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TITLE: Growth and seawater tolerance of fall chinook smolts (*Oncorhynchus tshawytscha*) after prerelease treatment with formalin and potassium permanganate.

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Introduction

For many years Federal, State and private salmon and trout hatcheries have used various medications to treat the many maladies incurred by stocks of fish in crowded rearing systems. Among the more common drugs used for therapeutic and prophylactic treatments are formalin and potassium permanganate ($KMnO_4$)

Formalin (37% by weight formaldehyde) is used extensively for the control of ectoparasites. Although formalin toxicity is infrequent in young salmonids, treatment with a 1:4000 dilution in water warmer than 10°C can cause a loss if the fish have bacterial gill disease (Wood 1974). Piper and Smith (1973) found, after conducting an extensive survey of 74 United States hatcheries, that few problems developed from formalin toxicity if the chemical was properly used. Wedemeyer (1971) observed that the prescribed dosage of 1:6000 for ectoparasitic treatment caused a significant drop in blood Cl^-, Ca^{++}, total CO_2, and tissue vitamin C levels in rainbow trout (Salmo gairdneri). However, coho salmon smolts (Oncorhynchus kisutch) were less affected. Bouck and Johnson (1979) found that formalin treatments produced low level mortality in coho salmon smolts upon direct transfer to seawater and no mortality after four days delayed transfer to seawater.

$KMnO_4$ has been used quite regularly since 1904 for the treatment of numerous parasitic outbreaks. Use of the drug has also been effective against bacterial gill disease, providing certain precautions are taken; i.e., allowing for temperature variations and changing of rearing water (Wood 1974). Tucker and Boyd (1977) stated that varying organic load levels caused inconsistencies in effectiveness against bacterial gill disease, and that the value of the compound in the aquatic environment is inversely proportional to the load of the oxidizable organic matter in the water. Jee and Plumb (1981) observed that the effectiveness of $KMnO_4$ was much greater when treating fathead minnows (Pimephales promelas) infected with Flexibacter columnaris in organically depleted tap water vice organically enriched pond water. Bouck and Johnson (1979) indicated that presecribed treatments with $KMnO_4$ caused an 80% mortality in coho salmon smolts when the fish were transferred to 28 parts per thousand (ppt) seawater immediately after treatment. However, if the fish were held for four days post-treatment in freshwater, the mortality rate dropped to 12%.

At present, formalin is registered with the FDA as a parasiticide and $KMnO_4$ has been exempted from registration (Schnick and Meyer 1979).

Our objective in these studies was to determine if timing and medication treatment effects smoltification as measured by gill Na^+K^+ adenosine triphosphatase (ATPase) activity, seawater survival and growth.

Methods and Materials

Experimental Animals

In both the 1981 formalin study and the 1982 $KMnO_4$ study, eyed fall chinook eggs were transported to the USFWS, Seattle National Fishery Research Center's Marrowstone Field Station in October, disinfected in a 1:100 solution of Argentine[1/] (an iodine base disinfectant) and reared to swim-up in an eight tray Heath-Tecna incubator with a fresh water flow rate of approximately 3 gallons per minute (gpm).

In February, the fry (weighing approximately 450/lb) were placed in 68-liter rectangular glass aquaria in lots of 50 with each aquarium receiving about 1 liter per minute fresh water. The fry were allowed to acclimate to their new surroundings for about two weeks prior to commencing treatments.

Experimental Design

For the 1981 formalin study, the aquaria were randomly divided into two groups; 103 for formalin and "sham" treatment controls and ATPase analysis and 10 for unhandled, untreated controls. Of the 58 aquaria in the $KMnO_4$ study, 36 were randomly divided into two groups; 18 each for $KMnO_4$ treatments and "sham" treatment controls. Of the remaining 22 aquaria, four were used as untreated, unhandled controls and 18 were used to monitor ATPase activity. Bi-weekly treatments were begun in February and continued through May (Figs. 1 and 2). ATPase analysis was conducted according to Zaugg (1982).

Once smolting was determined by gil ATPase activity analysis, the fish were acclimated to 28 ppt seawater by exposure to 12-15 ppt seawater (isotonic) for 48 hrs for formalin treated fish and 24 hrs for $KMnO_4$ treated fish. Once the acclimation period was complete, the fresh water was turned off and the seawater flow rate increased to approximately 1 liter/minute. In both studies, smoltification occurred in May. Mean loading densities at the time of seawater entry in 1981 and 1982 were 4.1 gm fish and 4.6 gm fish per liter of water respectively. Lighting was simulated to approximate the natural photoperiod by weekly adjustments to overhead florescent lamps.

The 1981 formalin treatments were conducted according to Wood (1974) using a 1:6000 dilution in static fresh water for one hour. $KMnO_4$ treatments in 1982 were also according to Wood (1974) using 2 mg $KMnO_4$ per liter static fresh water for one hour each on three consecutive days. In both studies the aquaria were not drained at the end of the treatment, but thoroughly flushed with fresh water. In both studies, "sham" treatments were conducted in static situations and stirred as if the medications had been used.

[1/]Reference to trade names is for identification only and does not imply U.S. Government endorsement of commercial products.

All study fish were fed once daily to satiation with OMP II. However, feed was withheld for 24 hrs prior to $KMnO_4$ treatments to reduce the organic load.

Microbial Examination

In both studies, all post treatment mortalities, other than those which were lost to system failures, were examined microbiologically for pathogens using sterile brain heart infusion agar and asceptically removed kidney material.

Results

Na^+K^+-ATPase Activity Analysis

Some significant differences at the 95% confidence level did occur in formalin treated fish when compared to their "sham" treated controls using a "t" test for comparison. ATPase activity was analyzed for those fish treated 60 days or less prior to seawater entry (Figs. 3-7). However, the enzymes' activity was not consistent in those groups; i.e., when Group 2's activity was monitored on May 7, one day before seawater entry (Fig. 4), the treatment group was lower than the control but when Group 6 was monitored on May 7, the treatment group was higher than the control (Fig. 7). When Group 6 was again analyzed five days after seawater entry, the treatment group was lower than the control.

ATPase activity in $KMnO_4$ treated fish responded similarly to the formalin treated fish. Significant differences at the 95% confidence level occurred between "sham" treatments and unhandled, untreated controls (Fig. 8) and between treatments and unhandled, untreated controls (Fig. 9). However, the treatment and "sham" treatment groups were consistently lower than the untreated, unhandled controls.

Growth and Seawater Survival

No significant differences occurred in growth and seawater survival (20 day challenge) in the formalin and $KMnO_4$ treated fish when compared to their respective "sham" treated groups and unhandled, untreated controls.

Mortalities, post seawater exposure, were practically none in both studies for treatment and control groups. Formalin treated fish demonstrated $\geq$ 97% survival and $KMnO_4$ treated groups were $\geq$ 99% (Figs. 11 and 12).

Discussion

The inconsistencies observed in the ATPase activity of the formalin treated fish indicates that something may be occurring during the smoltification process with recently treated fish, but given the good growth and seawater performance, it may not be enough to cause alarm. We feel the important point to remember is that the ATPase levels, when compared between treatments, "sham" treatments and unhandled, untreated controls over time, did rise as expected, the fish did convert to 28-29 ppt seawater and did survive very well (97% overall) for at least 20 days (Fig. 11).

$KMnO_4$ treated fish were also determined to be unaffected by the treatments and demonstrated a 99% survival after 20+ days on seawater. Even when technical problems were included in the losses, the survival rate was 94% (Fig. 12). The 12 deaths in the $KMnO_4$ group that did occur post seawater acclimation were determined to be those fish not able to cross the fresh to seawater threshold. The mean fork length of the 12 was 20 mm less than that of the survivors. Again, the point to remember is that even though a few significant differences did occur in ATPase activity, the young smolts did very well when acclimated to seawater. However, a possible latent ATPase rise may be occurring if the fish are treated with $KMnO_4$ 20 days or less prior to seawater entry.

In conclusion we feel that when properly used, formalin and $KMnO_4$ will not affect the ability of smolts to enter seawater. However, care should be taken when using the chemicals because if improperly used, both chemicals could cause unnecessary damage to the health of the workers and the fish. Also of importance is the fact that this was a laboratory situation and the environmental factors at various rearing facilities may cause somewhat varied results when using the drugs.

Literature Cited

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Figure 1. Experimental procedure to test the effect of formalin treatment on $Na^+ - K^+$ gill ATPase activity, growth, and freshwater-seawater transition of fall chinook salmon.

TREATMENT PHASE			DATE COLLECTION PHASE						
			Date and # Tanks Sampled for fish $Na^+ - K^+$ gill ATPase activity and Growth[d]						Date and # Tanks converted to seawater inflow
Date	Treatment	# Tanks Treated[a]	Feb 26	Mar 2	Mar 26	Apr 9	Apr 23	May 7	May 17 – May 19[e]
Feb 26	Formalin[b]	11	1	1	1	1	1	1	5
	Control[c]	11	1	1	1	1	1	1	5
Mar 2	Formalin	10		1	1	1	1	1	5
	Control	11		1	1	1	1	1	5
Mar 26	Formalin	9			1	1	1	1	5
	Control	9			1	1	1	1	5
Apr 9	Formalin	8				1	1	1	5
	Control	8				1	1	1	5
Apr 23	Formalin	7					1	1	5
	Control	7					1	1	5
May 7	Formalin	6						1	5
	Control	6						1	5
	Non-Treatment, Unhandled Control	10							10

[a] Each tank contained approximately 50 fish.

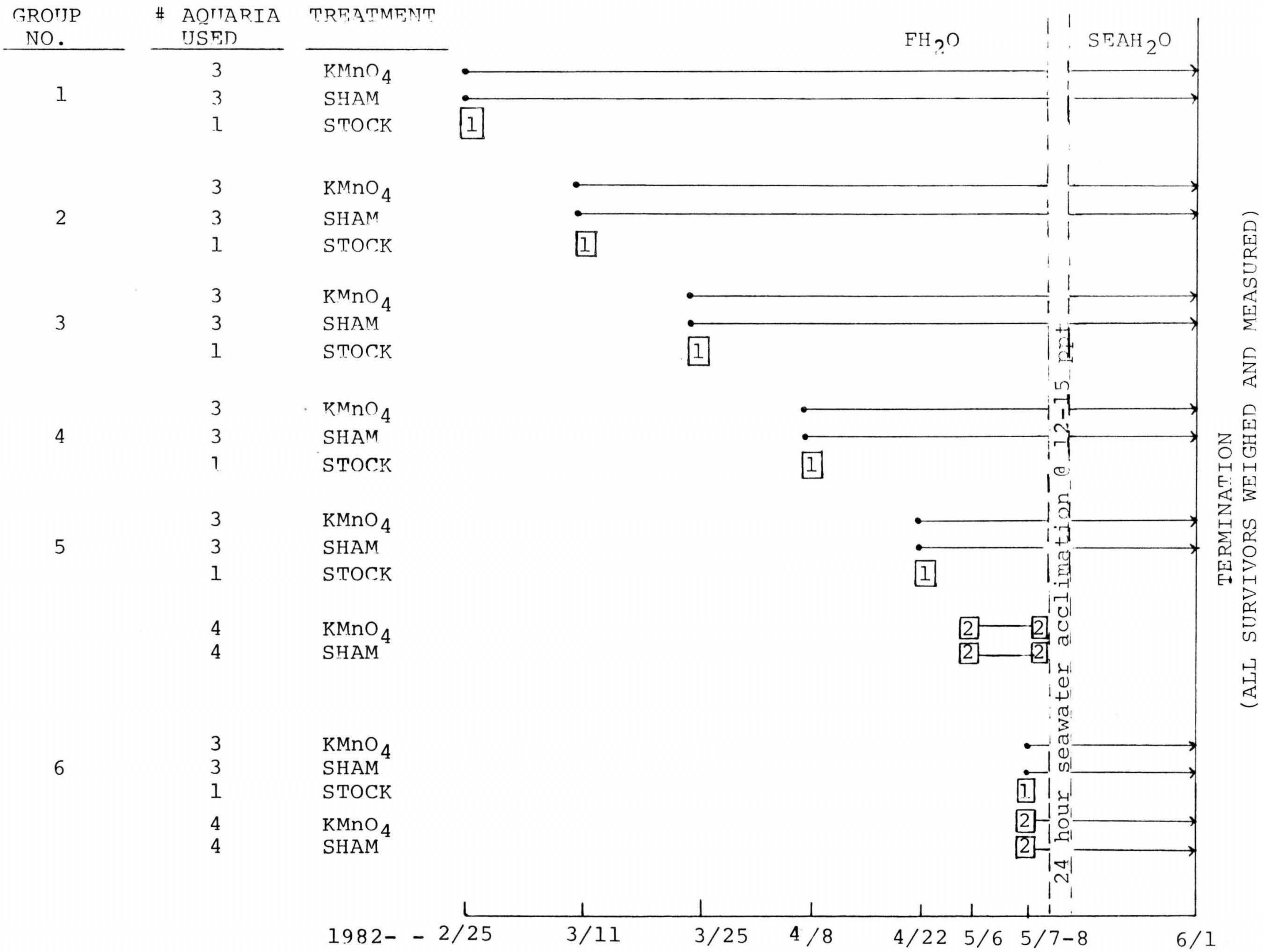
[b] Treatment procedures were according to Wood (1979).

[c] Control fish were handled exactly the same as fish exposed to formalin.

[d] Thirty fish, ten pools of three, were sampled for $Na^+ - K^+$ gill ATPase activity. Each fish was weighed to the nearest 0.1 gm and measured (fork length) to the nearest mm.

[e] Fish were exposed to 12-15 ppt salinity on May 17 and converted to 29 ppt salinity on May 19. Seawater survival was monitored for 20 days.

FIGURE 2.
EXPERIMENTAL DESIGN, AQUARIA ALLOCATION AND SAMPLING TIMES TO DETERMINE THE IMPACT OF $KMnO_4$ TREATMENTS ON FALL CHINOOK SALMON SMOLTIFICATION AND SEAWATER SURVIVAL



□: INDICATES ALL FISH IN NUMBER OF AQUARIA INDICATED WERE TERMINALLY SAMPLED FOR FORK LENGTH, WEIGHT AND GILL Na^+K^+-ATPase @ 50 FISH PER AQUARIA

FIGURE 3.
COMPARISON OF GILL Na^+K^+-ATPase ACTIVITY BETWEEN TREATMENT AND CONTROL GROUPS TREATED WITH FORMALIN. VALUES ARE MEANS ± STANDARD DEVIATION. 30 FISH WERE SAMPLED FOR EACH VALUE INDICATED.

■ : CONTROL
▢ : TREATED WITH FORMALIN

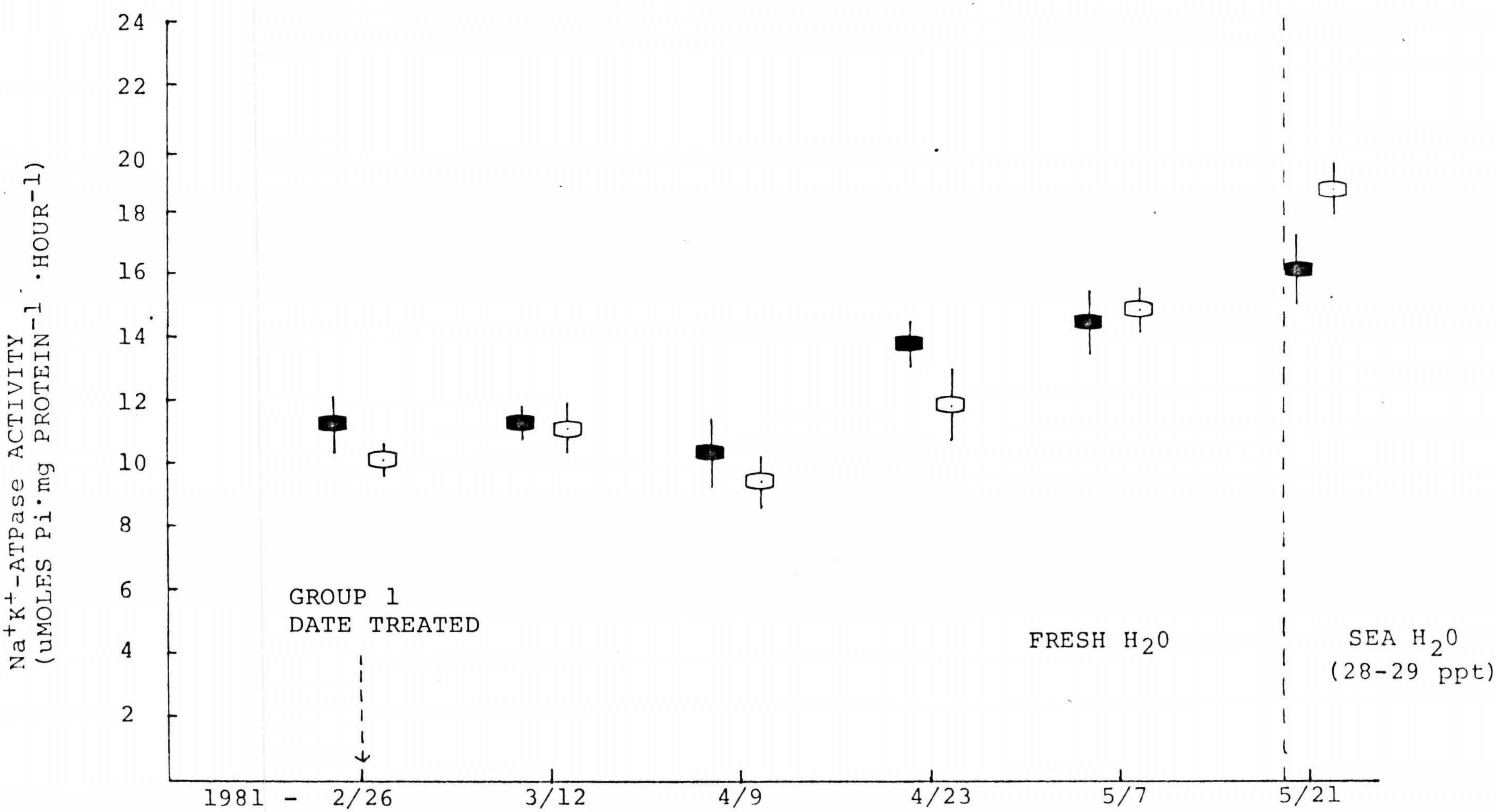


FIGURE 4
COMPARISON OF GILL Na^+K^+-ATPase ACTIVITY BETWEEN TREATMENT AND CONTROL GROUPS TREATED WITH FORMALIN. VALUES ARE MEANS ± STANDARD DEVIATION. 30 FISH WERE SAMPLED FOR EACH VALUE INDICATED.

■: CONTROL
□: TREATED WITH FORMALIN

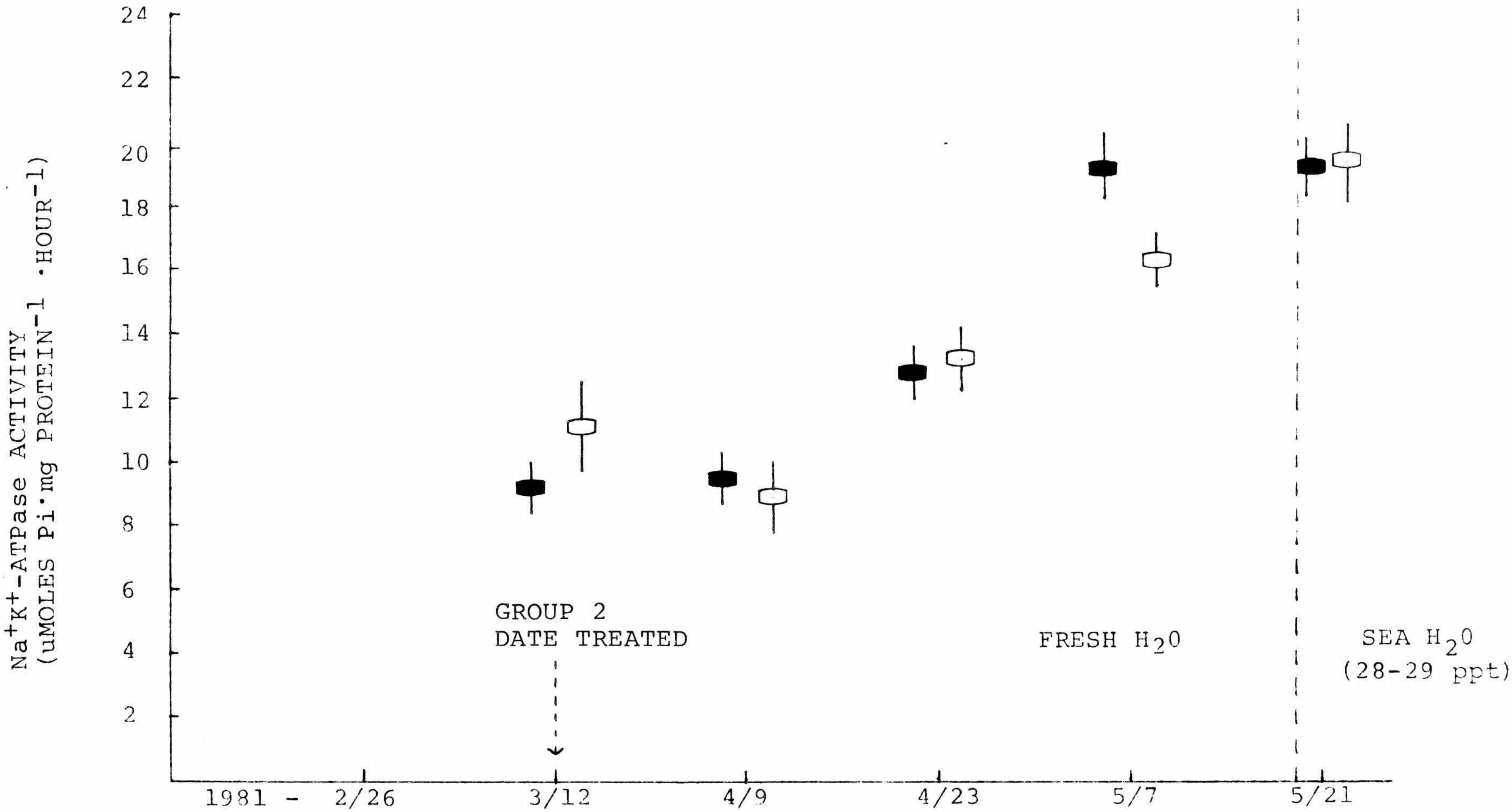


FIGURE 5.
COMPARISON OF GILL $Na^{+}K^{+}$-ATPase ACTIVITY BETWEEN TREATMENT AND CONTROL GROUPS TREATED WITH FORMALIN. VALUES ARE MEANS ± STANDARD DEVIATION. 30 FISH WERE SAMPLED FOR EACH VALUE INDICATED.

■: CONTROL
▢: TREATED WITH FORMALIN

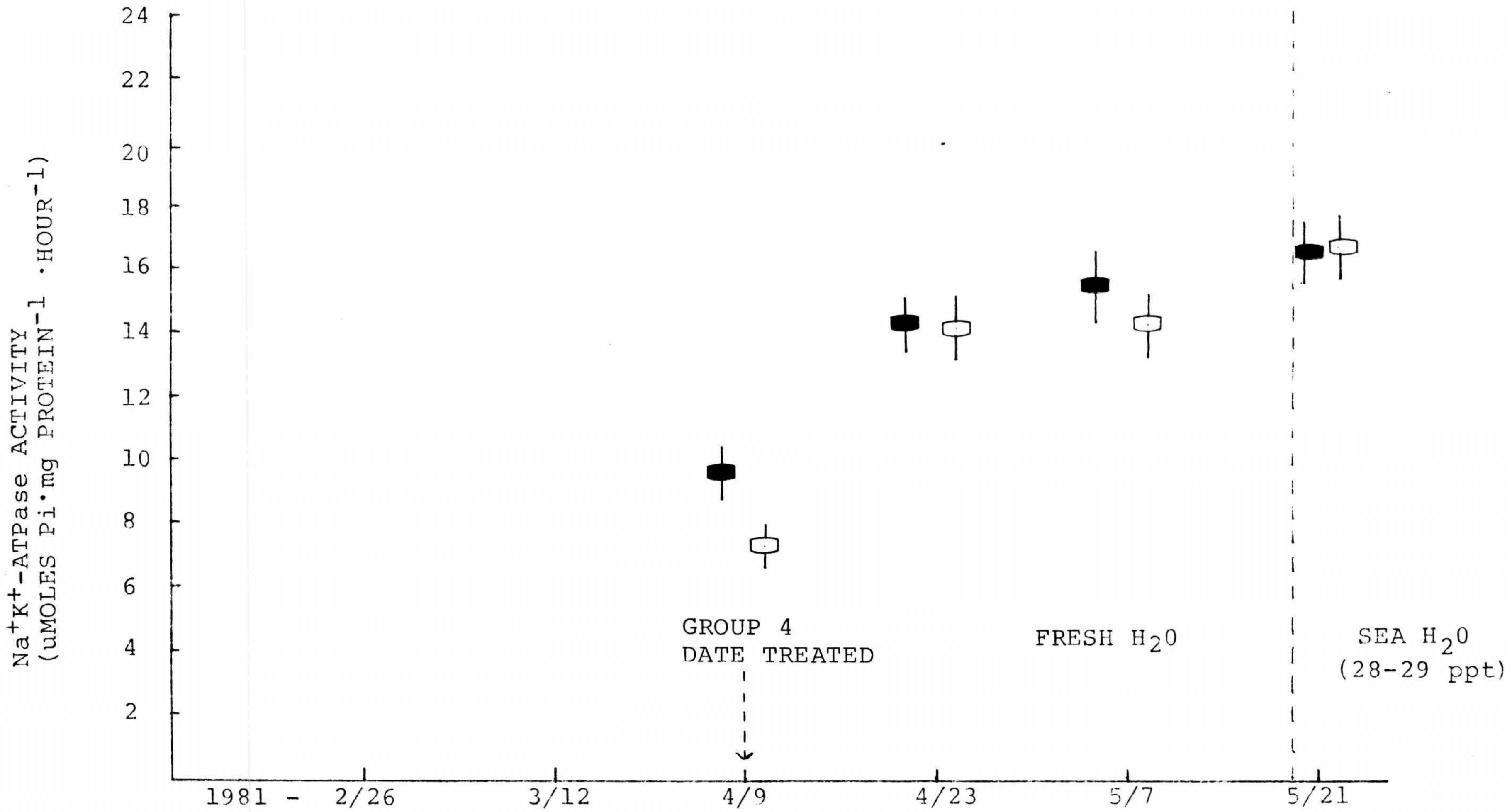


FIGURE 6.
COMPARISON OF GILL Na^+K^+-ATPase ACTIVITY BETWEEN TREATMENT AND CONTROL GROUPS TREATED WITH FORMALIN. VALUES ARE MEANS ± STANDARD DEVIATION. 30 FISH WERE SAMPLED FOR EACH VALUE INDICATED.

■: CONTROL
□: TREATED WITH FORMALIN

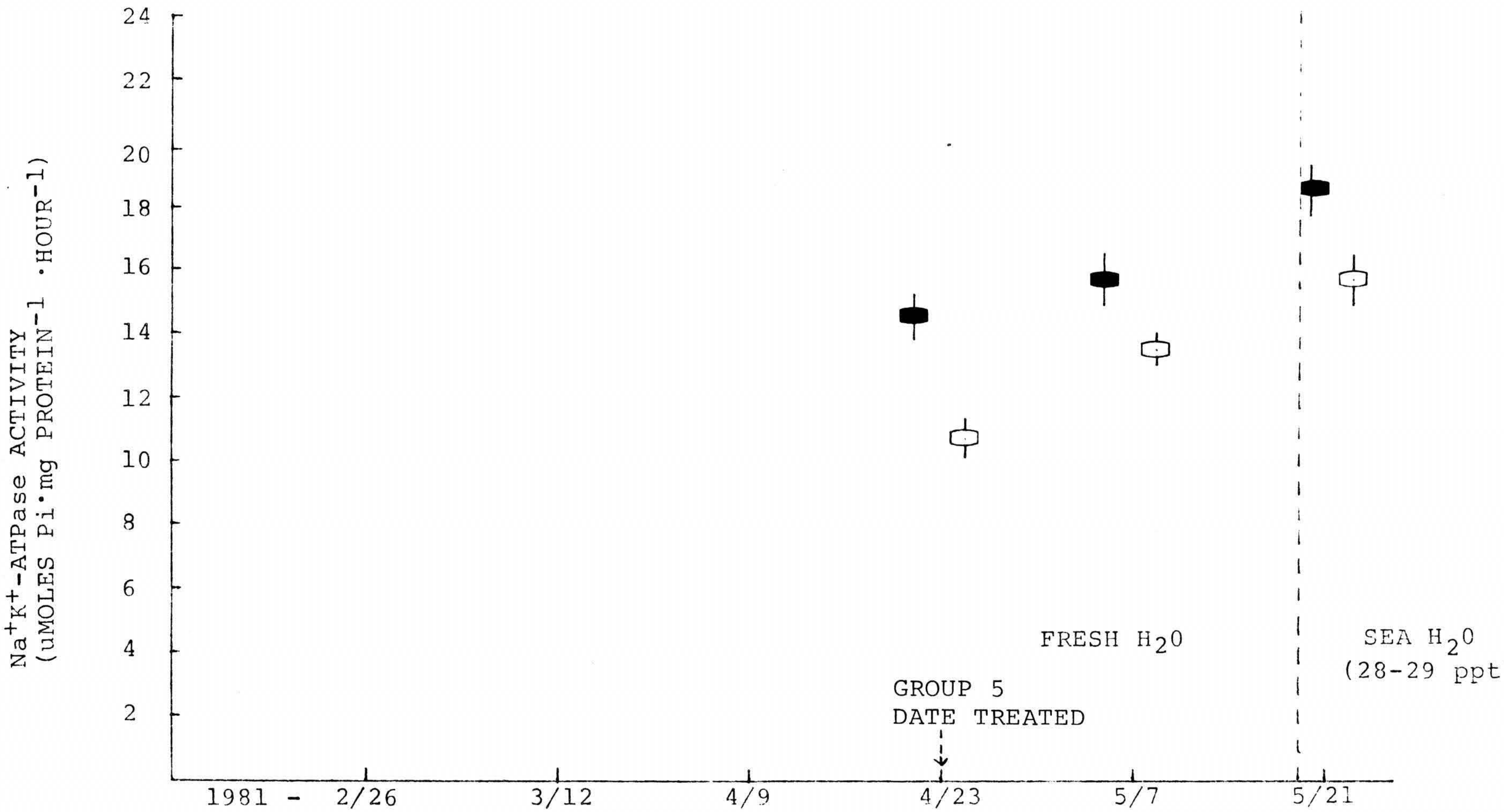


FIGURE 7.
COMPARISON OF GILL Na^+K^+-ATPase ACTIVITY BETWEEN TREATMENT AND CONTROL GROUPS TREATED WITH FORMALIN. VALUES ARE MEANS ± STANDARD DEVIATION. 30 FISH WERE SAMPLED FOR EACH VALUE INDICATED.

■: CONTROL
□: TREATED WITH FORMALIN

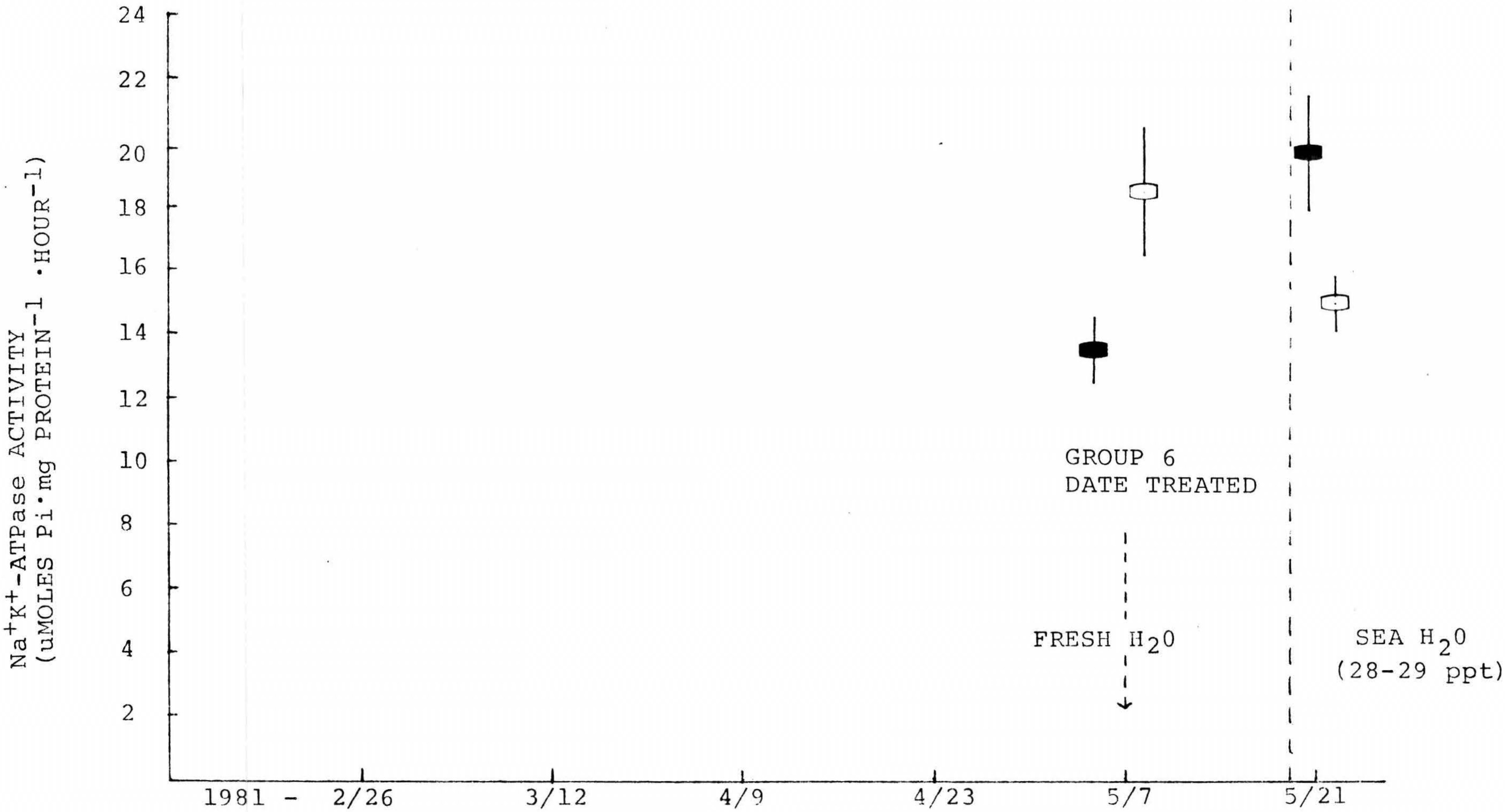


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FIGURE 8.
COMPARISON OF GILL Na^+K^+-ATPase ACTIVITY BETWEEN TREATMENT AND CONTROL GROUPS TREATED WITH $KmNO_4$. VALUES ARE MEANS ± STANDARD DEVIATION. 10 SAMPLES WERE USED FOR EACH VALUE INDICATED.

■ : CONTROL
▨ : TREATED WITH $KmNO_4$ (SHAM)

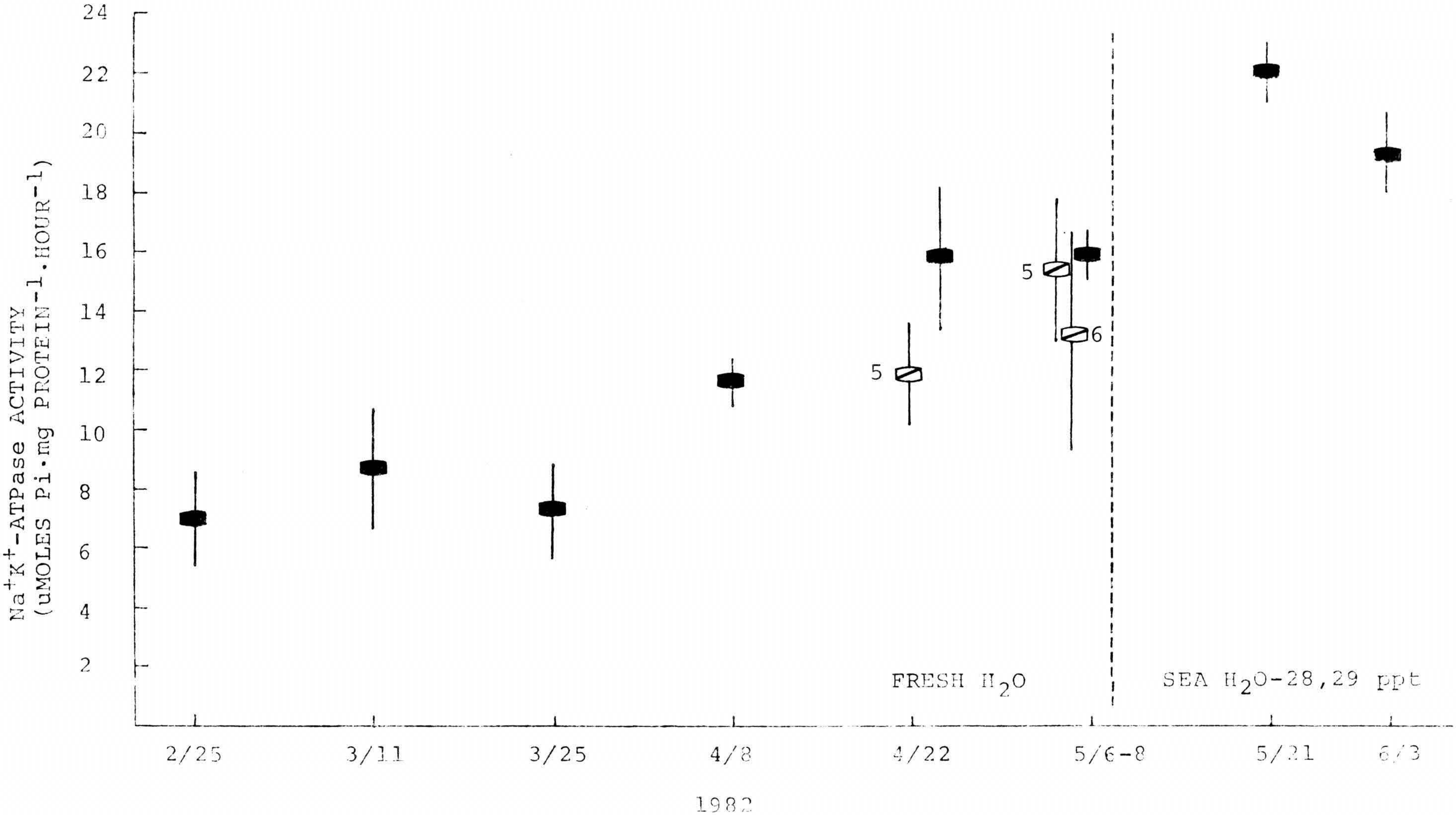

FIGURE 9.
COMPARISON OF GILL Na^+K^+-ATPase ACTIVITY BETWEEN TREATMENT AND CONTROL GROUPS TREATED WITH $KmNO_4$. VALUES ARE MEANS ± STANDARD DEVIATION. 10 SAMPLES WERE USED FOR EACH VALUE INDICATED.

■ : CONTROL
□ : TREATED WITH $KmNO_4$

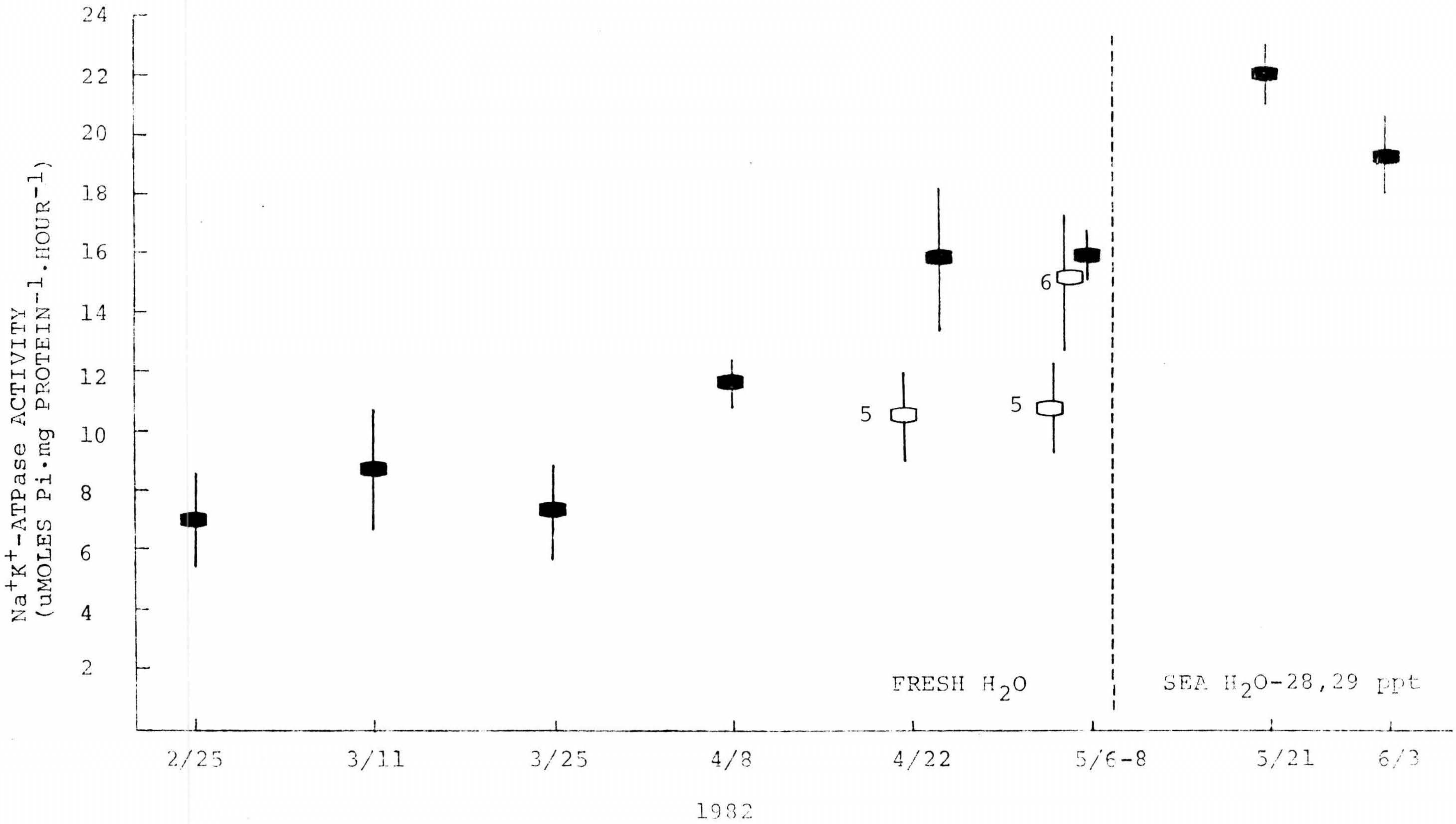

FIGURE 10.
COMPARISON OF GROWTH (FORK LENGTH) BETWEEN $KMnO_4$ TREATED, SHAM TREATED AND CONTROL FALL CHINOOK SMOLTS. SAMPLES SIZES ARE ≥20 WITH MOST ≥30.

GROUP NO. 6

[a] Indicates Significant Difference at the 0.95 Confidence Level

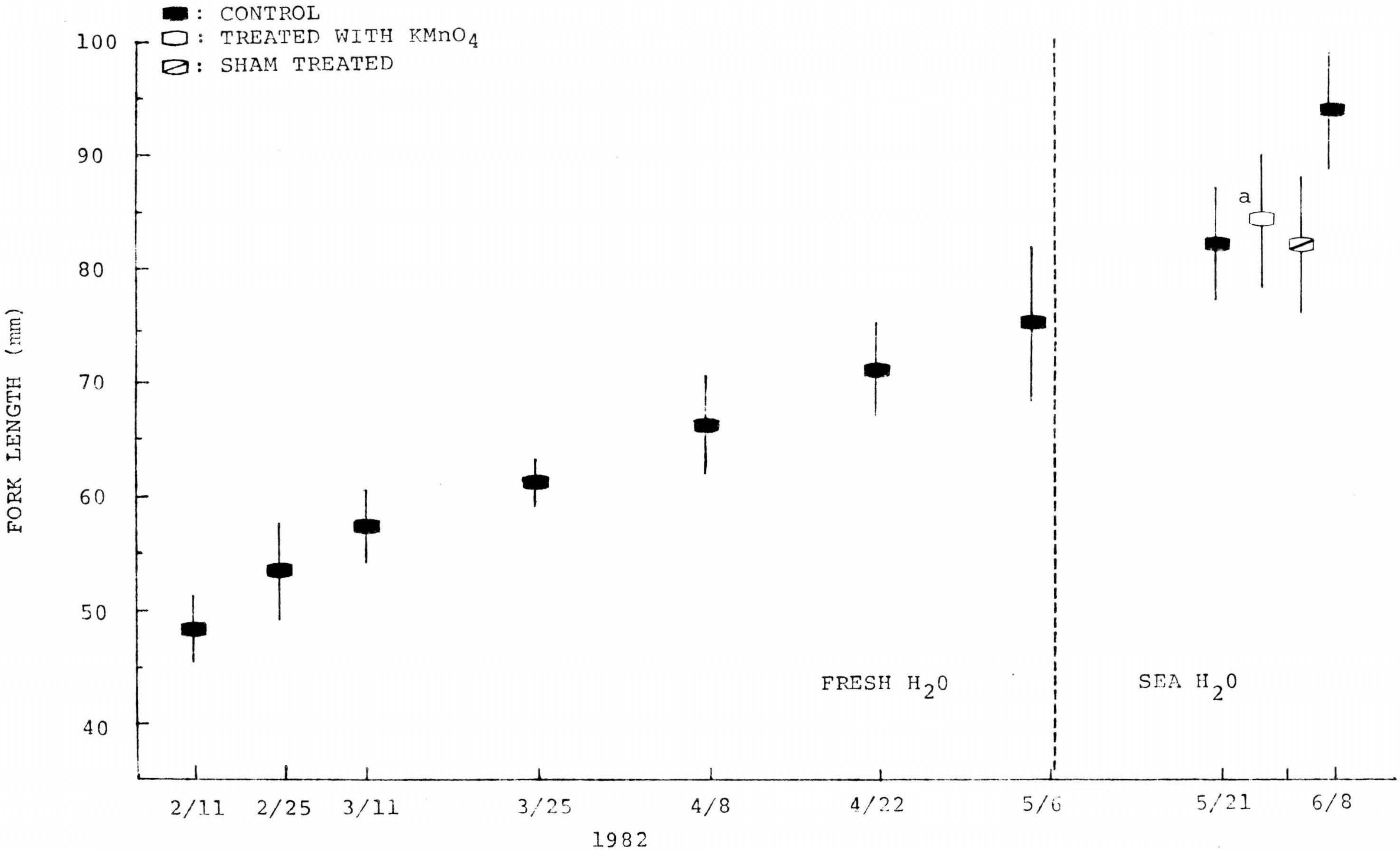

Figure 11. Seawater challenge survival (20d exposure) of fall chinook salmon treated or sham-treated (control) with formalin at different times prior to seawater entry.

Treatment Date (81)	Treatment	Number Tanks per Treatment	Total Number of Fish Challenged[b]	Total Number of Survivors	Range in Percent Survival Between Tanks	Total Percent Survival
Feb 26	Formalin	5	239	234	100%-96%	98%
	Control	5	239	234	100%-95%	98%
Mar 2	Formalin	5	239	233	100%-94%	97%
	Control	4[a]	181	174	98%-94%	96%
Mar 26	Formalin	5	245	234	98%-93%	96%
	Control	5	249	242	100%-90%	97%
Apr 9	Formalin	4[a]	204	195	100%-94%	96%
	Control	5	266	255	98%-92%	96%
Apr 23	Formalin	5	216	211	100%-94%	98%
	Control	4[a]	200	197	100%-96%	99%
May 7	Formalin	5	245	239	100%-94%	98%
	Control	5	244	240	100%-96%	98%
NO TREATMENT		10	481	465	100%-93%	97%

[a] Fish in tank were lost due to accidental water shut-off.

[b] Total numbers of fish in the tanks not equalling multipler of 50 are attributed to experimental (counting) error and "jump-outs."

FIGURE 12.
SEAWATER CHALLENGE SURVIVAL (20+d EXPOSURE) OF FALL CHINOOK SALMON TREATED OR "SHAM" TREATED (CONTROL) WITH $KMnO_4$ AT DIFFERENT TIMES PRIOR TO SEAWATER ENTRY.

TREATMENT DATE (82)	TREATMENT	NUMBER TANKS PER TREATMENT	TOTAL NUMBER OF FISH CHALLENGED[b]	TOTAL NUMBER OF SURVIVORS	RANGE IN PERCENT SURVIVAL BETWEEN TANKS	TOTAL PERCENT SURVIVAL
Feb 25	$KMnO_4$	3	149	149	-	100%
	Control	3	149	149	-	100%
Mar 11	$KMnO_4$	3	150	147	100%-98%	98%
	Control	3	150	150	-	100%
Mar 25	$KMnO_4$	3	149	148	100%-98%	99%
	Control	2[a]	128	124	100%-94%	97%
Apr 8	$KMnO_4$	2[a]	137	137	-	100%
	Control	3	149	141	98%-90%	95%
Apr 22	$KMnO_4$	3	150	147	100%-96%	98%
	Control	3	150	145	100%-90%	97%
May 6	$KMnO_4$	2[a]	133	133	-	100%
	Control	3	150	148	100%-96%	99%

[a] Fish in tank lost due to system failures post treatment date.

[b] Total number of fish in tanks not equalling multiplier of 50 are attributed to experimental (counting) error and "jump-outs".