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Title: Physiological and Hematological Changes in Chum Salmon (Oncorhynchus keta) Infected with Erythrocytic Necrosis Virus

Abstract approved: Redacted for Privacy

James R. Winton

Chum salmon (Oncorhynchus keta) were artificially infected with erythrocytic necrosis virus (ENV) to study the physiological and hematolgical consequences of ENV-infection. Infected and control fish were held in pathogen-free seawater and sampled weekly for five weeks. Physiological tests included plasma cortisol, glucose, protein, and osmolality, blood lactic acid, and liver glycogen concentrations. In general, fish infected with ENV had higher liver glycogen values, and lower plasma glucose and blood lactic acid levels than control fish. Hematological tests included red and white blood cell

(RBC and WBC) counts, hematocrits, blood hemoglobin concentrations, and erythrocyte fragility. Infected fish had lower RBC counts, hematocrits, and hemoglobin concentrations, higher WBC counts, and more fragile erythrocytes than control fish. Other blood parameters calculated from the hematology data indicated that the erythrocytes of infected fish had higher mean corpuscular volumes, depressed mean corpuscular hemoglobin concentrations, and slightly lower mean corpuscular hemoglobin. Erythrocytic inclusions were observed in the cytoplasm of RBC of infected fish, and unnucleated cells were observed by week 2. In this experiment, severity of infection progressed steadily through week 4, after which the fish appeared to be recovering. In a second study, fish were infected with ENV, held for three weeks, and recovery from exercise was measured. Plasma glucose and osmolality were higher in infected fish, while plasma cortisol and blood lactate levels were only slightly elevated. These studies indicate that chum salmon were able to withstand the effects of ENV-infection without irreversible physiological consequences. However, when subjected to exercise, infected fish recovered more slowly than controls and seemed to have increased osmoregulatory difficulties.

Physiological and Hematological Changes in Chum Salmon (*Oncorhynchus keta*) Infected with Erythrocytic Necrosis Virus

by

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INTRODUCTION

Viral Erythrocytic Necrosis (VEN) is a disease which has been observed in many species of marine and anadromous fish (WALKER and SHERBURNE, 1977; EVELYN and TRAXLER, 1978; ROHOVEC and AMANDI, 1981). Infections have been reported in fish collected from the North Atlantic Ocean off the coast of North America and the United Kingdom, as well as from the coastal waters of the Eastern North Pacific Ocean (ROHOVEC and AMANDI, 1981).

While it has not been possible to isolate the virus in cell culture, ultrastructural studies of infected red blood cells (RBC) from several species of fish have been described (WALKER and SHERBURNE, 1977; RENO et al., 1978). The virus has been tentatively placed in the family Iridoviridae on the basis of its size and morphology (RENO et al., 1978). Presumptive identification of VEN is based on examination of Giemsa stained blood smears for the presence of cytoplasmic inclusion bodies in infected RBC (RENO et al., 1978; MACMILLAN and MULCAHY, 1979).

The viral pathogenesis of the disease is not well understood, yet the infection can produce a severe and

chronic anemia (EVELYN and TRAXLER, 1978; MACMILLAN and MULCAHY, 1979). Mature erythrocytes (RBC) appear to be destroyed first, causing the increased synthesis and release of immature erythroblasts into the circulatory system (MACMILLAN, 1980; MACMILLAN et al., 1980). Few external signs are seen in infected fish, although pale gills and hemorrhaging of the body surface have been reported (ROHOVEC and AMANDI, 1981).

Although the anemia produced by VEN may be very severe, with hematocrits as low as 2% (EVELYN and TRAXLER, 1978), the disease has not been associated with massive mortalities. However, VEN infected fish have been shown to be sensitive to certain secondary challenge stressors. MACMILLAN et al., (1980) have shown that infected chum salmon (Oncorhynchus keta) had a decreased ability to regulate sodium and potassium in seawater, a significantly decreased tolerance to oxygen depletion, and a threefold greater mortality rate from Vibriosis. Thus, losses of fish infected with VEN may often be attributed to other causes.

The research reported here describes some of the physiological and hematological consequences of erythroyctic necrosis virus (ENV) infection in chum salmon. Fish were first tested under conditions whereby no stressor other than the virus was present. Then a study of recovery time following exercise was

performed. The results from these investigations will help assess whether or not infections with ENV are detrimental to natural populations of salmonids.

MATERIALS AND METHODS

Chum salmon for use in these experiments were obtained as eggs from Whiskey Creek hatchery, Netarts, Oregon and reared in pathogen free freshwater until attaining a weight of approximately 1g. They were then removed, placed in pathogen-free seawater, and fed a diet of Oregon Moist Pellet formula until reaching 75-150 g (wet weight), when they were used in this study.

Erythrocytic necrosis virus was obtained from chum salmon artificially infected with blood that had been identified as ENV-positive from stained blood smears. Blood cells from heavily infected fish were homogenized and centrifuged at 1000g for 10 minutes. The supernatant was diluted 1:3 with Hanks' balanced salt solution (BSS) and 0.1 ml was injected intraperitoneally into anesthetized chum salmon. Control fish were injected with 0.1 ml BSS. The salmon were placed by groups of ten into 75 liter tanks of pathogen-free, ambient temperature seawater and fed daily with Oregon Moist Pellet formula.

Twenty control and twenty infected fish were sampled weekly for a 5 week period. At each sampling interval the fish were euthanized with 200 ppm MS-222. The caudal peduncle was severed and blood was collected into heparinized culture tubes, spun at 2000 rpm for 15 minutes, and frozen at -80 C for subsequent plasma

cortisol, glucose, protein and osmolality analyses. Blood for determining the hematocrit of each fish was collected in heparinized capillary tubes, which were sealed and centrifuged for 15 minutes in a hematocrit centrifuge. Whole blood was also collected in heparinized calibrated hemocap tubes for cell counts, lactic acid, and hemoglobin analyses. Immediately following blood collection, 100 mg of liver tissue was removed for measurement of liver glycogen content.

Physiological Tests

Selected tests were performed in order to assess the physiological status of infected animals. The tests were chosen on the basis of their ability to measure the physiological responses of the fish to stress.

Plasma cortisol was determined by a radioimmunoassay developed by REDDING et al., (1984). Plasma glucose was measured by reacting blood plasma with orthotoluidine in glacial acetic acid, with absorbance of the resulting blue-green color read at 635 nm (WEDEMEYER and YASUTAKE, 1977). Liver glycogen was measured by extracting liver tissue into potassium hydroxide, purified by precipitation, and hydrolysed to glucose, which was then quantified with anthrone reagent (WEDEMEYER and YASUTAKE, 1977). Plasma protein was measured using the Biuret reagent and reading the absorbance of the stable blue color at 540 nm (WEDEMEYER and YASUTAKE, 1977). Plasma

glucose, protein and liver glycogen values were obtained by interpolation from a curve generated by running a series of standards. Lactic acid was determined by heating blood lactate with concentrated sulfuric acid to form acetaldehyde. Acetaldehyde reacts with p-phenylphenol to form a compound in which the absorbance at 570 nm is proportional to the lactic acid concentration (WEDEMEYER and YASUTAKE, 1977). Plasma osmolality was determined by use of a Vapor Pressure Osmometer (Wescor, Model 5100C).

Hematological Tests

Cell counts were made by adding 10ul whole blood to 1.0 ml phosphate buffered saline (PBS) plus 1.0 ml 0.005% crystal violet to give a 1:200 dilution. Red and white blood cells (WBC) were counted at the same time by standard procedure using a hemocytometer. Hemoglobin was measured using the cyanomethemeglobin procedure (WEDEMEYER and YASUTAKE, 1977). Hematocrits (Hct) were determined as previously described. When fish were killed, blood smears were made, air dried, fixed in 100% methanol and stained with Giemsa stain. Smears were examined for macrophages, abnormal RBC morphology (size, shape, color, maturity, inclusions), and to determine the WBC differential. Osmotic fragility was determined by adding one drop of blood from a heparinized capillary tube to 1.0 ml of each of the following saline solutions:

0.50, 0.45, 0.35, 0.30, and 0.25 % NaCl. The tubes were then visually inspected for lysis and the results recorded as the lowest saline concentration in which the cells were stable.

Other parameters were determined from the blood values. Mean corpuscular volume (MCV) indicates the average size of the RBC and was calculated by (Hct x 10)/(RBC count). Mean corpuscular hemoglobin (MCH) indicates the weight of hemoglobin in the average RBC and was determined by (Hgb x 10)/(RBC count). Mean corpuscular hemoglobin concentration (MCHC) shows the relationship between the size of the RBC and its hemoglobin content and was calculated by (Hgb x 10)/(Hct). The ratio of white blood cells to red blood cells (WBC:RBC) was also determined.

Recovery From Exercise

Fish were infected with ENV as described previously and held for three weeks. Infected and control fish were exercised by being held in a net in air for 30 seconds, and then placed by groups of five in a series of seawater tanks for sampling at six intervals. Fish were sampled immediately following exercise and at 30 minutes, 1 hour, 3 hours, 6 hours, and 24 hours post exercise. Blood was collected as described for making blood smears and determining plasma cortisol, plasma glucose, blood lactic acid, plasma protein, and hematocrit.

RESULTS

Physiological Tests

The results of all physiological tests are shown in Table 1. Unpaired t-tests were performed on the data in order to determine if the differences between the means were significant at the 95% Confidence level (P=0.05). The severity of the infection appeared to peak during weeks 3 and 4. The data obtained at week 5 indicate that the VEN-infected fish had begun to recover, although large fish to fish variation among infected fish was still being seen in some of the tests.

The variation in control fish cortisol concentrations at week 1 was due to an accidental crowding, and for this reason is not considered significant. Plasma cortisol levels were significantly higher in infected fish at the third week. However, this was due more to a reduction in the cortisol levels of the control fish than an increase in the cortisol levels of the infected fish, so may not be an indication of a change caused by the disease. Infected fish cortisol levels were higher than the controls in week 4 and week 5 as well, but not significantly so.

Plasma protein and plasma osmolality values were not significantly different between control and infected fish at any period during the five week study. The changes in plasma protein values seen were due to

Table 1. Means and standard deviations of selected physiological values for control and VEN-infected chum salmon sampled at weekly intervals.

		Type	Cortisol ng/ml	Glucose mg/ml	Glycogen mg/ml	Protein g/ml	Lactate mg/100ml	Osmolality mmol/kg
Mean	Week 1	Control	148.05	0.82	0.003	0.051	34.88	380.25
Std. Dev.			60.13	0.15	0.002	0.007	15.03	20.15
Mean	Week 2	Control	50.35	0.84	0.007	0.046	24.94	347.65
Std. Dev.			29.49	0.14	0.005	0.010	9.65	19.89
Mean	Week 3	Control	11.89	0.73	0.025	0.033	35.83	334.89
Std. Dev.			9.63	0.27	0.008	0.008	9.46	21.77
Mean	Week 4	Control	20.12	0.77	0.022	0.040	32.19	299.39
Std. Dev.			8.92	0.11	0.008	0.008	12.39	19.47
Mean	Week 5	Control	23.77	0.73	0.027	0.046	37.65	332.10
Std. Dev.			11.75	0.15	0.010	0.011	14.69	13.82
Mean	Week 1	Infected	42.59	0.73	0.007	0.042	22.86	370.45
Std. Dev			43.49	0.14	0.003	0.009	9.70	18.53
Mean	Week 2	Infected	45.14	0.83	0.012	0.047	21.88	352.60
Std. Dev.			35.07	0.14	0.007	0.012	9.23	18.05
Mean	Week 3	Infected	25.09	0.67	0.023	0.045	33.83	331.55
Std. Dev			24.86	0.10	0.009	0.011	9.55	17.44
Mean	Week 4	Infected	30.02	0.70	0.027	0.039	23.70	300.35
Std. Dev.			33.17	0.11	0.008	0.008	13.28	18.50
Mean	Week 5	Infected	25.85	0.78	0.025	0.040	30.67	334.30
Std. Dev			30.94	0.01	0.010	0.007	10.42	12.23

variations in the control fish rather than experimentally induced variations in the infected fish, and are therefore not significant.

Changes in plasma glucose concentration were statistically significant at week 4, when the glucose concentration of the VEN-infected fish was less than that of the controls. Week 1 and week 3 values were also depressed in the infected animals, but were only slightly significant ($P = 0.10$).

Differences in liver glycogen concentration between the control and infected fish were significant at weeks 1, 2, and 4, when the infected fish had higher liver glycogen concentrations. These liver glycogen values increased in both the control and infected fish over the five week period. This may have been due to growth of the fish during the course of the experiment.

Blood lactic acid levels were lower in the VEN-infected fish throughout the entire 5 week study. This difference was statistically significant only in week 1, although the week 4 and week 5 data were weakly significant ($P = 0.10$).

Hematological Tests

The results of all hematological tests are shown in Table 2. Both RBC and WBC counts changed dramatically in infected fish during the course of the five week period. While the RBC count stayed relatively constant in the

Table 2. Means and standard deviations of selected hematological values for control and VEN-infected chum salmon sampled at weekly intervals.

		Type	WBC x 10 E4	RBC x 10 E6	WBC:RBC	Hgb g/100ml	Hct %	MCV g/dl	MCH pg	MCHC %	Fragility % NaCl
Mean	Week 1	Control	2.25	1.14	1/52	8.69	44.83	404.75	78.75	19.20	0.50
Std. Dev.			0.77	0.21		0.96	3.81	78.36	18.15	1.21	
Mean	Week 2	Control	2.99	1.20	1/39	7.67	42.10	358.80	64.35	17.70	0.45
Std. Dev.			1.02	0.25		2.38	7.07	71.04	19.96	3.89	
Mean	Week 3	Control	3.38	1.08	1/38	7.64	35.71	344.88	67.33	20.06	0.35
Std. Dev.			0.81	0.32		2.89	10.19	73.83	21.37	6.58	
Mean	Week 4	Control	3.68	1.14	1/29	8.31	39.33	348.28	73.33	21.17	0.45
Std. Dev.			0.95	0.15		1.57	4.07	36.78	12.80	2.52	
Mean	Week 5	Control	3.17	1.07	1/41	7.52	38.50	371.95	72.26	19.42	0.40
Std. Dev.			0.72	0.21		1.35	4.62	77.54	17.02	2.09	
Mean	Week 1	Infected	2.70	1.26	1/44	7.95	41.30	336.90	64.70	19.30	0.45
Std. Dev			0.77	0.23		0.98	4.47	56.17	10.27	1.52	
Mean	Week 2	Infected	3.07	1.07	1/37	7.58	40.40	391.30	72.75	18.65	0.50
Std. Dev.			0.83	0.26		1.16	5.27	68.41	13.03	1.53	
Mean	Week 3	Infected	7.74	0.96	1/15	6.35	34.80	370.15	67.15	18.35	0.35
Std. Dev			3.31	0.17		1.59	4.43	68.81	18.07	4.46	
Mean	Week 4	Infected	6.73	0.63	1/7	4.78	30.90	523.65	79.11	14.84	0.30
Std. Dev.			2.00	0.15		1.58	5.10	149.05	33.38	3.66	
Mean	Week 5	Infected	4.90	0.73	1/15	3.38	28.50	415.20	44.22	11.06	0.35
Std. Dev			1.24	0.19		1.57	4.26	132.49	16.03	4.01	

control fish, it dropped significantly in infected fish by weeks 4 and 5 (Figure 1). Similarly, WBC counts in VEN-infected fish were significantly elevated over the control values by weeks 3 through 5 (Figure 2).

Hemoglobin and hematocrit values decreased steadily in infected fish over the five week period, while control values remained relatively constant. These differences were statistically significant by weeks 4 and 5 for both tests (Figures 3 and 4). Results of the osmotic fragility test also indicated that the RBC in VEN-infected fish were more susceptible to lysis during weeks 4 and 5.

Other differences were observed in blood values between control and infected fish. Mean corpuscular volume, while quite variable in the VEN-infected fish, was significantly higher than the control fish values by week 4, indicating that the average size of the RBC was greater in VEN-infected fish. The values for MCH were similar in both control and infected fish except at the week 5 sampling period. By this time the VEN-infected fish had developed significantly lower levels than the controls, suggesting that the average weight of hemoglobin in the RBC had decreased. Similarly, MCHC was significantly depressed in infected fish by weeks 4 and 5.

Cytopathic changes in RBC were also observed over the course of the experiment in VEN-infected fish. During

Figure 1. Red blood cell counts for control (x) and ENV-infected (o) chum salmon sampled at weekly intervals for five weeks.

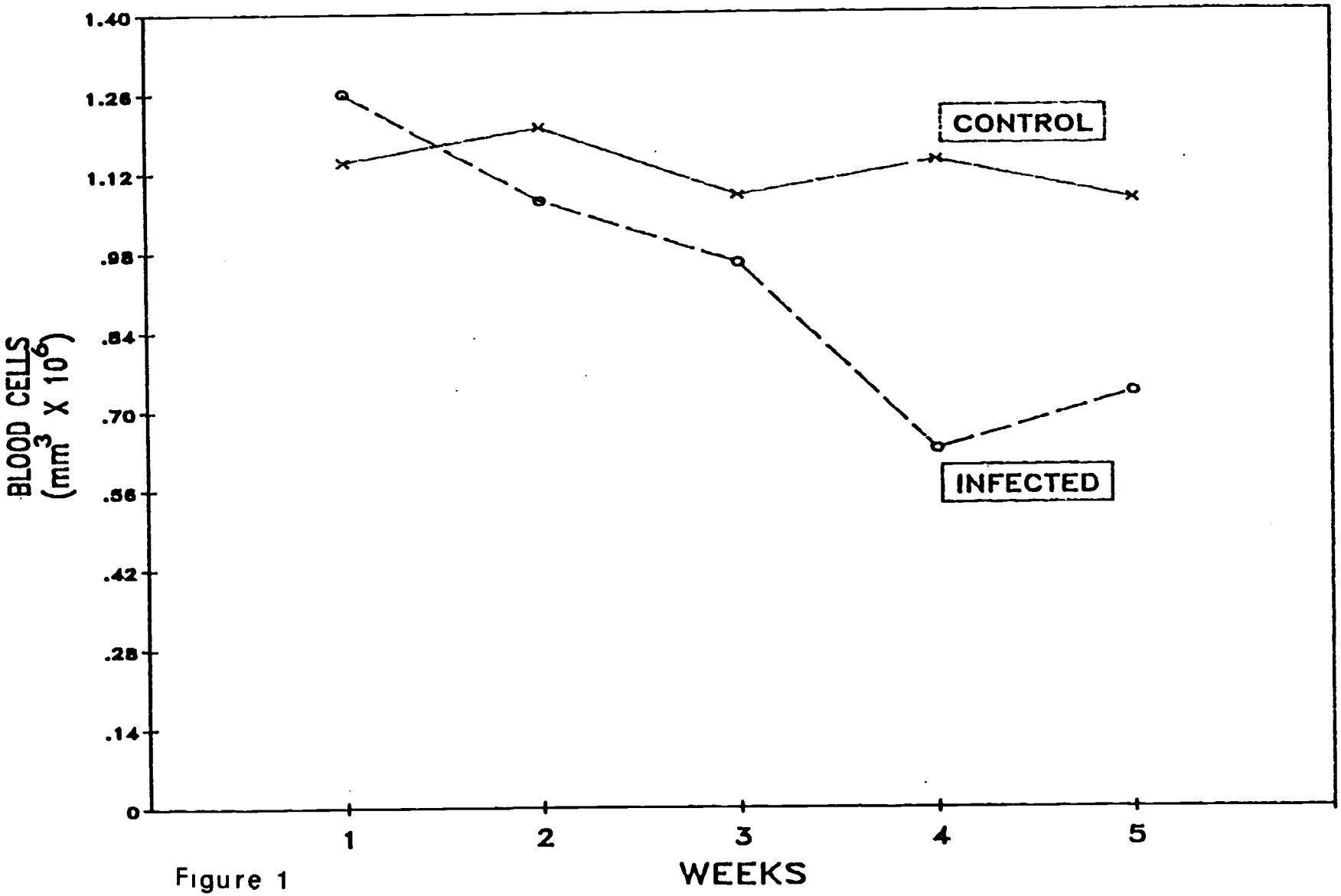
1.40
1.26
1.12
.98
.84
.70
.56
.42
.28
.14
0
BLOOD CELLS
(mm3 X 10^6)
CONTROL
INFECTED
1
2
3
4
5
WEEKS

Figure 1

Figure 2. White blood cell counts for control (x) and ENV-infected (o) chum salmon sampled at weekly intervals for five weeks.

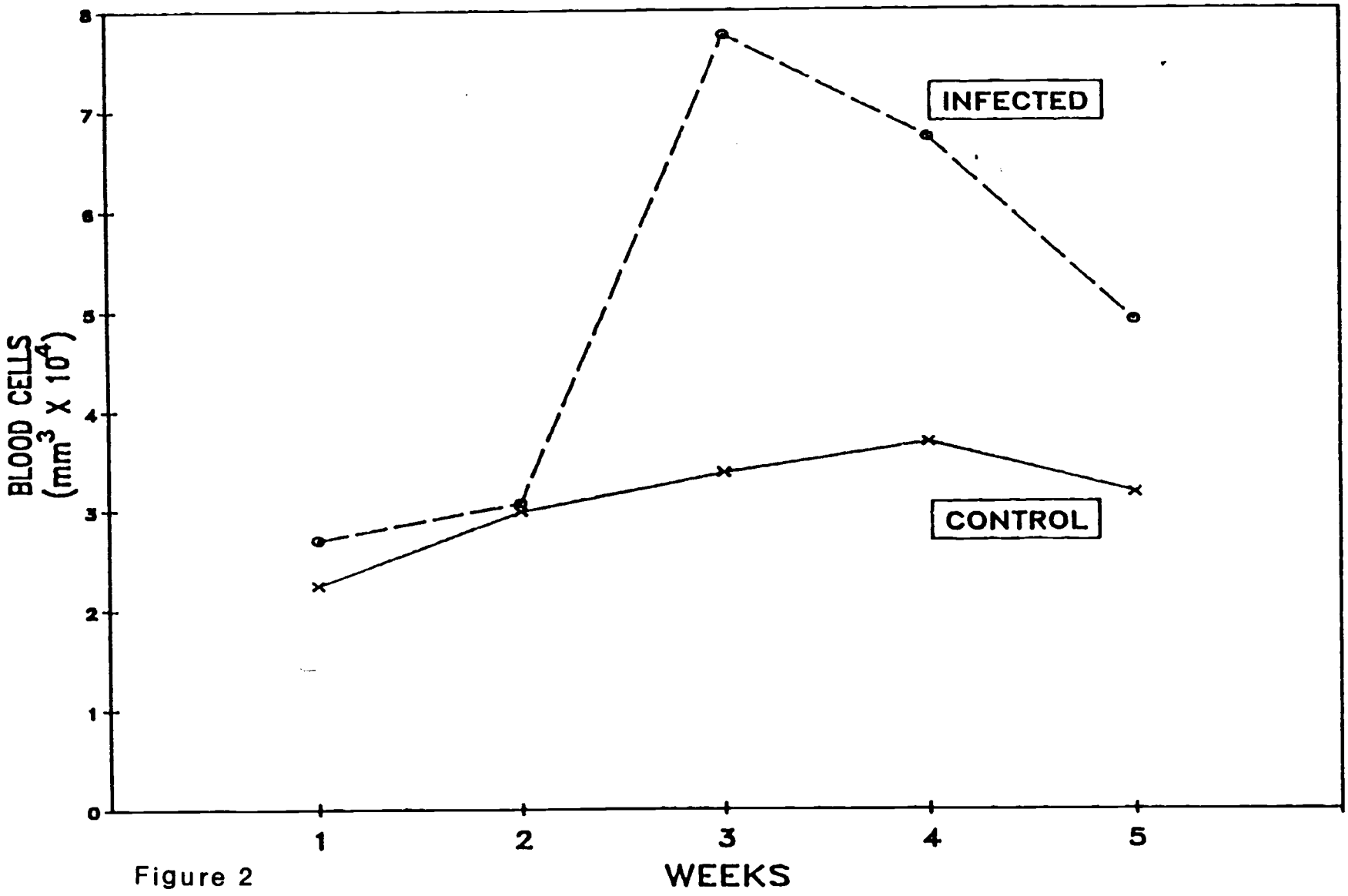
BLOOD CELLS
(mm3 X 10^4)
0
1
2
3
4
5
6
7
8
INFECTED
CONTROL
1
2
3
4
5
WEEKS

Figure 2

Figure 3. Blood hemoglobin concentrations for control (x) and ENV-infected (o) chum salmon sampled at weekly intervals for five weeks.

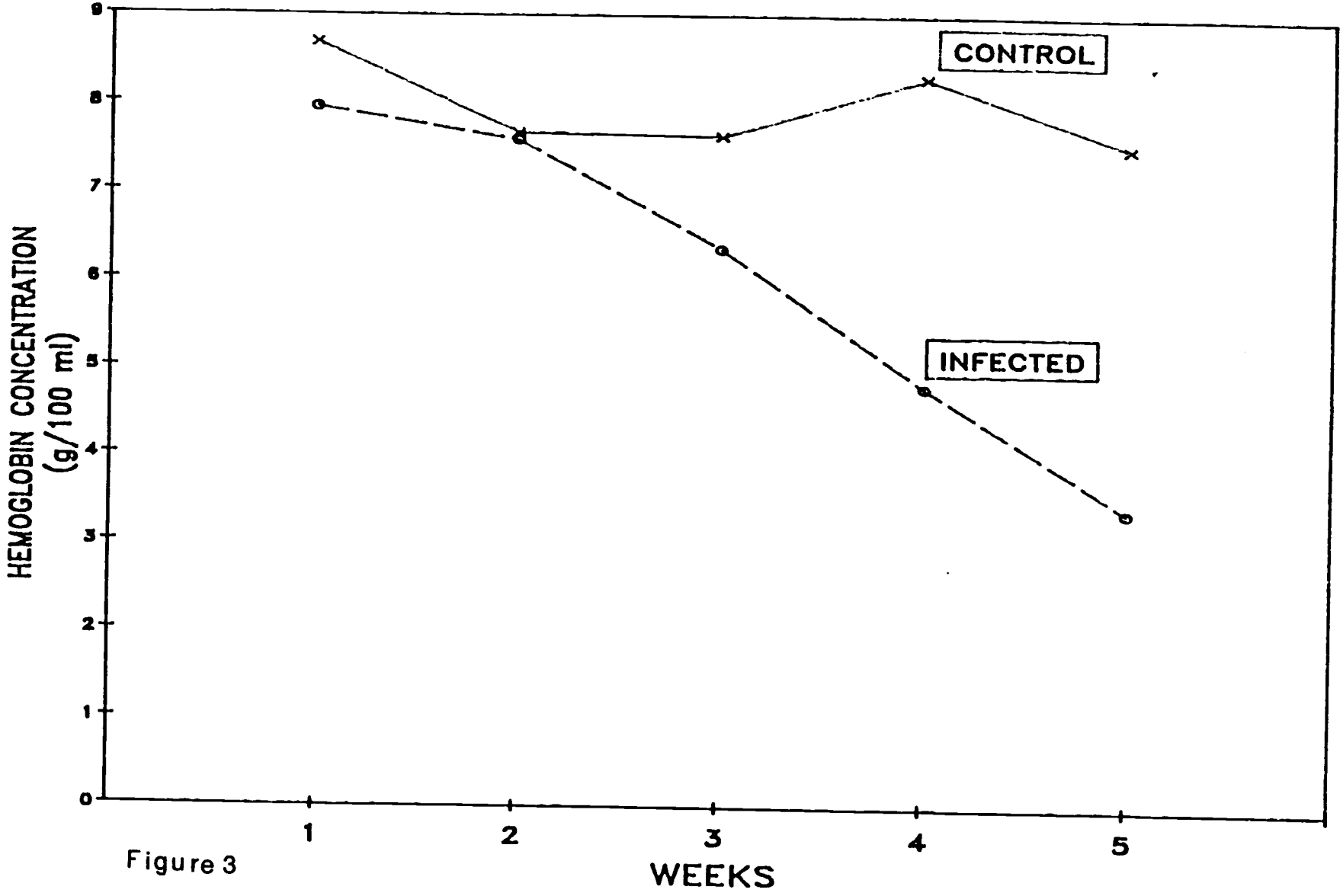
CONTROL
INFECTED
HEMOGLOBIN CONCENTRATION
(g/100 ml)
0
1
2
3
4
5
6
7
8
9
1
2
3
4
5
WEEKS

Figure 3

Figure 4. Hematocrit values for control (x) and ENV-infected (o) chum salmon sampled at weekly intervals for five weeks.

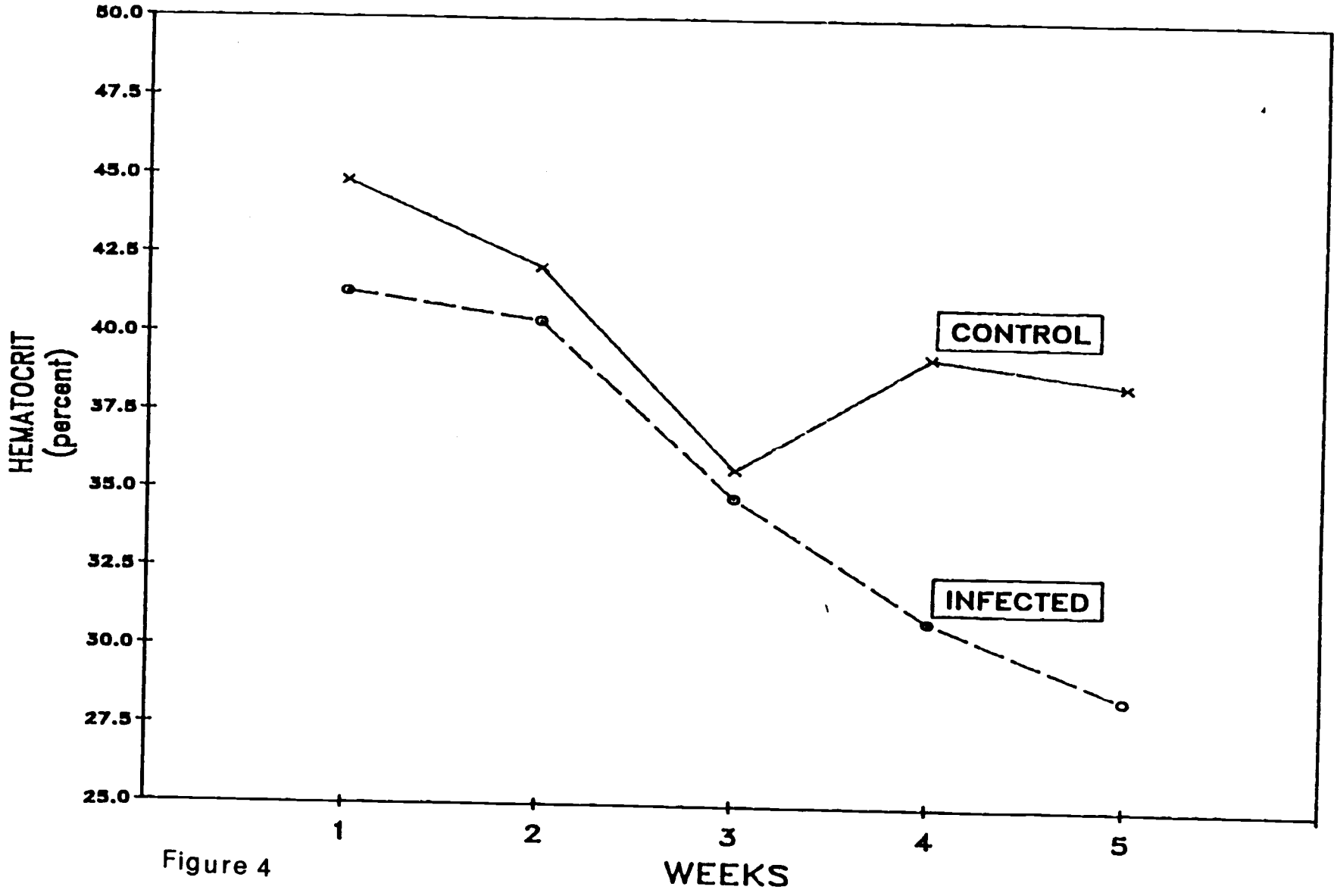
50.0
47.5
45.0
42.5
40.0
37.5
35.0
32.5
30.0
27.5
25.0
HEMATOCRIT
(percent)
CONTROL
INFECTED
1
2
3
4
5
WEEKS

Figure 4

week 1, infected and uninfected RBC were predominately mature. By week 2, one third of the infected fish contained varying numbers of unnucleated RBC, and immature RBC were becoming infected. By week 3, 70% of the fish had unnucleated RBC and approximately half of the immature RBC were now infected. At this time, the majority of the VEN-infected fish were showing abnormal, dense, compact WBC. By week 4, almost all of the fish contained some unnucleated RBC. Many immature RBC were present, but few were infected. By week 5, only 30% of the fish had unnucleated RBC. Many immature RBC were still present with only a very few being infected. This seemed to indicate that the VEN-infected fish were beginning to recover by week 5.

Cytoplasmic inclusion bodies were seen in all VEN-infected fish, with the highest prevalence occurring during week 4. No cytoplasmic inclusion bodies were seen in any control fish. Hemorrhaging of the body surface was also seen in most VEN-infected fish from weeks 2 through 4. No other external signs of the disease were present.

Recovery From Exercise

The results of the tests performed to study recovery time after a secondary exercise stressor are shown in Table 3. In general, these parameters took longer to return to normal in VEN-infected fish. Plasma glucose levels were elevated in infected fish and were still

Table 3. Means and standard deviations of selected tests performed on control and VEN-infected chum salmon at six intervals following an exercise stress.

	Hours	Type	Glucose mg/ml	Lactate mg/100ml	Cortisol ng/ml	Osmolality mmol/kg	Hct %
Mean	0	Control	0.74	70.84	117.87	363	41
Std. Dev.			0.09	9.14	3.3	7.35	5.66
Mean	0.5	Control	0.78	109.05	239.08	381.4	40.6
Std. Dev.			0.19	21.6	24.04	35.52	12.59
Mean	1	Control	0.86	88.33	225.68	399	38
Std. Dev.			0.02	9.56	52.35	17.17	5.37
Mean	3	Control	1.02	60.8	137.63	342.8	40.8
Std. Dev.			0.25	15.87	34.21	7.08	1.72
Mean	6	Control	1.1	36.15	84.95	320.6	36
Std. Dev.			0.27	6.81	50.21	10.19	7.98
Mean	24	Control	0.93	37.61	18.34	339.2	38.2
Std. Dev.			0.32	8.96	9.22	20.26	5.31
Mean	0	Infected	0.56	86.75	84.46	344.4	34.4
Std. Dev.			0.01	16.58	19.76	9.29	10.78
Mean	0.5	Infected	1.05	136.92	155.25	425.4	39.6
Std. Dev.			0.18	19.6	56.35	25.67	2.87
Mean	1	Infected	0.9	84.12	233.34	397.2	43.2
Std. Dev.			0.07	26.05	31.33	22.55	2.48
Mean	3	Infected	1.06	68.32	167.91	376	30.5
Std. Dev.			0.15	19.21	11.17	17.56	2.28
Mean	6	Infected	1.07	42.91	94.54	372.3	35
Std. Dev.			0.25	7.41	54.03	15.97	5.76
Mean	24	Infected	1.14	48.07	40.83	330.8	37.6
Std. Dev.			0.03	16.36	26.7	17.28	4.54

rising at 24 hours. However, control fish seemed to be more nearly recovered within 24 hours, although glucose concentrations were significantly lower only at 30 minutes post exercise.

Neither blood lactate nor plasma cortisol values were significantly higher in infected fish than in the controls at any interval following the exercise. However, both lactate and cortisol were somewhat higher at most of the intervals tested.

Plasma osmolality was found to be significantly different between control and infected fish. Osmolality increased greatly in VEN-infected fish, with recovery to normal levels taking a full 24 hours. While osmolality increased briefly in control fish, recovery was made within 3 hours, resulting in significant differences between control and infected fish by 3 and 6 hours post exercise. (see Figure 5).

Hematocrit values were determined in order to evaluate the extent of the infection. As the data indicate, the VEN-infected fish were not particularly anemic at any time, and these values were not significantly different from the controls. Hemorrhaging of the body surface was evident in virtually all VEN-infected fish.

Figure 5. Plasma osmolality values for control (x) and ENV-infected (o) chum salmon subjected to a secondary exercise challenge. Fish were sampled at six intervals following the exercise stress.

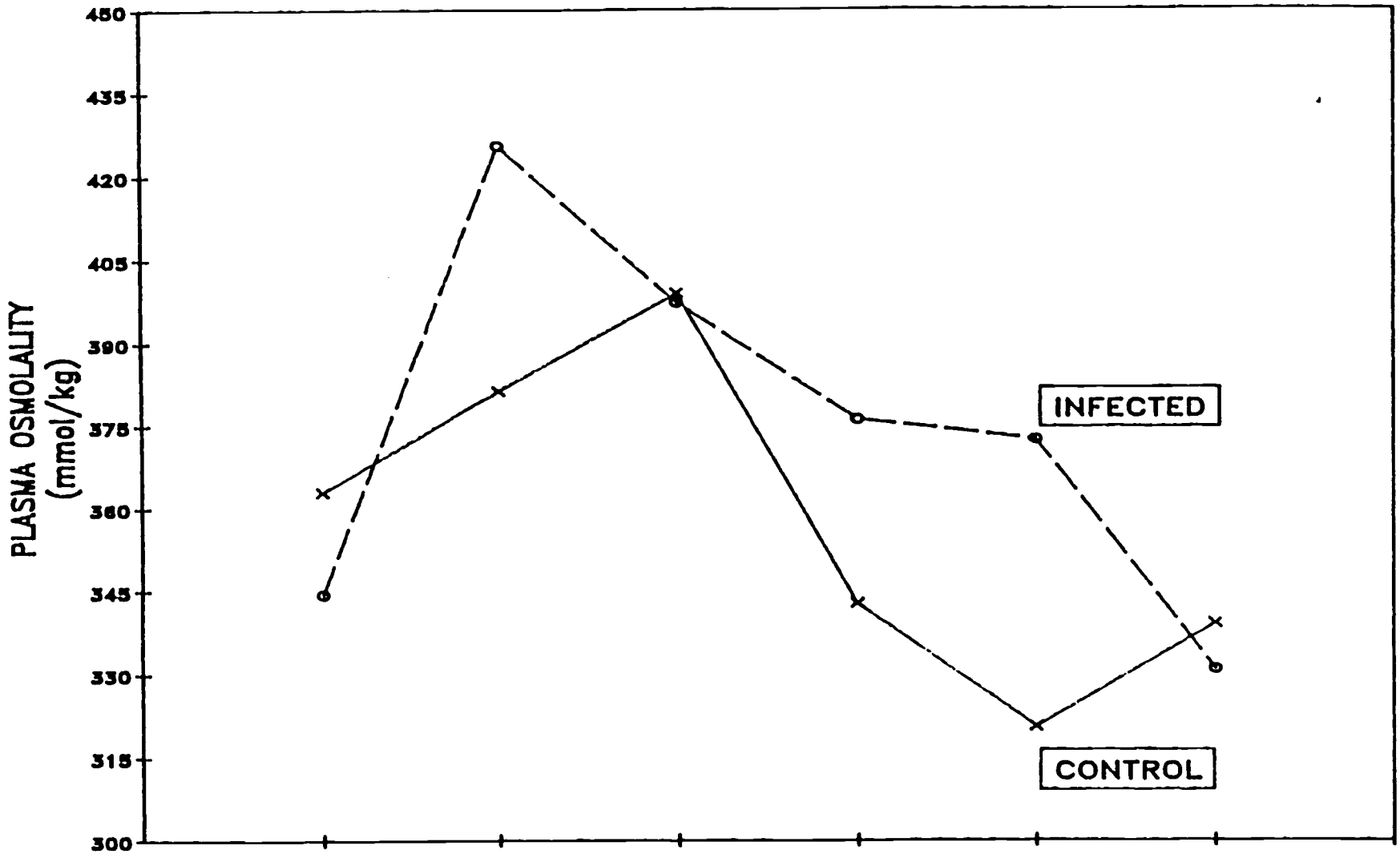
PLASMA OSMOLALITY
(mmol/kg)
450
435
420
405
390
375
360
345
330
315
300
INFECTED
CONTROL
0
0.5
1
3
6
24
HOURS FOLLOWING EXERCISE

Figure 5

DISCUSSION

The homeostatic mechanisms of fish are continually being tested by the normal demands of the aquatic environment. Superimposed on these natural stresses may be the affects of adverse environmental conditions including disease. Stress can be defined as the host response to environmental alterations or forces (stressors) that extend the homeostatic processes beyond their normal limits (WEDEMEYER and MCLEAY, 1981; PICKERING, 1981). Thus, a fish's survival in the face of an environmental stress depends upon its ability to adjust physiological processes so as to maintain homeostasis (WEDEMEYER, 1977). This study was designed to determine if infection with ENV is a significant stressor that causes measurable effects on the physiological maintenance of homeostasis. The results of the present study indicate that VEN, in isolation, does not lead to a significant breakdown in the homeostatic control systems of chum salmon.

Plasma osmolality and protein levels were not altered significantly by VEN at any time. Increased cortisol levels, which have been documented as being strong indicators of stress in fish (WEDEMEYER and MCLEAY, 1981; MAZEAUD et al., 1977), were slightly higher in infected fish. Increases in plasma osmolality, which are known to occur in marine fish subjected to

environmental stresses (MAZEAUD et al., 1977; EDDY, 1981), were not evident. Increases in total plasma protein due to the destruction of RBC and the resultant release of their cell contents into the blood stream did not occur. The reasons for this may have been efficient phagocytosis of infected RBC by leukocytes or effective clearance of proteins in the kidneys.

Plasma glucose and blood lactate levels were expected to rise over the course of the experiment since hyperglycemia and hyperlactemia, due to stress, has been observed by a number of researchers (MAZEAUD etal., 1977; BLACK et al., 1960; GEORGE, 1977). Such changes did not occur in response to VEN-infection. Similarly, expected decreases in liver glycogen concentrations were not seen.

Results from this study seem to indicate that chum salmon are able to maintain their homeostatic mechanisms, even in the face of a severe ENV-infection.

The dramatic increase in WBC count indicates that an extensive leucocytosis was occurring in the infected fish, presumably as a response to infection. Decreased RBC counts, hematocrits, and hemoglobin concentrations all indicate that RBC were being destroyed by the virus, resulting in an erythrocytic anemia, with subsequent erythroblastosis. One reason why hematocrits were not even lower may be due, in part, to the premature release of erythroblasts in an attempt to keep the oxygen

carrying capacity of the system at normal levels (MACMILLAN et al., 1980).

Other data indicative of a shift towards immature RBC included MCV values which were elevated at week 4. This suggests that there were more erythroblasts present, as they are larger, on average, than the mature erythrocytes (MACMILLAN, 1980).

Blood values obtained indicated that the fish were severly infected with VEN; the data are comparable to those obtained by MACMILLAN et al., (1980). The physiological and hematological data suggest that the severity of infection was greatest sometime during weeks 3 and 4, with a partial recovery by week 5.

A natural population of fish has to contend with many environmental factors such as predation, changes in pressure, temperature and dissolved gases, and in the case of anadromous fish, severe osmotic challenges. In a second experiment, netting of the fish, and the resultant struggling (exercise) was used to induce stress. The results of that study suggest that VEN-infected fish did have more difficulties maintaining homeostasis than control fish.

Changes in plasma glucose and osmolality were seen at various intervals following exercise in both control and infected fish. However, VEN-infected fish had significantly higher levels at 30 minutes post exercise,

and in each case recovery time was much slower. The plasma osmolality results were particularly striking, showing that infected fish had increased difficulty in osmoregulation. This was probably due to an increase in the circulating concentrations of norepinephrine, which leads to an increased water permeability in the gills (MAZEAUD et al., 1977).

While plasma cortisol levels were not significantly higher in VEN-infected fish at any interval following exercise, their slight elevation further supports the view that these fish were undergoing a more severe stress response compared to control fish. Contrary to expectations, lactic acid levels were not elevated in VEN-infected fish following exercise. Because these animals were not as anemic as expected, their oxygen carrying capacity may not have been greatly reduced. Thus, it is not surprising that the infected fish had only slightly more difficulty paying off their oxygen debt.

The results of the exercise challenge indicate that VEN-infected fish had more difficulty than control fish in maintaining homeostasis when presented with a secondary challenge stressor. It is noted that observed changes occurred in a population of fish that were not extremely anemic nor heavily infected at the time of the experiment. Severely infected fish would be likely to

have even more significant problems.

It would be interesting to test the effects of other stressors on VEN-infected fish. For example, challenge by a secondary chronic stressor may provide information concerning problems these fish may encounter in the natural environment. An osmotic challenge, subjecting seawater acclimated fish to freshwater, would be of particular interest, since few fish may actually be able to osmoregulate efficiently in the face of this virus.

If no other stressor is present, laboratory-maintained chum salmon seem to be able to maintain physiological homeostasis when infected with VEN. However, a secondary challenge of exercise caused a deterioration of the homeostatic systems. A severe infection and an additional stress may require adjustments which are beyond the ability of the fish to generate, possibly resulting in death.

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