

**An Outbreak of Infectious Hematopoietic Necrosis in the Baker River System Affecting Two Year Classes of Sockeye**

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**INTRODUCTION**

Sockeye salmon (*Oncorhynchus nerka*) fry are particularly susceptible to infectious hematopoietic necrosis (IHN). During the spring of 1994 the first known outbreak of IHN in Baker River sockeye occurred dramatically, affecting two brood-years. Although IHN virus (IHNV) has been detected in the adults of this stock previously, this is the first year that a noticeable outbreak in juvenile fish has occurred.

The Baker River is a tributary to the Skagit River which drains into Puget Sound in northwestern Washington State. The Baker River has two hydroelectric dams with two resulting reservoirs, the upper named Baker Lake and the lower named Lake Shannon. These lakes contain numerous species of resident fish including trout and kokanee as well as anadromous populations of sockeye, coho, chinook and pink salmon. All the anadromous fish receive truck transportation around the two dams for both their downstream and upstream migrations. The sockeye program involves trucking some of the returning adults to Baker Lake while the rest are put into artificial spawning beaches at two locations which compensate for the lack of lake spawning habitat. These rectangular gravel beaches are supplied with upwelling spring water. Adults placed in the beaches in July spawn during the fall and winter and unfed fry migrate out from February until June. As fry emerge from the newest beach at the upper end of Lake Shannon, they are trucked to Baker Lake where they grow for a year until they smolt. Fry from the older beaches migrate into Baker Lake

on their own. During the adult season mortalities and spawned fish are removed from the beaches daily. Between brood years the beaches are disinfected prior to restocking with new adults.

Because of the relatively low fry to smolt survival in Baker Lake, there is a supplemental program of rearing 120,000 fry to smolts in net pens located at the lower end of Lake Shannon.

Over the years there has been no testing for IHNV in the fry, and testing of the adults has been limited and sporadic. IHNV was found in about 1/3 of the kidney pools tested in 1987 (with no evident resultant fry outbreak), but in none of the 51 kidneys tested in 1993.

**EVENTS OF 1994**

1993 was the first year the newer larger beach was stocked to its rated capacity of 3000 adults. At the same time one of the older beaches (rated at half the capacity) was stocked with about 900. Adults were evenly distributed between the 2 beaches as they arrived. One small virus sample was taken from fresh spawn-outs from each beach with no virus detected.

Higher than normal mortality and a premature emergence condition were noticed from the beginning of fry outmigration in March at the larger beach. IHNV was quickly confirmed and subsequent weekly testing revealed a steadily increasing prevalence of infected fish until the beach was shut down in mid-May (See table). Weekly 60-fish samples were also taken from the smaller beach but virus was never found there.

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By the time it was shut down, nearly three million fry from the infected beach had been trucked in normal fashion to Baker Lake for release, hoping for the survival of those not yet infected. However, due to the sensitivity of sockeye fry to this virus and the high concentration of virus in the dying fish, the question arose whether the uninfected fry were becoming infected during the trucking process. Bill Batts of the National Biological Survey at Seattle, Washington used tangential flow filtration (Batts, 1989) to concentrate water samples taken on March 30 to assay for viable IHNV. Whereas he found about 20 PFU/mL in the beach effluent, there were about 1000 PFU/mL in the transport water at the end of the 30 minute truck ride. Attempts were made to estimate the post-transport mortality of the fry by live-boxing weekly representative samples of 60-100 fry. They were put into a low density, high flow, 50-gallon container from which mortalities were removed and recorded daily. Typically 10-20% of them died within four days and mortality continued toward 100% by 10-14 days. It is unknown how closely the live-box mortality reflects the actual post-transport mortality in the lake.

In an effort to minimize horizontal transmission of the virus during fry trucking we started adding 0.25ppm iodine to the transport water (Batts, 1991). An attempt was made to evaluate the effectiveness of this by counting PFUs in the transport water and by live-boxing post-transport fry. It wasn't possible to do a controlled test by arranging identical loads with and without iodine or to split a load into identical replicates. However, when two similar loads on consecutive days were tested for virus concentration, the one with iodine showed 98% fewer PFU/mL at the end of the ride than the one without iodine in the water (6 PFU/mL compared to 300 PFU/mL). The live-box mortality results were equivocal.

The previous year class became involved when the yearling sockeye smolts that were in net pens at the lower end of Lake Shannon came down with IHN. This happened six weeks after the beginning of infected fry emergence from the beach at the upper end of Lake Shannon and the hauling of infected fry to the upper end of Baker Lake. A cause and effect relationship with the virus seeding of the lake about seven miles upstream is suspected but not proven. The lake level was quite low at the time which would mean a faster transit from one end to the

other. In addition, some of the "wild" yearling smolts migrating out of Baker Lake were shown to have IHNV infections; 5 out of 12 five-fish pools of kidney+spleen had 200-50,000 PFU/g. All 60 of these fish looked healthy.

Just before the net pen smolt outbreak, fry from the IHN-free beach were put into eight net pens a quarter mile from the smolt pens. Two weeks later they also broke with IHN.

Time and testing will reveal how long this amplification of IHNV will be impacting the Baker system. There are resident trout and kokanee as well as numerous other species in both lakes which may serve to carry on the infection. A record number of adults returned to the Baker river this summer shortly after the juvenile episodes. At least 12,000 of them have been released into Baker lake to try to find spawning habitat.

#### COMPARISON TO OTHER REPORTS

Alaska's successful IHN control strategy (FRED Special Report, 1988) consists of three fundamentals, a virus free water supply, rigorous disinfection, and compartmentalization of eggs and fry. Only the virus free water supply is possible with the Baker spawning beaches.

Garth Traxler of the Pacific Biological Station at Nanaimo, British Columbia, (Traxler, 1989) relates experience with their spawning channels indicating that the density of adults is a better predictor of fry outbreaks than finding IHNV during adult testing. This year's Baker experience is in agreement with that observation in two ways: virus was not found in the small sample of adults we took, and the outbreak occurred only in the more heavily stocked beach even though the broodstock was uniformly distributed between the two beaches. He also reported a reduction in IHN prevalence with time, but we saw a prevalence increase.

#### ACTIONS TAKEN

>The method of controlling virus amplification by destroying infected populations was used in only one instance in these outbreaks. All the other fry and smolts were released in hopes of obtaining the survival of those not yet terminally infected. The one instance of destruction was three of the eight fry net pens that became epizootic.

**SOCKEYE FRY IHNV PREVALENCE AND SEVERITY INCREASE OVER TIME AT LARGE BEACH**

Each titre from individual fish except where noted

Date sampled:	3/23/94	3/30/94	4/6/94	4/13/94	4/20/94	4/27/94	5/4/94	5/11/94	5/18/94
No. Fish Tested:	60	60	57	58	59	55	60	60	68
% $\leq 10^2$ PFU/gram	57	57	63	On these days samples tested in pools of five					4
% at $10^3$ PFU/gram	15	15	10						6
% at $10^4$ PFU/gram	7	2	2						3
% at $10^5$ PFU/gram	7	0	2						2
% at $10^6$ PFU/gram	0	3	4						2
% at $10^7$ PFU/gram	0	2	4						19
% at $10^8$ PFU/gram	5	10	5						32
% at $10^9$ PFU/gram	10	10	9						24
% at $10^{10}$ PFU/gram	0	2	2						9
% High Pos. ( $\geq 10^7$ )	15	23	19						42
% Low or Neg. ( $\leq 10^3$ )	72	70	74	42	33	18	0	8	10
Total % Positive	43	43	37	100	92	100	100	100	96

Test system: PEG pretreated EPC cells overlaid with methyl cellulose, incubated at 15°C for 14 days. Typical rhabdovirus plaques counted.

- ▶The beaches, net pen site and handling equipment have been disinfected prior to restocking with the next brood year.
- ▶The stocking density for three beaches for the '94 brood year has been kept to half the density of the beach that broke this spring.
- ▶There will be increased viral testing of adults and the resulting fry in both the beaches and lake spawning grounds this year.
- ▶Smolts resulting from this year's fry will be evaluated for survival and IHNV infection.

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**AVAILABLE: Graduate Research Assistantships in the molecular basis of fish diseases (MS or PhD).**

Graduate research will involve applied molecular biology in fish health. This laboratory is currently involved in development of recombinant live vaccines, studies on the molecular pathogenesis of infectious disease, and development of diagnostic tests. Applicants should have a strong background in aquaculture, biochemistry or microbiology. Stipends are competitive and include tuition remission.

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## Plasmacytoid Leukemia in an Adult Chinook Salmon from Salmon River

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During August 1993, an adult chinook salmon (*Oncorhynchus tshawytscha*) that returned to the South Fork trap on the Salmon River, Idaho, was found to have a large growth on its liver. The tumor was classified (RTLA Accession No. 5973) by Dr. John Harshbarger, Director of the Registry of Tumors in Lower Animals, as a granulocytic leukemia (Harshbarger, 1984) or plasmacytoid leukemia (Kent, 1990a). Classification of the tumor was confirmed by Dr. Mike Kent, Fisheries and Oceans, Nanaimo, B.C., Canada, as a plasmacytoid leukemia. Histologically, the leukemia is a generalized invasion of visceral tissue by plasmacytoid cells, but has also been reported in retrobulbar tissue (Kent 1990a).

This form of leukemia was first detected in chinook salmon collected in 1974 by Dr. Yasutake and identified by Dr. Harshbarger and Clyde Dawe (personal communication from Dr. Harshbarger, June, 1994; RTLA Accession Nos. 1022 & 1023). This leukemia has been implicated as the cause of mortality in chinook salmon fingerlings reared at a freshwater hatchery in the state of Washington (RTLA Accession Nos. 1022 & 1023) and in pen-reared chinook salmon in British Columbia (Kent, 1990a). The tumor reported here was not infected with *Enterocytozoan salmonis*, which has been implicated as a cause of similar manifestations of leukemia in chinook salmon in Washington and California (Morrison et al., 1990; Hedrick et al., 1990). This report constitutes the first finding of this form of leukemia in a hatchery-reared chinook salmon which returned to spawn.

According to Kent (1990b), the condition is transmittable experimentally in chinook salmon, sockeye salmon (*O. nerka*), Atlantic salmon (*Salmo salar*), and rainbow trout (*O. mykiss*). A more recent report provides evidence of a retroviral etiology for the disease (Eaton and Kent, 1992).

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## Viral examination of ovarian fluid from Chinook Salmon, *Oncorhynchus tshawytscha*, in Lake Sakakawea, North Dakota.

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The state of North Dakota, in negotiating with the Canadian Province of Manitoba concerning a water project, diverting water from the Missouri River Drainage to the Hudson Bay Drainage, was asked, "Are fish in the upper Missouri River infected with infectious hematopoietic necrosis virus?" The question has persisted since the 1976 publication (Lloyd, et al.), "International Garrison Diversion Study Board Report" was submitted to the International Joint Commission. In that report it was indicated that "Infectious hematopoietic viral necrosis (IHN) and enteric redmouth (ERM), a bacterial disease, have a high probability of being introduced to Manitoba waters." One hundred and fifty fall Chinook salmon, *Oncorhynchus tshawytscha*, five years of age from Lake Sakakawea were examined for IHN at the Fish Disease Control Center (USFWS), Fort Morgan, Colorado (personal communication, 1994) in 1991 and found to be negative. Chinook salmon from Washington, Michigan, Oregon, Wisconsin and Minnesota were stocked in Lake Sakakawea during 1976-1987 (Lee, personal communication, 1994). Since then, 1988-1992, eggs and smolts were produced by artificial spawning. During 1970-1982 Coho salmon, *O. kisutch* from Michigan, Washington and Oregon were stocked in Lake Sakakawea. The north Dakota Water Resource Research Institute contracted with us to re-examine the question in light of changes since 1976.

### Procedures:

Ovarian fluid samples were collected aseptically October 7, 9 and 12, 1992 from Chinook salmon captured in lake Sakakawea, North Dakota. Samples were

pooled, five fish per pool, for a total of thirty pools. Pooled samples were stored at -110 C. Thawed samples were tested for viral activity by inoculation of CHSE-214 cultures according to Blue Book procedures. Cultures were inoculated with 0.4 ml ovarian fluid per 25 cm<sup>2</sup> flask, incubated for one hour at 17 C, then flooded with Tris-buffered MEM supplemented with 5% fetal bovine serum and antibiotics. Cultures were incubated at 17 C in normal atmosphere to allow plaque formation and viewed periodically under phase contrast to identify plaque-positive cultures. Cultures devoid of plaques after three weeks of incubation were concluded to be virus-negative. Supernatants were drawn off plaque-positive cultures and stored at -110 C. Supernatant and control IHN (isolate 039-82) were used to infect CHSE-214 cultures in order to assay for titer and observe plaque morphology. Sample viruses were tested by serum neutralization using an anti-IHN (RO83`19) or anti-Chum salmo reovirus serum (obtained from J.L. Fryer, Oregon State University). Virulence tests were conducted on rainbow trout, *O. mykiss*, (Mean weight 3.5 g) that were intraperitoneally (ip) injected or waterborne exposed to approximately 10<sup>4</sup> TC<sub>10</sub><sub>50</sub>. Mortality was monitored for 28 days.

### Results:

Of the thirty ovarian fluid samples, four were found to contain virus, twenty-two were found to be virus negative and four yielded inconclusive results (due to bacterial contamination or unexplained cell death). Plaque morphology was consistent between samples but distinctly different from control IHN plaques. IHN plaques were large lightly stained

regions filled with rounded cell debris (Figure 1). Ovarian fluid sample plaques were small unstained regions devoid of cells with darkly staining amorphous plaque margins (Figure 2). These differences in plaque morphology suggested that the sample viruses appeared to be serologically identical to the Chum salmon reovirus (Winton et al., 1981) and the cytopathic effect was also similar. Dead fish were not detected in either intraperitoneal or waterborne exposed groups.

### Conclusions:

Survey of 150 Chinook salmon from Lake Sakakawea, North Dakota has not detected evidence of IHNV within the population. Due to the small sample size, occurrence of unexplained death in some cultures and choice of ovarian fluid as source of virus, we cannot definitively state that IHNV is not present in the population but merely that if IHNV exists in the Chinook salmon population of Lake Sakakawea, it has not yet been detected. However, a virus that was serologically identical to a reovirus isolated from chum salmon was detected. The isolate was shown to be avirulent in small rainbow trout after waterborne exposure or ip injection; in agreement with the results of previous studies that examined the virulence of other reoviruses isolated from salmonids. Reoviruses from adult salmonids have had an increased frequency of detection in the Pacific Northwest in recent years. Further epizootiological research is planned on the Chinook salmon population in Lake Sakakawea along with comparing this reovirus isolate to other recent isolates.

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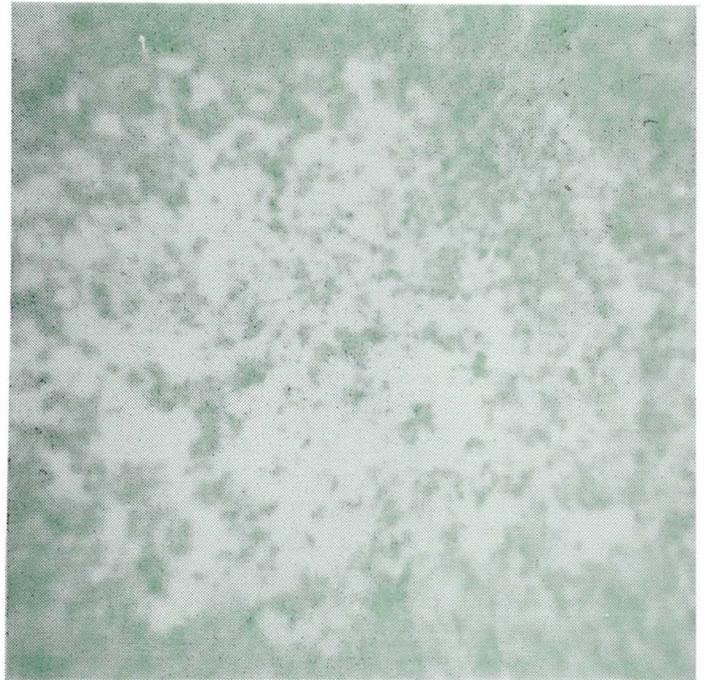


Figure 1. IHNV plaque on CHSE-214 cells with lightly stained margin and cellular debris in center. The plaque selected for photography was unusually small, 68X.

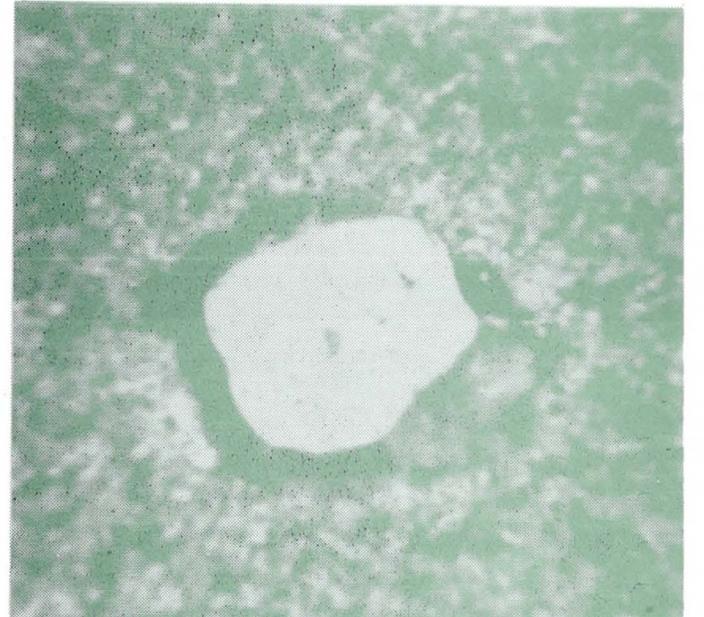


Figure 2. Ovarian fluid plaque on CHSE-214 cells containing little debris and having an amorphous darkly staining margin. The plaque size presented is typical and shows that plaque size is smaller than that of the smallest IHNV plaques, 68X.

## Isolation of *Flexibacter maritimus* from California.

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*Flexibacter maritimus* was described by Wakabayashi et al. (1986), as the agent causing disease in cultured marine fish in Japan. The organism has also been isolated from diseased marine fish in Scotland (Campbell and Buswell 1982) and Spain (Pazos et al. 1993). Although marine gliding bacteria have been isolated from diseased salmonid fish (Borg 1960, Kent et al. 1988) and oysters (Dungan 1989) on the Pacific coast of North America, cultures were either lost, characterization was not complete, or the isolates were found to be distinct from *F. maritimus* (Kent et al. 1988). In a study of diseases affecting captive or cultured marine fish in southern California, we have isolated what appears to be *F. maritimus* from white seabass *Atractoscion nobilis*, Pacific sardine *Sardinops sagax*, and northern anchovy *Engraulis mordax*, at three separate netpen sites.

### Materials and Methods:

Field diagnosis consisted of phase-contrast examination of wet mounts prepared from skin lesions. Fish tissues were fixed in 10% neutral-buffered formalin for sectioning by standard histological methods. We used 50% seawater Hsu-Shotts (SW-HS) medium (per liter: 2 g tryptone, 0.5 g yeast extract, 3 g gelatin, 15 g agar, pH 7.4) for routine culture, supplemented with neomycin sulfate (4 µg/ml) and polymyxin B (200 IU/ml) for primary isolation. Cell shape and size were determined from gram-stained 48 hr cultures. Motility was observed in hanging-drop wet mounts by phase-contrast microscopy. Isolates were grown aerobically in 100% SW broth

containing 1% tryptone, 0.008 M MgSO<sub>4</sub> and 0.5 M glycerol (Dworkin and Gibson 1964) to test for microcyst formation. Cultures inoculated into microcyst broths were examined by phase-contrast and gram-stain at two and four weeks for the presence of microcysts, and viability of the cultures was tested by inoculating a loopful of broth (0.01 ml) onto 50% SW-HS agar. Formation of fruiting bodies was examined by inoculating small pieces of autoclaved fish tissue and incubating in 100% SW (Ordal 1946). After three weeks, the fish tissue was examined visually and by phase-contrast (100X) for the presence of fruiting bodies. The methods described by Lewin and Lounsbery (1969) and Bernerdt et al. (1990) were used to determine biochemical characteristics using 50% SW-HS broth or agar as basal media. The typestrain *F. sancti* served as a positive control for biochemical tests, using media made without added sea salts. Bacterial colonies growing on Marine Agar (Difco) were tested for flexirubin pigments by adding 20% KOH and observing the presence or absence of a violet pigment. A 1% Congo Red solution was added to colonies on 50% SW-HS agar to test for retention of the dye. Primary isolation, routine culture and biochemical testing were performed at 22-25 C, but growth was also determined at 15 C and 37 C. Each isolate was also tested with the API-ZYM system incubated for 12 h at 22 C. Seawater requirements were determined by inoculating isolates into HS broths made with 0, 33, 66 and 100% SW. Antibiotic sensitivity was evaluated by swabbing cultures to cover a 50% SW-HS plate and placing either a oxytetracycline (30 mcg)

or Romet (Ormetoprim 1.2 mcg and sulfadimethoxine 23.8 mcg) disc on the plate. The diameter of the zone of inhibition was measured after 5 days of incubation at 25 C.

### Results:

Long, thin rod-shaped bacteria with the ability to flex or glide were observed on lesions of diseased juvenile chinook salmon, northern anchovy, pacific sardine and juvenile white seabass held in netpens in a saltwater harbor at Ventura from 1990-1993. Motile, curved short rods were also present in the lesions. Chinook salmon reared in 1991 had severe white-pale pink eroded areas of the gills leading to rapid mortality. An infestation of *Trichodina* spp. was also found on chinook salmon gills. Northern anchovy displayed eroded, hemorrhagic lesions of the fins, body and snout, associated with rapid mortality. Pacific sardine were found with a tan-colored membranous coating of gliding bacteria over wide areas of the body. White seabass had lesions of the head, eyes, and fins, but lesions of the body ranging from white-colored areas of scale loss to open ulcers extending into muscular tissue were predominant. Lesions of white seabass sampled at Redondo Beach and Newport Beach in 1993 and 1994 were similar to those found at Ventura. A small number of gliding bacteria were observed in skin scrapings of white seabass which did not exhibit lesions or mortality at a marine hatchery in San Diego.

Long, thin, basophilic staining bacteria were abundant in hematoxylin and eosin-stained sections of lesion material from northern anchovy and white seabass sampled at Ventura. Areas of complete epithelial loss on the snout of anchovies were associated with matted growth of bacteria that extended into the subdermal connective tissue of the head. Mats of long, thin bacteria were also found in areas of ulceration and scale

loss in white seabass, invading the dermis and subdermal layers, and growing in scale pockets and between scales. Infiltration of inflammatory cells into affected areas was mild or absent in both fish species.

Attempts to culture gliding bacteria from lesions of chinook salmon and northern anchovy in 1991 and 1992 were not successful but improvements in media and technique led to recovery of gliding bacteria from each disease outbreak investigated in 1993-1994 (Table 1). Numerous rhizoid colonies of white or light tan-colored bacteria appeared after 2-4 days of incubation. The size of the gram-negative rods were 0.5  $\mu\text{m}$  in width and ranged from 3-9  $\mu\text{m}$  in length, with extended culture on 50% SW-HS agar tending to produce shorter and more uniform lengths.

No large (8  $\mu\text{m}$ ) cysts or microcysts similar to those observed by Ostland et al. (1994) were seen in our isolates or the *F. maritimus* reference strain. In all cultures tested (Table 2), non-refractile spheroplasts of 1.0  $\mu\text{m}$  maximum diameter were abundant after two and four weeks in microcyst medium. Vegetative rods were few in number but still present after four weeks in microcyst medium. *Flexibacter maritimus* and isolates V2 and R2 were not viable after four weeks, so the spheroplasts were not a resting stage (Table 2). Macroscopically visible or colored fruiting bodies were not seen after 3 weeks of growth on sterile fish tissue. The sensitivity of the isolates in Table 1 to oxytetracycline and Romet was equivalent to *F. maritimus*, but some growth was noted in the inhibition zone of Romet with all strains tested.

Representative isolates (Table 1) were compared to reference strains to determine biochemical and physiological similarity (Table 2). Bacterial isolates V2, N1-2 and R2 from diseased white seabass were virtually identical to the *F.*

*maritimus* typestrain with the exception of R2 having a stronger salinity requirement. Isolates V5 and V6 from diseased anchovy and sardine were also similar to the *F. maritimus* typestrain except that these two isolates did not reduce nitrate. Isolate SD1a from healthy white seabass differed from *F. maritimus* in five tests, testing gelatinase negative as well as having a stronger salinity requirement. Pellicle formation in broth culture was especially strong in 100% SW-HS for all isolates and *F. maritimus*.

The production of 19 enzymes, as determined by the API-ZYM method is shown in Table 3. All isolates from diseased fish were very similar in number of enzymes detected and in level of enzyme activity to the *F. maritimus* typestrain. The isolate from healthy fish (SD1a) was also similar in enzymes detected to *F. maritimus* and the other isolates but displayed some lower activity levels and also was the only isolate to hydrolyse a carbohydrate, N-acetyl-B-glucosamine.

#### Discussion:

The visible lesions seen in this study generally corresponded to known sources of physical trauma to the fish. Anchovies and sardines used as live bait are subject to net abrasion from capture and transfer. The snout is abraded from bumping into enclosure walls. Netpen-reared white seabass are cannibalistic and display antagonism at feeding periods. Severe outbreaks of flexibacteriosis in white seabass often followed interruption of feeding due to climatic conditions or mechanical failures. The outbreak of gill flexibacteriosis in chinook salmon coincided with a large flexibacter-associated dieoff of anchovies in a bait-pen located about 70 meters upcurrent from the salmon. We believe that pieces of anchovy tissue containing flexibacters were lodging in salmon gill filaments, causing gill lesions. However, the *Trichodina* may also have predisposed salmon to gill

flexibacteriosis. Although motile, curved rod-shaped bacteria could also be seen in wet mounts of lesions and could be cultured and identified as *Vibrio* spp., histopathology showed that only long-thin flexibacter-like bacteria were invasive, appearing under the surface of ulcerated lesions and were probably the primary pathogens.

Biochemical tests, morphological measurements and the lack of resting cells or fruiting bodies showed that gliding bacteria recovered from diseased fish were isolates of *F. maritimus* and represent the first isolations of this species on the Pacific coast of North America. The gliding bacteria isolated from asymptomatic fish, SD1a differed in several important aspects from the *F. maritimus* typestrain and the other isolates and was clearly not the same species. However, the results of our API-ZYM testing did not show dramatic differences between SD1a and the *F. maritimus* isolates, so this technique may not be needed for routine identification of clinical isolates. Two isolates in this study showed a slightly greater percent seawater requirement than the typestrain. While this may have affected the results of biochemical testing somewhat, it did not prevent identification of the isolates as *F. maritimus*. Our results and those of Bernardet and Grimont (1989) suggest that presumptive tests for the identification of *F. maritimus* from lesions of fish include flexirubin, Congo red, catalase, agar hydrolysis, gelatinase and a requirement for seawater. Production of antisera for serotyping and rapid identification is in progress. **From this study, the following recommendations were made for netpen culture of white seabass:**

1. White seabass netpens should be located as far from live bait pens as possible.
2. Sufficient feed should be provided frequently to avoid cannibalism and antagonism.
3. Antibiotics administered in food should

be examined as a means of control of marine *Flexibacter* outbreaks.

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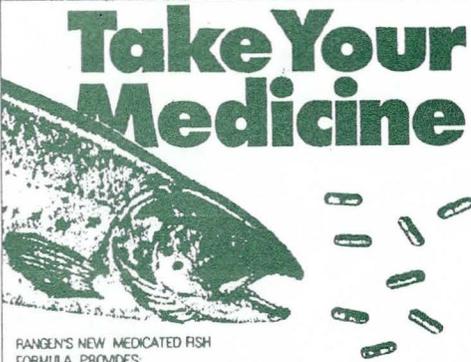
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Table 1.-Origin of gliding bacteria isolated from southern California marine fish and origin of reference isolates.

Isolate	Host species	Location	Date isolated
Southern California isolates			
V2	White seabass <sup>a</sup>	Ventura	8/1993
V5	Pacific sardine <sup>b</sup>	Ventura	8/1993
V6	Northern anchovy <sup>c</sup>	Ventura	8/1993
N1-2d	White seabass	Newport Beach	2/1994
R2	White seabass	Redondo Beach	3/1994
SD1a	White seabass	San Diego	3/1994
Reference isolates			
ATCC 43398	Red sea bream <sup>e</sup>	Japan	1986
<i>(Flexibacter maritimus)</i>			
ATCC 23092	unknown	Argentina	1969
<i>(Flexibacter sancti)</i>			

<sup>a</sup> *Atractoscion nobilis*, <sup>b</sup> *Sardinops sagax*, <sup>c</sup> *Engraulis mordax*, <sup>d</sup> two isolates, <sup>e</sup> *Pagrus major*.

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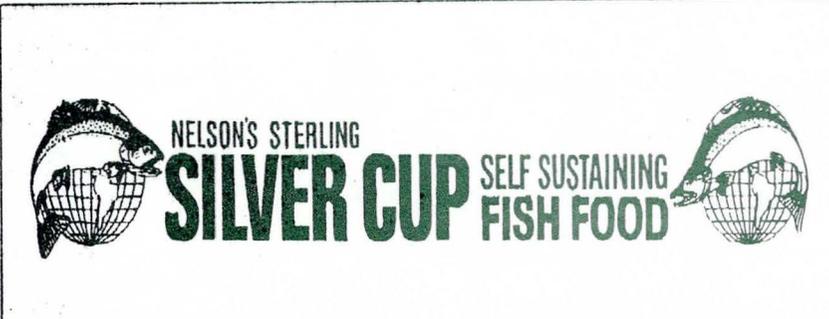


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Table 2.—Differential characteristics of marine gliding bacteria isolated from southern California fishes and reference isolates.

Characteristic	Bacterial isolates						
	V2	V5	V6	N1-2	R2	SD1a	ATCC 43398 <sup>a</sup> 23092 <sup>b</sup>
Flexirubin pigments	-	-	-	-	-	-	- +
Congo red absorption	+	+	+	+	+	-	+ +
Catalase	+	+	+	+	+	-	+ +
Cytochrome oxidase	+	+	+	+	+	+	+ +
Nitrate reduction	+	-	-	+	+	-	+ -
H <sub>2</sub> S production	-	-	-	-	-	(+) <sup>c</sup>	- -
ONPG <sup>d</sup> test	-	-	-	-	-	-	- +
Hydrolysis of							
CMC <sup>e</sup>	-	-	-	-	-	-	- +
Starch	-	-	-	-	-	-	- +
Agar	-	-	-	-	-	-	- -
Gelatin	+	+	+	+	+	-	+ +
Trypsine	+	+	+	+	(+)	+	+ -
Pigment on tyrosine agar	+	+	+	+	+	-	+ -
Precipitate on egg yolk agar	+	+	+	+	+	-	+ NT <sup>f</sup>
Growth							
in TSB <sup>g</sup>	-	-	-	-	-	-	- +
in 50% SW <sup>h</sup> -TSB	+	+	+	+	-	-	(+) (+)
at 15°C	+	+	+	+	+	+	+ +
at 37°C	-	-	-	-	-	-	- -
in 0% SW-HS <sup>i</sup>	-	-	-	-	-	-	- +
in 33% SW-HS	(+)	(+)	(+)	(+)	-	-	(+) (+)
in 66% SW-HS	+	+	+	+	+	+	+ -
in 100% SW-HS	+	+	+	+	+	+	+ -
Viability in microcyst broth <sup>j</sup>							
at two weeks	+	+	+	+	+	+	+ NT
at four weeks	-	+	+	+	-	+	- NT

<sup>a</sup> *Flexibacter maritimus* typestrain, <sup>b</sup> *Flexibacter sancti* typestrain, <sup>c</sup> (+) weak positive, <sup>d</sup> ONPG, o-Nitrophenyl-B-D-galactopyranoside, <sup>e</sup> CMC, carboxymethylcellulose, <sup>f</sup> NT, not tested, <sup>g</sup> TSB, trypticase soy broth, <sup>h</sup> SW, Seawater, <sup>i</sup> HS, Hsu-Shotts medium, <sup>j</sup> Viability was tested by plating 0.01 ml broth on 50% SW-HS.

Table 3.—Production of 19 enzymes by the typestrain *Flexibacter maritimus* and six isolates of marine gliding bacteria isolated from southern California marine fish, as determined by the API-ZYM micromethod<sup>a</sup>.

Enzymes	Bacterial isolates						
	V2	V5	V6	N1-2	R2	SD1a	ATCC 43398 <sup>b</sup>
Control	0	0	0	0	0	0	0
Alkaline phosphatase	5	5	5	5	5	5	5
C4 Esterase	2	2	2	2	2	2	2
C8 Esterase Lipase	3	3	3	3	3	2	3
C14 Lipase	1	1	1	1	1	1	1
Leucine arylamidase	5	5	5	5	5	4	4
Valine arylamidase	5	5	5	5	5	5	5
Cystine arylamidase	2	3	3	3	1	2	2
Trypsin	3	3	2	3	3	1	3
a-Chymotrypsin	1	1	1	1	1	1	1
Acid phosphatase	5	5	5	5	5	3	5
Naphthol-AS-BI-phospho-							
hydrolase	5	5	5	5	5	2	5
a-Galactosidase	0	0	0	0	0	0	0
B-Galactosidase	0	0	0	0	0	0	0
B-Glucuronidase	0	0	0	0	0	0	0
a-Glucosidase	0	0	0	0	0	0	0
B-Glucosidase	0	0	0	0	0	0	0
N-acetyl-B-glucos-							
aminidase	0	0	0	0	0	1	0
a-Mannosidase	0	0	0	0	0	0	0
a-Fucosidase	0	0	0	0	0	0	0

<sup>a</sup> One drop of cultures with an O.D. of 1.0 at 530 nm in PBS was added to each well. Tests were incubated for 12 h at 22°C.

<sup>b</sup> Typestrain *Flexibacter maritimus*.

### Additional Observations on the Epizootiology of WSIV Among Cultured White Sturgeon in the Pacific Northwest

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Previous investigations have reported the presence of the white sturgeon iridovirus (WSIV) in cultured white sturgeon *Acipenser transmontanus* from the lower Columbia River in Oregon, the Snake River in southern Idaho and the Kootenai River in northern Idaho. In Oregon, WSIV was consistently detected in young sturgeon that were progeny from Columbia River adults and cultured in river water but not detected in sturgeon cultured in well water. In Idaho, WSIV was detected in sturgeon that were progeny from wild Snake River and Kootenai River adults after being subjected to stressful conditions of low spring water flows and high fish densities. When densities were reduced and water flows increased, mortality subsided. These observations suggested that WSIV may occur in wild sturgeon and that the virus may be present in many Northwest populations due to the long life span of the species, migratory patterns, and continuity of the river systems. Additionally, since the disease appeared size(age)-specific and stress-mediated, fish culture management strategies could potentially be used to avoid or minimize epizootics. A study was conducted at the College of Southern Idaho (CSI) in Twin Falls to examine the effects of density on the manifestation of WSIV disease using the 1993 brood year Snake River white sturgeon. Replicate groups of sturgeon (mean weight 134 fish/lb or 3.4 g, 5 months old) were stocked at three densities in fiberglass aquaria with 11.1 ft<sup>3</sup> of volume and spring water flows of 8 gpm (Table 1).

Table 1. Total fish, cumulative weight, and loadings of sturgeon in duplicate groups cultured at three different densities.

	Density Designation		
	Low	Medium	High
Number of fish	330	600	1000
Total weight (lbs)	2.2 (1020 g)	4.5 (2040 g)	7.5 (3400 g)
lbs/ft <sup>3</sup>	0.2 (91 g)	0.4 (182 g)	0.7 (318 g)
Sturgeon/ft <sup>3</sup>	27	54	90

Sturgeon mortality was monitored daily and specimens were fixed in neutral buffered formalin for histologic analysis. When mortality increased, additional morbid animals were collected and submitted for viral isolation. Mortality ranged between 1-4% during the first 6 weeks and densities increased as sturgeon grew (Table 2).

In the following 20 d period WSIV was diagnosed in all groups and mean cumulative mortality increased to 7, 20%, and 57% in the low, medium, and high density groups, respectively. Cumulative percent mortality of the replicates was analyzed by analysis of variance on transformed (arcsin percentage) data. Significant ( $p > 0.05$ ) differences were not observed among sturgeon held at different densities during the initial six week period. However, 20 d later cumulative mortality in the high density group was significantly ( $p > 0.05$ ) different than mortality detected in the other treatments. The high and medium density groups were subsequently divided into additional aquaria in an attempt to alleviate stressful conditions and minimize mortality. Test groups were monitored for an additional four weeks. Cumulative mortality in the medium and high density groups increased to 64% and 94%, respectively.

Mortality also increased in the low density groups that had not been divided but was less (26%). Although mortality did not subside in the medium and high density groups after the disease appeared and densities were reduced, sturgeon maintained at the lowest density throughout the test period exhibited substantially less mortality to WSIV. These results suggest that maintaining low sturgeon densities in fish younger than one year may be a prudent strategy for minimizing mortality caused by WSIV.

Table 2. Fish size, mortality, loadings and densities in sturgeon after being cultured for 42 d.

	Density Designation		
	Low	Medium	High
Mean weight	10.3 g	9.4 g	8.6 g
Cumulative mortality	2.7 %	1.3%	3.4%
lbs/ft <sup>3</sup>	0.5 (227 g)	1.1 (499 g)	1.6 (726 g)
sturgeon/ft <sup>3</sup>	22	53	84

In cooperation with Idaho Department of Fish and Game and the Kootenai Tribe of Idaho another density study using a similar experimental design and protocol was initiated at the Clear Springs Foods (CSF) Research Laboratory using the 1993 brood year Kootenai River white sturgeon. Groups of sturgeon (mean weight 126 fish/lb or 3.6 g, 0.5 months old) were stocked at high (0.7 lb/ft<sup>3</sup>) or low (0.2 lb/ft<sup>3</sup>) densities, previously tested at CSI. Fish were cultured in aquaria that received ultraviolet-light disinfected, spring water. Triplicate high density groups and replicate low density groups were tested. Sturgeon exhibited no signs of WSIV or abnormal mortality immediately after transfer from the Kootenai Hatchery. However, after 36 d mortality increased to 10-18% (7/69 - 14/80) and 14 - 18% (39/281 - 49/272) in the low and high density groups, respectively, and WSIV was detected in morbid fish examined from each treatment. The presence of the virus was confirmed by electron microscopy. Sturgeon left at the Kootenai Hatchery (Bonners Ferry, ID) showed no evidence of WSIV. Although fish density did not appear to effect occurrence of WSIV or cumulative mortality, the results again suggested that a stressor (e.g. handling and transport) in subyearling sturgeon may enhance manifestation of WSIV disease. Five months later the remaining fish at the Kootenai Hatchery were moved to the University of Idaho Aquaculture Research Institute. Approximately 1,840 sturgeon (mean weight 151 fish/ lb or 3 g, 10.5 months old) with no detectable WSIV or abnormal mortality were transported at 8 C and acclimated to 15 C upon receipt. These fish were divided into six aquaria on a recirculation system supplied with chlorinated - dechlorinated well water and cultured at very low densities (0.05 - 0.07 lb/ft<sup>3</sup>). During the first 10 d mortality among the six groups ranged from 1-7% and cumulative mortality was 2.7% (50/1829). However, over the next 60 d temperature ranged from 12 - 19 C and cumulative mortality increased to 58% (1064/1838). Morbid animals exhibited signs of abdominal distention and emaciation and were diagnosed as WSIV-positive. In this case, densities again did not appear to be a factor in predisposing fish to a WSIV epizootic but handling, transport, and temperature may have been involved. Temperature stress may also have been a factor in manifestation of WSIV disease in siblings studied at CSF. These fish were transported approximately 14 h at 2-6 C, acclimated to 14 C over a 6 h period, and cultured at a constant temperature of 15 C.

As observed in previous studies, subyearling sturgeon appear to be predisposed to WSIV disease by stressors including densities, adverse environmental conditions (e.g. acute temperature fluctuations), and handling. Results from these studies further substantiate the potential for egg-associated and waterborne transmission of WSIV. Although the pathogen may be present, disease may not occur until certain stressors at critical life stages are confronted. We suspect that in each case described in this and previous reports the source of virus infection in cultured juvenile sturgeon was wild sturgeon either caught from the river and used for broodstock or present in the hatchery water supply. However, definitive evidence supporting this hypothesis has yet to be obtained.

## Purification of the Causative Agent of Erythrocytic Inclusion Body Syndrome (EIBS) in Salmonids.

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Erythrocytic inclusion body syndrome (EIBS) was first reported by Leek (1987) and is frequently found infecting chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon throughout the Pacific Northwest. Epizootics are characterized by the presence of inclusion bodies in the cytoplasm of erythrocytes, accompanied by anemia and supervening bacterial infections. The disease is evidently caused by an essentially uncharacterized viral agent. Although the virus has been observed using transmission electron microscopy (TEM), attempts to replicate the virus in continuous cell lines have not been successful (Piacentini et al., 1989). The disease can be transmitted to a variety of salmonids by direct injection of infected blood into susceptible fish, but the virus etiology has not been unequivocally determined due to the difficulties in obtaining virus to study. At our laboratories at Oregon State University, we have succeeded in devising a procedure for purification of the EIBS viral agent from infected red blood cells. Transmission electron microscopy of the resulting viral suspension demonstrates a high degree of purity.

### Methods

EIBS infected blood containing approximately 5% infected erythrocytes was collected from chinook salmon into heparinized syringes, pooled into 5 ml aliquots and frozen at -70 C until processed. The blood was thawed at room temperature (RT) and mixed 1:2 with Tris buffer (0.002 M Tris, pH 9.0), to osmotically rupture the cells (Verwoerd, 1969). The cell/virus suspension was sonicated three times in 5 s bursts, frozen at -70 C for 15 min, followed by thawing

again at RT. The crude virus preparation was then centrifuged at 3000 g for 10 min. The resulting supernatant was layered onto a 20-40% (w/w) linear CsCl gradient prepared in Tris-NaCl-EDTA (TNE) buffer, pH 8.0, and centrifuged at 143,000 g, 14 C for 3 h. Fractions of 750 ml were removed from the top of the tube with a fractionator. A density and protein profile of the gradient was obtained by plotting the spectrophotometric readings of each fraction at 260 nm and refractive indices for each fraction. The fractions were then diluted five-fold with TNE buffer and centrifuged at 126,000 g, 4 C for 1.25 h to wash the virus. The supernatant was decanted and the resulting pellet was resuspended in 200  $\mu$ l of TNE buffer. Each fraction was observed on the transmission electron microscope, after staining with 2% phosphotungstate acid (PTA), pH 7.0. Additionally, each fraction was injected intraperitoneally (IP) into groups of nine coho salmon, approximately 4 g in weight. Blood samples were taken from fish at three different intervals after injection. Blood smears were fixed in methanol, stained with pinacyanol blue, and observed for cytoplasmic inclusion bodies in the RBCs characteristic of EIBS (Piacentini et al., 1989).

### Results

A clean preparation of virus, as determined by TEM, was consistently observed in the fraction with a density of 1.33 g/cm<sup>3</sup>. Overnight runs in the CsCl did not result in a change of the density of the virus, and therefore, the centrifugation was isopycnic. The viral particles were observed to be unenveloped, icosahedral, approximately 65-75 nm in diameter with visible capsomeres (Figures 1 & 2). No

band was ever visible in the CsCl gradient at the location corresponding to this fraction. However, this fraction also consistently produced a heavy EIBS infection when injected IP into coho salmon. Cytoplasmic inclusion bodies were first observed in RBCs from 6-14 days after injection. A second fraction, with a density of 1.18-1.20 g/cm<sup>3</sup> also produced a mild EIBS infection when injected IP into fish. However, no virus was observed in this fraction by TEM, due to the large amount of contaminating material present in this fraction. Most of the extracellular debris from the RBCs concentrated in this area of the CsCl gradient.

### Discussion

TEM observations of the fraction of interest indicate that a fairly large concentration of purified virus was obtained by the method described. The purified virus can now be used in future experiments to determine the exact characteristics of the causative agent of EIBS in salmon. Data gathered to date indicate the possibility of a whole new class of virus, although research to confirm or deny this is ongoing at the moment. In any case, the availability of purified virus will allow experiments on EIBS to be more consistent and reproducible than in the past.

EIBS is found in approximately one-third of the salmon culture facilities in Oregon, Washington and Idaho. Yet, virtually nothing is known about the pathogen. Purification of the virus that causes EIBS will allow characterization of this agent and understanding of the transmission of the disease, which is the first step towards attempting to control the disease both in feral stocks and in culture facilities.

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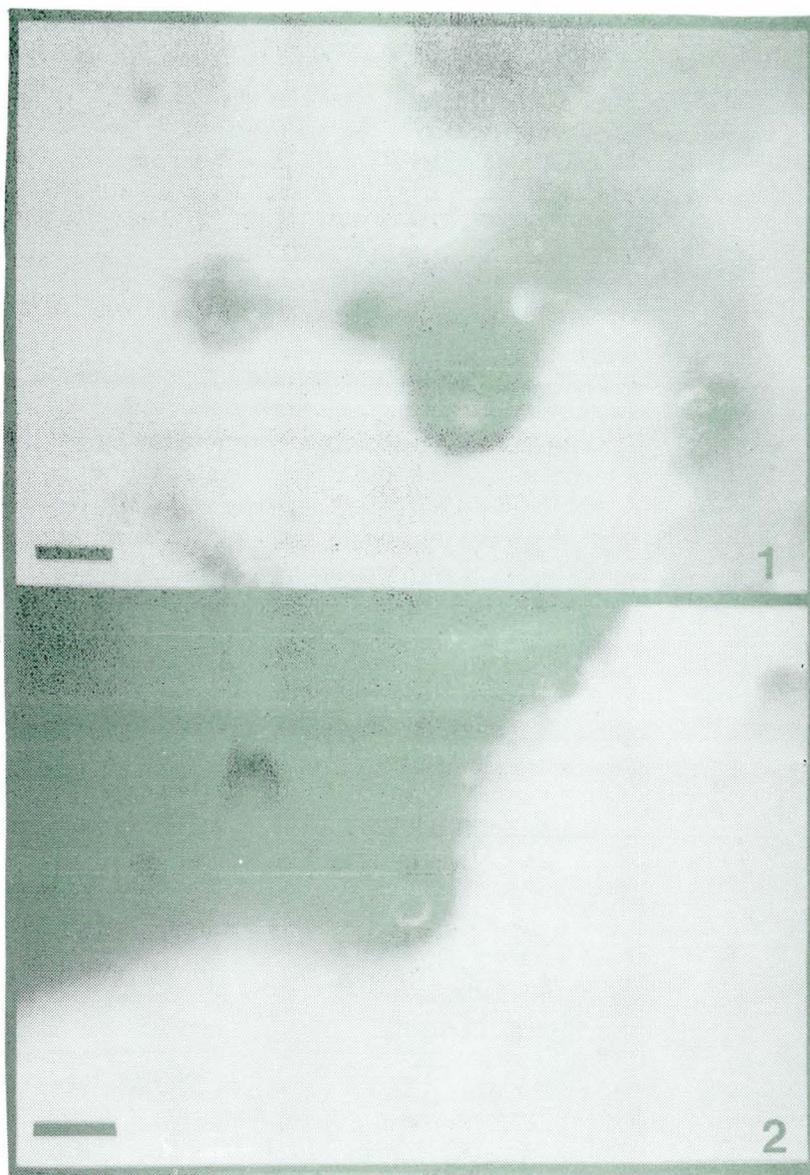


Figure 1. High-magnification TEM of a full ca 64 nm diameter nucleocapsid of the EIBS virus. Scale bar = 100 nm. Figure 2. High-magnification TEM of an empty ca 65 nm diameter nucleocapsid of the EIBS virus. Scale bar = 100 nm.

## Isolation of *Bacillus thuringiensis* from feed and intestines of channel catfish experiencing severe anemia

Jill A. Jenkins and John R. Morrison

In September 1992 and May 1993, field observations were made on two outbreaks of severe anemia affecting channel catfish, *Ictalurus punctatus*, cultured in ponds on one farm in west central Alabama. Affected fishes displayed clinical signs of "no-blood" disease: fluid-filled intestines, pale gills, kidney, liver and spleen, and hematocrits below 10%. Because the disease has been attributed to feed (Butterworth et al. 1986), the intestinal contents from affected fishes and feed samples were cultured in media using folic acid as the sole source of carbon and nitrogen (Levy and Goldman 1967). Gram positive and negative species of bacteria occurred in the intestinal contents and feed. The predominant Gram negative species was *Aeromonas*. Gram positive spore-forming rods were the predominant bacteria from feed. These bacteria were subtyped by dendrogram analysis of cellular fatty acids using gas liquid chromatography (Analytical Services, Essex Junction, VT). From the first incident of anemia, *Bacillus thuringiensis* (B.t.) was found in the feed and from the second incident another strain of B.t. was found in the luminal contents of the lower portion of the intestines. The second strain contained parasporal crystals and both B.t. strains were positive for lecithinase.

The catfish feed from which one strain of B.t. was derived was fed to 350 g - 500 g channel catfish in tanks for twenty weeks beginning September 1992, and within two weeks, two mortalities occurred which displayed clinical signs of "no-blood" disease. A control group receiving feed not associated with any known health problems suffered no mortalities. Each group consisted of 200 fish, and 20 fish chosen at random were used at each sampling period. Hematocrits and hemoglobin values of the test fish were significantly lower than those from fish in the control tank after six weeks of feeding (Table 1). From the test group during the final week of the study, seven morbid fish occurred with external *Flexibacter columnaris*, fluid-filled intraperitoneal cavities, and pale gills. A mixed population of *Aeromonas* and *B. thuringiensis* was derived from blood and livers from most morbid fish.

Anemia may be defined by a reduction in the total number of red blood cells (RBC), or in packed cell volume, or in hemoglobin. There may also be RBC of abnormal size and shape. Cytology of peripheral blood smears from the final sampling date revealed a significantly higher number of phagocytes and thrombocytes in the test group. Many immature RBC and rounded RBC were present in the test fish. Dividing RBC were seen in both the control and test fish, but many dividing thrombocytes and some bilobed RBC were observed from the test fish. Bacterial rods were observed in some smears from the test group, and *Bacillus* spp. were cultured on blood agar from the blood of two individuals.

This is the first report of *B. thuringiensis* derived from morbid fish, although B.t. is widespread in the environment (Martin and Travers 1989). Because *B. thuringiensis* strains produce toxins lethal to many insects, strains of this bacterial species have been formulated as commercial products and used worldwide since 1962 (Burgess 1981) for biological control of pest insects in the orders Lepidoptera, Diptera, and Coleoptera. The crystalline inclusions of B.t. contain proteins that, after ingestion, solubilization, and proteolytic processing, are hemolytic and cytolytic. Six additional toxins from B.t. have been described (Kurstak 1982), including phospholipase C which assists bacteria in translocating the gut to enter circulation. A  $\beta$ -exotoxin produced by some strains is toxic to some vertebrates, therefore these strains have not been used for pest control in Western countries. There has been a limited range of vertebrate aquatic species against

which *B. thuringiensis* has been tested for toxicity (Burges 1981) and there are few data available.

That *Bacillus* spp. are found in trout and salmon feed (Barbash 1992) or in the gastrointestinal tracts of channel catfish (Macmillan and Santucci 1990) are not new findings. In catfish experiencing a feed-related anemia, accumulated fluid in the gut may result from a toxin acting as an enterotoxin, interfering with electrolyte transport and stimulating the secretion of electrolytes and water. Histopathological lesions of the intestines of channel catfish affected with an idiopathic anemia revealed epithelial desquamation, submucosal edema, and necrosis of the mucosa and muscularis (Noyes et al. 1991).

This feed-related anemia has been diagnosed in Alabama since 1983 (Lovell 1988). Management practices include withholding feed and switching batches of feed. Butterworth et al. (1986) associated this disease with folic acid degradation in the feed, where pseudomonads were implicated (Plumb et al. 1991). However, this severe anemia has not been consistently associated with any single pathogen. Facultative anaerobic *Bacillus* spp. may thrive in the low oxygen environment of the gut. Our data suggest that B.t. was associated with "no-blood" disease. The epidemiology of severe anemia and the pathogenesis of infection need further elucidation. Accordingly, Koch's postulates are being tested with the *Bacillus* strains found from the field studies. Both B.t. strains isolated were able to utilize folic acid as a nutrient source and persist in the blood stream as seen in month-long challenge studies (data not shown). In this laboratory, to understand the possible bacteria-host relationship, efforts focus on isolation of purified cultures, analysis of the red blood cell cycle progression, and blood cell responses. Surveillance and basic research are complementary approaches in identifying possible emerging opportunistic pathogens.

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Table 1. Mean hematocrit and hemoglobin values for channel catfish receiving a control diet or feed from a commercial farm which experienced losses due to a severe anemia ( $P > 0.001$ ).

Sampling Period Dates (1992)	Mean Hematocrit (Percent RBC's)		Mean Hemoglobin (g/dl)		Mean Water Temperature (C)
	Test Diet	Control Diet	Test Diet	Control Diet	
Pre-study	31.2	32.1	7.8	7.8	29.4
9/1 - 9/15 <sup>a</sup>	28.0	30.0	7.9	8.1	27.3
9/16 - 9/29	28.3	30.5	5.8*	7.0	25.0
9/30 - 10/13	25.8*	31.4	5.0*	6.3	20.4
10/14 - 11/17	34.8	35.9	6.8*	8.4	20.0
11/18 - 1/25/93	39.3	40.2	8.3*	9.3	20.2

\* Measured blood parameters are significantly different ( $P \leq 0.001$ ).

<sup>a</sup> Fish began receiving the respective diets 9/1/92, and were sampled 9/1, 9/16, 9/30, 10/13, 11/18, and 1/26/93.

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#### ANNOUNCEMENT-CONTINUING EDUCATION SESSION

Submitted by: Craig Olson, Northwest Indian Fisheries Commission, 6730 Martin Way E., Olympia, WA 98506. (206)-438-1181 ext. 343

The continuing education session on viral CPE offered at the Western Fish Disease Workshop in Bozeman last June was well received. Thirty of the sixty-some people at the workshop attended the continuing education session. These participants received certificates recording their attendance in the session as well as continuing education credit which they may apply toward professional recertification by the Fish Health Section. The enthusiasm for this session immediately carried over to plans for a session on histology at next year's Western Fish Disease Workshop. We're looking forward to instruction from John Morrison and Charlie Smith at that meeting.

Drs. Jim Winton and Ron Hedrick presented an excellent summary of 15 viruses and *Piscarickettsia salmonis* that should be familiar to fish health professionals. For each agent they showed informative quality slides of typical CPE and discussed tissue cultural parameters, the CPE appearance, and the primary identification methods. In order to help participants take home what they had seen, copies of the slide collection were made available for their own future reference. Over half ordered a set, and a limited number of additional sets have been made and are hereby offered the rest of you.

The 36 transparencies include one or more CPE of each of the sixteen agents plus the appropriate uninfected cell line monolayers for comparison. You can get all 36 slides for only \$30. The easiest way to order is to just call me and ask for a set. You'll be sent an order blank which you can return with payment. One of the aspirations of the Continuing Education Committee is to accumulate a collection of educational materials for sale or loan. This viral CPE slide set will be in that collection until it is sold out.

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## Detection of *Ceratomyxa shasta* in Alaskan Chum Salmon, *Oncorhynchus keta*

J. E. Follett, J. L. Geesin, T. M. Burton

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Spores of *Ceratomyxa shasta* (Noble 1950), have been found in adult chum salmon from the Sushana River, Alaska which is the first finding of this parasite in Alaska. The adult chum salmon were being examined for certain disease agents as a requirement for use of their gametes in a fisheries enhancement program. Wet mounts of hindgut contents from 150 chum salmon, each diluted with two ml PBS, showed the presence of spores characteristic of *C. shasta* in nine of the specimens. No inflammation of the hindguts was noted. All positive fish appeared to be lightly infected. The spores were stained with methylene blue or Ziehl-Nielsen stain for further examination. Spores averaged  $17.8 \pm 1.6 \mu\text{m}$  in length by  $7.2 \pm 0.5 \mu\text{m}$  in width while polar capsules had a mean diameter of  $2.3 \pm 0.1 \mu\text{m}$ .

The Sushana River is a tributary of the Toklat River that flows into the Kantishna River before joining the lower Yukon River. The parasite had been reported previously from the upper Yukon River near Minto, in the Yukon Territory, Canada (McDonald, 1984). Our finding is additional confirmation of the presence of *C. shasta* in the Yukon River system but within Alaska boundaries. The infectious stage is most likely present in the lower part of the river. However, prior examinations of fish from Alaskan rivers including 150 chum salmon adults from this same site in 1992 have not detected spores of *C. shasta*. Screening of other stocks in the lower Yukon River has not been done.

The life cycle of *C. shasta* is not well understood but is suspected to involve an intermediate host (Bartholomew *et al* 1989; Heckmann 1993). The spores are not infective but infective stages are probably present in the sediments or water. The Yukon River water temperatures vary from

10-17°C during the chum salmon migration and adult fish have been in freshwater for at least 40-45 days upon reaching the spawning beds in the Sushana River (pers. comm., Louis Barton, Alaska Dept. of Fish and Game). This is adequate time for *C. shasta* infection to have occurred upon reentry to fresh water. The low level of the infection would indicate that either the fish were not in contact with the infectious stage for an extended period of time, the disease had not progressed, or this stock was somewhat refractory to the parasite. Progress of the disease is reported to be temperature dependent (Bartholomew *et al* 1989; Lom, J. and I. Dykova 1992).

The enhancement of the Sushana chum salmon stock involves transfer of fertilized eggs to a hatchery where they are incubated and reared before being planted back into the river as fry. Since only gametes are transferred, this positive finding should not adversely affect the enhancement program as long as the eggs are thoroughly surface-disinfected and contaminated water or sediments are not transferred to the facility.

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## Brown trout (*Salmo trutta*) loss to Infectious Hematopoietic Necrosis Virus (IHNV)

H. Mark Engelking and John Kaufman

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This report documents the first known loss of fingerling brown trout (*Salmo trutta*) from IHNV infection in Oregon. Although Wolf's book, "Fish Viruses and Fish Viral Diseases" (1) describes brown trout as having experienced losses to IHNV, no other written records of such losses have come to our attention. The "Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens" (2) reports brown trout to be susceptible to IHNV. This was documented by *in vitro* challenges of brown trout to two isolates of IHNV (3). Personal communications from several fish health sources suggests brown trout losses due to IHNV have not occurred in the last twenty years, if ever, in the United States. The brown trout loss, virus typing and the virulence of this isolate as determined by an *in vitro* challenge will be described.

Round Butte Hatchery is situated at the base of Round Butte Dam in central Oregon on the Deschutes River. Lake Billy Chinook is formed behind this dam and is fed from the flow of the Deschutes, Metolius and Crooked rivers. Salmonid fish including a naturally reproducing kokanee salmon (*Oncorhynchus nerka*) population known to be infected with IHNV reside in these watersheds. The hatchery water supply are springs by the base of the dam. In early May, 1994 summer steelhead trout (*O. mykiss*) fry, descendants of IHNV positive adults, at Round Butte Hatchery began experiencing losses. Losses from IHNV occur almost annually at this facility in the summer steelhead trout. These dying fry were determined to be infected with Type 1 IHNV. The isolate was typed by electropherotyping and lack of reactivity in an IFAT assay with the Type 2 selective monoclonal antibody 2NH105B (DiagXotics, Inc., 4 and 5). The loss had continued in 70% of the groups in fish weighing about 3 grams or less at a low chronic loss level of about 0.3% per day through June. Total losses were about 54% of the fry.

On June 17 the virus lab received five dead brown trout from Round Butte Hatchery. The fish were darkly pigmented but had no other specific signs of IHNV infection. Losses were very low (0.05% per day). Because of the proximity of these fish to the dying summer steelhead trout and the common water supply, the manager, Bill Nyara, requested a virus examination be performed. The very low loss and chronic nature of the outbreak in the brown trout at Round Butte Hatchery could have been overlooked had not a severe loss been taking place among the summer steelhead. All fish examined were determined to have IHNV infections, but no evidence of bacteria or parasites. The virus was determined by electropherotyping to be a Type 1 strain (5).

During June and July losses continued at the same level in the eight month old brown trout fingerlings. Samples of morbid and dead fish were obtained on two more occasions. Infectious hematopoietic necrosis virus was detected in all samples. The average size of dead fish was 7.9 cm in fork length with a range of 4.5 to 10.3 cm. The average weight of the dead fish was 7.1 grams with a range of 2.0 to 14.2 grams. The fish are older and larger than the common susceptible size range of other IHNV host species (2). Virus titers were determined in gill, kidney and spleen pooled samples of five dead fish and two live dark fish. of the dead fish examined, the titers ranged from  $1 \times 10^5$  TCID<sub>50</sub> to  $2.8 \times 10^8$  TCID<sub>50</sub> per gram of tissue. of the live fish examined, the titer was from less than 10 to  $7.9 \times 10^3$  TCID<sub>50</sub> per gram of tissue (Table 1). The high titers found in the dead fish indicates that

IHNV was most likely responsible for the death of the fish.

**Table 1.**

**IHNV Titer in Brown Trout**

Fish	Weight (g)	Length (cm)	Titer <sup>a</sup>
Mortality 1	9.35	9.5	1.1 X 10 <sup>5</sup>
Mortality 2	7.38	8.5	5.0 X 10 <sup>6</sup>
Mortality 3	7.98	9.0	5.4 X 10 <sup>5</sup>
Mortality 4	14.25	10.0	1.1 X 10 <sup>7</sup>
Mortality 5	13.92	10.3	2.8 X 10 <sup>8</sup>
Live 1	6.9	8.5	<10
Live 2	8.5	8.7	7.9 X 10 <sup>3</sup>

<sup>a</sup> Titer expressed as TCID<sub>50</sub> per gram of gill, kidney, spleen pooled tissue.

The virulence of these isolates was examined by *in vitro* challenges. A chronic low loss occurred in susceptible summer steelhead trout (McKenzie River stock) challenged for two hours at various concentrations with Round Butte IHNV isolates from either the brown trout or that from the summer steelhead trout (6). The challenge was terminated at thirty days post infection. No challenged group experienced more than 50% mortality. There was no peak of mortality as exhibited in typical acute infections (Figure 1). The mortalities were confirmed to be IHNV positive by viral examination of all dead fish. Loss in control groups infected with Type 2 IHNV from Leaburg Hatchery summer steelhead trout was acute and more than 90% of the fish died at similar virus challenge levels within two weeks post infection with a peak loss between 10-12 days post infection. Uninfected control fish had no losses (data not shown). The laboratory infection with the Round Butte isolates paralleled the type of loss experienced at the hatchery, however the losses subsided with time. In the hatchery, constant re-infection of the fish from the water may result in the continued losses. The losses have continued for three months among the steelhead trout and for two months in the brown trout pond. The similar low level chronic losses caused by this virus isolate in two species of fish and two stocks of steelhead trout both in the hatchery and in laboratory challenges suggests that this aspect of virulence is determined by the virus not the host.

A similar situation was reported in brook trout (*Salvelinus fontinalis*) at Summerland Trout Hatchery in British Columbia in 1991 (7). An epizootic occurred in rainbow trout at the facility at the same time two month old brook trout were on site. No loss occurred in the brook trout, but they were shown to be susceptible by *in vitro* challenge. Asymptomatic four month old brook trout survivors of the artificial challenge still carried IHNV at a low prevalence. Continued monitoring of the fish at Round Butte will determine if the survivors of this outbreak also carry detectable levels of IHNV.

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1. Wolf, K. 1988. Fish Viruses and Fish Viral Diseases. Cornell University Press. p 476.
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The support and assistance in obtaining these samples by Bill Nyara, hatchery manager, and crew if the Oregon Department of Fish and Wildlife at Round Butte Hatchery is gratefully acknowledged.

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**\*\*\*\*JOINT MEETING OF THE FISH HEALTH SECTION OF THE AMERICAN FISHERIES SOCIETY AND THE EASTERN FISH DISEASE WORKSHOP.**

**JULY 19-22, 1995.**

**SYRACUSE, NEW YORK. ABSTRACT DEADLINE: MAY 31, 1995.**

THE GENERAL MEETING SCHEDULE HAS BEEN ARRANGED TO ALLOW AIR-TRAVELERS TO ARRANGE FOR SUPERSAVER AIRFARE. THE PROGRAM WILL HAVE WEDNESDAY JULY, 19, SERVE AS A TRAVEL DAY WITH A RECEPTION IN THE EARLY EVENING. FORMAL PRESENTATIONS WILL BE ON THURSDAY, FRIDAY AND IN THE MORNING ON SATURDAY, JULY 20-22.

AN OPTIONAL FIELD TRIP WILL BE ARRANGED TO TOUR THE ONEIDA FISH HATCHERY OF THE NEW YORK STATE DEPARTMENT OF ENVIRONMENTAL CONSERVATION ON SATURDAY AFTERNOON, JULY 22. THIS STATE-OF-THE ART WALLEYE HATCHERY WENT THROUGH ITS FIRST FULL PRODUCTION YEAR IN 1994.

THE 1995 MEETINGS WILL BE COORDINATED BY JOHN SCHACHTE (315-337-0910) AND PAUL BOWSER (607-253-3365), THE LOCAL ARRANGEMENTS CO-CHAIRMEN. FRANK HETRICK (301-405-1035) WILL SERVE AS THE PROGRAM CHAIRMAN AND ABSTRACTS SHOULD BE SENT DIRECTLY TO HIM. MORE INFORMATION ON THE JOINT MEETING, INCLUDING REGISTRATION AND HOTEL ACCOMMODATIONS, WILL APPEAR IN THE NEXT ISSUE OF THIS NEWSLETTER.  
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**MEETINGS**

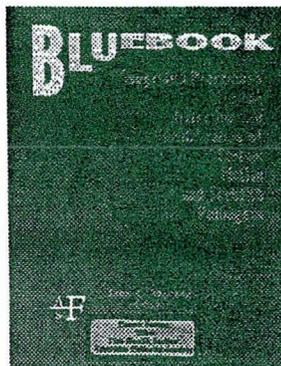
*Salmon Ecosystem Restoration: Myth and reality." 1994 Northeast Pacific Chinook and Coho Salmon Workshop. November 7-10, 1994.* Eugene, OR. Contact: Jeff Dose, U.S. Forest Service, Umpqua National Forest, P.O. Box 1008, Roseburg, OR 97850. (503)-672-6601

*Aquaculture '95. February 1-4, 1995.* San Diego, Ca. Contact: Aquaculture '95, c/o Sea Fare expositions, Inc., 850 N.W. 45<sup>th</sup> street, Seattle, WA 98107.

*East Coast Trout Management and Culture Workshop II. May 31-June 2, 1995.* Penn State University, State College, PA. Contact: Marty Marcinko, 450 Robinson Lane, Pennsylvania Fish Commission, Bellefonte, PA 16823. (814)-359-5223.

*Modulators of Immune Responses: Hiking up the Evolutionary Trail. July 8-15, 1995.* Breckenridge, CO. Contact: Joanne Stolen, SOS Publications, 43 DeNormandie Avenue, Fair Haven, NJ 07704-3303. (908)-530-3199.

*Fourth Asian Fisheries Forum. October 16-20, 1995.* Beijing, China. Contact: the China Society of Fisheries, 31 Min Feng Lane, Xidan, Beijing, CHINA. (861) 602-0794.



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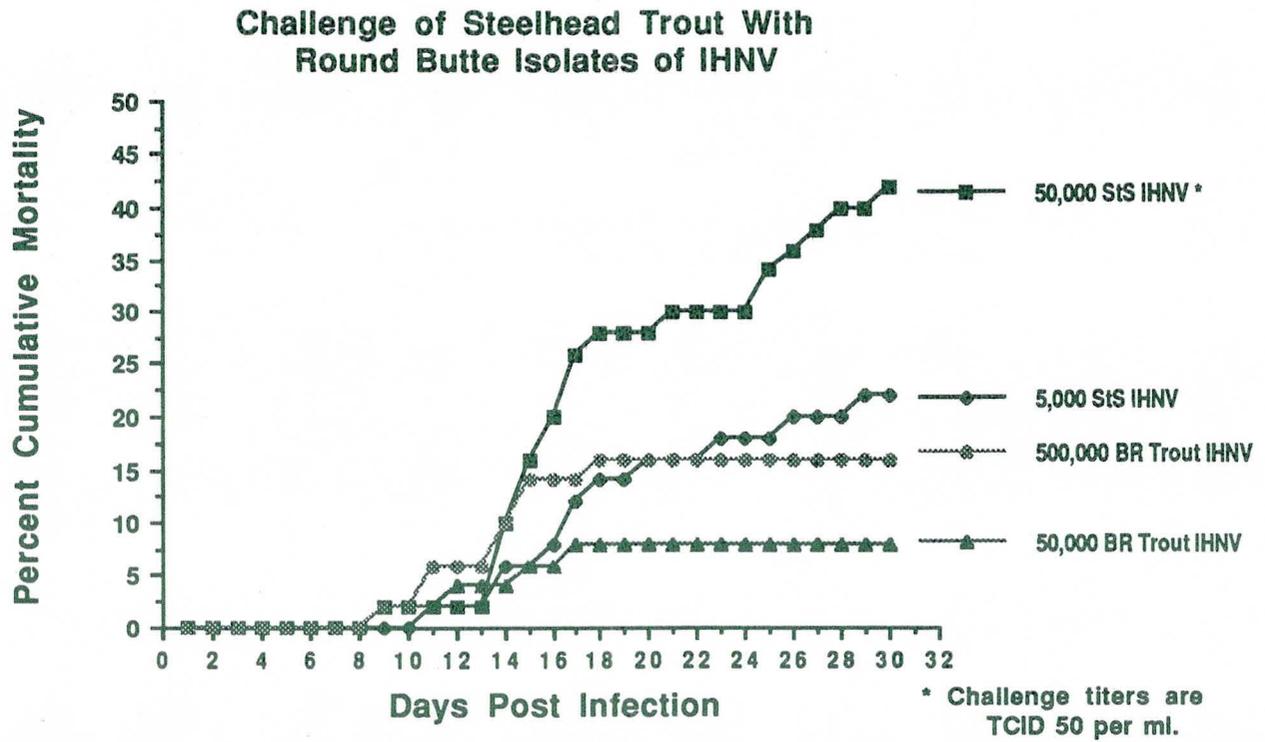
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Figure 1. Engelking and Kaufman



**Fish Health Section Newsletter**

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