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13. ABSTRACT

Studies on the virucidal properties of seawater indicate that microbial agents are involved since(a) both autoclaving and filtration remove the activity and (b) the activity can be restored to the filtrate if the filter pads are immersed back in the filtrate. Studies on high temperature (62°C) inactivation indicate that all 3 enteroviruses were inactivated within 30' at pasteurization temperatures.

Studies of the effects of temperature and salinity on the survival of three enteric viruses (poliomyelitis type 1, echovirus-6, and coxsackievirus B-5) under controlled laboratory conditions and in situ indicate that temperature rather than salinity is the critical factor affecting their stability, in that the higher the temperature the more rapid was the loss of viral infectivity. In the laboratory studie all three viruses were quite stable at 4°C, with infectious virus still detectable after 46 weeks of incubation. In situ studies on virus survival in free-flowing estuarine or marine waters showed that, although the viruses were more labile in natural waters than in the laboratory studies, they persisted for several months, in some cases during the winter months. At all temperatures and salinities, coxsackie-virus B-5 was the most stable, echovirus-6 was intermediate, and poliovirus 1 was the least stable of the viruses tested.

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Human enterovirus survival						
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Viruses Employed

Stocks of poliovirus type 1 (Mahoney strain), Coxsackie B-5 (Faulkner strain), and ECHO-6 (D'Amori strain) viruses were obtained from the Reference Reagents Branch of NIAID, Bethesda, Maryland. All viruses were propagated in a continuous line of African green monkey kidney cells (BGM line) kindly supplied by Dr. D. R. Dahling, EPA Laboratory, Cincinnati, Ohio. The cells were routinely propagated with Eagle's Minimum Essential Medium with Earle's salts (EMEM) supplemented with 10% fetal calf serum.

Virus Assay Procedure

Confluent BGM cultures were grown in 60 x 15 mm culture dishes. Serial 10-fold dilutions of virus were absorbed to the monolayers for 30 min. after which 3 ml of an overlay consisting of equal volumes of 1% agarose (Grand Island Biological, electrophoresis grade) and a 2X concentration of EMEM were added. After the overlay solidified, 3 ml of EMEM with 2% fetal calf serum were added to each dish. Following a 48 hr (polio) or 72 hr (ECHO and Coxsackie) incubation at 35 C in 5% CO_2, 2 ml of full strength (40%) formaldehyde were added to each dish. After standing for 30 min., the agarose and formaldehyde were decanted and the remaining cell sheet stained for 2 min. with a 1% methylene blue solution and the plaques counted. Results are expressed as the number of plaque forming units (pfu's) per ml.

1. Effect of salinity and temperature on enterovirus survival under controlled laboratory conditions

(A) Methods

For the controlled laboratory studies, synthetic seawater was prepared by the addition of Marine Mix (Utility Chemical Co., Paterson, New

Jersey - composition described in their technical bulletin #156), to glass distilled water. The water was adjusted to the desired salinity by means of a salinity meter (Model 33, Yellow Springs Instrument Co., Yellow Springs, Ohio); the salinities employed were 10, 20, and 34 parts per thousand. The solutions were sterilized by autoclaving and the pH adjusted to 7.5 - 8.0 if necessary.

Replicate flasks, containing 100 ml each of the various salinity waters, were seeded with 10^5 - 10^6 pfu's/ml of the respective viruses. Virus stocks were centrifuged at 5,000 g for 10 min. to remove cellular debris prior to their inoculation into the test flasks or dialysis bags. Four incubation temperatures were employed (4 C, 15 C, 25 C and 37 C) with the flasks being immersed in shaker water baths operated at 150 cycles per minute. Samples were taken immediately after adding the virus (Time 0) and at 7 day intervals thereafter until viable virus was no longer detectable in the undiluted samples.

(B) Results

Although not a natural temperature for estuarine or marine waters, we included an incubation temperature of 37 C in our studies as a control to determine the lability of these viruses in water at the normal incubation temperature for most biological assays. No results are given in the ensuing tables because no viable virus was detectable with any of the viruses at any salinity after 7 days of incubation at this temperature.

Poliomyelitis Virus

The inactivation rates for polio type 1 virus incubated at 4 C are shown in Table 1. At a salinity of 10 parts per thousand, only a one log

reduction in viral infectivity occurred within 8 weeks and it took 18 weeks for a 99% loss in viability. Similar findings were made with the virus suspended in salinities of 20 and 34 parts per thousand in that a 2 log drop in titer occurred gradually during the first 12 weeks of incubation. Infectious virus was still detectable after 40 weeks incubation at all salinities.

Table 1. Effects of salinity and incubation temperatures on the survival of poliomyelitis type 1 (Mahoney) virus

	4 C			15 C			25 C		
Weeks	10[a]	20	34	10	20	34	10	20	34
0	5.4[b]	5.3	5.2	4.7	4.5	4.8	5.0	5.5	4.8
2	5.2	4.5	4.1	4.7	3.3	4.2	3.2	2.6	3.7
4	5.0	3.9	3.4	4.4	3.0	3.6	2.1	0.5	2.5
6	4.8	3.7	3.3	4.0	2.9	2.8	1.0	0.0[c]	0.8
8	4.3	3.6	3.3	3.5	2.8	2.5	0.0		0.0
10	4.0	3.4	3.1	3.9	2.5	2.3			
12	3.8	3.3	3.3	4.3	2.7	2.3			
14	3.9	2.6	3.2	4.1	2.3	1.9			
16	3.7	3.0	3.5	4.3	2.5	2.3			
18	3.1	2.6	3.4	3.8	1.7	1.8			
20	2.8	2.2	2.7	3.9	0.6	1.3			
22	2.4	1.8	2.2	3.4	0.0	0.0			
24	2.8	2.3	2.4	2.8					
32	2.6	1.9	1.5	3.1					
40	1.4	1.7	1.9	2.2					
46	0.0	0.0	0.7	1.1					
53			0.0	0.0					

Letter on file

[a]Salinity in parts NaCl per thousand.

[b]Log_{10} of the number of pfu's/ml.

[c]No plaques detected with the undiluted sample.

A

At 15 C, polio 1 was inactivated more rapidly than at 4 C and the salinity employed did seem to have an effect in that the virus seemed to lose infectivity at a faster rate at salinities of 20 and 34 parts per thousand (no infectious virus was detectable after 20 weeks of incubation) when compared to a salinity of 10 ppt where viable virus was noted after 46 weeks. At 25 C, total loss of infectivity occurred within 9 weeks at all salinities tested.

ECHO-6 Virus

The results obtained with ECHO-6 virus are shown in Table 2. This virus seemed to be more stable than poliovirus; however, the differences noted may not be significant. Again, the temperature of incubation, rather than the salinity of the diluent, was the main factor affecting the survival time.

Coxsackie B-5 Virus

This agent proved to be the most stable of the three viruses tested (Table 3). Although its infectivity decreased steadily with prolonged incubation at 25 C, it survived 2 to 4 weeks longer than the other two viruses at this temperature. Similarly, at 15 C, the agent proved to be more stable in that low levels of infectious virus were detected after 40 weeks at all salinities and after 46 weeks at 10 and 20 ppt. While all three viruses were fairly stable at 4 C, Coxsackie B-5 was again the most stable in that infectious virus was demonstrable at all salinities after 53 weeks of incubation, which was the last sampling time.

Table 2. Effects of salinity and temperature on the survival of ECHO-6 (D'Amori) virus

	4 C			15 C			25 C		
Weeks	10[a]	20	34	10	20	34	10	20	34
0	4.5[b]	5.0	4.7	4.7	4.8	5.0	4.5	4.8	4.7
2	4.4	4.5	4.8	4.4	4.8	4.3	4.1	3.7	3.1
4	4.7	4.7	4.2	4.0	4.5	4.0	3.3	2.7	2.3
6	4.5	4.5	4.0	3.5	3.9	3.5	1.8	0.7	0.0[c]
8	4.3	4.4	4.1	3.6	3.8	3.6	1.0	0.0	
10	4.3	4.2	3.8	3.3	3.0	3.3	0.0		
12	4.1	4.0	3.6	2.3	2.3	2.0			
14	3.8	4.0	3.3	2.3	2.7	2.3			
16	3.4	4.1	3.4	2.4	2.7	2.4			
18	2.5	3.3	2.4	0.9	1.9	0.9			
20	2.9	2.6	2.9	0.8	0.7	0.3			
22	2.8	3.0	2.8	0.3	1.0	0.8			
24	2.7	3.4	2.7	0.0[c]	1.0	0.3			
32	2.8	2.8	2.7		0.0	0.0			
40	2.6	3.3	2.5						
46	0.0	0.3	0.7						
53		0.0	0.0						

[a]Salinity in parts NaCl per thousand.

[b]Log_{10} of the number of pfu's/ml.

[c]No plaques detected in the undiluted sample.

Table 3. Effects of salinity and temperature on the survival of Coxsackie B-5 (Faulkner) virus

	4 C			15 C			25 C		
Weeks	10[a]	20	34	10	20	34	10	20	34
0	5.6[b]	5.4	5.5	4.8	4.9	4.7	5.2	6.0	4.7
2	5.0	4.9	5.0	4.5	4.4	4.4	4.1	4.4	3.6
4	5.4	4.9	4.7	4.7	4.2	4.6	3.7	2.6	3.1
6	4.9	5.1	4.8	4.7	4.6	4.6	2.6	2.6	2.3
8	5.1	4.6	4.9	4.5	4.1	4.0	2.2	1.3	1.0
10	4.9	4.7	4.8	4.5	4.1	4.3	1.1	0.0[c]	0.0
12	5.3	4.9	4.8	4.6	4.2	4.3	0.0		
14	4.9	5.2	5.1	4.3	4.0	4.1			
16	5.3	5.8	5.4	4.8	4.3	4.4			
18	5.2	5.0	5.1	4.6	4.3	4.1			
20	4.8	4.2	4.5	4.0	2.9	3.3			
22	3.9	3.8	3.9	3.5	2.8	2.9			
24	4.5	4.4	4.5	3.5	3.2	3.1			
32	3.3	4.5	4.8	3.3	3.1	2.6			
40	2.1	4.0	4.6	3.0	2.3	1.3			
46	1.4	3.4	3.8	1.6	0.3	0.0			
53	1.6	2.6	3.1	1.2	0.0				

[a]Salinity in parts NaCl per thousand.

[b]Log_{10} of the number of pfu's/ml.

[c]No plaques detected in the undiluted sample.

2. In situ studies on virus survival

(A) Methods

The in situ survival studies were conducted at the University of Delaware Marine Laboratory, Lewes, Delaware and at the State of Maryland Natural Resource Institute Laboratory at Solomons, Maryland. Dialysis bags, fitted with Neoprene stoppers with a sampling port were filled with autoclaved water samples (approximately 250 ml each) taken at the two locations. The samples were then seeded with 10^5 - 10^6 pfu's/ml of the respective viruses and immersed in plastic tanks which were continuously being charged (6 - 7 liters per minute) with free-flowing marine or estuarine water. The ocean water had a salinity of 26 - 30 parts per thousand (ppt) and a pH of 7.8 - 8.0, whereas the estuarine water ranged in salinity from 8 - 12 ppt and was in the pH range of 7.6 - 7.8. Experiments were conducted during both the summer and winter months at two locations. At Lewes, the water temperature varied between 21 and 26 C in the summer and from 4 - 16 C in the winter. At Solomons, the bay water temperature ranged from 4 - 19 C during the winter and 24 to 28 C during the summer experimentation.

(B) Results

Figures 1-3 show the survival characteristics of the enteroviruses in free-flowing ocean water. As in the laboratory studies, water temperature proved to be the important factor since the viruses were more labile during the summer months. Poliovirus 1 again proved to be the most labile agent tested in that all infectivity was lost by 27 days during the summer and by 65 days during the winter (Fig. 1). ECHO-6 survived slightly longer than polio, i.e., 31 days in the summer and 70 days in the winter (Fig. 2), although the differences noted may not be significant. As in the laboratory

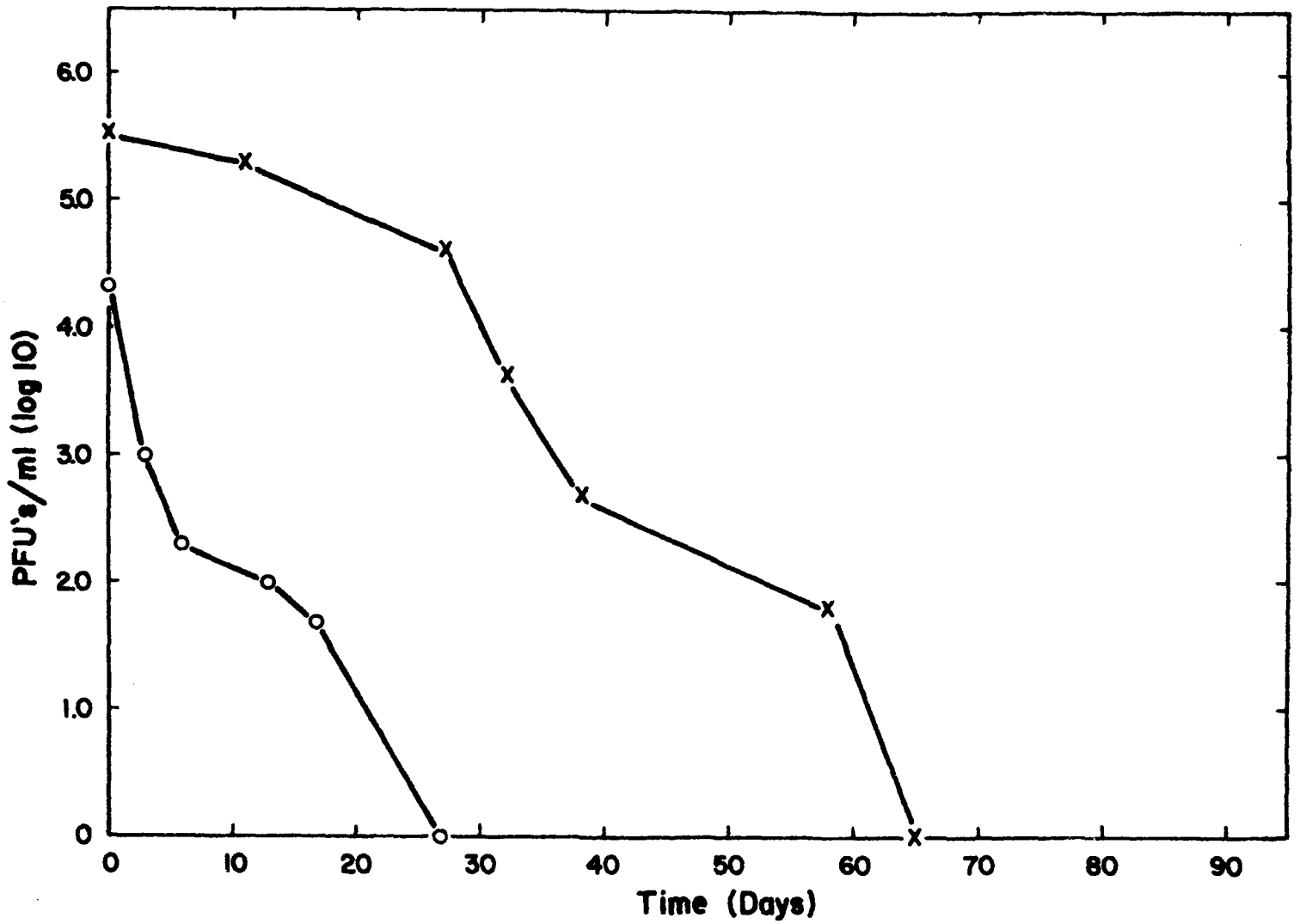

Fig. 1. Survival of poliomyelitis virus *in situ* in ocean water. X - winter months; O - summer months.

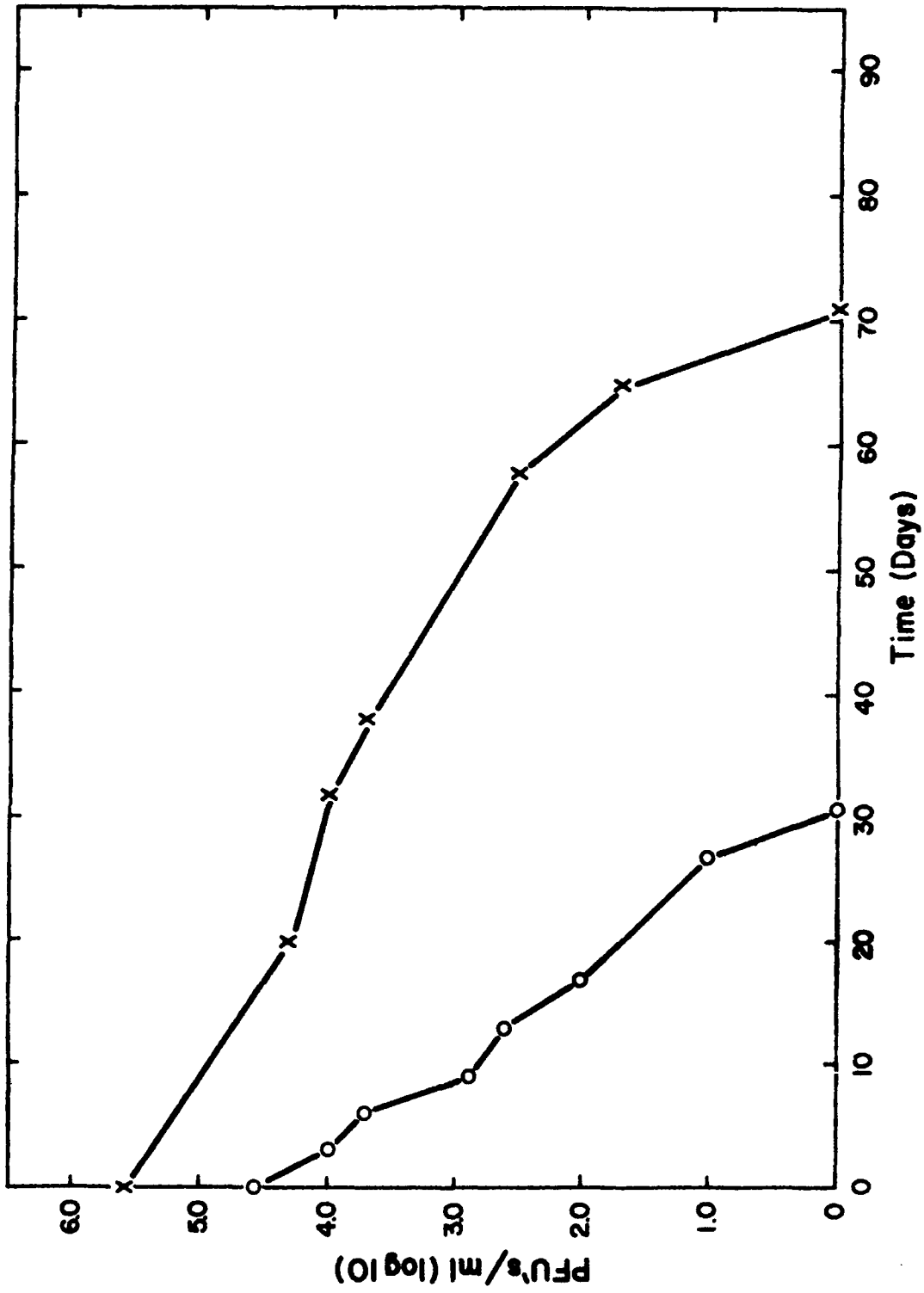

Fig. 2. Survival of ECHO-6 virus in situ in ocean water. X - winter months; O - summer months.

studies, Coxsackie B-5 virus proved to be the most stable one tested in that it took 7 weeks for its total inactivation in the summer and only a 2 log drop in infectivity occurred over an 80 day period during the winter months (Fig. 3).

Similar results were obtained when the viruses were exposed to estuarine waters in that their survival times were longer during the winter (Fig. 4) than during the summer months (Fig. 5). Also, as found in previous studies, the order of the viruses in terms of increasing stability was polio 1, ECHO-6, and Coxsackie B-5 viruses.

3. Effect of temperature on the virucidal activity of seawater

(A) Methods

(1) Virucidal studies

The ocean water employed was collected offshore of Lewes, Delaware and its salinity was 28 parts per thousand. The sample was divided into 3 portions: one was autoclaved at 121 C for 20 min.; one was filtered through a 0.45 μm Millipore filter; and the third portion was untreated. Replicate flasks of each type of seawater (100 ml each) were seeded with approximately 10^4 - 10^5 pfu's/ml of polio 1, ECHO-6, and Coxsackie B-5 viruses, respectively. Three incubation temperatures were also employed, i.e., 4, 15, and 25 C. The flasks were placed in shaker baths (150 cycles per min.) held at the various temperatures and samples were withdrawn at weekly intervals for assay.

(2) Virucidal factor

When it was determined that both filtration and autoclaving greatly diminished the virucidal activity of seawater, attempts were made

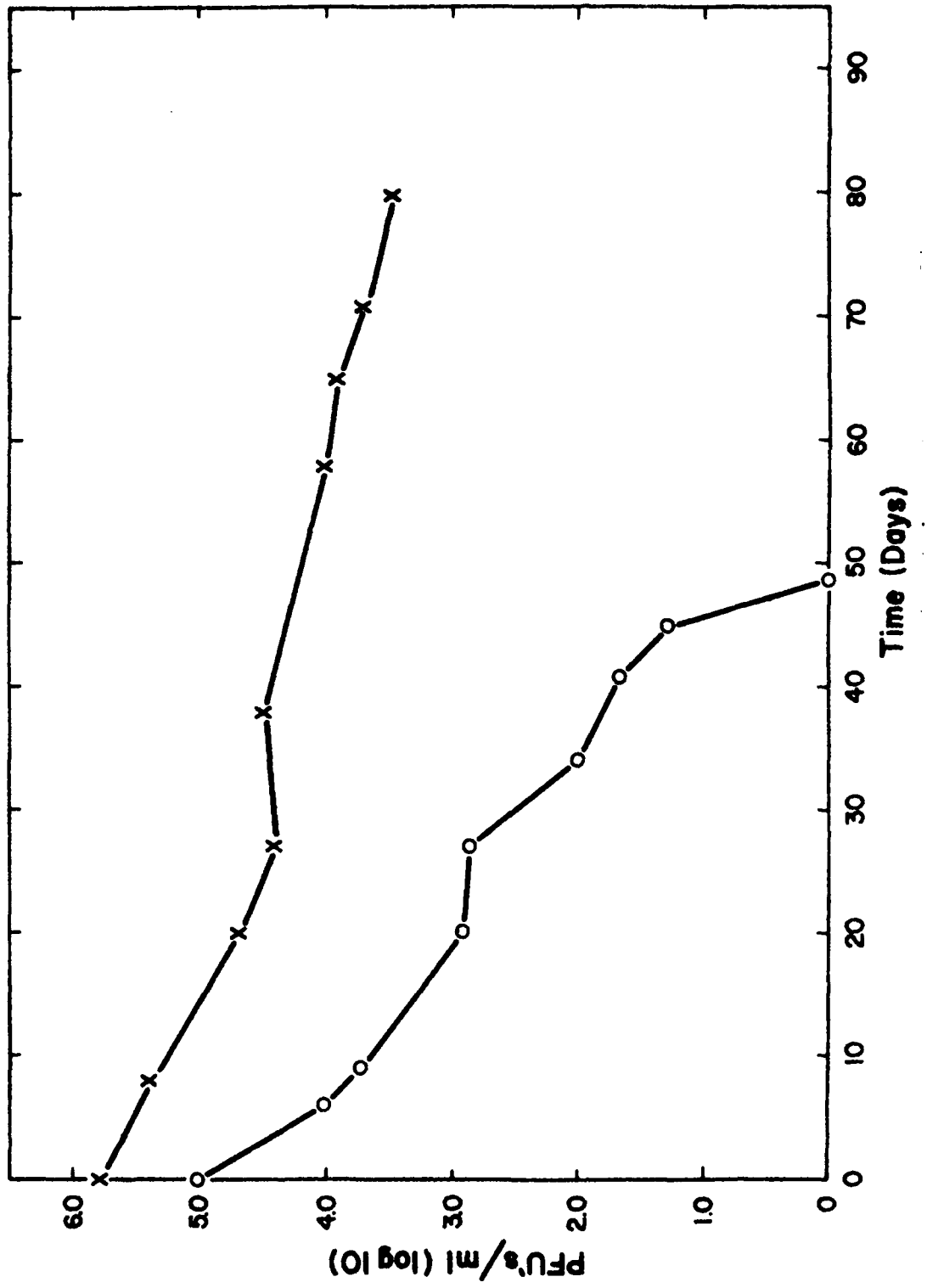

Fig. 3. Survival of Coxsackie B-5 virus in situ in ocean water. X - winter months; O - summer months.

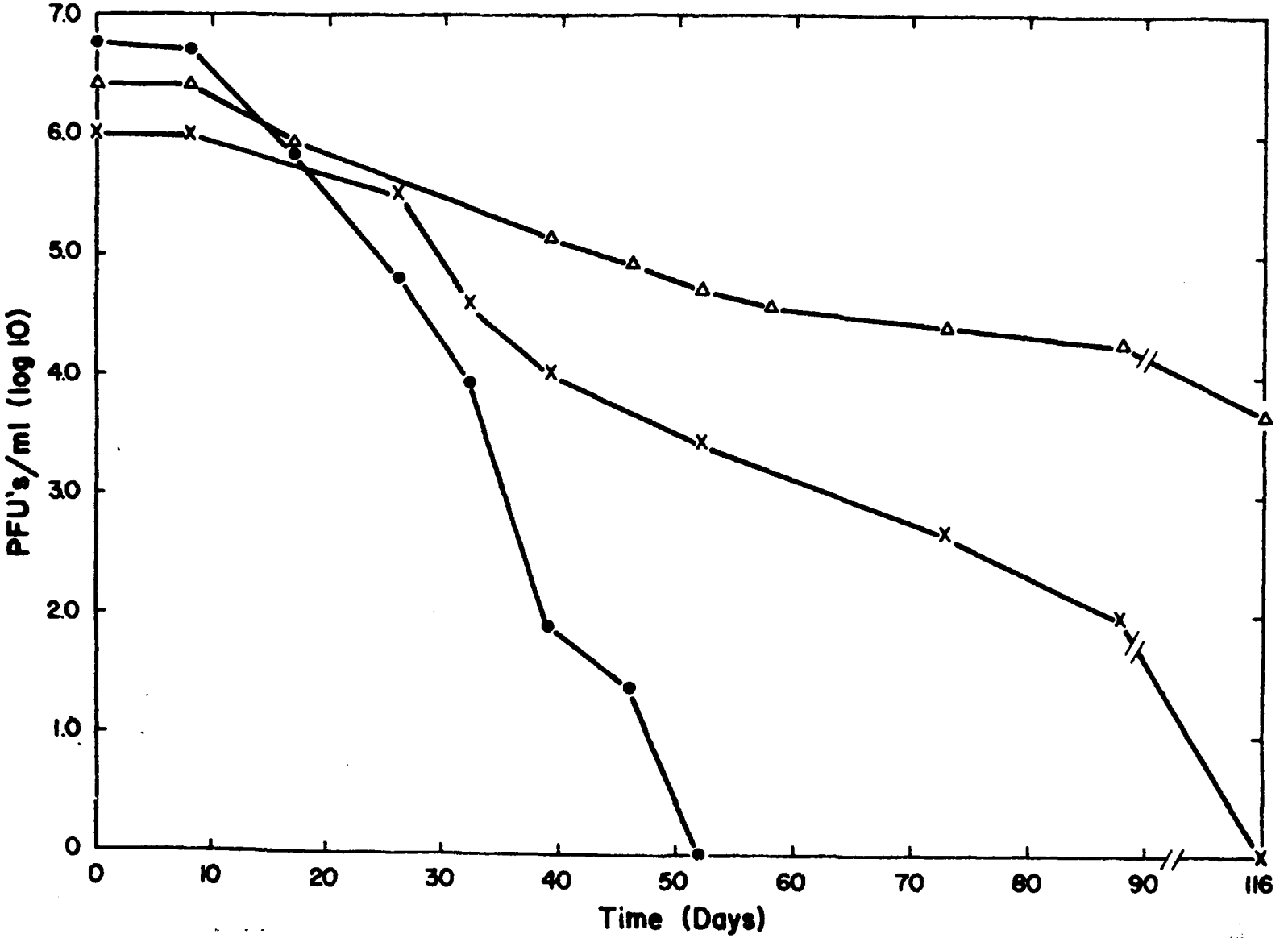

Fig. 4. Survival of enteroviruses in situ in estuarine water during the winter months. O - polio 1; X - ECHO-6; Δ - Coxsackie B-5.

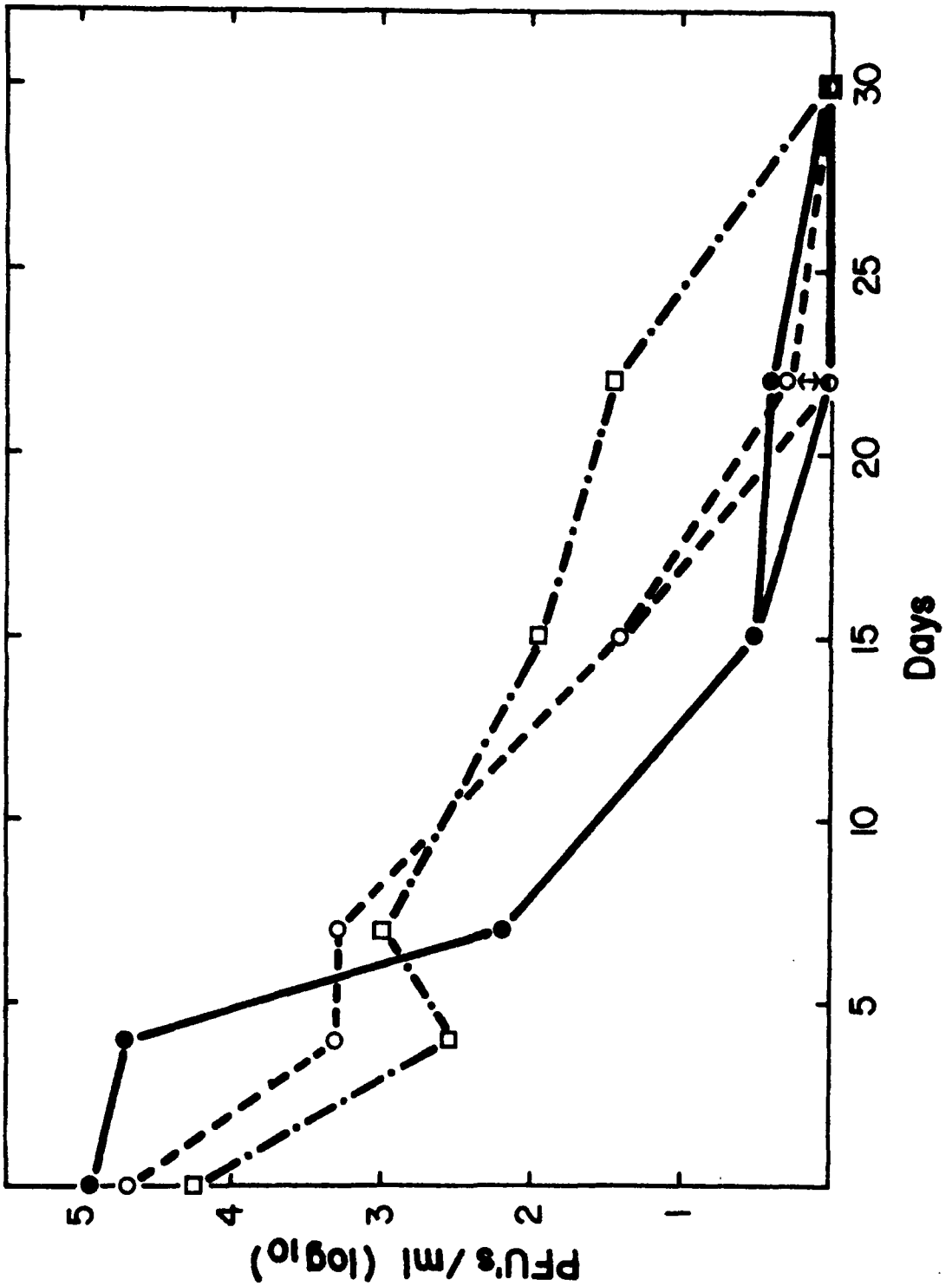

Fig. 5. Survival of enteroviruses in situ in estuarine water during the summer months. O - polio 1; X - ECHO-6; Δ - Coxsackie B-5.

to determine if the activity could be restored by reintroduction of the substance removed by filtration. For this study, 100 ml aliquots of seawater were filtered through 0.45 μm Millipore pads. One of the filter pads was washed in 100 ml of autoclaved seawater and another was washed in 100 ml of the seawater filtrate. Each flask was then seeded with approximately 10^5 pfu's/ml of poliovirus 1. Autoclaved, filtered, and untreated waters from the same ocean water sample were also seeded with 10^5 pfu's/ml and served as controls. Two incubation temperatures (15 and 25 C) were employed and samples were taken for virus assay at 2-4 day intervals until virus was no longer detectable in the undiluted samples.

(B) Results

(1) Virucidal activity of seawater at 4 C

Poliovirus 1 suspended in untreated seawater underwent a 3 log drop (99.9%) in infectivity within 14 days and was totally inactivated within 4 weeks (Fig. 6). This virus was much more stable in the autoclaved or filtered samples and although the infectivity titers were markedly reduced, infectious virus was still detectable after 11 weeks' incubation (Fig. 6). With ECHO-6 virus, comparable results were obtained (Fig. 7) except that this virus appeared to be considerably more stable in the filtered water than in the autoclaved sample.

Coxsackie B-5 virus was the most stable of the three viruses tested at 4 C in that infectious virus was still detectable in the untreated seawater sample after 63 days and only negligible drops in infectivity occurred in the autoclaved or filtered samples after 12 weeks' incubation (Fig. 8).

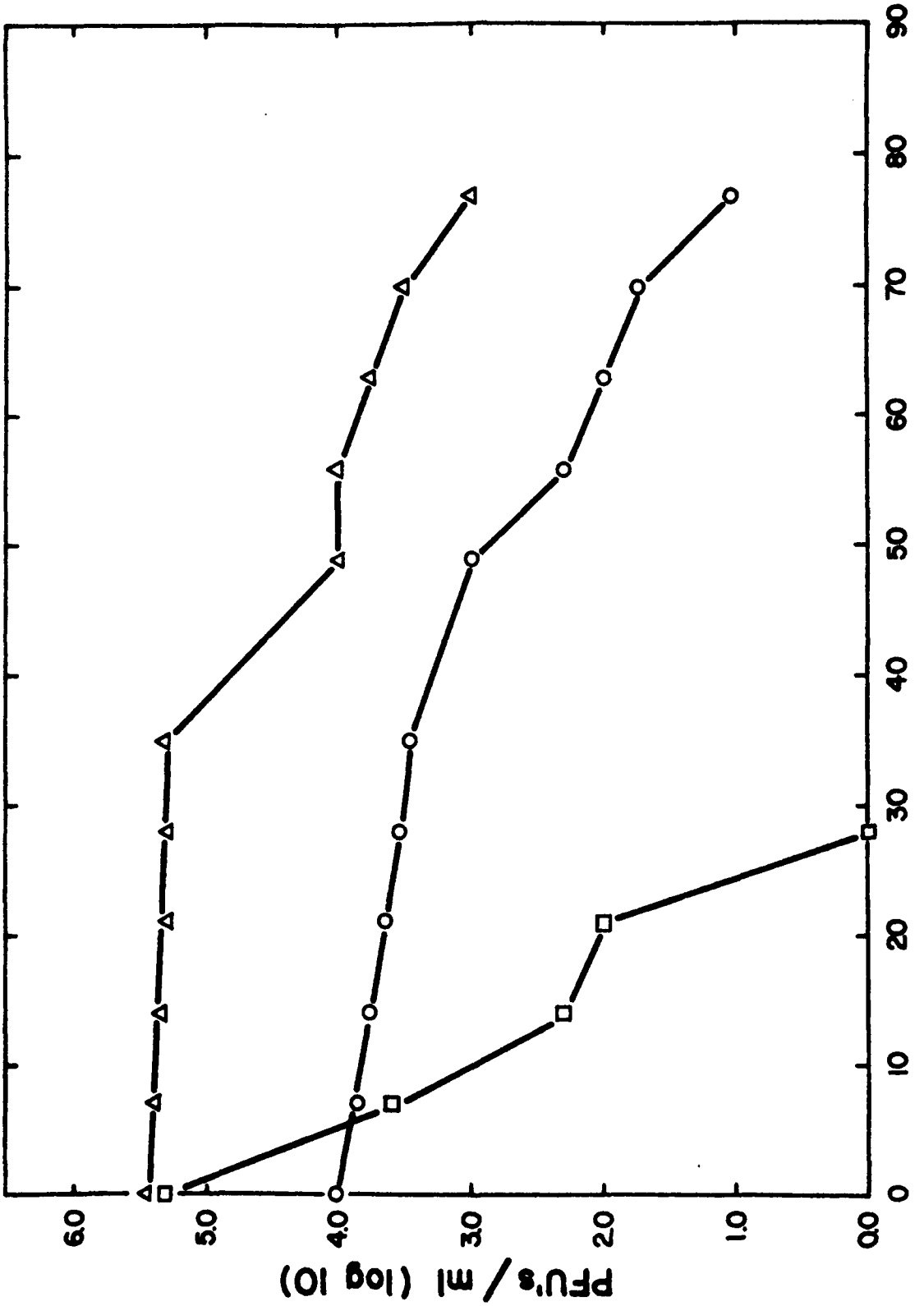

Fig. 6. Inactivation of poliovirus in seawater at 4 C. □ - untreated; Δ - filtered; O - autoclaved seawater.

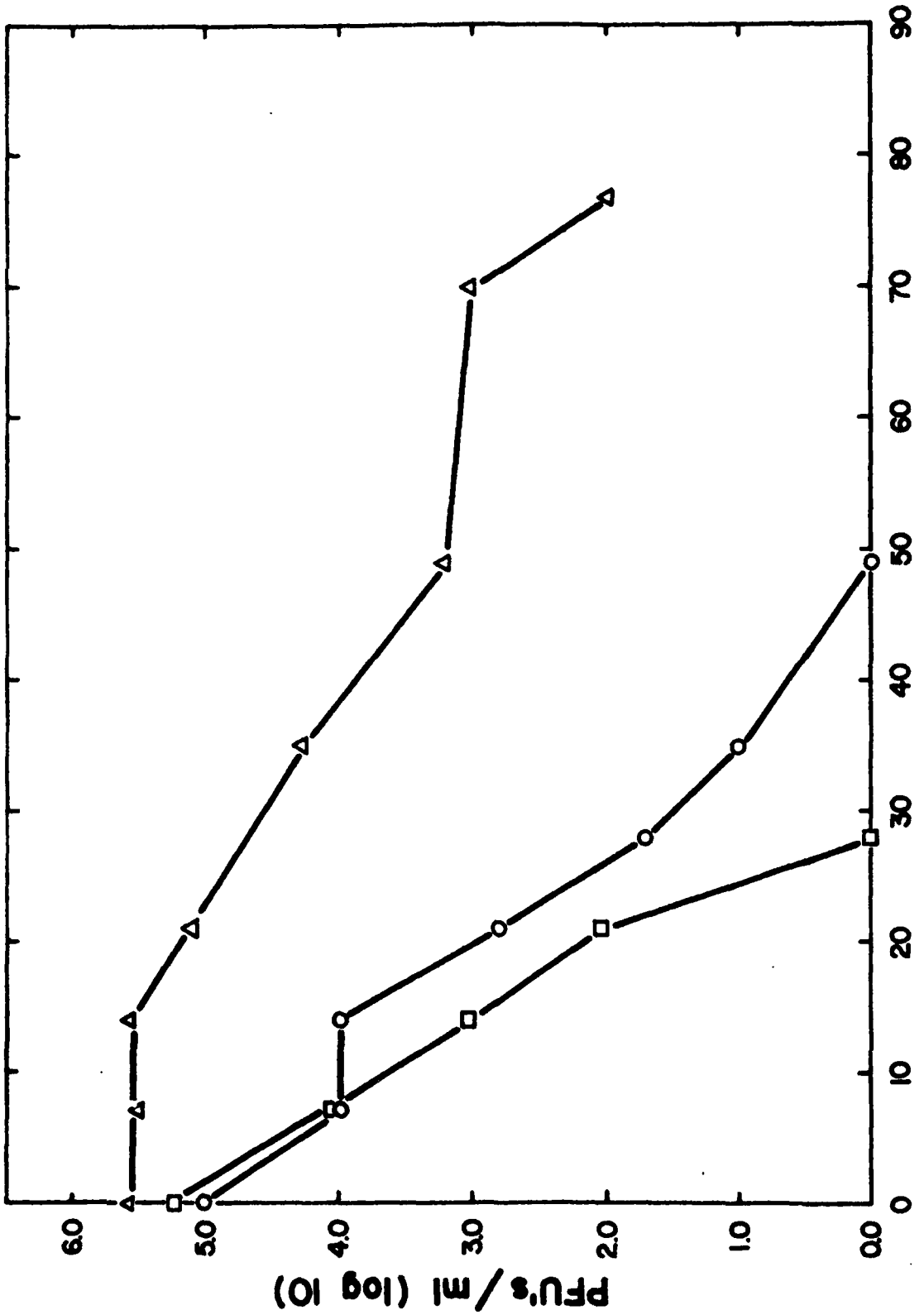

Fig. 7. Inactivation of ECHO-6 virus in seawater at 4 C. □ - untreated; Δ - filtered; O - autoclaved seawater.

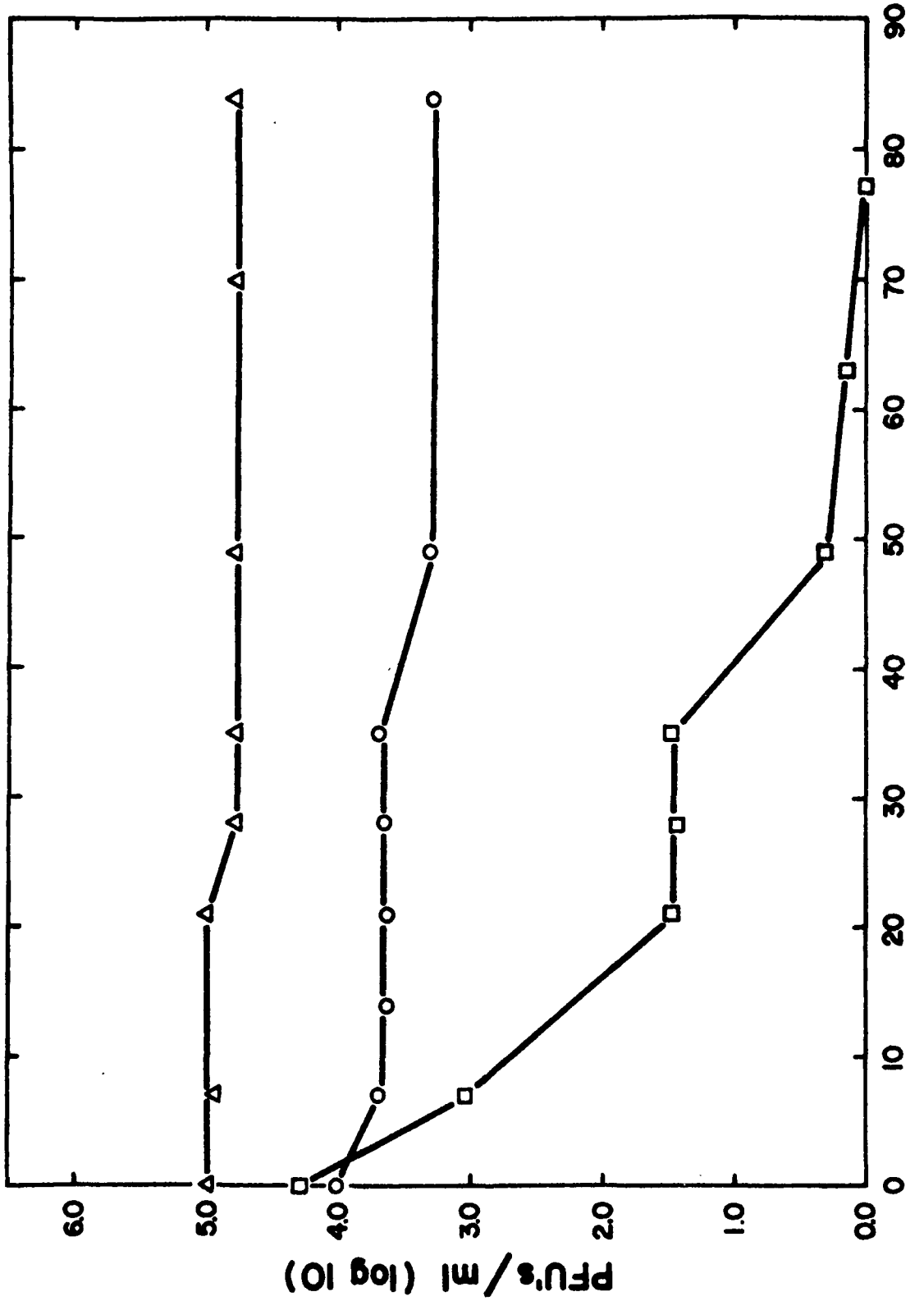

Fig. 8. Inactivation of Coxsackie B-5 virus in seawater at 4 C. □ - untreated; Δ - filtered; ○ - autoclaved seawater.

(2) Virucidal activity at 15 C

At this temperature, all three viruses were inactivated more rapidly than at 4 C. In the untreated water, polio 1 lost 3 logs of infectivity within 5 days and was totally inactivated after 7 days (Fig. 9). Although infectious virus was still detectable after 11 weeks in the autoclaved and filtered samples, the amount present was considerably less than that detected in similar samples held at 4 C. The results with ECHO-6 virus followed the same general pattern in that at 15 C the virus was much more labile than at 4 C (Fig. 10). Likewise, Coxsackie B-5 lost its infectivity more rapidly in all three samples of water (Fig. 11) at 15 C when compared to the results obtained at 4 C but, as in the previous experiments, it proved to be the most stable virus tested.

(3) Virucidal activity of seawater at 25 C

Of the three temperatures studied, virus inactivation was most rapid at 25 C both in untreated and treated water samples. In these experiments, virus survival in distilled water was followed for comparative purposes. All three viruses were inactivated within one week in the untreated water samples at this temperature. Poliovirus 1 lost all infectivity within 5 weeks in both the filtered and autoclaved samples (Fig. 12) as compared to only a one log drop of viral activity in the distilled water. Similar findings were observed for Coxsackie B-5 virus (Fig. 13) in that all viral infectivity had been lost after 4 weeks in the seawater samples (treated or not) whereas considerable infectivity remained in the distilled water sample after 11 weeks of incubation.

ECHO-6 virus proved to be the most labile virus at this temperature in that all infectivity was lost within 2 weeks in the seawater samples (Fig. 14) and within 4 weeks in the distilled water sample.

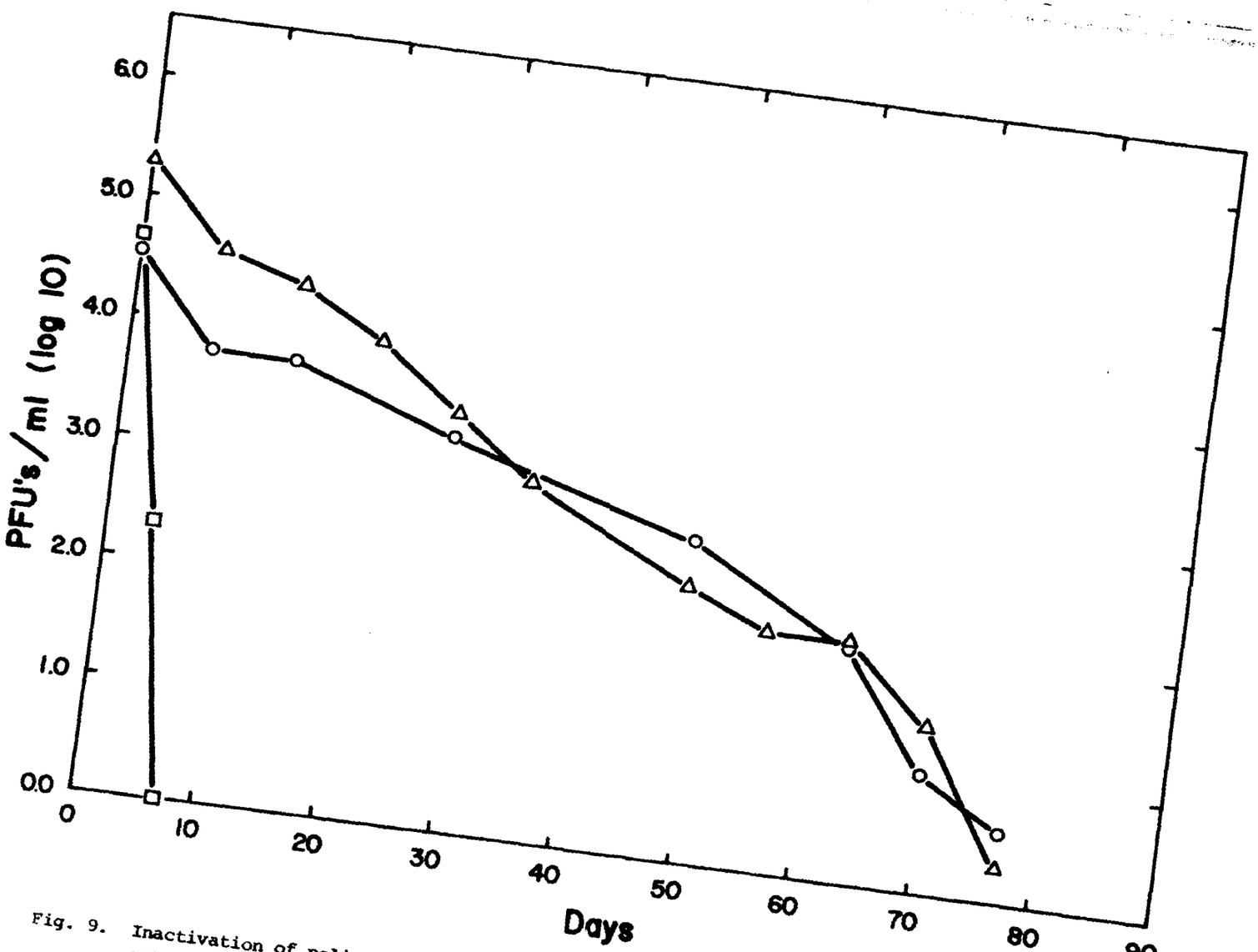

Fig. 9. Inactivation of poliovirus in seawater at 15 C. □ - untreated; Δ - filtered; O - autoclaved seawater.

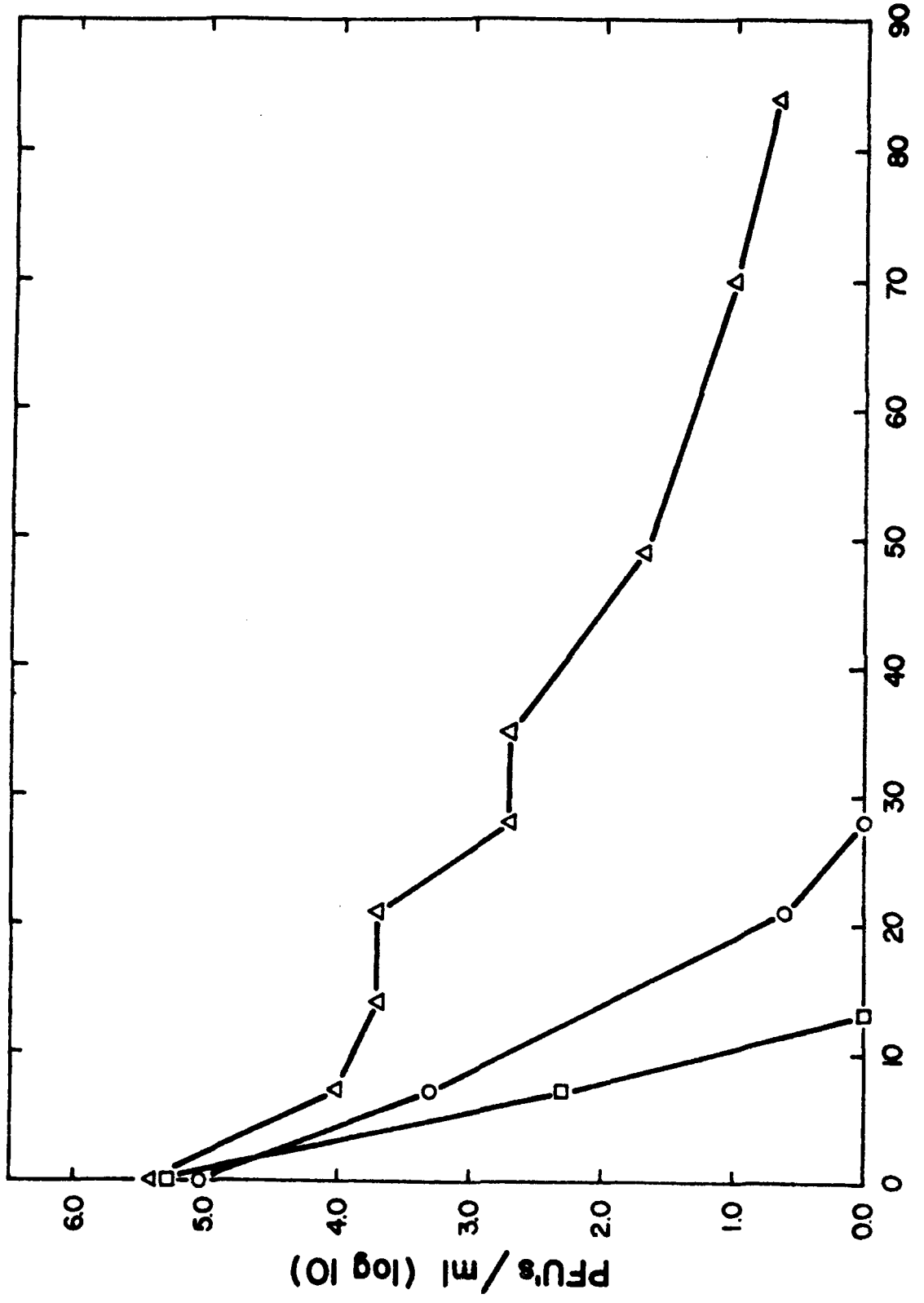

Fig. 10. Inactivation of ECHO-6 virus in seawater at 15 C. □ - untreated; Δ - filtered; o - autoclaved seawater.

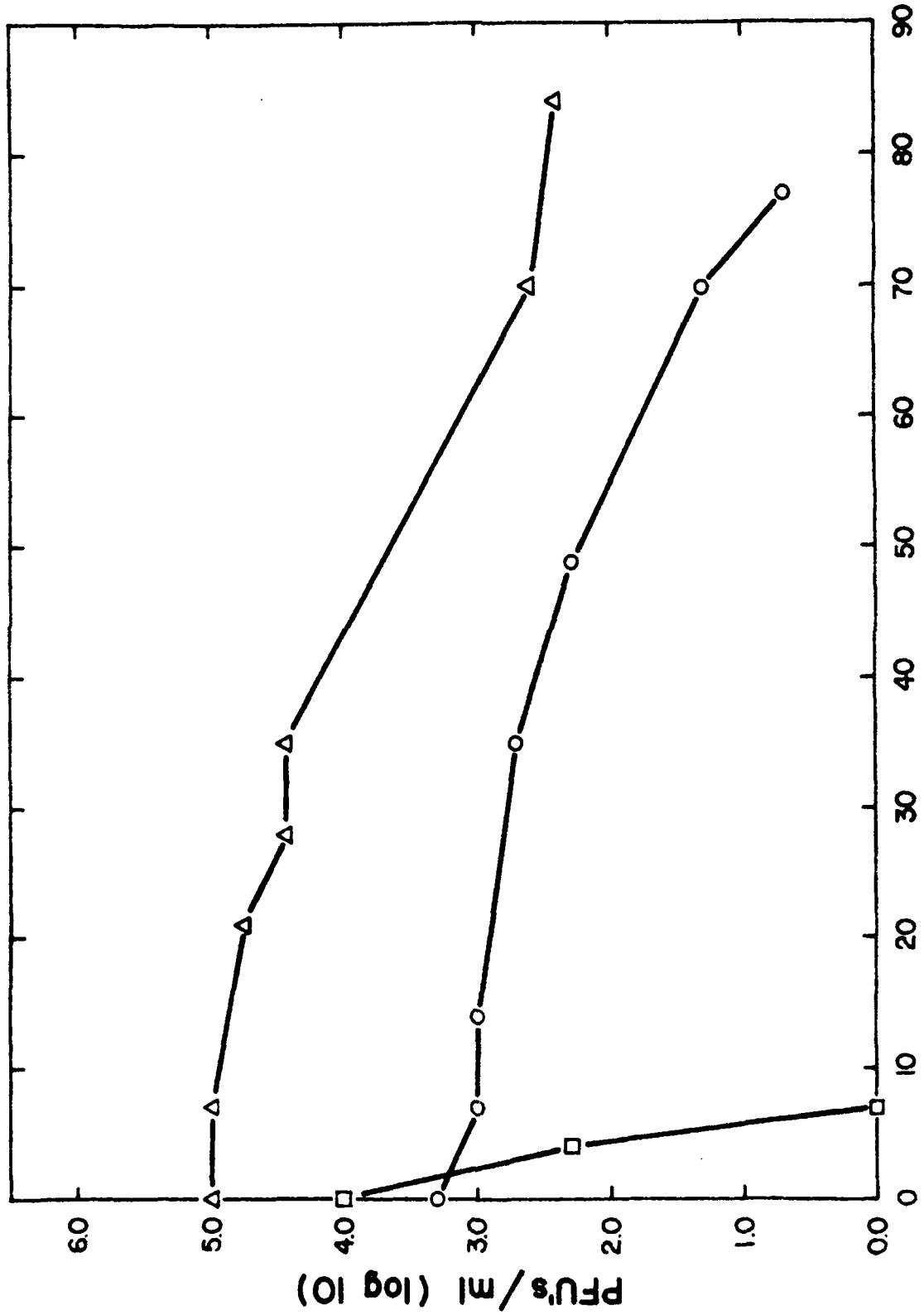

Fig. 11. Inactivation of Coxsackie B-5 virus in seawater at 15 C. □ - untreated; Δ - filtered; O - autoclaved seawater.

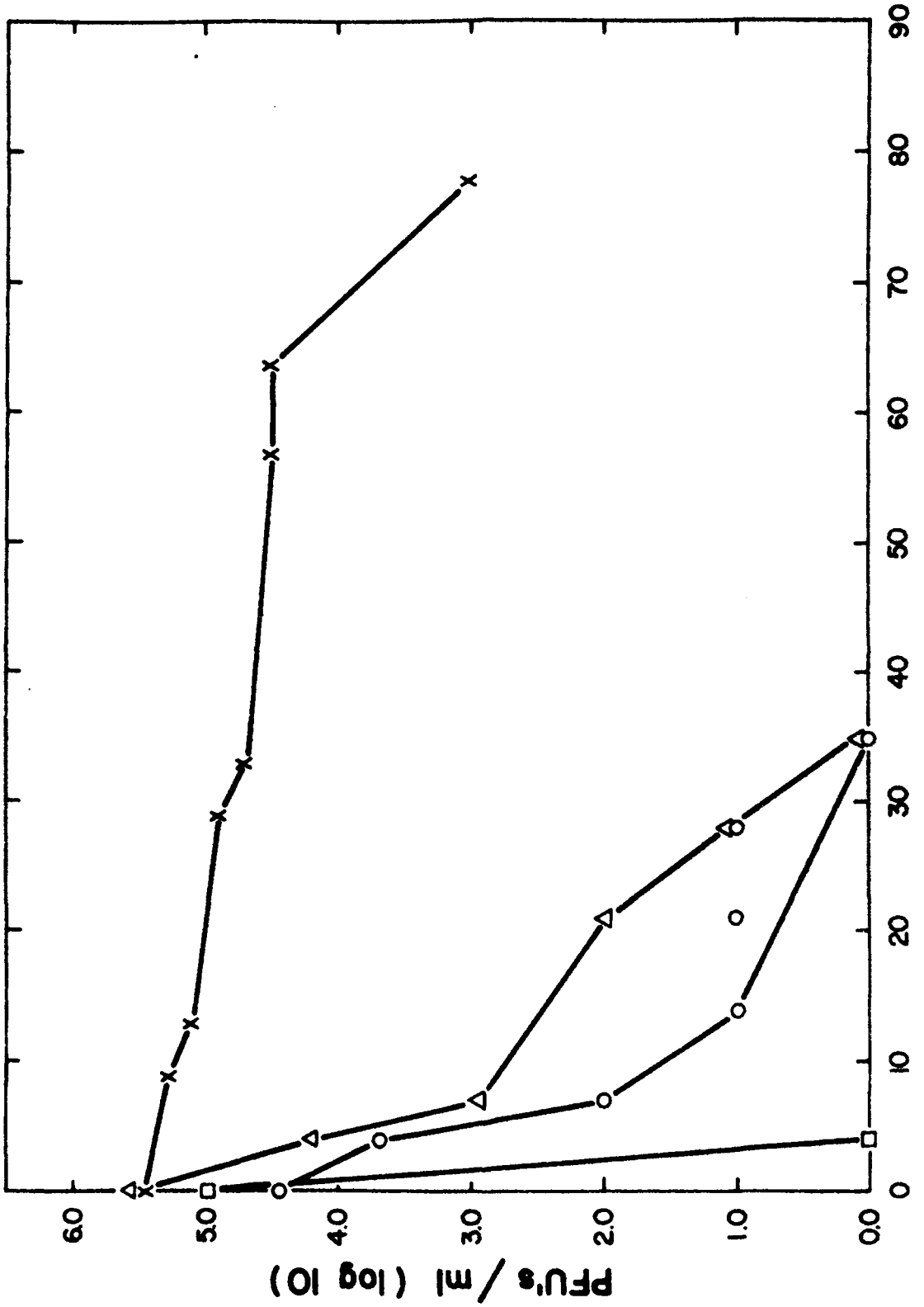

Fig. 12. Inactivation of poliovirus in seawater at 25 C. □ - untreated; Δ - filtered; O - autoclaved seawater.

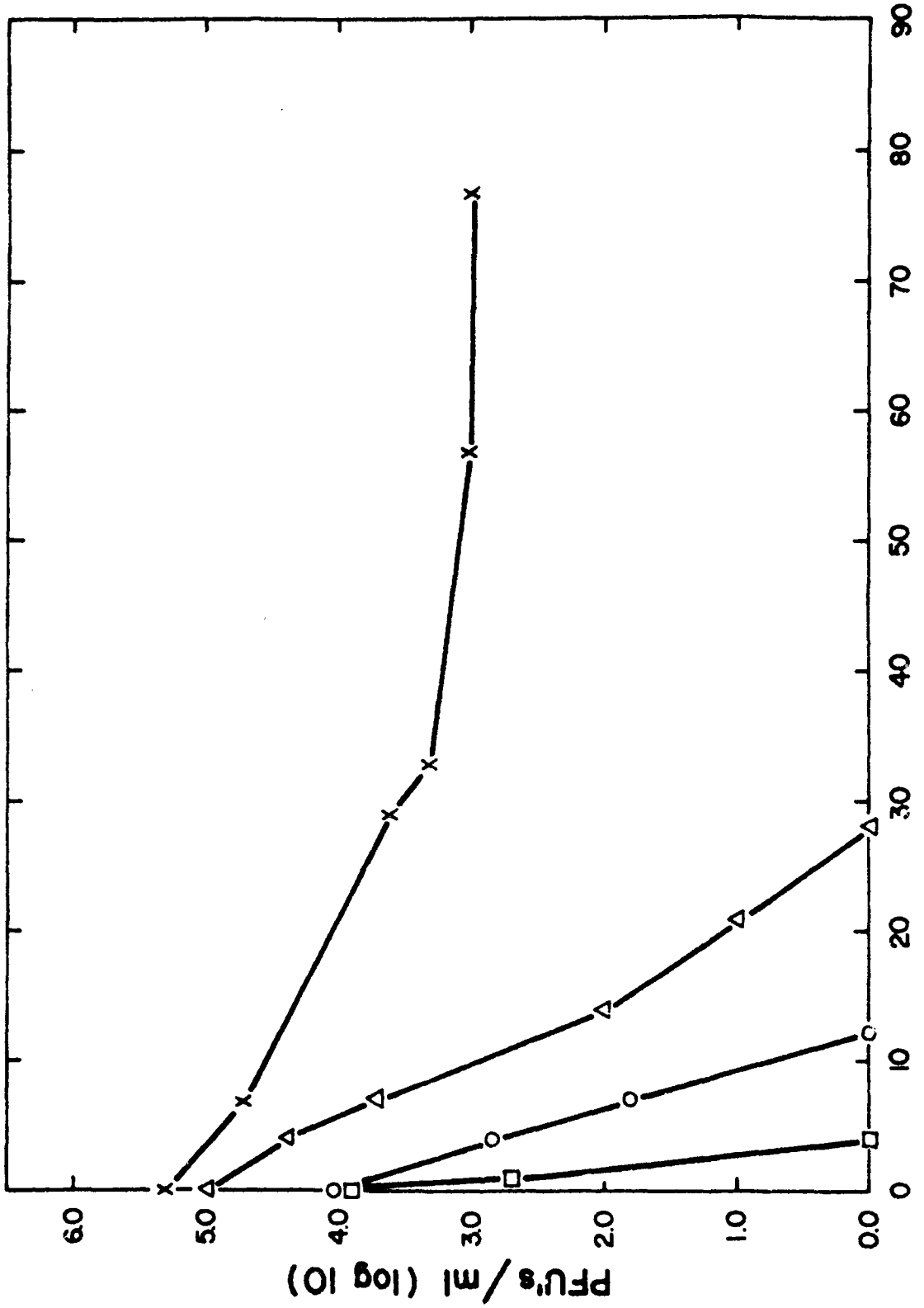

Fig. 13. Inactivation of Coxsackie B-5 virus at 25 C. □ - untreated; Δ - filtered; O - autoclaved seawater.

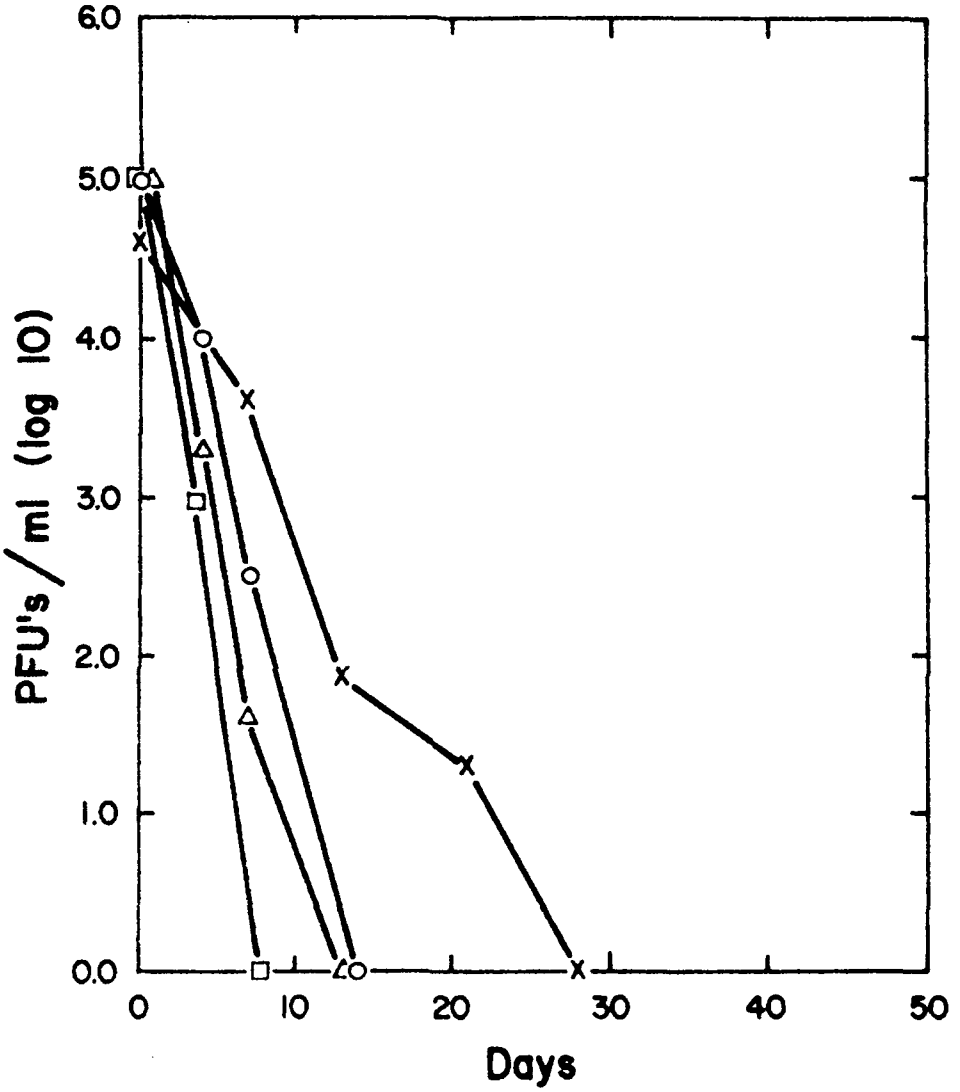

Fig. 14. Inactivation of ECHO-6 virus in seawater at 25 C. □ - untreated; Δ - filtered; O - autoclaved seawater.

(4) Studies on the virucidal properties of seawater

Aliquots, 100 ml each, of fresh seawater were filtered through a 0.45 µm Millipore pad. One of the filter pads was washed in a 100 ml sample of autoclaved seawater, another pad was washed back on a 100 ml sample of seawater filtrate. Each flask was then seeded with 10^5 pfu's/ml of polio 1 virus. Autoclaved, filtered, and untreated seawater samples were also seeded with virus as controls. Two incubation temperatures (15 and 25 C) were employed with the flasks being immersed in shaker water baths.

The results of this study are shown in Figs. 15 and 16. It is apparent that the virucidal activity of seawater, which is removed by filtration, is readily restored to either filtered or autoclaved seawater by elution of the substance from the filter pads back into the samples.

4. Inactivation of enteroviruses in seawater at pasteurization (62 C) temperature

Since our data indicate that enteric viruses survive for considerable periods of time in high salinity waters, the onboard sewage treatment process that the Navy selects for its vessels must reduce or eliminate virus levels in the effluent prior to its return to the water. One obvious treatment could involve the application of heat to the effluent to thermally inactivate the viruses. In order to provide some baseline data in this regard, we have determined the kinetics of virus inactivation in various salinity waters when exposed to pasteurization temperatures.

(A) Methods

Natural waters of three salinities were obtained from Solomons,

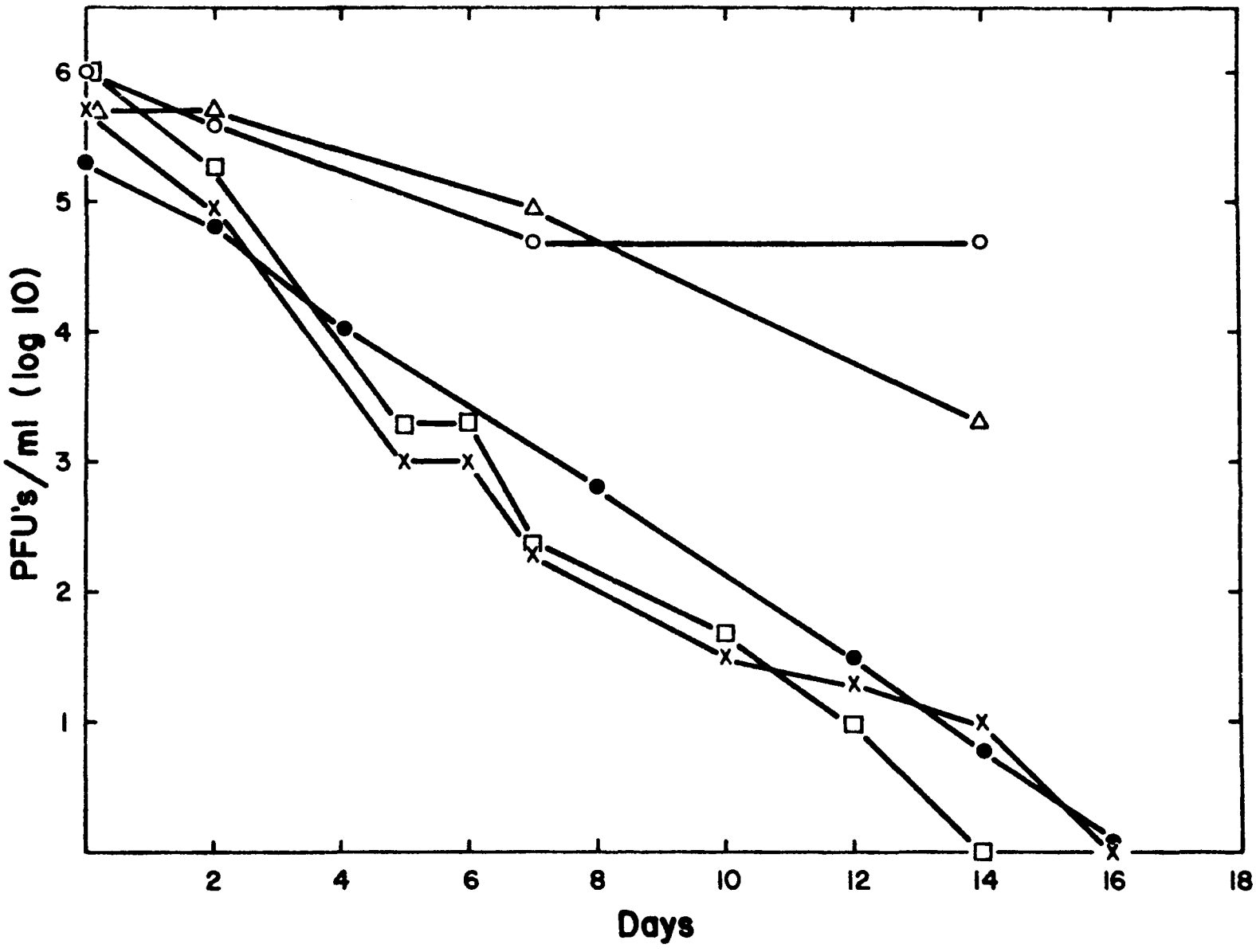

Fig. 15. Inactivation of poliovirus in ocean water at 15 C. X - untreated; Δ - filtered; O - autoclaved seawater; □ - filtered seawater plus washing from a filter pad; ● - autoclaved seawater plus washing from a filter pad.

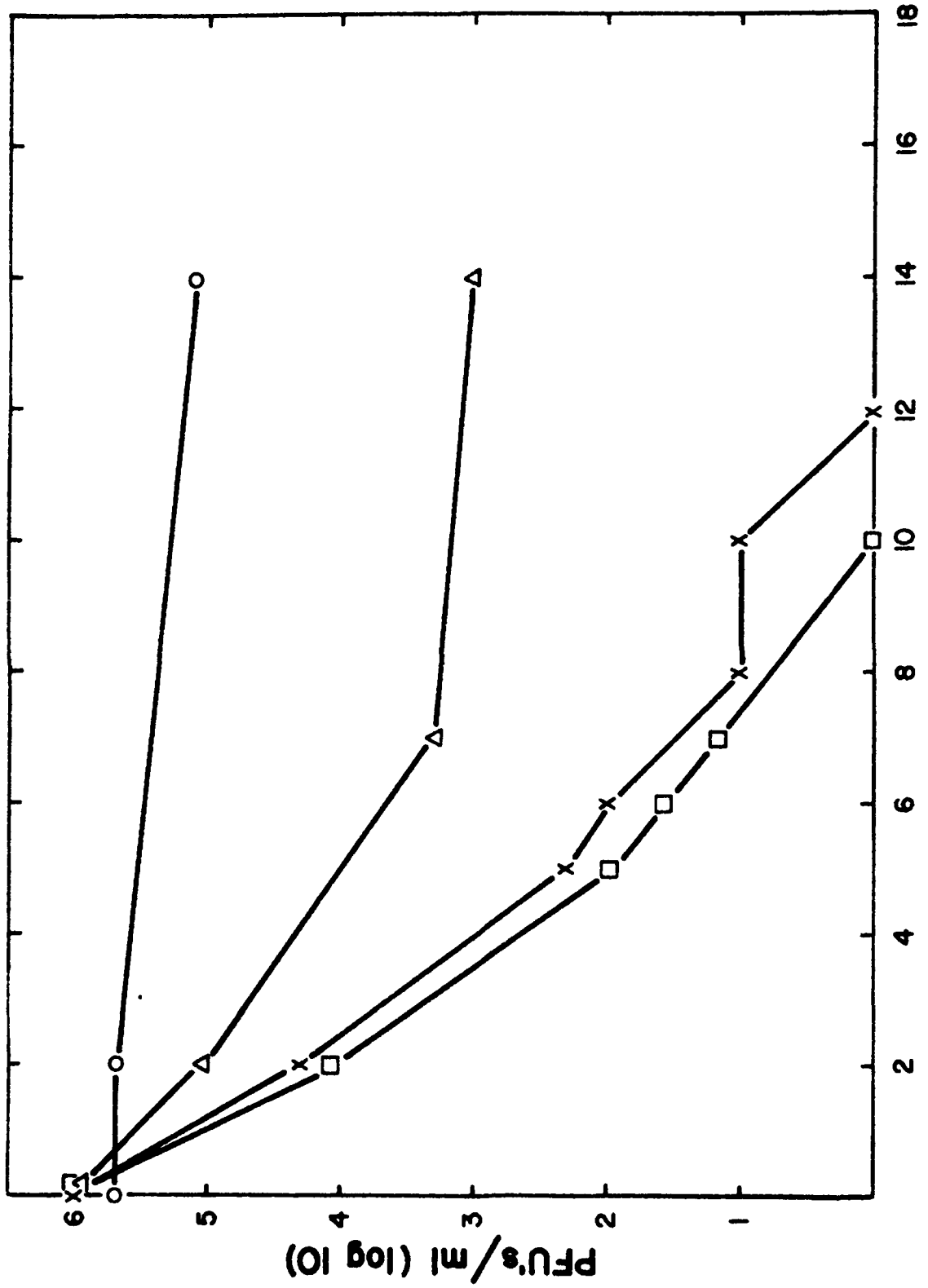

Fig. 16. Inactivation of poliovirus in ocean water at 25 C. X - untreated; Δ - filtered; O - autoclaved; □ - filtered water plus washing from a filter pad.

Maryland (estuarine, 10 °/oo salinity), Lewes, Delaware (coastal marine, 28 °/oo salinity) and off the coast of North Carolina over the continental shelf (deep ocean marine, 34 °/oo salinity). Nine experiments were conducted using the three viruses in the three water samples. In each experiment, untreated water was employed, as well as samples either filtered through a 0.45 μM membrane filter or autoclaved.

Aliquots of the respective filtered, autoclaved, or untreated water was seeded with virus to contain approximately 10^6 pfu's/ml. The test flasks were immersed in a water bath equilibrated at 62 C and samples taken after 0, 1, 3, 5, 10, 20 and 30 minutes of incubation. The samples were cooled in an ice bath and then assayed for viral infectivity.

(B) Results

In general, it can be seen that Coxsackie virus is more resistant to heat (Figs. 20-22) than is poliovirus (Figs. 17-19) or ECHO virus (Figs. 23-25) regardless of the salinity of the water used. Poliovirus is generally more stable at 62 C than ECHO virus except in 10 °/oo salinity water where poliovirus (Fig. 17) is inactivated more rapidly than ECHO virus (Fig. 23).

Poliovirus appears to be most stable to heating in the higher salinity waters. It is undetectable within 10 minutes in 10 °/oo salinity (Fig. 17) and 28 °/oo salinity (Fig. 18), but remains viable after 30 minutes in autoclaved 34 °/oo salinity (Fig. 19) waters.

Coxsackie virus is unactivated within 20 minutes in 10 °/oo salinity water, except in the filtered sample which remains detectable through the duration of the experiment (Fig. 20). In 28 °/oo salinity water, all samples were negative within 30 minutes (Fig. 21), while in 34 °/oo salinity water, the autoclaved sample remained detectable at 30 minutes, while the other

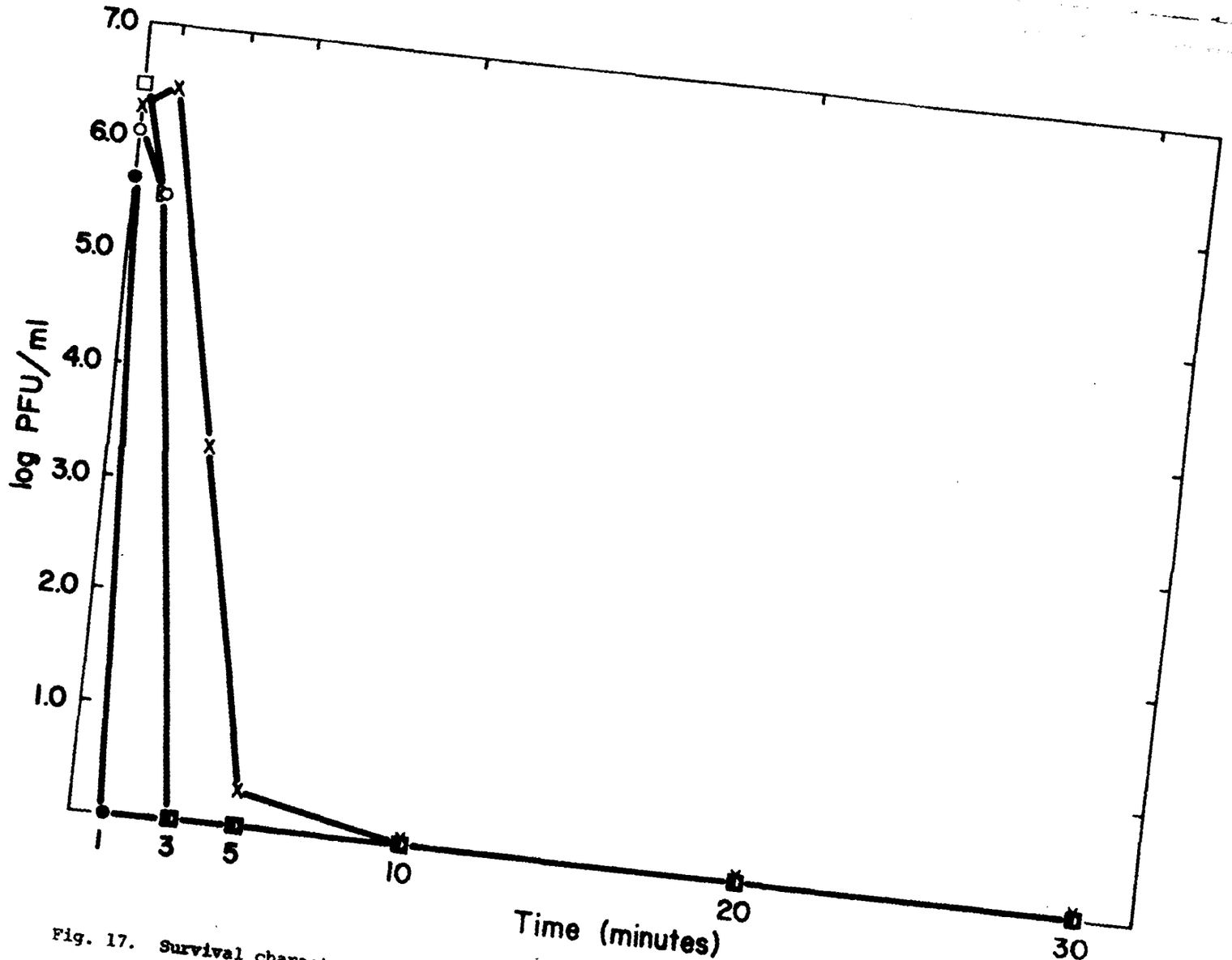

Fig. 17. Survival characteristics of poliovirus type 1 at 62 C in Solomons water (10 °/oo salinity). ●—● untreated; O—O autoclaved; X—X filtered; □—□ distilled water.

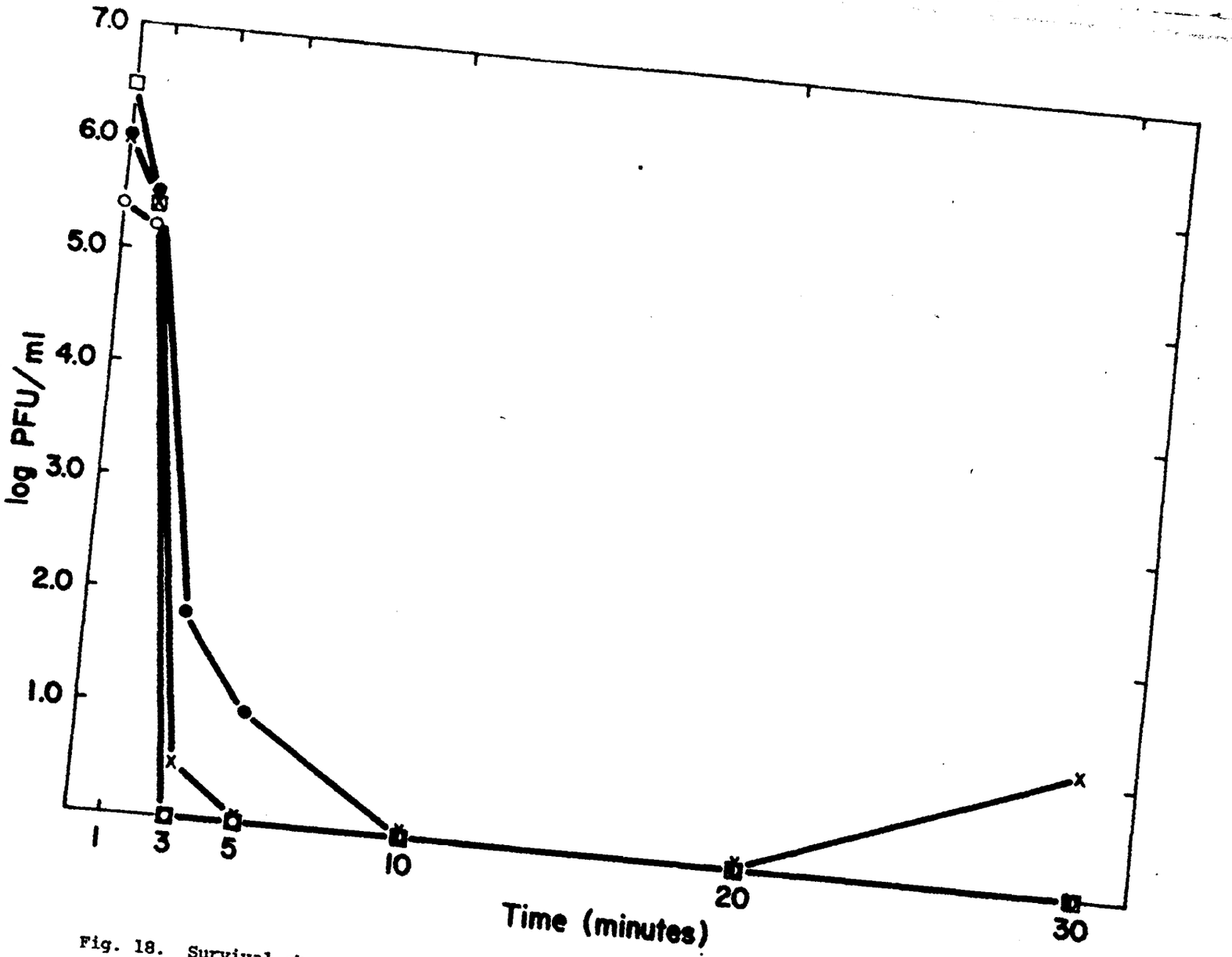

Fig. 18. Survival characteristics of poliovirus type 1 at 62 C in Lewes water (28 o/oo salinity). ●—● untreated; O—O autoclaved; X—X filtered; □—□ distilled water.

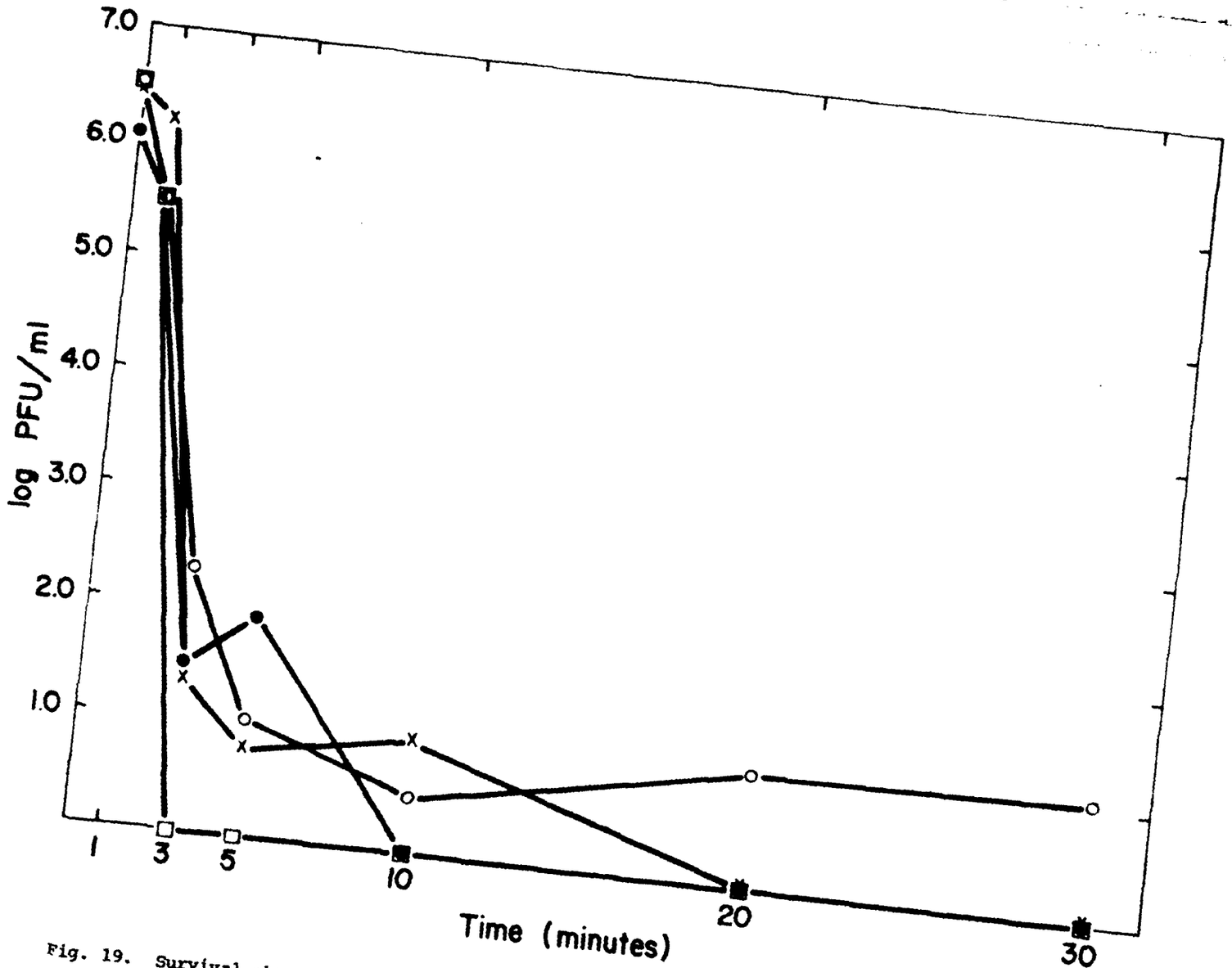

Fig. 19. Survival characteristics of poliovirus type 1 at 62 C in North Carolina water (34 o/oo salinity). ●—● untreated; O—O autoclaved; X—X filtered; □—□ distilled water.

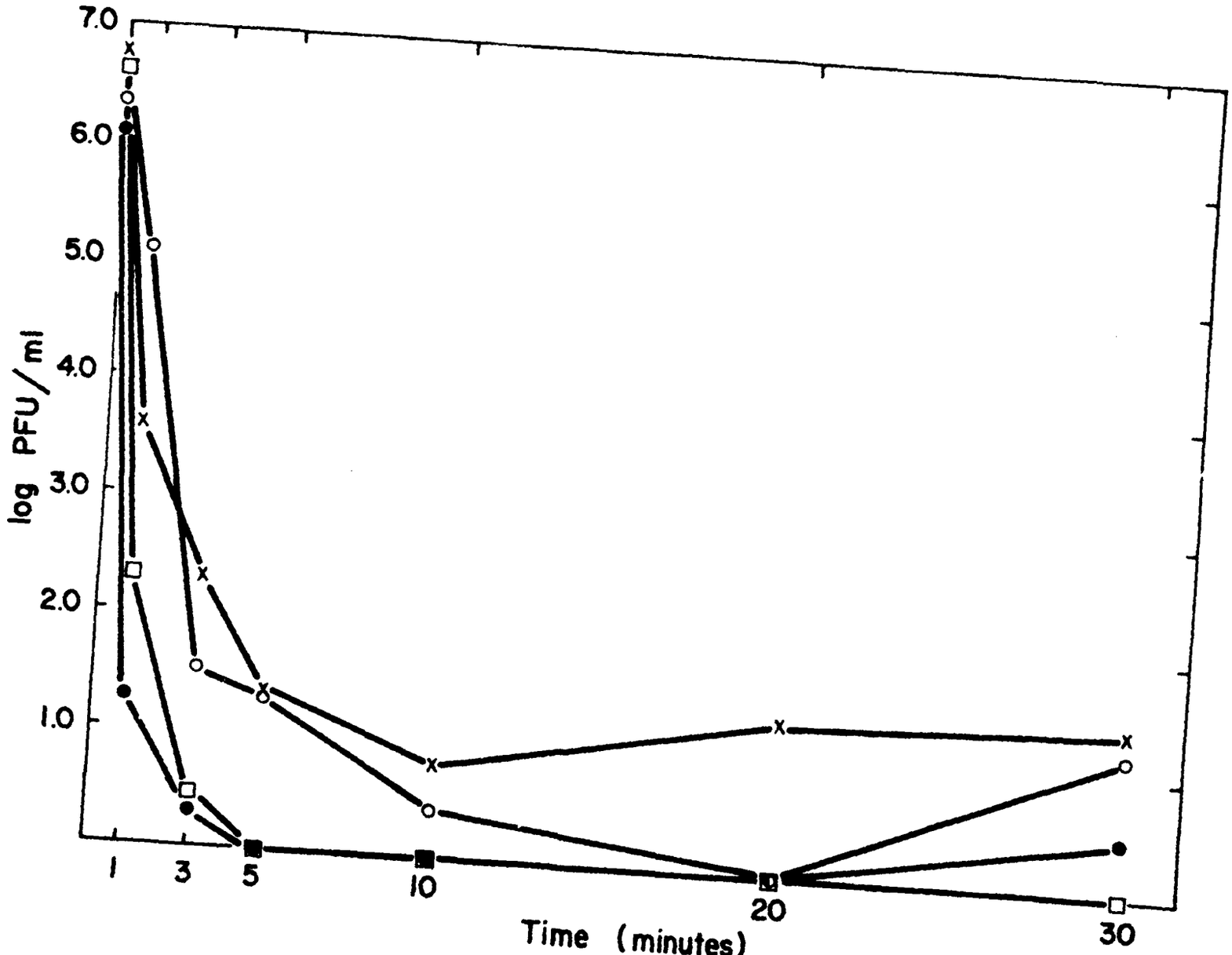

Fig. 20. Survival characteristics of Coxsackie virus type B-5 at 62 C in Solomons water (10 °/oo salinity). ●—● untreated; O—O autoclaved; X—X filtered; □—□ distilled water.

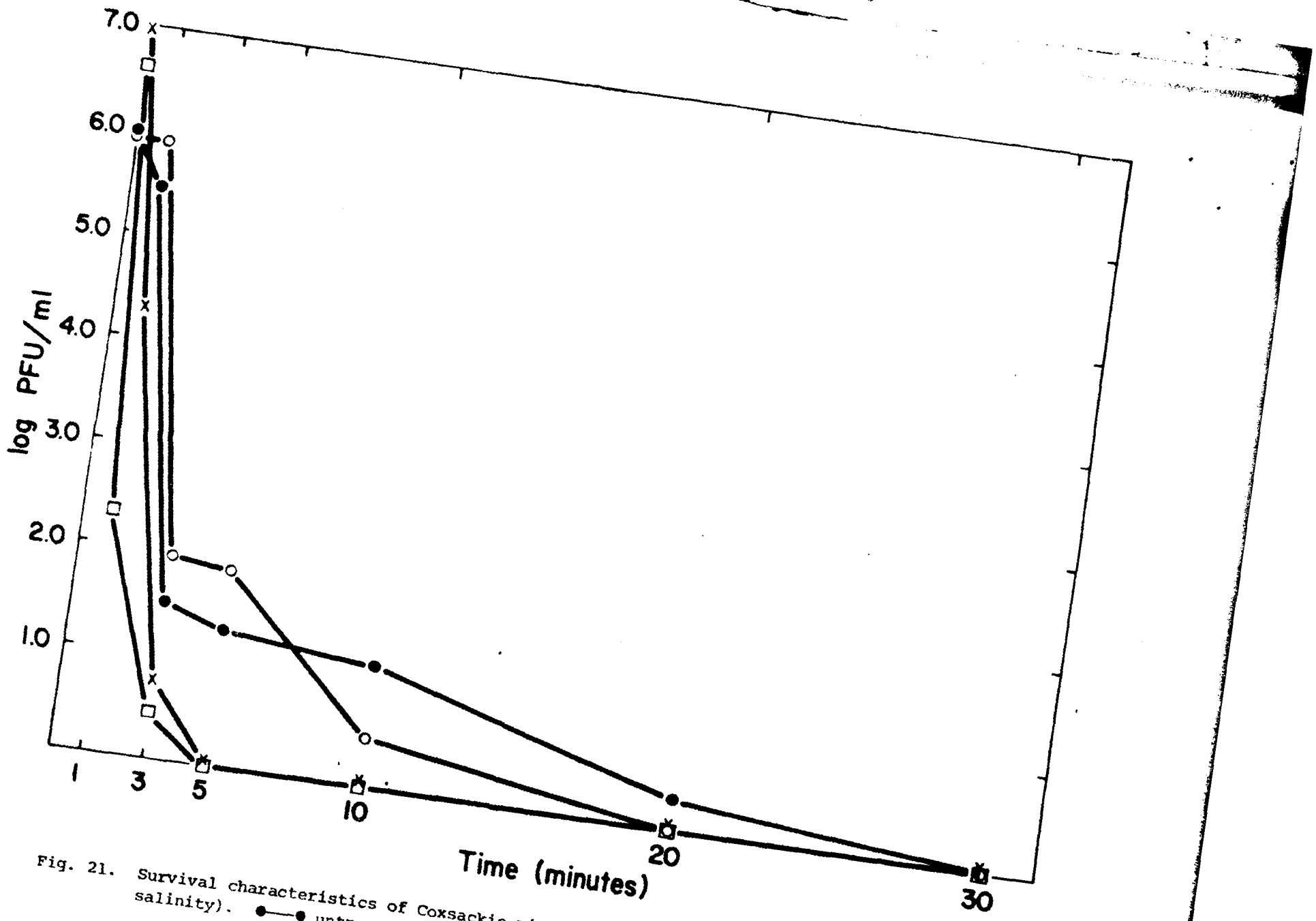

Fig. 21. Survival characteristics of Coxsackie virus type B-5 at 62 C in Lewes water (28 o/oo salinity). ●—● untreated; O—O autoclaved; X—X filtered; □—□ distilled water.

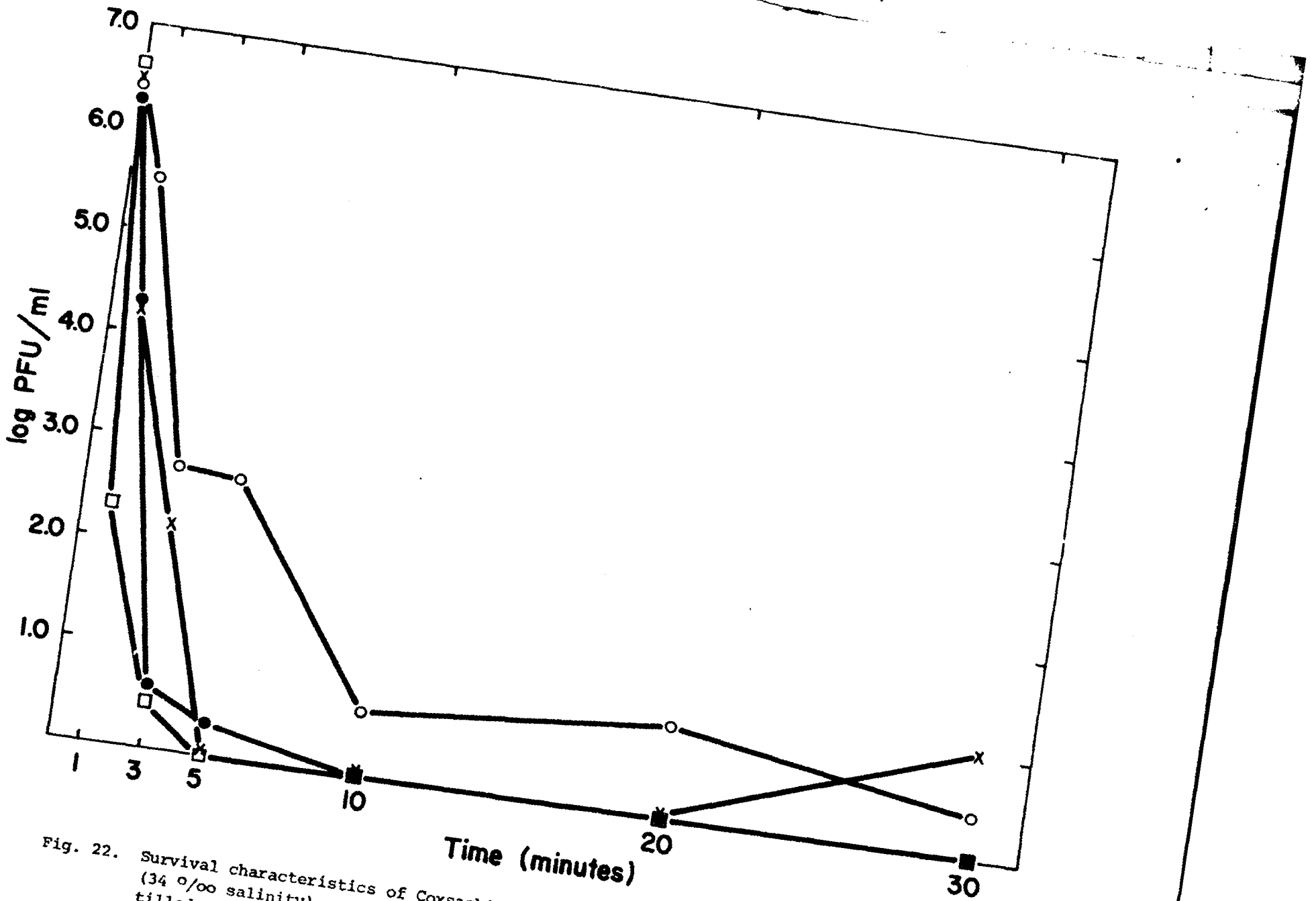

Fig. 22. Survival characteristics of Coxsackie virus type B-5 at 62 C in North Carolina water (34 o/oo salinity). ●---● untreated; O---O autoclaved; X---X filtered; □---□ distilled water.

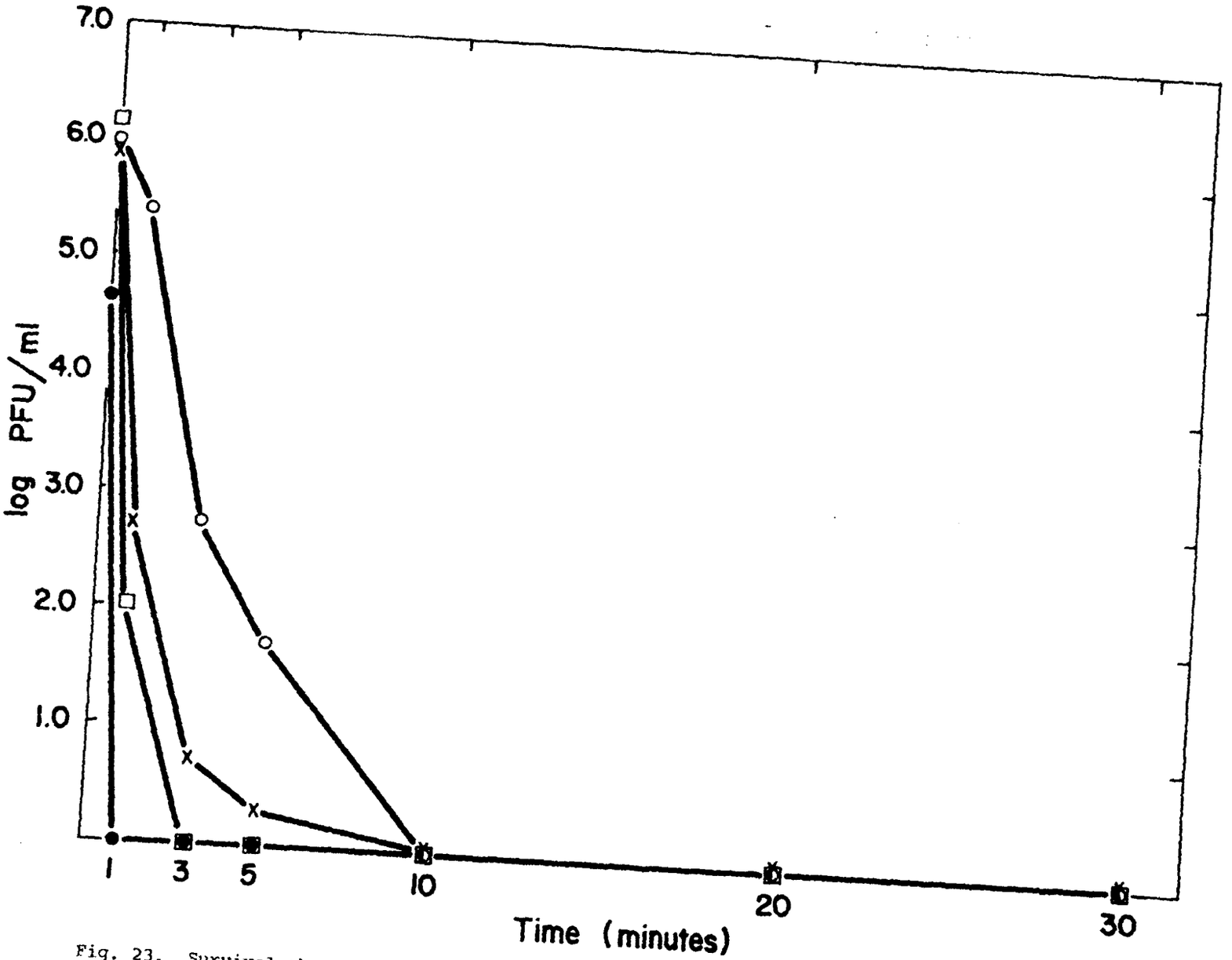

Fig. 23. Survival characteristics of ECHO virus type 6 at 62 C in Solomons water (10 o/oo salinity). ●—● untreated; O—O autoclaved; X—X filtered; □—□ distilled water.

samples were inactivated within 10 minutes (Fig. 22).

ECHO virus, generally the least stable of the three viruses tested, was inactivated within 10 minutes in 10 °/oo water (Fig. 23), within 5 minutes in 28 °/oo water (Fig. 24), and within 3 minutes in 34 °/oo water (Fig. 25).

It is interesting to note that all viruses were as stable or more stable in the natural waters than they were in distilled water with one exception. All three viruses appeared to be more rapidly inactivated in untreated 10 °/oo salinity water than in distilled water (Figs. 17, 20, 23).

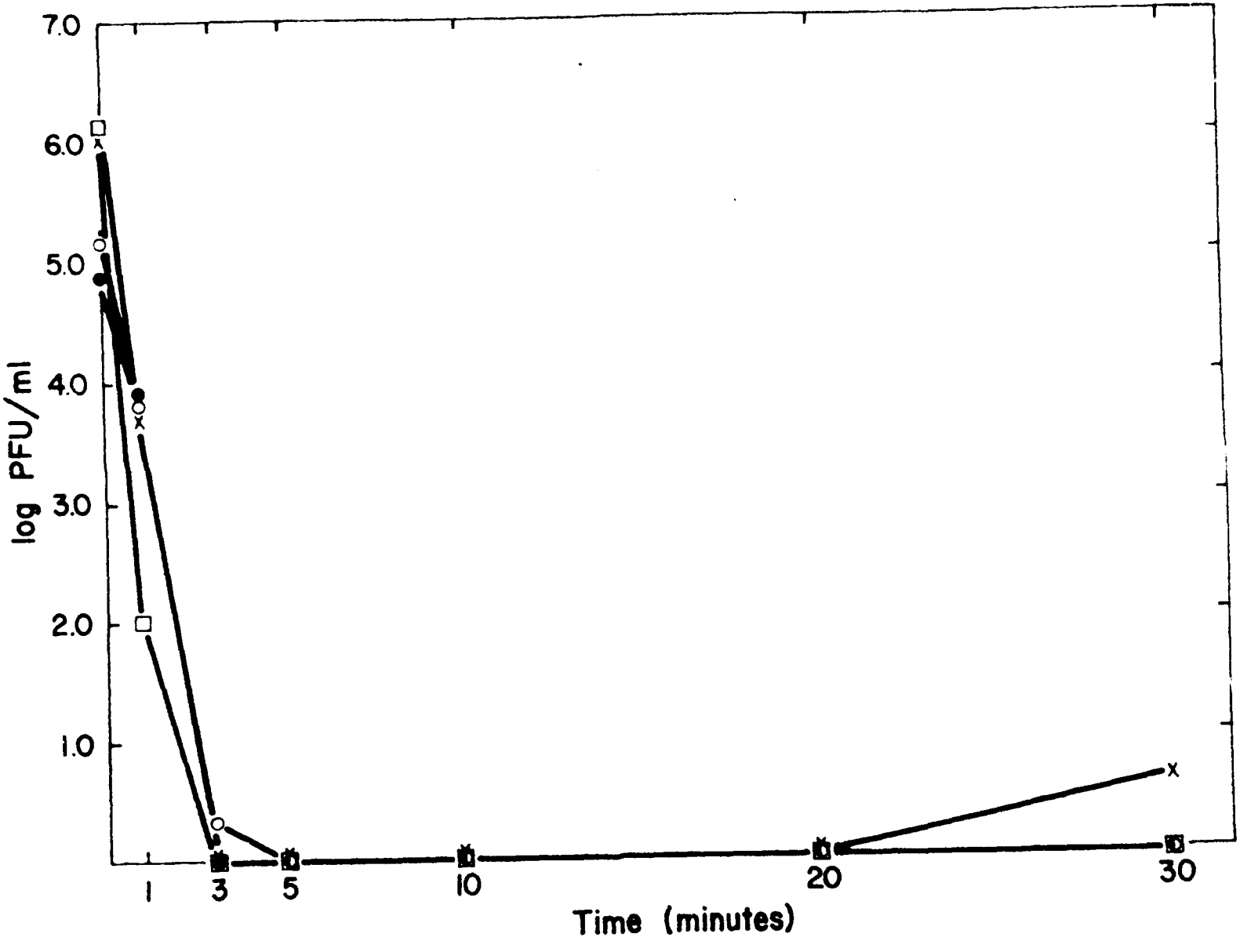

Fig. 24. Survival characteristics of ECHO virus type 6 at 62 C in Lewes water (28 °/oo salinity). ●—● untreated; O—O autoclaved; X—X filtered; □—□ distilled water.

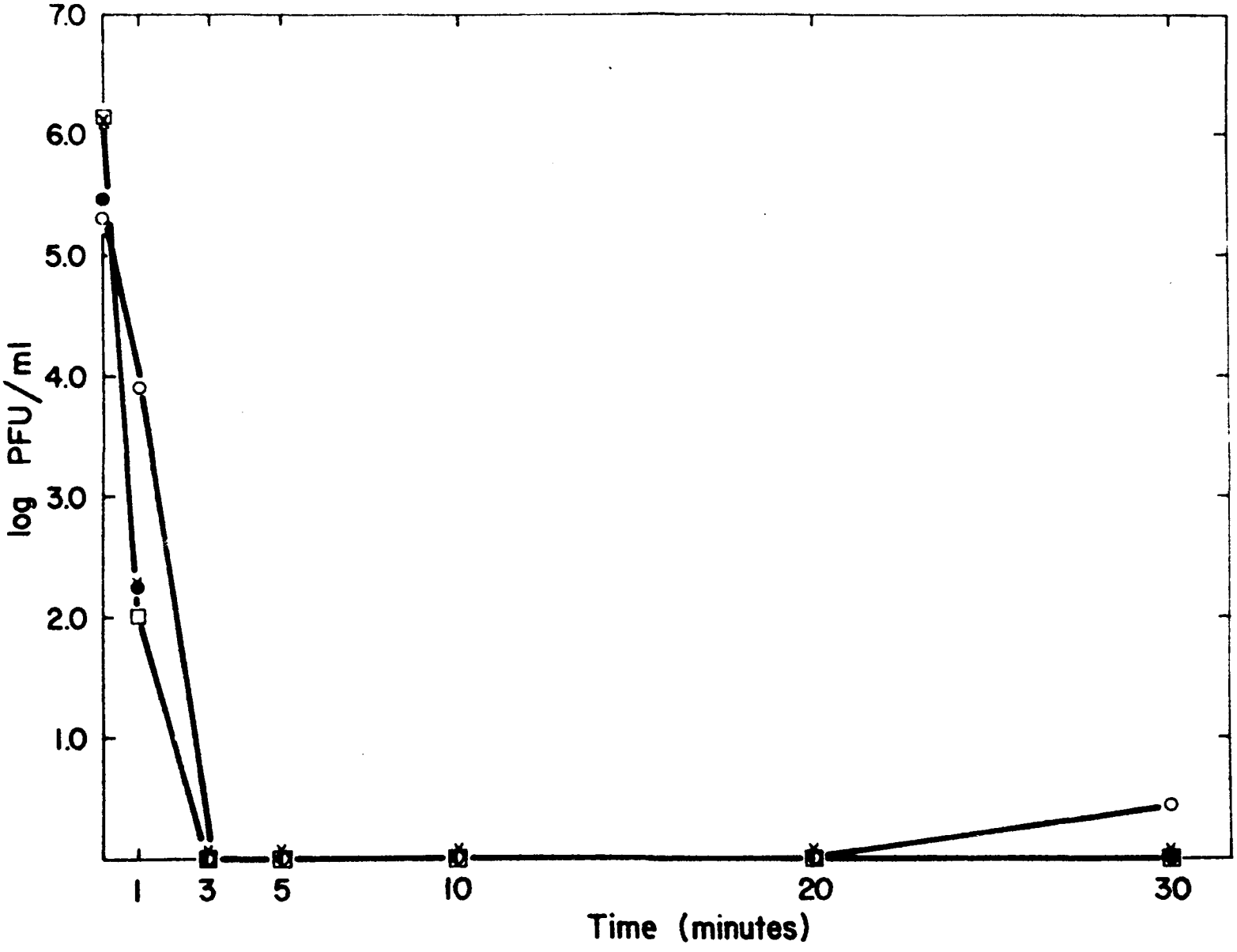

Fig. 25. Survival characteristics of ECHO virus type 6 at 62 C in North Carolina water (34 °/oo salinity). ●—● untreated; O—O autoclaved; X—X filtered; □—□ distilled water.

5. Antibiotic resistance in marine bacteria.

Antibiotic resistant bacteria were isolated from seawater samples collected in the Atlantic Ocean off the Southeastern Coast of the United States. Large numbers of antibiotic-resistant bacterial strains were found to be present in harbor and inshore waters. However, the percentage of antibiotic-resistant bacterial strains was higher in several seawater samples collected off-shore than for those collected near shore. Bacteria resistant to tetracycline, chloramphenicol, and streptomycin were found in nearly all samples collected, including samples from 200 miles off-shore and at depths to 8,200 M. Sediment samples, in general, were found to contain smaller populations of resistant bacterial strains, compared with the seawater samples examined. Antibiotic-resistant bacteria exhibiting phenetic characteristics common to autochthonous marine bacterial species were examined in detail and several of the isolates exhibited unstable resistance, with antibiotic resistance transferable to recipient Escherichia coli cells. Deoxyribonucleic acid preparations from ten strains were examined using ethidium bromide-cesium chloride gradients. Six of the strains were found to contain covalently closed circular plasmid DNA.

Antibiotic resistant strains of coliforms and salt-requiring bacteria were isolated from the holding tank of an ocean-going vessel at sea. Plasmid-mediated antibiotic resistance was discovered in four strains of Vibrio spp. isolated from water samples collected from the tank.

Two papers describing this work in detail have been submitted to Applied and Environmental Microbiology: "Plasmids Carried by Antibiotic-resistant Marine Bacteria"; and "R Factors in a Seawater-operated Shipboard Holding Tank" - both papers authored by R. K. Sizemore and R. R. Colwell.