeder, 1977). There is an annual cycle, with low salinities predominant in the spring during freshwater flooding (Schroeder, 1977, 1979) which can lower salinities to below 1 o/oo. If the condition persists for weeks, extensive oyster mortality may result (Galtsoff, 1930; May, 1972). Significant diurnal cycling on the bottom is unusual, but shifts in salinity may be rapid and unpredictable.

As is typical for marine bivalves, *C. virginica* is an osmoconformer (Anderson and Anderson, 1976) with an apparent capacity for cell volume regulation with free amino acids (Lynch and Wood, 1966). Laboratory studies with this species show a low salinity lethal limit around 3 o/oo (Loosanoff, 1952; Castagna and Chanley, 1973) which corresponds well with ecological observations on the Gulf Coast (Galfsoff, 1930; Gunter 1953; May, 1971). While oysters may avoid extremely low salinities for several days by valve closure, the oystrs in Mobile Bay appear to function normally from 5 o/oo to over 30 o/oo total salinity (May 1971, 1972).

It is well documented for marine organisms that compensatory mechanisms serve to regulate physiological functions within narrow limits in the face of broadly fluctuating environmental and internal parameters such an osmotic and ionic concentrations (Mangum and Towle, 1977). When an osmoconforming organism such as *C. virginica* is exposed to a change in salinity, an alteration of physiological function is expected, followed by a return to normal activity as a result of internal compensatory mechanisms. This has been shown to be the case for pumping and feeding rates in *C. gigas* (Hopkins, 1936).

The experiments reported here make use of quantitative measurements of the activity of cilia on the surface of ctenidial filaments of the oyster (oyster ctenidial structure is described by Nelson, 1960). Two such measurements were used: the percentage of filaments whose lateral cilia display a metachronal wave and the transport rate of small and large particles by the frontal cilia and laterofrontal cirri. These cilia and cirri are involved in the generation of water flow through the mantle cavity and the capture of suspended particles, so changes in their activity will directly affect feeding and oxygen consumption.

This study examines the effect of rapid salinity change on ciliary activity *in vitro*. The activity was highly sensitive to salinity change, but compensatory responses were observed that permitted acclimation of the ciliated system within hours or days. These responses were mediated primarily by changes in the calcium ion concentration of the medium, not the total osmotic concentration.

MATERIAL AND METHODS

Oysters (*Crassostrea virginica*) were collected from a shallow subtidal oyster bed on the north side of Dauphin Island, Alabama, USA during late summer, fall and winter 1981-82. Salinities ranged from 10 to 30 o/oo during this period.

Preliminary experiments were performed at the Dauphin Island Sea Lab; the experiments reported here were performed at Southern Illinois University, Carbondale, Illinois, UDA. Oysters were collected in Alabama and shipped to Illinois in insulated containers, arriving within three days. No mortality resulted from shipping.

Oysters were acclimated for two weeks to 15 o/oo artificial sea water in an Instant Ocean Aquarium (temperature 21° C., pH 7.5-8.0). Before each experiment, oysters were placed in finger bowls containing the artificial sea water, the posterior and anterior adductor muscles were cut and each ctenidium with its branchial nerve, visceral ganglion and piece of posterior adductor muscle for support was isolated. This ganglion/nerve/ctenidium preparation was pinned to rubber mats glued to the bottom of a petri dish containing sea water and the dish was placed in a holder fastened to the adjustable stage of a microscope. Under a 10-power objective the ctenidium was seen to consist of numerous parallel filaments. Three major types of ciliated cells were clearly distinguished: frontal, latero-frontal, and lateral (Nelson, 1960; Ribelin and Collier, 1977). This study was concerned with the rate of beating of the lateral cilia which beat in such a way that metachronal waves (Aiello and Sleigh, 1972) appear to travel in opposite directions along each side of each filament. A field of view was selected which contained 50 filaments. By moving the microscope stage each filament could be followed from its dorsal attachment at the axis to its free ventral margin. Changes in the metachronal wave activity over time were determined by the use of a calibrated sub-microscopic stroboscopic light source. A total of 12 readings were obtained for each preparation; and five preparations were tested at each test salinity. The percentage metachronal wave was determined for each preparation by calling the highest reading equal to 100% and normalizing all subsequent readings against this value.

Particle transport rates were measured with the same apparatus and identical tissue preparations. The tissue was exposed to suspensions of latex beads (Sigma) of two particle diameters at a concentration of 10^6 beads l^{-1}. The movement of individual particles on the grill surface was observed with an optical micrometer and the period of transit timed. Twelve determinations were made for each preparation and the transport rate calculated (10^{-6} m s^{-1}); five preparations were tested at each salinity.

A constant temperature of 21 ± 1.5°C was maintained on the adjustable stage containing the preparation by connecting tubing to a temperature-controlled circulator. A thermoprobe was placed in the petri dish to monitor temperature during the course of the experiment. Control measurements of percentage metachronal wave activity and particle transport rate were made at 0, 10 and 20 minutes in the acclimation medium. Exposure to the test media was accomplished by replacing the contents of the petri dish with the new solution and continuing to perfuse the dish through the experimental period.

Test solutions were made from reagent grade salts according to Harvey, 1957. When called for, Ca2+ (as $CaCl_2$) and Na (as NaCl) substituted for one another.

The data for maximum metachronal wave activity are expressed as a percentage of the maximum activity observed during the control period. The data for particle transport rate were similarly normalized:

$$\% \text{ maximum rate} = \frac{(\text{maximum control rate} - \text{rate at time } t) \times 100}{\text{maximum control rate}}$$

RESULTS

Oysters were divided into three groups (Table 1). Group A oysters were those acclimated to 15 o/oo artificial seawater. Group B oysters were transferred from 15 o/oo to 5 o/oo seawater for 24 hours before opening. Group C oysters were transferred from 15 o/oo to 5 o/oo for 24 hours and back to 15 o/oo for an additional 24 hours before measurements of ciliary activity wre made. All experiments were performed on these three groups.

Comparison of mean control values by analysis of variance indicated that in two of the three experiments there were significant differences between the three groups (Table 2). Since the percentage of filaments displaying a metachronal wave (metachronal wave activity) was normalized at 100 percent during the control period (see METHODS), the significant differences in the means observed in the constant Ca2+ experiment may be interpreted as a greater variability over the three control time points (Table 2).

Mean control values for the particle transport rates of the three groups were not significantly different in the variable salinity experiment, but were significantly different ($P < 0.001$) in the other two experiments. These results are interpreted as showing incomplete acclimation of the ciliary activity to the salinity treatment regimes described for groups B and C. The maximum rate in the control period was assumed to be 100 percent to normalize the experimental data. In all the figures, the experimental results are the change in the mean value for ciliary activity over the 30- to 40-minute time period. Standard deviations of the means during perfusion of the dishes with the test solutions were comparable to the control values, indicating uniform responses among individuals. For simplicity, standard deviations are not shown in the figures.

Table 1. *Crassostrea virginica*. Experimental design. Acclimation salinity refers to acclimation in the laboratory; treatment involved transferring intact oysters to aquaria of the indicated salinities.

GROUP	ACCLIMATION SALINITY (o/oo)	TREATMENT (o/oo)
A	15	15
B	15	5 for 24 hours
C	15	5 for 24 hours
		15 for 24 hours

A rapid change in medium salinity resulted in a loss of coordinated ciliary motion; the greater the change in salinity, the greater the reduction in the percentage of ctenidial filaments displaying a metachronal wave (Figure 1). The usual response was an immediate inhibition of ciliary activity followed by a partial recovery within the experimental period. The metachronal wave activity of group A oysters was unaffected by salinities above the treatment salinity of 15 o/oo, but was strongly inhibited by lower salinities (Figure 1A). Similar results were obtained with the group B oysters (Figure 1B). However, the range of salinity sensitivity has shifted. Maximum metachronal wave activity was measured at 5 o/oo, the treatment

Table 2. *Crassostrea virginica*. Mean control ciliary activities on the ctenidia. Percentage metachronal wave refers to the percentage of observed filaments displaying metachronal wave activity; transport rate refers to the movement of particles on the frontal surface of the ctenidia. Five individuals were tested at each salinity: mean values on table are the means of all mean control values from each group in each experiment. Test for significant differences between groups A, B and C by ANOVA; groups are as shown in Table 1.

				TRANSPORT RATE (10^{-6} m s^{-1})		
EXPERIMENT	GROUP	N	PERCENTAGE METACHRONAL WAVE	N	SMALL PARTICLES	LARGE PARTICLES
Salinity Change	A	21	98.0 ± 2.1	16	191.2 ± 14.3	128.8 ± 15.5
	B	21	97.4 ± 2.3	16	188.7 ± 14.8	115.4 ± 33.2
	C	21	98.0 ± 1.9	16	183.9 ± 17.6	116.7 ± 15.7
			N.S.		N.S.	N.S.
Ca^{2+} Change	A	21	97.6 ± 2.5	16	186.6 ± 20.2	127.2 ± 23.1
	B	21	97.4 ± 2.9	16	164.4 ± 17.8	138.1 ± 15.9
	C	21	97.8 ± 2.0	16	121.2 ± 16.5	75.6 ± 22.2
			N.S.		P<005	P<001
Salinity Change (Ca^{2+} constant)	A	18	92.7 ± 3.6	12	180.1 ± 31.0	151.2 ± 26.7
	B	18	95.8 ± 1.9	12	143.1 ± 28.2	125.8 ± 25.5
	C	18	93.2 ± 2.6	12	182.5 ± 24.3	161.0 ± 27.9
			P<005		P<005	P<01

salinity, while both lower and higher salinities inhibited ciliary activity. At 15, 20 and 30 o/oo test salinities, a more gradual loss, or decay, of ciliary activity was observed rather than the immediate inhibition seen with low salinity effects on groups A and B oysters (Figure 1B). The group C oysters showed that 24 hours after returning to 15 o/oo the oysters were regaining the range of sensitivity shown by the group A oysters; residual sensitivity to high salinity remained, seen as a decay in activity (Figure 1C). The effect of treatment salinity on salinity sensitivity is clearly revealed by "the spectrum of sensitivity" maximum inhibition of metachronal wave activity as a function of exposure salinity for the three groups (Figure 2).

The sensitivity to rapid salinity change observed could be a result of changes in the concentration of particular ions. Since changes in the intracellular concentration of Ca2+ are known to inhibit the ciliated cells in *Mytilus edulis* (Walter and Satir, 1978; Paparo, 1980), the effect of this ion was tested. Test solutions were made up with a total salinity of 35 o/oo but with Ca2+ concentrations equivalent to the salinities already tested. Any differences between the effects of these test solutions can be ascribed to changing levels of the cation. The results (Figure 3) show that for all three groups, the effect of varying the Ca2+ concentrations at constant high salinity virtually duplicates the effect of varying the total salinity; the sensitivity spectra are similar (Figure 4).

To confirm these results, the inverse experiment was performed: Ca2+ concentration was held constant at the level expected in seawater at 35 o/oo salinity (10^{-2} mol l^{-1}), but the total osmotic concentrations were varied as in the first experiment (Figure 1). The results (Figure 5) indicate that all three groups of oysters responded similarly to the rapid change in salinity. Rather than sudden inhibition, a gradual decay in the percentage of gill filaments displaying a metachronal wave was observed. At all salinities, the effect of the rapid change was minimized, as is shown in the sensitivity spectra (Figure 6). Departures from the treatment salinity exerted only a minor inhibitory effect.

In general, particle transport rates showed changes in response to test media that were similar to the changes in metachronal wave activity. Large particles (25.7 microns mean diameter) were transported at a slower rate than smaller particles (5.7 microns mean diameter) of the same density; the recovery of the normal rate following a salinity change was also slower for the larger particles. This may be seen in the results for group A oysters exposed to salinities of 5, 10 and 15 o/oo (Figure 7). The particle transport rate tended to decay over the experimental period even when measured at the treatment salinity (Figures 7, 8, 10 and 12) and the variability of the responses of individual oysters was high as can be seen in the standard deviations of the control values.

Particle concentration also affected the rate of particle transport. This effect was tested by exposing the ctenidial tissue preparations to increasing concentrations of particles beginning 60 minutes after exposure to the test medium when recovery should be underway. Concentrations ranged from 10^1 to 10^7 particles l^{-1}. In general, particle transport rate decreased with increasing particle concentration: at most test salinities, the rate was significantly negatively correlated with log particle concentration ($P < .05$). At some salinities for each group, this correlation was not observed, usually when the transport rates were maximal. When the effects of medium change on particle transport rates over time were measured, a high concentration (10^6 particles l^{-1}) was used. Hence transport rates are much below the maximum possible rates.

The particle transport rates changed in response rapid salinity change much as did the metrachronal wave activity (figure B). With all three groups of oysters an inhibition of the transport rate was followed by recovery, with maximum rates measured at the treatment salinity. Characteristic shifts in the sensitivity spectra were observed for groups B and C (Figure 9). These results for group A oysters were nearly duplicated by varying the Ca2+ concentration while holding the salinity constant at 35 o/oo as previously described (Figure 10A, 11). Group B oyster tissue displayed more variable responses with a minimum inhibition at 10 o/oo but maximum recovery at 5 o/oo, the treatment salinity Figure 10B). The response to test salinities by the group C oysters was highly variable, but the key elements of a return to low salinity sensitivity and residual but diminished high salinity sensitivity are present (Figure 10C).

The experiments measuring particle transport rate as a function of rapid salinity change with a constant high Ca2+ concentration show results comparable to those measuring metachronal wave activity (Figure 12). However, a substantial decline in the transport rate over the experimental period was observed even at the treatment salinities for all groups. The rate of decay of the transport rate was clearly related to the test and acclimation salinities. Although the pattern of response in the presence of constant calcium is different from both the variable salinity and variable calcium responses, the salinity sensitivity spectra are essentially the same for all three experiments (Figure 13).

DISCUSSION

Oysters have a highly complex ctenidial structure interpreted as an adaptation for pumping water through the mantle cavity and capturing suspended particles for food (Nelson, 1960; Ribelin and Collier, 1977; Jorgenson, 1980). The lateral cilia function primarily in moving water through the animal and display a characteristic metachronal wave (Aiello and Sleigh, 1972). The laterofrontal cirri and the frontal cilia generate the currents required for capturing the suspended particles and moving them down the gill surface to be ingested (Ribelin and Collier, 1977; Jorgenson, 1980). The present study has measured directly the activity of the lateral cilia as the percentage of filaments displaying a metachronal wave. Generally a loss of ciliary coordination is accompanied by a slowing of the beating rate, although variations in the beating rate are not always associated with a loss of the metachronal wave. The activity of the laterofrontal cirri and the frontal cilia was measured indirectly as the rate at which small and large particles were transported across the ctenidial surface.

The response of oysters to a change in environmental salinity may be expected to involve a complex of behavioral and physiological changes. When *Crassostrea gigas* was exposed to altered salinity in a laboratory aquarium system, the animal ceased pumping and closed their valves (Hopkins, 1938). Valve closure in response to salinity change has been termed an "isolation response" (Davenport, 1979) but it may be to some extent a laboratory artifact. Hand and Stickle (1977) monitored valve movements of *C. virginica* when exposed to changing salinities and found only very rapid shifts resulted in significant valve closure. Under natural conditions, oysters remain open most of the time (Loosanoff and Nomejko, 1946) and the ctenidia are exposed to changing osmotic and ionic concentrations in their environment. In organisms inhabiting fluctuating salinity environments, we expect strong selec-

tive pressure for compensatory mechanisms active in maintaining normal physiological functions.

Our studies using largely intact innervated oyster ctenidia show that shifts in salinity over the range occurring in the natural habitat of this species have an immediate inhibitory effect on the activity of the cilia on the ctenidia. The results show that the inhibition is largely reversible over the tolerance range of the oyster and that this species will rapidly acclimate to salinities as low as 5 o/oo.

Ciliary activity of oysters acclimated to 15 o/oo in the laboratory was very sensitive to low salinity exposure; salinities above 15 o/oo exerted no effect. This asymmetry in high and low salinity effects was preserved even after exposure of intact oysters to 5 o/oo for 24 hours. In this group, while salinities above 5 o/oo did indeed inhibit the activity, the pattern of inhibition was different: high salinity exposure resulted in a decay in activity rather than rapid inhibition (refer to group B in Figure 1 and 3). The explanation may lie with different compensatory mechanisms for dealing with the different stresses; similar results were obtained in testing the response of *Mytilus edulis* nerve action potentials to salinity change (Willmer, 1978).

Even 24 hours exposure in the laboratory to a lower salinity produced a shift in the sensitivity spectrum; the effect was almost completely reversed by a 24-hour re-exposure to 15 o/oo. Clearly in these animals the isolation response, if it exists, must have been relatively brief. These shifts are important for interpreting several earlier studies of ciliary activity in bivalves, where excised filaments were exposed to test salinities for 24 to 48 hours before measuring activity (Schlieper *et al.*, 1960; Vernberg *et al.*, 1963; Theede and Lassig *et al.*, 1967; and Schlieper *et al.*, 1967). Such preincubations would be sufficient for acclimation to all but the most extreme salinities so the investigators were measuring the extent of compensation of the ciliary systems, rather than acute sensitivity. Their results are consistent with our studies of *C. virginica*, in that ciliary responses were related to habitat salinity and could be shifted by exposure of intact animals to a new salinity regime (Theede and Lassig, 1967). Acute responses of the laterofrontal cirri and frontal cilia were measured in *C. virginica* and three other bivalve species by Van Winkle (1972) from 0.5 to 12 hours with results similar to those reported here.

Several studies have implicated Ca2+ in controlling ciliary activity on bivalve ctenidia. Paparo and Murphy (1975a) demonstrated ciliary arrest with *Mytilus edulis* tissue in Ca2+-free medium and increasing inhibition of ciliary activity as the Ca2+ concentration was raised beyond normal levels. An increase in the intracellular free Ca2+ level in the ciliated cells also results in ciliary arrest (Walter and Satir, 1978; Paparo and Satir, 1980) and alterations in intracellular free Ca2+ have been unambiguously associated with chemical, photic and electrical stimulation and inhibition at the level of the ciliated cell and the branchial nerve (Paparo and Murphy, 1975a, 1975b, 1976, 1980; Paparo, 1980).

Our study supports the significance of Ca2+ in controlling ciliary activity. When Ca2+ was varied but total medium salinity held constant at 35 o/oo, the results of the salinity variation study could be duplicated. These results suggest that the salinity effect is in fact a calcium effect, and explanations for the compensatory response will be found in mechanisms influencing the intracellular calcium pool.

Maintaining the Ca2+ concentration at a level expected for seawater at 35 o/oo did not completely eliminate the salinity effect. This can be most easily seen in the relationship of percent inhibition to test salinity in the constant calcium experiment

(Fig. 5). Other ions may exert a minor effect, or the change in osmotic pressure and the volume regulation response may play some role. It must be pointed out that the salinity level in the variable calcium experiment, and the calcium level in the constant calcium experiment were substantially above the acclimation level of 15 o/oo for these animals. This should not affect the within-group effects of different test salinities or calcium concentrations.

The results for particle transport rate tend to confirm the results for the percentage of filaments displaying a metachronal wave. Large particles were transported more slowly than small particles, and the results were consistently more variable. The measured transport rates were also sensitive to particle concentration, generally declining as concentration was increased. Since a high concentration (10^6 particles l^{-1}) was used in the time course experiments, the rates recorded were probably well below the maximum. It should also be noted that transport rates tended to decay over the 140-minute period even in individuals exposed to the control solution, perhaps a function of using dissected tissue. One important departure from the results for percentage metachronal wave was observed: transport rates continue to show substantial salinity sensitivity even when a consant Ca2+ concentration was maintained. The pattern of response was also different, with a progressive decay of the rate in place of inhibition and recovery. These results suggest a sensitivity of the ciliary systems involved in particle transport that is different from the lateral cilia.

The experimental system we have employed in this study offers several distinct advantages over those used by earlier investigators. First, we use relatively untraumatized tissue, maintaining full innervation of the ctenidia by the branchial nerve, unlike the excised filaments used by others (Schlieper *et al.*, 1960; Vernberg *et al.*, 1963; Theede and Lassig, 1967; Schlieper *et al.*, 1967; Van Winkle, 1972); the responses of innervated ctenidia should more closely resemble normal *in vivo* ciliary responses. Second, our system provides both quantitative and direct measurements of the activity of cilia of clear physiological significance to the intact organism. Alterations in the activity of the lateral cilia will alter the pumping rate of the animal, with concomitant effects on oxygen consumption and feeding expected. Earlier studies have relied either on qualitative scoring of the activity of "terminal cilia" (Schlieper *et al.*, 1960; Vernberg *et al.*, 1963; Theede and Lassig, 1967; Schlieper *et al.*, 1967) or on the rate of movement of gill fragments across a surface (Van Winkle, 1972). Our future experiments will include lateral cilia beating rates as well. Third, our technique resolves rapid and short term change in ciliary activity as well as the effect of acclimatory or longer term compensatory changes in salinity sensitivity. In addition to confirming results of earlier studies, this system permits a kinetic analysis study of rates of ciliary responses. Our results suggest that the ciliary response can be dissociated into several components: inhibition, recovery rate, and extent of recovery. These may ultimately be related to the kinetics of calcium flux in the ciliated cells or the neurons which innervate them. Given the physiological requirements for normal ciliary activity, these responses may prove to be a useful indicator of stress in this and other bivalve species.

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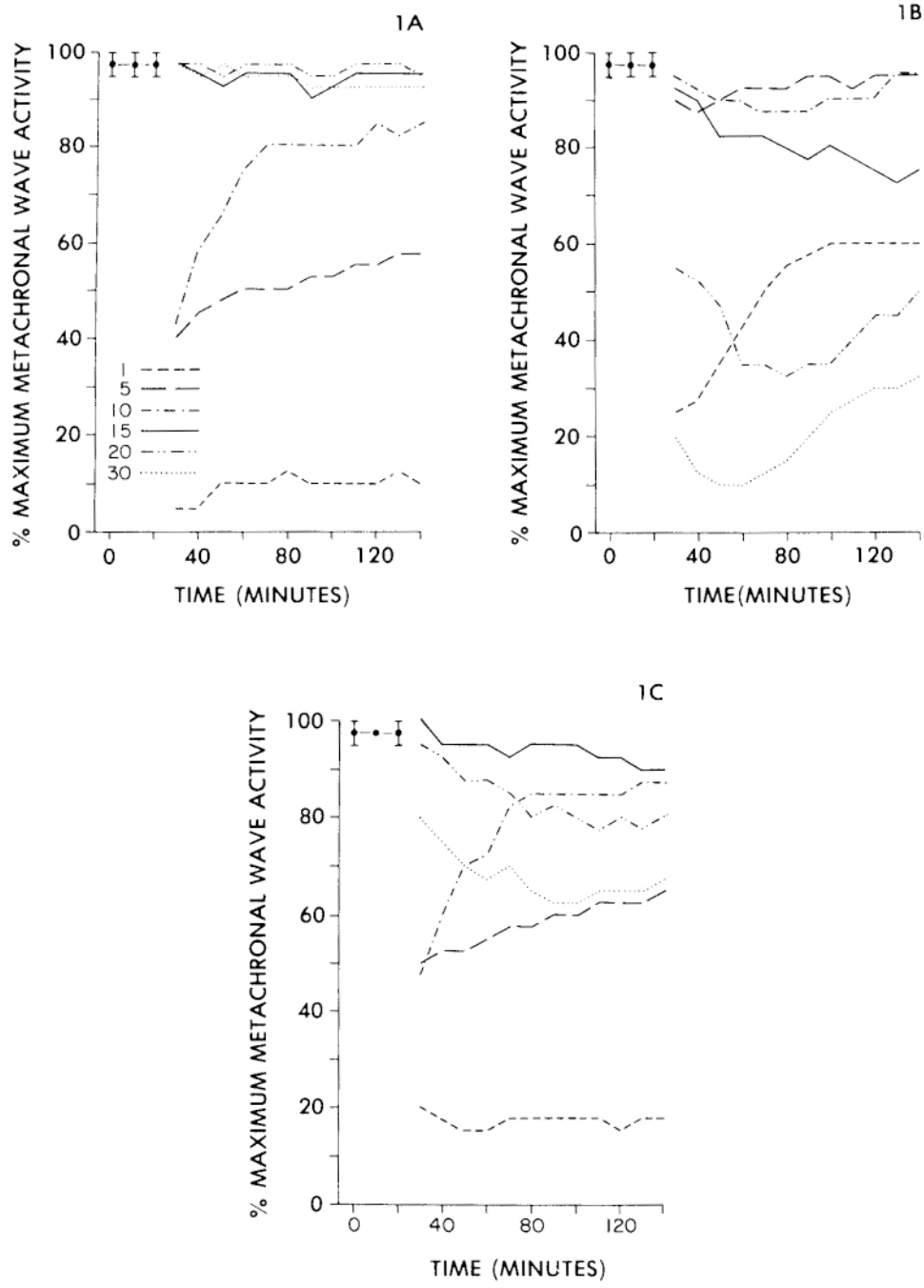

FIGURE LEGENDS

Figure 1. *Crassostrea virginica*. Percentage of maximum metachronal wave activity after exposure of oysters to rapid salinity change. A, B, and C refer to the three experimental groups (see text). Control measurements at 0, 10 and 20 minutes are mean percent maximum activity for all oysters ± standard deviation. Experimental results are mean values (n = 5 animals).

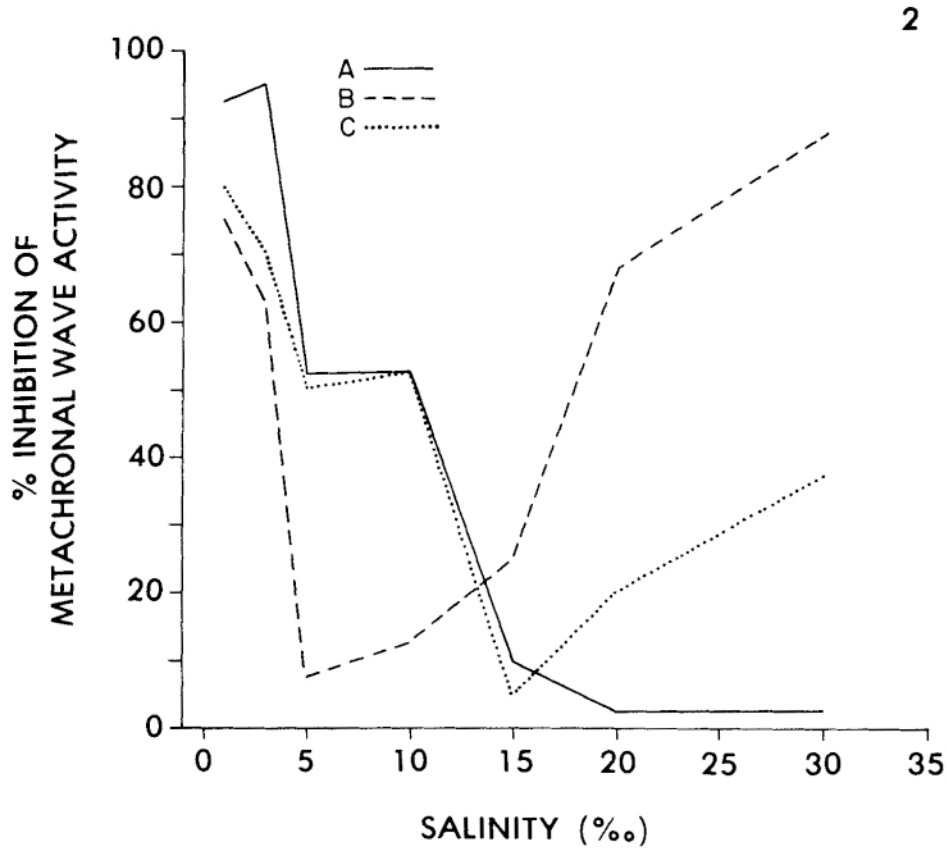

Figure 2. *Crassostrea virginica*. Salinity sensitivity spectra for metachronal wave activity for oysters of groups A, B, and C. Data from Fig. 1.

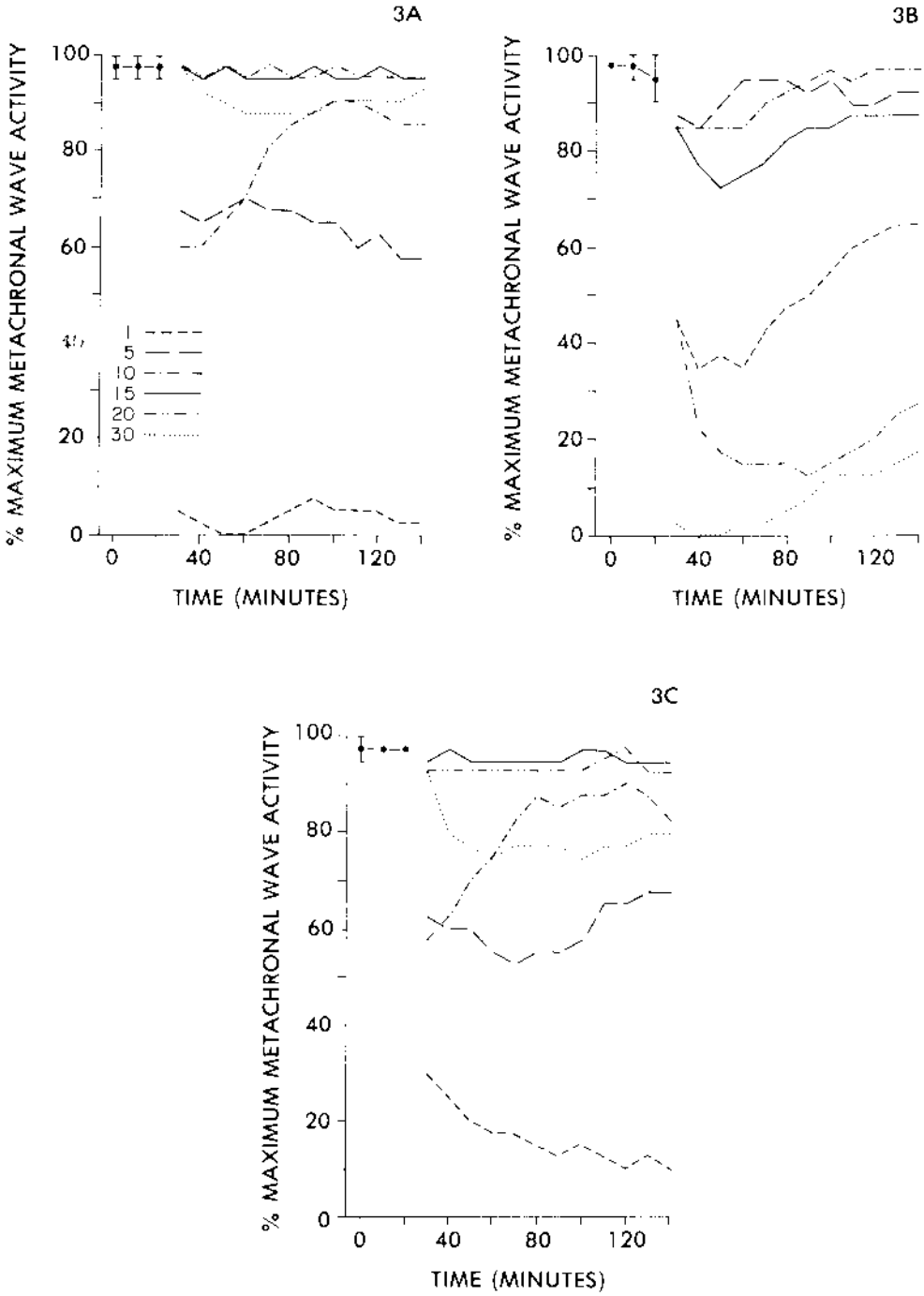

Figure 3. *Crassostrea virginica*. Percentage of maximum metachronal wave activity after exposure of oysters to a rapid change in Ca2+ concentration. Total salinity was held constant at 35 o/oo; salinities on ordinate refer to the Ca2+ concentration expected from dilution of 35 o/oo seawater. Other details as in Fig. 1.

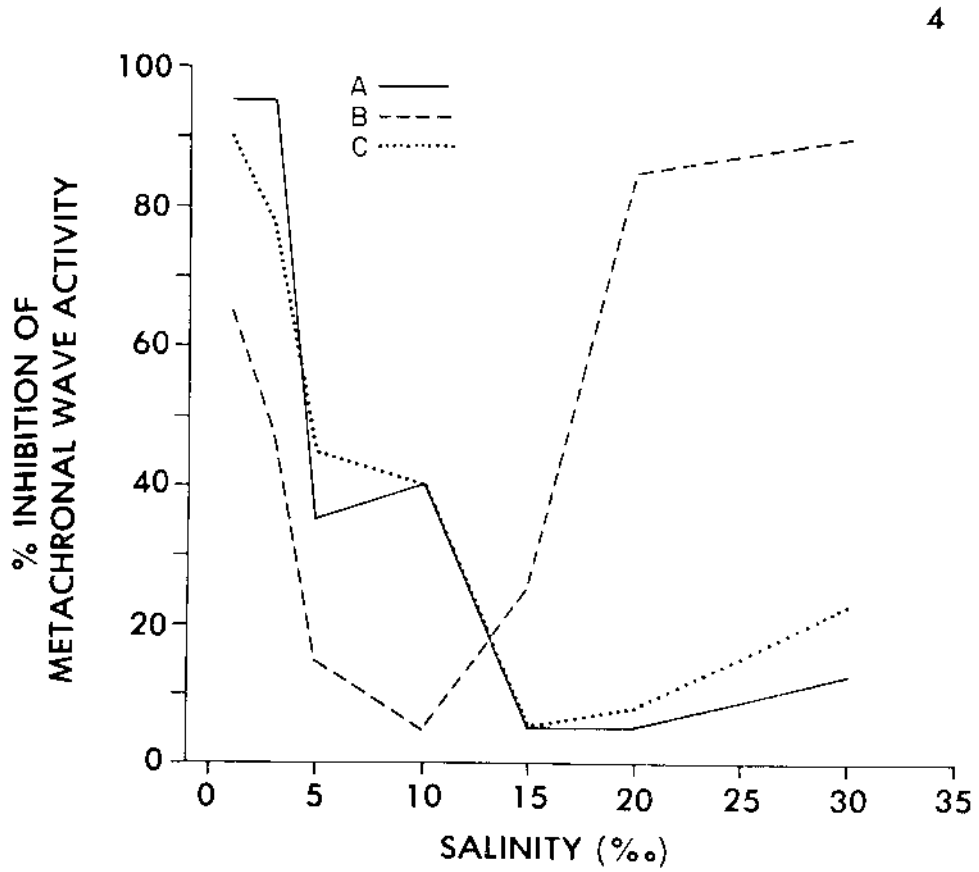

Figure 4. *Crassostrea virginica*. Ca2+ sensitivity spectra for metachronal wave activity spectra for metachronal wave activity for oysters of groups A, B and C. Data from Fig. 3.

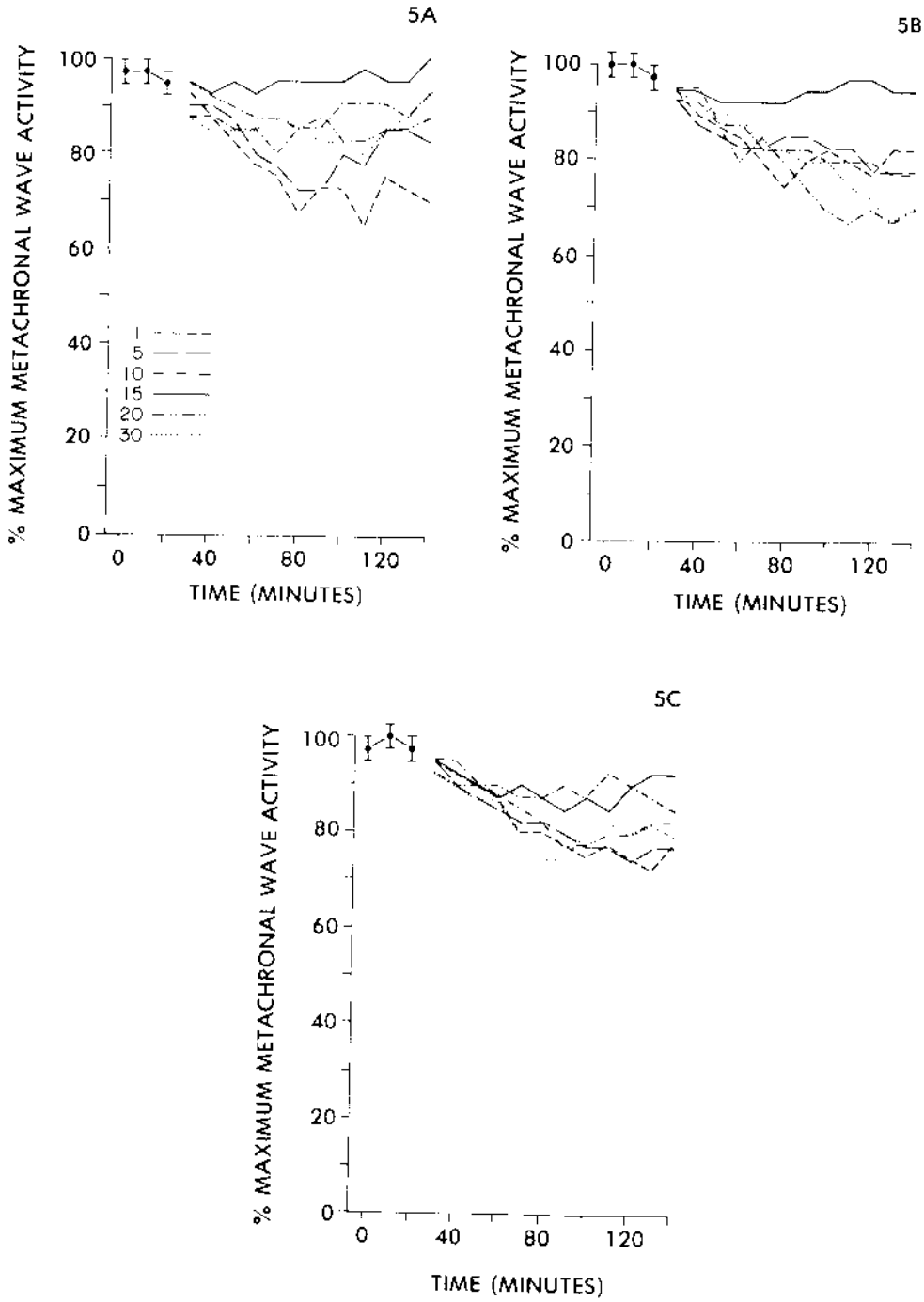

Figure 5. *Crassostrea virginica*. Percentage of maximum metachronal wave activity after exposure to rapid salinity change in the presence of a constant Ca2+ concentration (10^{-2} mol l^{-1}). Other details as in Fig. 1.

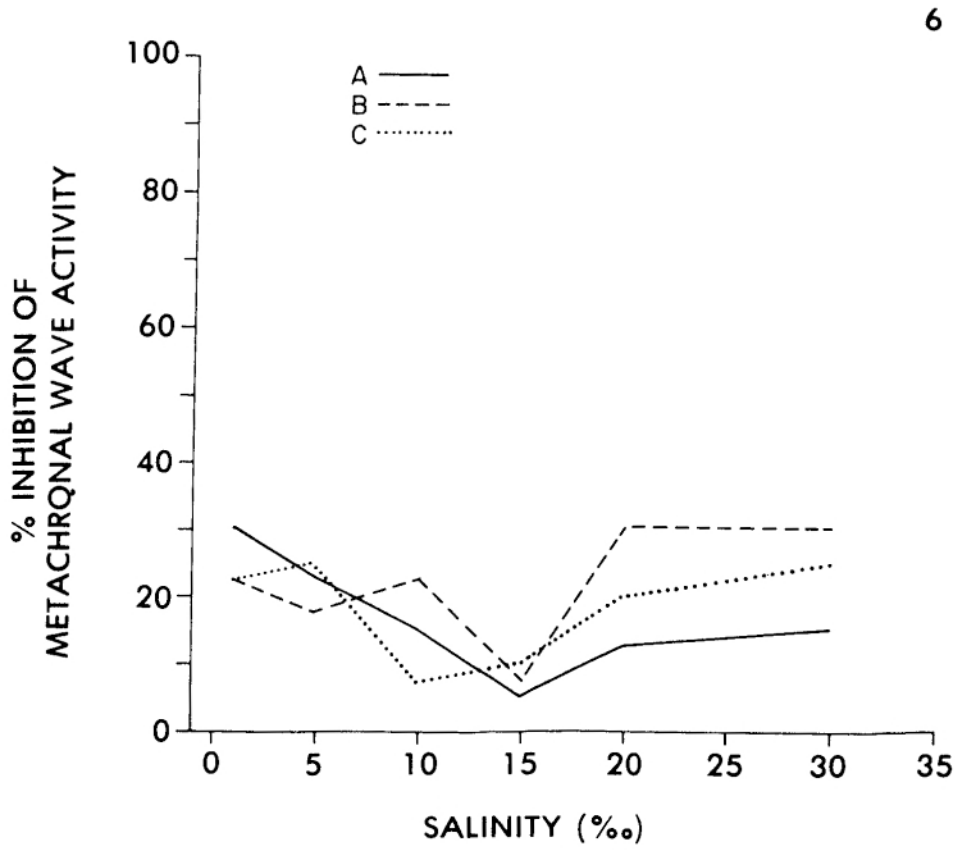

Figure 6. *Crassostrea virginica*. Salinity sensitivity spectra determined in the presence of 10^{-2} mol l^{-1} Ca2+ for oysters of groups A, B and C. Data from Fig. 5.

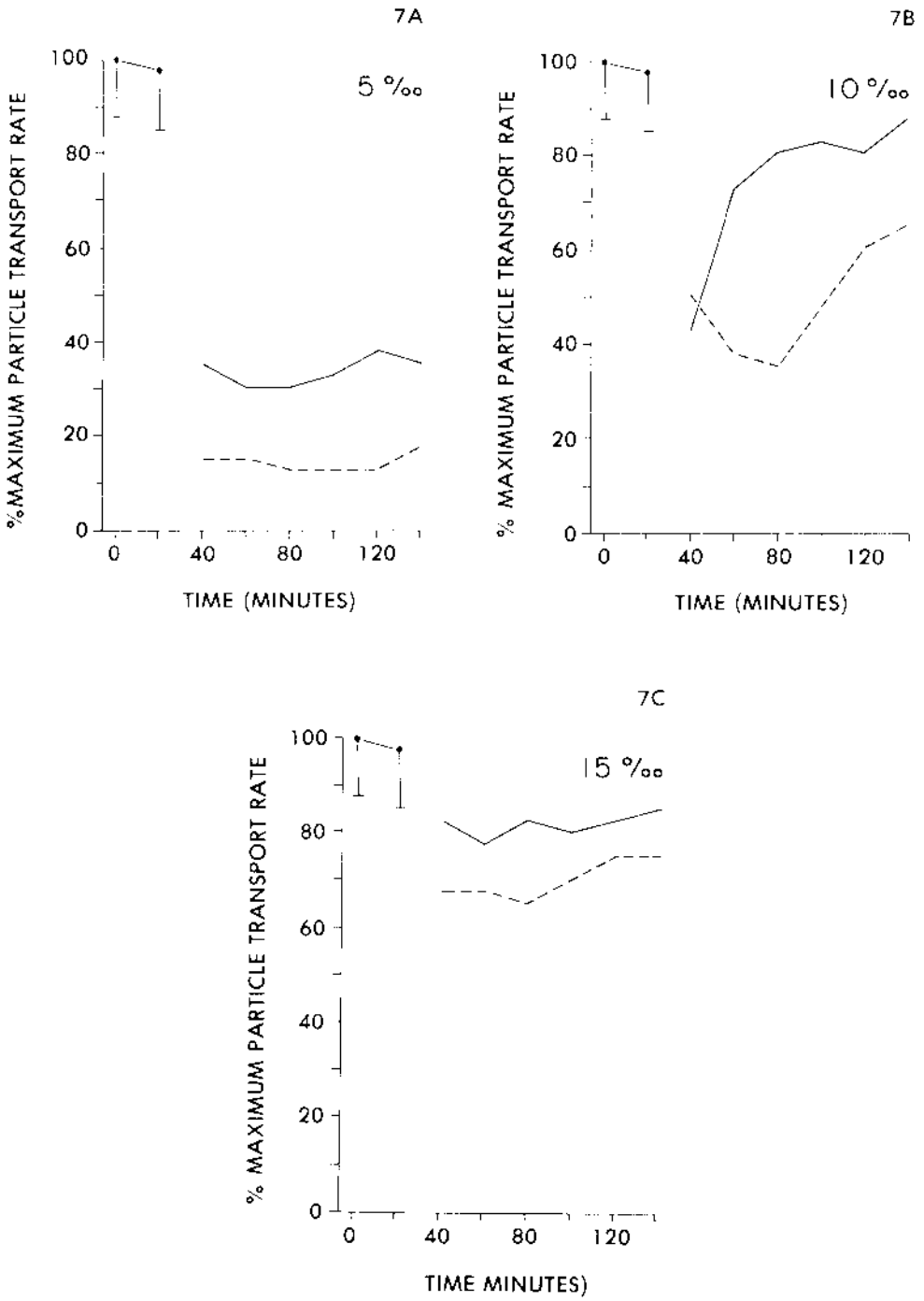

Figure 7. *Crassostrea virginica*. Particle transport rates of small (5.7 microns diameter) and large (25.7 microns diameter) on the frontal surface of group A oyster ctenidia at 5, 15 and 30 o/oo test salinities (——) small particles; (——) large particles.

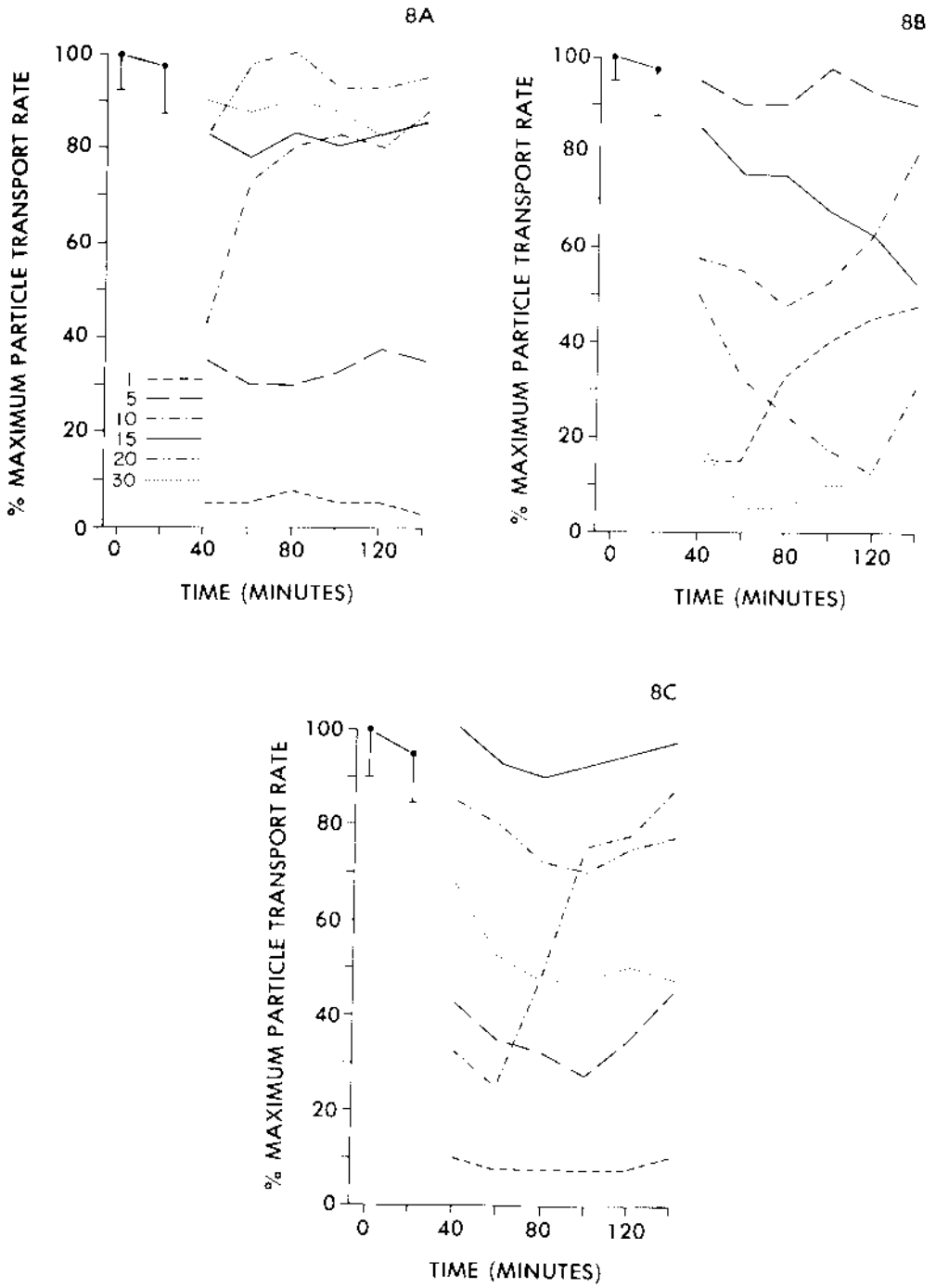

Figure 8. *Crassostrea virginica*. Small particle transport rates after exposure of oysters to rapid salinity change for oysters of groups A, B and C. Other details as in Fig. 1.

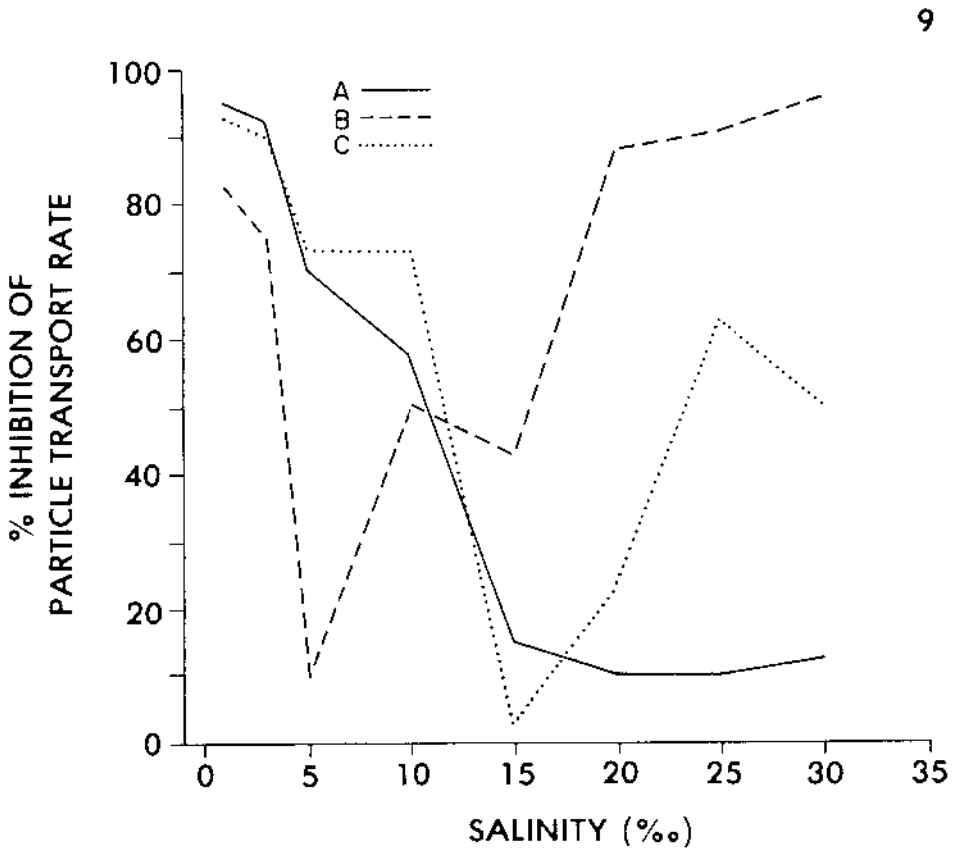

Figure 9. *Crassostrea virginica*. Salinity sensitivity spectra of small particle transport rate for groups A, B and C oysters. Data from Fig. 8.

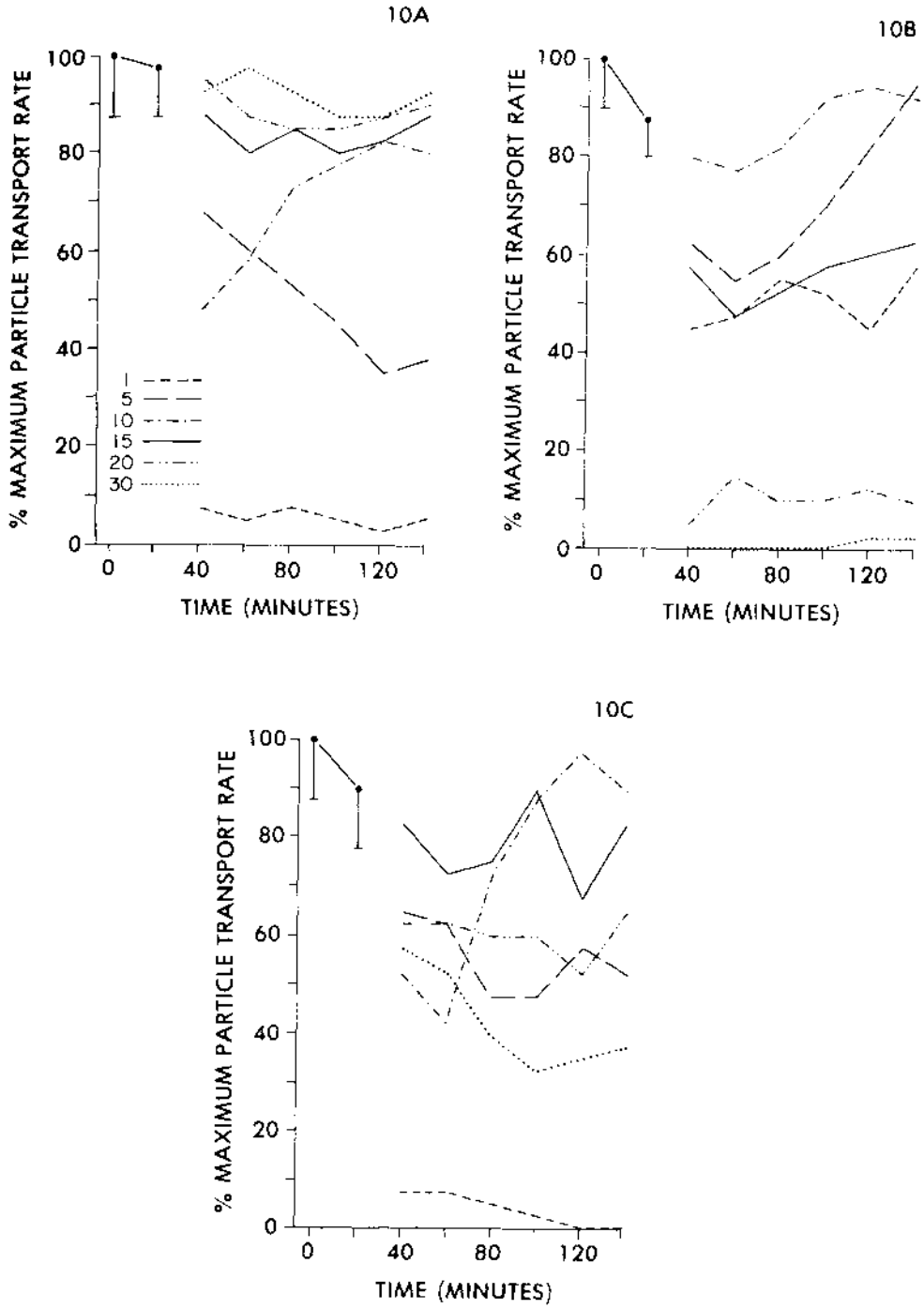

Figure 10. *Crassostrea virginica*. Small particle transport rate on the frontal surface of oyster ctenida of groups A, B and C oysters after exposure to rapid change in Ca2+ concentration at a constant salinity (35 o/oo). Salinities on the ordinate refer to the calcium concentration expected in equivalently diluted seawater. Other details as in Fig. 1.

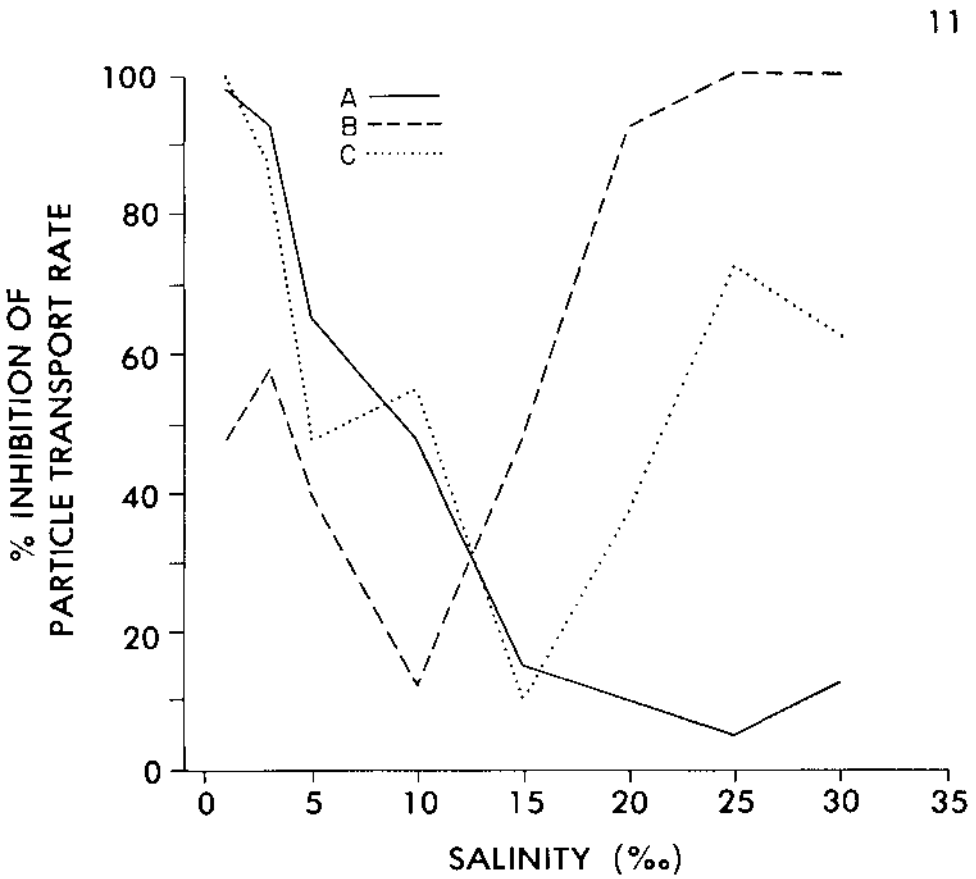

Figure 11. *Crassostrea virginica*. Ca2+ sensitivity spectra for small particle transport rate for groups A, B and C oysters. Data from Fig. 10.

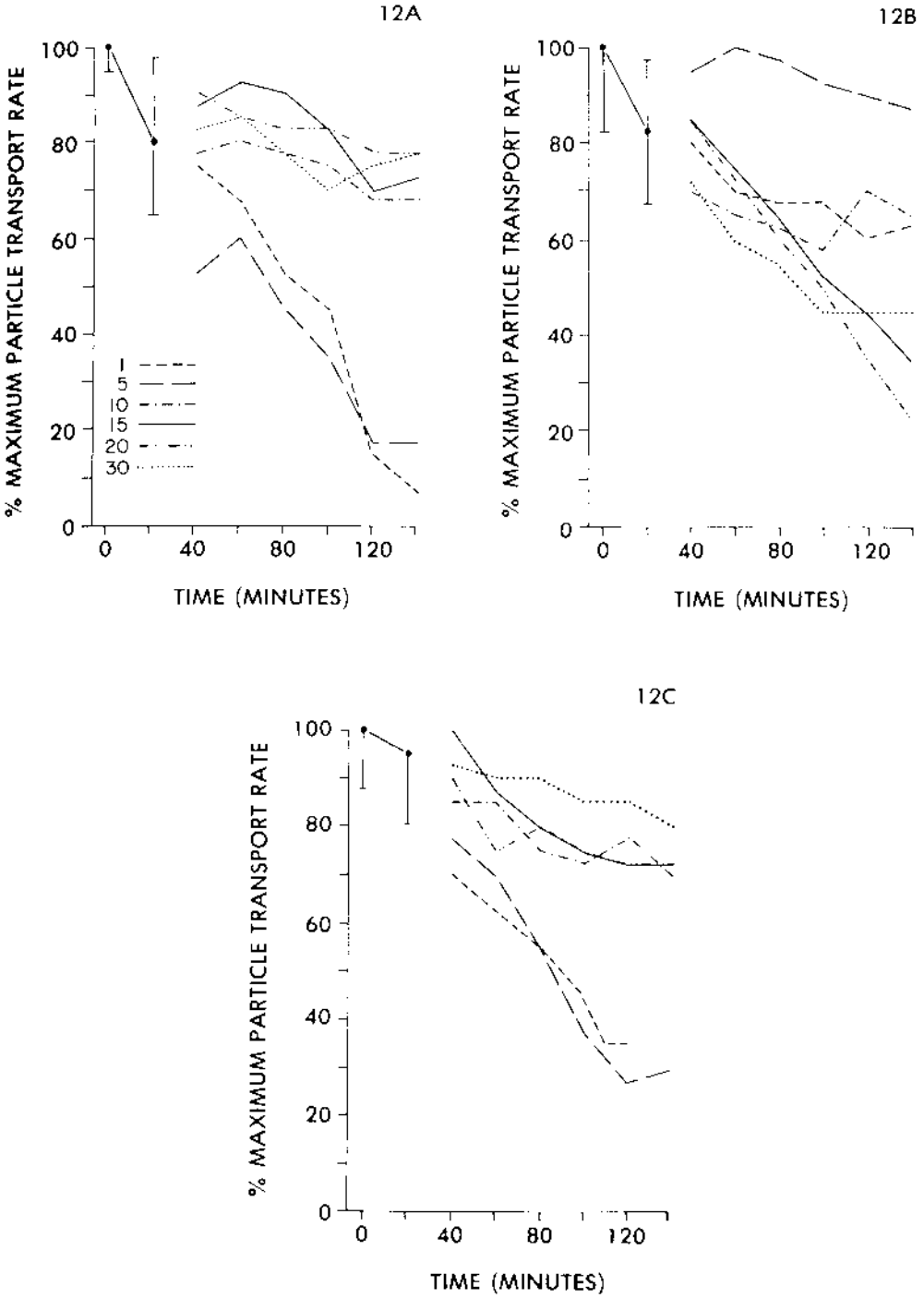

Figure 12. *Crassostrea virginica*. Small particle transport rate on the frontal surface of oyster ctenidia of groups A, b and C oysters after exposure to rapid salinity change at a constant Ca2+ concentration (10^{-2} mol l^{-1}). Other details as in Fig. 1.

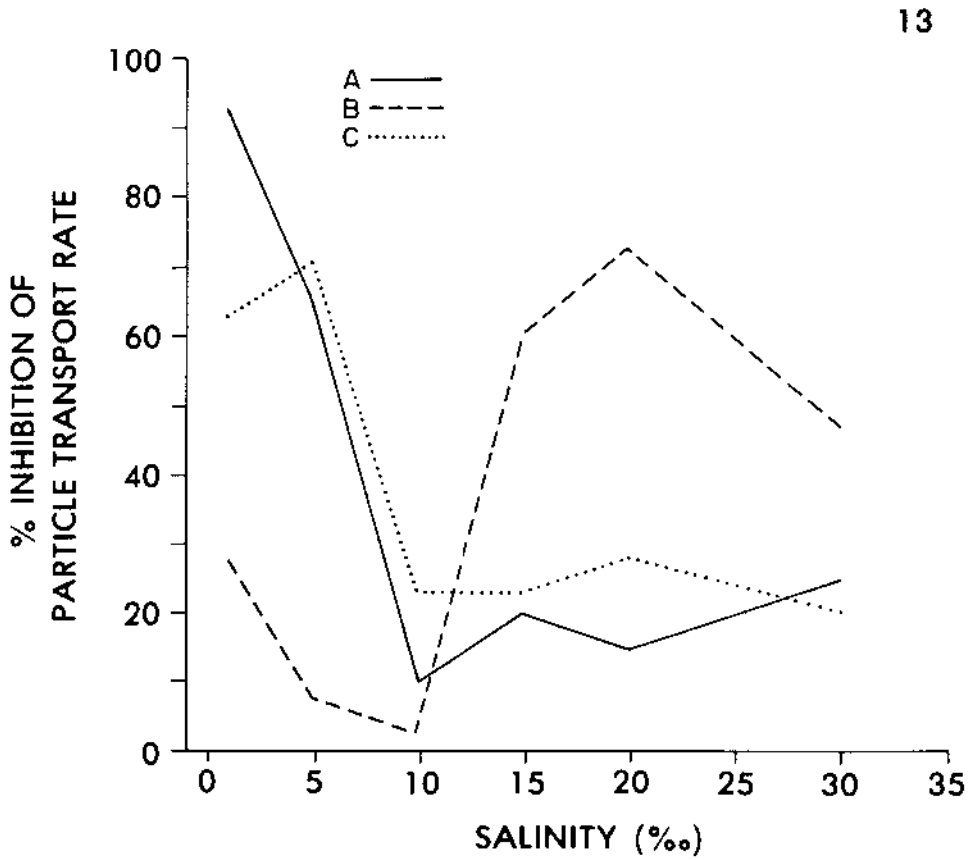

Figure 13. *Crassostrea virginica*. Salinity sensitivity spectra at a constant Ca2+ concentration (10^{-2} mol l^{-1}) for oysters of groups A, B and C. Data from Fig. 12.