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Epizootiology of viral hemorrhagic septicemia in confined Pacific herring

by

Paul Hershberger

A dissertation submitted in partial fulfillment of the requirements
for the degree of

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Approved by _____
(Chairperson of Supervisory Committee)

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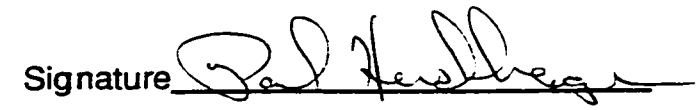
Signature 

Date Feb 3, 1999

University of Washington

Abstract

Epizootiology of viral hemorrhagic septicemia in confined Pacific herring

by

Paul Hershberger

Chairperson of the Supervisory Committee

Professor Richard Kocan

School of Fisheries

Little research has been directed towards understanding disease outbreaks among wild fish and little attention has been placed on fishery-related activities which may be involved in activation and perpetuation of overt disease among associated fish. Experiments reported in this dissertation were designed to describe the epizootics of viral hemorrhagic septicemia virus (VHSV) which often occur among wild Pacific herring confined in containment structures for the herring spawn-on-kelp fishery. Results indicated that wild herring confined in net pens and/or laboratory tanks underwent increased infections of VHSV, with both viral prevalences and titers increasing after confinement. The temporal occurrence, duration, and magnitude of infection were dependent on numerous factors including age and immune status of confined herring, crowding density, source of viral infection, and viral shedding intensity among VHSV positive herring. Additionally, waterborne virus was found in schools of wild, unimpounded herring displaying low-level infections. A mechanism to explain the epizootics which occurred among confined herring is proposed. This begins with impoundment and subsequent crowding of herring carrying low-level infections. Increased density and stress among the confined fish results in viral shedding by infected animals and increased exposure of other susceptible herring to unnaturally high levels of waterborne VHSV with a subsequent increase in VHSV prevalence among impounded herring. These findings have direct implications for management of the closed pound herring spawn-on-kelp fishery and alternative management options are discussed.

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Preface

Viral hemorrhagic septicemia virus (VHSV), the causative agent of the disease referred to as viral hemorrhagic septicemia (VHS), has historically been associated with mortalities among cultured rainbow trout in Europe. Recent discoveries of the virus in fishes on the west coast of North America have raised new management concerns and research opportunities regarding host range, virulence, molecular phylogeny, and management of susceptible finfish species. This dissertation was designed to investigate the epizootiology of VHSV in confined herring from the closed pound spawn-on-kelp (SOK) fishery and determine how the infection is amplified and spread among captured herring.

This dissertation will begin with a brief outline of the life history of Pacific herring (*Clupea pallasi*) along the Pacific coast of North America. An extensive description of herring fisheries including the relatively new SOK fishery is included because an in-depth understanding of the fishery and stressors associated with it are necessary to formulate conclusions from these studies. The introduction concludes with some physiological effects of environmental stressors and a literature review of VHSV. This is followed by descriptions of select studies, some of which are currently in submission for publication, which describe the author's findings in both Puget Sound, Washington and Prince William Sound, Alaska. The final chapter contains a summary of the results and suggests management options to mitigate the effects of VHS epizootics in the herring fisheries, while maintaining high SOK product quality.

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Chapter 1: Introduction

Herring

The geographic distribution of Pacific herring (*Clupea pallasi*) extends along the western coast of North America from northern Baja California into arctic Alaska, west to Russia, Japan, and into the Yellow Sea (Lassuy 1989). Discrete stocks of *C. pallasi* occur throughout this range and regional management authorities classify stocks differently. For example, the entire fishery in Prince William Sound, AK is managed as a single stock (ADF&G 1996) because only 2 groups of *C. pallasi* in the Gulf of Alaska are discernible with starch gel electrophoresis: 1) an eastern group which extends south of Kodiak Island and includes Prince William Sound (PWS) and 2) a western group which extends into the Bering Sea (Grant 1979). However, in Puget Sound, WA individual herring stocks are recognized for every discrete geographic spawning location (Stick 1994) even though larval herring distributions are geographically continuous throughout different spawning sites along the northern mainland coast of British Columbia (Hay and McCarter 1990).

Herring stocks along the western coast of North America are managed by several organizations. Each state within the U.S. has responsibility for adjacent coastal waters extending 0-3 nautical miles from shore, but the U.S. Department of Commerce maintains management authority in the Fishery Conservation Zone, which extends 3-200 nautical miles from shore. Entry into commercial herring fisheries in Puget Sound, WA is limited by Senate bill No. 2918, 1973 to those fishers who can document landings of herring between Jan. 1, 1971 and

April 1, 1973 (Trumble 1980). Annual herring allotment catches in Puget Sound are based on stock size which is determined by combining: 1) hydroacustic and midwater surveys of pre-spawn herring abundance, 2) escapement surveys from herring spawning grounds, and 3) a computerized catch-reporting system used to estimate landings. British Columbia herring fisheries are regulated by the Department of Fisheries and Oceans (Trumble and Humphreys 1985). Alaska herring fisheries are managed by Alaska Department of Fish and Game (ADF&G) and are regulated based upon geographic areas including southeast, Yakutat, Cook Inlet, PWS, Kodiak, Chignik, Alaska peninsula, Bristol Bay, Kuskokwim, and Bering Sea (ADF&G 1996).

Herring spawn in late winter / early spring when the water temperatures range between 3-9°C (Alderdice and Velsen 1971). Unlike Atlantic herring (*Clupea harengus*), which deposit eggs on rocky bottoms along open coastlines, *C. pallasi* generally spawn only in protected bays, inlets, sounds, and estuary regions and prefer gradual slopes containing suspended vegetation such as red algae, eelgrass, rockweed, and japweed (Scattergood *et al* 1959, Miller and Schmidtke 1956, Haegele and Schweigert 1985, Haegele *et al* 1981). Deposition intensity of the negatively buoyant eggs (Hay and Fulton 1983) varies with the blade-like, foliose, or filamentous nature of the submergent macrophyte species present (Alderdice and Hourston 1985) and is highest in the lower intertidal and upper subtidal regions (Lassuy 1989). Spawning events generally occur in "waves" lasting 1-3d with the larger fish within a stock tending to spawn first (Lassuy 1989, Hay 1986). Gamete maturation and release is regulated by gonadotropin releasing hormone

(Carolsfeld *et al* 1988) with initiation of a spawning event likely triggered by release of a sexual pheromone contained in the milt (Stacey and Hourston 1982). Hay and Fulton (1983) calculate that eggs and milt released in regions of high herring spawning biomass from the Straight of Georgia may contribute more carbon than is accounted for by annual primary production.

Discrete herring stocks have different physiological optima and differ in their preference for physical conditions (Alderdice and Hourston 1985). Minimum oxygen concentration on the egg surface necessary for viability is 2.5mg·L^{-1} (Alderdice and Hourston 1985), salinity optima for the euryplastic eggs is 12-16ppt (Alderdice and Velsen 1978), and temperature range for optimal hatch is between 5.5-8.7°C (Alderdice and Velsen 1971). Hatch viability is inversely correlated with thickness of the jelly coat encapsulating the eggs and thickness of the egg mass on the spawning substrate, with jelly coat thickness inversely proportional to salinity (Alderdice and Hourston 1985). Birds are the primary predators of herring eggs (Lassuy 1989), and may consume 40-60% of exposed eggs in the intertidal region. Egg loss is greater in shallow water and at sites where diving ducks congregate (Haegele and Schweigert 1990). Additional egg mortality may occur when major storms wash littoral eggs ashore (Alderdice and Hourston 1985), but Haegle *et al* (1981) estimated that total egg loss from predation and tidal flux is probably less than 10%.

Herring eggs average 1.2-1.5mm in diameter (Hart 1973) depending on salinity (Alderdice *et al* 1979b) and generally hatch in 2-3wk (Hay and Fulton

1983, Brown *et al* 1996); possibly less in environmentally stressful situations (Hay 1986). Egg bursting pressures rise to a maximum following fertilization and slowly decline to their lowest point immediately prior to hatch (Alderdice *et al* 1979b). After hatch, the yolk sac larval stage lasts approximately one week (von Westernhagen and Rosenthal 1979) and initial feeding must commence by 9d post hatch or starvation will occur (Blaxter 1968). Increased feeding incidence among larvae occurs with low-moderate levels of turbidity (Boehlert and Morgan 1985) and may be correlated with the spring phytoplankton and zooplankton blooms. Larval mortalities as high as 99.5% have been estimated, resulting primarily from passive transport of larvae by inshore currents to lethally high salinities in the open ocean (Alderdice *et al* 1979a, Stevenson 1962). Other researchers contend that the primary causes of mortality to the euryplastic, pre-recruit larval stages include starvation and predation, with disease, parasitism, and environmental conditions of salinity, temperature, and dissolved oxygen of only minor importance (Alderdice and Hourston 1985, Blaxter and Hunter 1982). Many marine organisms, including several species of hydromedusae (Needler and Hay 1982), ctenophores, chaetognatha (Stevenson 1962), amphipods (Alderdice and Hourston 1985), as well as juvenile and adult herring (Hourston *et al* 1981) are known consumers of larval herring.

Metamorphosis of larvae to juveniles occurs 2-4mo post hatch (Hourston and Haegele 1980, Blaxter 1968) after which the young herring form large schools and remain near inshore waters throughout the first summer (Hay 1985). Juvenile schools either move offshore during late fall and remain

separate from offshore schools of adults (Haist and Stocker 1985) or stay inshore and become resident populations within coastal inlets and bays (Stevensen 1955). Age at first maturity is 2-5yr, increasing with latitude (Hay 1985), at which time herring return to specific spawning grounds (Lassuy 1989). Mean fecundity is greater in older, larger fish, but fecundity of herring having the same body length decreases with latitude (Nagasaki 1958 and Paulson, Smith 1977). Fecundity decreases with gamete maturation (Hay and Brett 1988) but temporally decreased fertilization rates through the spawning season are attributed to reduced fertilization potential of the eggs (Alderdice and Velsen 1978). Food intake prior to the spawn results in heavier eggs, higher glycogen levels, earlier spawn, and more mature eggs than when food is not available (Hay *et al* 1988). Adult herring return to deeper water after completion of the spawn (Miller and Schmidtke 1956).

Numerous pathogenic organisms are known to infect *C. pallasi* including viral hemorrhagic septicemia virus (VHSV) (Meyers *et al* 1994), an undescribed, "*Vibrio*-like disease" among confined adult herring (Hay *et al* 1988), viral erythrocytic necrosis virus (MacMillan and Mulcahy 1979), *Ichthyophonus hoferi*, herring worms (Anisakidae), an intestinal coccidian (*Goussia* sp.), a hepato-coccidian (*Goussia* (*Eimeria*) *clupearum*), a myxosporean in large ducts of the kidney (*Ortholina orientalis*), a myxosporean of the gall bladder (*Ceratomyxa auerbachi*) (Marty *et al* 1995), as well as an unknown agent causing severe necrotizing enteropathy among laboratory-reared hatchlings (Elston and Pearson 1986). Additionally, mortalities of

Atlantic herring (*C. harengus*) have been associated with toxic algae blooms (White 1980).

Interest in the susceptibility of different life stages of herring to petroleum exposure arose following the *Exxon Valdez* oil spill (EVOS) in Prince William Sound, AK which coincided with the 1989 herring spawn (Brown *et al* 1996). Subsequent petroleum exposure studies indicate that:

1) Prevalence of VHSV may be correlated with oil concentration (Carls *et al* 1995), although Kocan *et al* (1996a) found no correlation.
2) Larvae collected from oiled sites at the time of the EVOS had significantly more morphological deformities and cytogenetic abnormalities than larvae from unoiled sites (Hose *et al* 1996)
3) Larvae captured from areas oiled by the EVOS exhibited clinical signs associated with oil exposure in the laboratory (Norcross *et al* 1996)
4) Oil had its greatest effect on the blastodisc and gastrula stages of the herring egg (Kocan *et al* 1996b)
5) The life history stage that was most susceptible to the water-soluble fraction of crude oil is the larval stage (Lassuy 1989)
6) Adult herring, from a site oiled by the EVOS, collected 3 yr after the spill exhibited a lower percent hatch and fewer morphologically normal larvae than fish from a previously unoiled site (Kocan *et al* 1996c)
7) Mean egg-larvae mortality rates were twice as high in areas oiled by the EVOS than in unoiled areas (McGurk and Brown 1996).
8) Enlarged livers and reduced ovary size were observed among herring following a spill of crude oil in the Gulf of Finland (Urho 1990).

Herring Fisheries

Herring fisheries are among the oldest fisheries in the world, historically influential in the development of European coastal ports from the late middle ages (1400 AD) (Blaxter 1985). Herring, cod, and eel bones have been found in excavations of human settlements along the Danish coast dating to 3000 BC and the existence of a herring fishery near Yarmouth, England has been traced to 500 AD (Hardwick 1973).

Although landings of Atlantic herring (*C. harengus*) have always been greater than those of *C. pallasi* (Blaxter 1985), the latter has played a significant role in the development of cultures along the Pacific rim. Herring and herring spawn were used for food and barter by Pacific coast natives whose settlements go back to 800 BC (Hourston and Haegele 1980). The Japanese herring fishery started in 1447, but records of the fishery were not kept until 1871 (Morita 1985). The first reported commercial catch of Pacific herring in North America was made off Vancouver Island in 1877 (Hourston and Haegele 1980). Herring fisheries in California date from the mid 1800's, but no catch records were kept during this time (Spratt 1981). Prior to the 1900's, the catch of *C. pallasi* remained low due to the use of primitive gear. The market for dry, salted herring in the Orient increased from 1904-1907 and annual catch by Canadian fishers rose to 27,216 metric tons with the advent of the drift net in 1919. The Canadian catch rose sharply to 77,111 metric tons in 1927 when the purse seine was introduced (Hourston and Haegele 1980). Herring were reduced to meal and oil or canned and sold to European markets during the same time in

California (1917-1919), but the practice ended with the State Reduction Act of 1919 (Hardwick 1973). Demand for commercial bait herring in Puget Sound was high prior to World War II (WWII), but gradually decreased after halibut fishers found sources of bait herring in British Columbia and Alaska. Additionally, bait fish were no longer needed for the shark fishery because synthetic oils replaced the shark liver oil market. A lesser market for smaller, sport-bait herring became established in Puget Sound after WWII. The market for canned herring had a slight boom around 1948 when San Francisco canners attempted to fill the demand created by the scarcity of sardines (Hughs 1949), but demand has steadily decreased since 1952 (Hardwick 1973). Canadian fishers, stimulated by the absence of a herring meal and oil (reduction) fishery in California, began catching herring to supply the world demand. The herring catch then increased from 90,718 metric tons in 1938 to 239,497 metric tons by 1962. A subsequent population crash forced the closure of the last Alaskan reduction plant in 1966 (Reid 1971) and elimination of the B.C. reduction fishery in 1968. Fish meal production is now limited to utilization of residues from other forms of herring products (Hourston and Haegele 1980).

Extinction of the Japanese coastal stocks in 1950 forced Japanese fishers to join Soviet trawlers in the eastern Bering Sea to fish offshore, targeting mixed stocks of herring. Peak harvest in this fishery occurred in 1970 and steadily declined until the fishery was eliminated with the establishment of the Fishery Conservation Zone in 1980 (Fried and Wespestad 1985). Japanese herring fisheries were virtually eliminated after the USSR banned Japanese herring fishers from the Sea of Okhotsk in 1971 (Spratt 1981). Failure of the

Japanese herring fisheries and high demand for herring roe products forced Japanese buyers to seek international sources of high quality herring roe products. Japanese demand for herring products was realized in 1972 when wholesale prices for roe reached $\$1.36 \cdot kg^{-1}$ (Spratt 1981).

Herring as a food fish generally brings only 1/5 the price of sac roe herring (Hourston and Haegele 1980) which comprises the largest commercial herring harvest (Trumble and Humphreys 1985). Harvest estimates from Alaskan herring sac roe fisheries during the spring of 1994 totaled 43,401 metric tons (Funk 1994). The price of sac roe peaked at $\$3,630 \cdot metric\ ton^{-1}$ in 1979 then dropped to $\$900 \cdot metric\ ton^{-1}$ in 1980 after Japanese companies drove wholesale prices to $\$12.20 \cdot kg^{-1}$ and the market collapsed from consumer outrage (Spratt 1981). Sac roe gift packs in Japan can still sell for the equivalent of $\$100$ - $\$125 \cdot kg^{-1}$ during the holiday seasons (Pynn 1991).

Product from the spawn-on-kelp (SOK) fishery may be worth twice as much as sac roe (Mundy *et al* 1997). The first North American SOK permits were issued by California in 1965 (Hardwick 1973) when the California Department of Fish and Game permitted harvest of wild SOK in both San Francisco and Tomales Bays (Spratt 1981). Permits for this fishery are granted to divers who swim through known herring spawning sites and cut kelp blades containing attached herring eggs. Harvesting is not permitted until 4-5d post-spawn to allow time for the eggs to cement to the kelp. Only a narrow picking window exists since the eggs begin to eye after 9-10d which renders the product valueless (Pleschner 1986a).

Efficiency in the SOK fishery increased in 1985 when San Francisco Bay SOK fishers began using open harvest platforms (open pounds), and attainment of the 4.5 metric ton quota was realized for the first time in 1986. These platforms are constructed of log or wood frames containing suspended *Macrocystis* blades and are pushed into areas of active herring spawn (Shields *et al* 1985). Product quality was optimized after the introduction of open pounds and the corresponding decline in fishery effort resulted in increased involvement. New restrictions by California Fish and Game in 1989 fixed the number of permits at 10 and the amount of product · permit^{-1} at 7.5t (Mundy *et al* 1997).

The Washington Department of Fisheries drafted a 5yr plan in 1988 to study the feasibility of producing SOK from closed pounds (Washington Department of Fisheries 1988). Fishing efforts varied depending on the herring stock size, but generally consisted of 6 -11 permits, split between tribal and non-tribal fishers (Mundy *et al* 1997). All kelp for the Washington SOK fishery is imported from British Columbia and is subjected to quarantine regulations since *Macrocystis* culture in WA is illegal.

The SOK fishery in British Columbia (BC) started in 1971 with an experimental permit granted for the Queen Charlotte Island region (Shields *et al* 1985). The fishery expanded and grew through the 1970's until 28 license holders participated in 1979, each of which was allowed to produce 7.25 metric tons of product (Mundy *et al* 1997). Opposition to the SOK fishery has been

raised by those who feel that the fishery may interfere with the natural herring spawning process and with the natural harvest allowed to natives (Dickson 1979). The BC SOK fishery further expanded after the introduction of open pounds in 1985 (Mundy *et al* 1997), until 39 SOK licenses were granted in 1997. The SOK fishery in BC is currently conducted throughout the coast, but is concentrated on the east side of the Queen Charlotte Islands and along the north coast of the mainland (Shields *et al* 1985). Currently, 25% of the SOK in the Queen Charlotte Islands is produced by open harvest platforms (Mundy *et al* 1997). Management of the BC SOK fishery is based on market conditions and each licensee is allotted a quota which comprises an equal share of the total pre-determined harvest (Dickson 1979).

Wild SOK collection in Alaska began in Prince William Sound (PWS) during 1969 and in Bristol Bay in the 1970's, when divers collected *Fucus* and *Laminaria* species containing attached herring eggs (Mundy *et al* 1997). Two closed pound SOK permits were issued for PWS in 1979 and the fishery steadily increased until ADF&G declared the resource fully utilized in the late 1980's, at which time participation was based on a limited entry system. Two recent additions to SOK in Alaska were the Hoonah Sound SOK fishery in 1990 and the Craig / Klawock fishery in 1992. Over $1.5 million of product consisting of 36.3 metric tons was harvested from SOK fisheries in Hoonah Sound and Craig, AK in 1994 (Funk 1994).

Numerous techniques from different disciplines are incorporated into the closed pound SOK fishery to produce a more consistent, superior quality

product which is valued 5-10 times higher than wild SOK (Pleschner 1988b). Herring pounds are typically composed of foam blocks or commercially manufactured floatation bladders held together with an aluminum frame and wooden walkway (Brady 1985). Most pound frames in British Columbia are rectangular and average 18.3 x 9.1m (Dickson 1979). New premanufactured, walk-around systems cost approximately $10,000 (Pleschner 1986b). Webbing is hung below the pound frame and is normally 25-35mm knotted herring seine. An additional section of seine webbing is sewn into a corner of the pound to serve as a gate which can be dropped to allow herring to swim from the seine or push pound into the closed pound. Lead lines are placed at the inside corners of the closed pound to prevent the webbing from floating up and crowding the herring (Shields *et al* 1985). Closed pounds are anchored to the bottom and/or tied to the shore in deep water bays which are sheltered from the prevailing winds. Open pounds are often positioned next to closed pounds because free-ranging herring are attracted to the spawn emitted from the herring within closed pounds (personal observation).

Macrocystis is currently the only type of kelp used in closed pound SOK fisheries because other types such as *Laminaria* and *Agarum* are either too smooth for egg attachment or are so thin that they are subject to tearing (Whyte 1979). Apical meristems and immature kelp fronds exude a polysaccharide which prevents roe attachment and, thus cannot be used (Shields *et al* 1985, Whyte 1979). Availability of good quality *Macrocystis* is a limiting constraint in both the B.C and Puget Sound SOK fisheries (Shields 1987). Kelp for the Puget Sound fishery is imported from British Columbia and the supply for

California comes from the Channel Islands. *Macrocystis* in Prince William Sound is harvested in southeast Alaska, where 4-41 metric tons have been harvested annually since the 1980's (Mundy *et al* 1997). Kelp harvest regulations require that macrophytes can be harvested from no more than 0.3m below the surface by hand so harvesting is conducted using skiffs at low tides (Shields *et al* 1985). *Macrocystis* can be transported to the pounds at low temperatures (5-10°C) for up to 24h without adverse effects (Whyte 1979). Kelp fronds are tied to weighted lines within the pounds, suspended vertically from ropes, and usually harvested before 8-10d, after which they begin to rot (Shields *et al* 1985).

Herring are seined as close to the impoundments as possible to decrease the possibility of tow-related injuries. After a successful set, the seine and herring are slowly towed (1 knot · hr^{-1} maximum in Alaska) by a skiff to the pound, where the gate is opened and the herring are allowed to swim into the pound (Shields *et al* 1985). Alternatively, herring may be transferred to the closed pound in a portable, smaller net pen or push pound. Approximately 54 - 91 metric tons of herring are used to produce 7.25 metric tons of SOK product.

Impounded herring spawn within 1-10d following capture, after which the egg-laden kelp blades are harvested. Processing the SOK product involves removal of the blades from the pounds, trimming rotted edges and bare spots from the blades, stacking the blades in regulation-size containers (1.07m square x 60cm deep), adding 100% brine to the containers and transporting the brined product to shore (Shields *et al* 1985). The container is drained at the

processing plant and refilled with 100% brine. The containers are drained again for 1-12hr, and the product is graded (Shields *et al* 1985, Mundy *et al* 1997). Grade I kelp, or "Ichiban" (Pleschner 1986b) contains complete egg coverage with at least 3 egg layers · side^{-1}. Grade II product has complete egg coverage with a thin layer on each side, or the blade may be cracked. Product containing eggs which are not evenly distributed or are not tightly attached to the kelp constitutes grades III and IV (Dickson 1979) and is often rejected. Rotten kelp, kelp without roe, and product contaminated with sand or silt are unacceptable. The graded product is packed and salted in buckets, which are then filled with 100% brine and stored at 5°C (Shields *et al* 1985). Shelf life for the product is approximately 2yr (Pleschner 1986b). Two market opportunities are currently available for the product in Japan: the traditional year-end gift product is produced by closed pounds, and the year-round restaurant and sushi bar product is produced by open pounds and collected by divers (Mundy *et al* 1997).

The SOK impoundment process is prone to numerous pitfalls which may result in zero profit or substantial monetary loss to the operators. A significant amount of bruising to the fish, scale loss, or seine rupture can occur if large quantities of herring are towed long distances, if the tow seine gets tangled in an underwater obstruction such as a rock or pound anchor cable, or if the seine is towed too quickly (Shields *et al* 1985). Mortalities may reach levels as high as 33% in pounds containing high herring loading densities (Brett and Solmie 1982). Shields *et al* (1985) speculated that the cause of such mortality may be density-related stress, but they were unable to discern the pathological cause.

Additional injuries that can occur with high loading densities include bruising of the opercula and snout, fin-base hemorrhage, fin deterioration, open sores on the body and snout, as well as broken and mangled snout and jaw. The percentage of females that spawn is low (Shields *et al* 1985) and as much as 41% of the spawned eggs adhere to the pound webbing rather than to the kelp (Morstad *et al* 1992 and Morstad and Baker 1995). The weight of the dead fish in the bottom of the pound as well as the eggs on the web and kelp is often sufficient to either sink the pound or rip the bottom of the pound open, thus spilling all the dead herring and releasing the live fish (Shields *et al* 1985).

Stress

Brett (1958) defines stress to be "a state produced by any environmental factor which extends the normal adaptive responses of an animal, or which disturbs the normal functioning to such an extent that the chances of survival are significantly reduced." For the purposes of this dissertation, the definition will be altered slightly to encompass anthropogenic as well as environmental factors and, as Pickering (1981) suggested, "stress" will also be used to describe the stimulus responsible for the response rather than the response itself. Based upon these definitions, operations associated with the closed pound SOK fishery are inherently stressful to the associated herring. Effects of purse seine capture, transport to a pound, confinement and crowding within the pound for extended periods, and pseudo-natural spawning conditions may either combine, or act singularly to stress the impounded herring.

Fish protect themselves from pathogenic organisms with an immune system comparable to that of humans and other vertebrates (Anderson 1990). All vertebrate immune systems share common characteristics such as possession of lymphocytes, production of immunoglobulins containing fundamentally similar structure, and ability to mount T-cell functions (Saad 1988). Selye (1936) defined several morphological criteria of stress coined the term General Adaptation Syndrome, a condition characterized by enlarged adrenal glands, atrophy of lymphoid tissues, and ulcers in the gastrointestinal tract. Stress causes immunosuppression, which (in concert with other factors) leads to decreased disease resistance (Schreck 1990).

Stress alters the immune system indirectly by stimulating increased levels of circulating plasma corticosteroids (CS's) such as cortisol and cortisone. CS's are regulated *in vivo* through a negative feedback system, beginning with corticotropin releasing factors (CRF's) being secreted from the hypothalamus. CRF's stimulate the pituitary to release adrenocorticotropic hormone (ACTH) into the bloodstream which, in turn, triggers corticosteroid (cortisol and cortisone) release from the interrenal region of the head kidney (Donaldson 1981). To date, two explanations have been proposed to account for the action of CS's within the cell. First, corticosteroids pass freely through the cell membrane into the cytoplasm where they combine with CS receptors. This combination is then transported to the nucleus where it forms a triad with a nuclear receptor. The triad then interacts with DNA and gives rise to mRNA which is then transported back to the cytoplasm and directs the synthesis of selected proteins which influence a number of metabolic pathways including

glucose uptake and transport (Pearson *et al* 1978). The second proposed mode of CS function within the cell operates through alteration of a transcription factor that activates immunoregulatory genes. The nuclear factor NF-*k*ß is kept as an inactive cytoplasmic complex by I*k*ß inhibitory proteins in unstimulated cells. The I*k*ß's are rapidly degraded after cell stimulation and free NF-*k*ß translocates to the nucleus where target genes are activated. Corticosteroids inhibit NF-*k*ß activation by inducing expression of the I*k*ß proteins. Following CS exposure, newly synthesized NF-*k*ß is either sequestered in the cytoplasm or translocates to the cell nucleus where it is sequestered and dissociation of DNA-bound NF-*k*ß is promoted (Auphan *et al* 1995, Scheinman *et al* 1995).

Plasma corticoid concentrations increase rapidly immediately after the onset of stressors such as handling, and continue to slowly rise for at least 24hr (Strange *et al* 1977). Inhibition of monocyte-macrophage differentiation (Baybutt and Holsboer 1990), monocytopenia, prevention of lysosomal exocytosis through stabilization of lysosomes, suppression of mature macrophage activity (Ellis 1981), decreased immunoglobulin synthesis (Pearson *et al* 1978), and reduced numbers of circulating lymphocytes (Pegg *et al* 1995) result from CS stimulation. Following cortisol exposure, remaining blood lymphocytes no longer respond to mitogenic stimuli (Ellsaesser and Clem 1987).

Immunosuppression caused by stress-induced CS release, in concert with other factors, may lead to decreased disease resistance (Schreck 1990). Increased growth of the ectofungal parasite *Saprolegnia* occurs on the dermis

of suckers (*Catostomus commersonii*) following intravenous administration of corticosteroids (Roth 1972). Similarly, coho salmon (*Oncorhynchus kisutch*) are more susceptible to infections of the copepod *Lepeophtheirus salmonis* after implantation with cortisol (Johnson and Albright 1992). Synthetic corticosteroids administered in concert with infectious agents have proven successful in producing overt furunculosis in rainbow trout (*Oncorhynchus mykiss*) (Bullock and Stuckey 1975), ichthyopthiriasis in carp (*Cyprinus carpio*) (Houghton and Matthews 1986), and enteric septicemia of catfish in channel catfish (*Ictalurus punctatus*) (Antonio and Hedrick 1994). Similarly, administration of synthetic CS to rainbow trout (*O. mykiss*) immunized against *Yersinia ruckeri* results in reduced numbers of splenic antibody producing cells, lower circulating antibody titers, and lower numbers of splenic lymphocytes (Anderson *et al* 1982).

Crowding, transfer, and handling stresses, similar to those experienced by herring in SOK fisheries, cause increased plasma cortisol concentrations in Atlantic salmon (*Salmo salar*) (Mazur and Iwama 1993). Crowding of fish may suppress the specific immune system, resulting in increased probability of disease if exposure to a pathogen occurs. Crowded fish display decreased blood lactate levels, decreased coefficient of condition (K), prolonged decreases in numbers of circulating lymphocytes (Pickering and Pottinger 1987), increased plasma glucose levels, decreased plasma chloride, decreased total serum protein, decreased serum lysozyme activity, decreased bactericidal complement (Yin *et al* 1995), and decreased growth rate (Pickering and Stewart 1984). High stocking densities, handling , transport, and elevated

temperature result in increased prevalence of iridovirus disease among cultured white sturgeon (LaPatra *et al* 1996).

Viral Hemorrhagic Septicemia Virus

Classification

Viral hemorrhagic septicemia virus (VHSV), the causative agent of the disease referred to as viral hemorrhagic septicemia (VHS), is a member of the family *Rhabdoviridae*, in the Order mononegavirales. Rhabdoviruses are among the most widely distributed viruses in nature with at least 44 known to infect plants and at least 70 known to infect animals (Wagner and Rose 1996). The family *Rhabdoviridae* includes: the *Cytorhabdovirus* and *Nucleorhabdovirus* genera of plant viruses, and *Vesiculovirus*, *Ephemerovirus*, *Lyssavirus* , and *Novirhabdovirus* (including VHSV and other economically important fish viruses), genera of animal viruses (Kurath, pers. comm., Bjorklund *et al* 1996, Dietzschold *et al* 1996, Bernard and de Kinkelin 1985). Rhabdoviruses known to infect fish include viral hemorrhagic septicemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV), spring viremia of carp virus (SVCV), hirame rhabdovirus (HIRRV), and pike fry rhabdovirus (PFRV) (Bjorklund et al 1996, Giorgetti 1980, Lenoir and de Kinkelin 1975) and VHSV has been termed "the most important viral disease of salmonid fish in European aquaculture..." (Jorgensen 1992, Hill 1991).

History

The original strain of VHSV was termed "Egtved virus," after the small village in the eastern part of Jutland where it was first described (Jensen 1965). The disease has been referred to as "nierenschwellung" or "kidney swelling", "infektiose nierenschwellung und leberdegeneration" (INuL) or "infectious kidney swelling and liver degeneration" (Schaperclaus 1954a,b), "bauchwasswesucht der forellen" (Heuschmann 1952, Liebmann 1956), "die neu forellenkrankheit" or "new trout disease" (Numann and Deufel 1956, Klinger 1957), "forellenseuche" or "trout plague" (Tack 1957), "la lipoidosi epatica" (Scolari 1954), "l'anemie infectieuse de la truite" (Besse 1955), "l'anemie pernicieuse des truites" or "pernicious anemia of trout" (Besse 1956), "Egtvedsygen" (Rassmussen 1959), and "syndrome enterohepato-renale" or "entero-hepatic-renal syndrome" (Bellet 1958). A decision was made at the first European symposium on fish disease in Turin, 1962 that the disease should be referred to as "viral hemorrhagic septicemia" (VHS) (Ghittino 1965, Wolf 1988). The disease is still often referred to by different names such as "Egtved disease" (Rasmussen 1965) or "popeye sickness" (Bellet 1965) and has also been incorrectly identified as "ulcus-syndrome in cod" (Jensen and Larsen 1979, Jensen *et al* 1979) prior to subsequent virological identification (Jorgensen and Olesen 1987).

Etiology

The VHSV virion is helically symmetrical with a typical, bullet shape (Baroni *et al* 1982) containing a single strand of negative-sense RNA (Robin and Rodrique 1977). Virions of VHSV range in size from 180 x 60 - 70nm

(Zwillenberg *et al* 1965), 240 x 75nm (de Kinkelin and Scherrer 1970), 165 x 75nm (Olberding and Frost 1975), 177 x 60nm (Cohen and Lenoir 1974), and 165 x 57nm (Baroni *et al* 1982). Regardless of the reported size discrepancies, which may be a result of the fragile nature of the virus or different methods of fixation, observation, and preparation of electron microscopy materials, all reported sizes are within the size range for rhabdoviruses; 45 - 100nm in diameter x 100 - 430nm in length (Wunner *et al* 1995).

Inactivation of VHSV results from ultraviolet irradiation (254nm at 5cm after 10min.), gamma irradiation ($1.7 \cdot 10^5$rads), chlorine (500ppm after 2 min.), I_2 (0.01% after 5min.) (Ahne 1982 a,b, Ahne and Held 1980), x-ray irradiation (Dietzschold *et al* 1996), formalin (2% after 5min.), pH (2.5 after 60min.), quaternary ammonium compounds (125ppm after 5min.) (Dorson and Michel 1987). Infectivity is only reduced in half after drying (1-2wk) or exposure to pH 12.2 (120min) (Jorgensen 1973a).

Rhabdoviruses possess a lipid-containing envelope in which are imbedded glycoproteins forming surface spikes or peplomers (Cohen and Lenoir 1974) 5 - 10nm long and about 3nm in diameter (Dietzschold *et al* 1996, Wunner *et al* 1995). The infectious component of all rhabdoviruses is the nucleocapsid, or ribonucleoprotein core (Wagner and Rose 1996) which is helically symmetrical with a diameter of 30 - 70nm (Wunner *et al* 1995). Viral RNA contained within the core accounts for 1 - 4% of the total virion weight (Dietzschold *et al* 1996) and base composition of the RNA is 29.3% cytosine, 23.6% adenine, 14.5% uridine, and 32.6% guanine (Robin and Rodrique 1977).

The VHSV genome codes for at least 6 proteins; L (large protein) with a molecular weight of 190 kd, G (transmembrane glycoprotein) with a molecular weight of 70kd, N (protein associated with the nucleocapsid) with a molecular weight of 41kd, P (phosphoprotein) with a molecular weight of 28kd, NV (non-virion protein) with a molecular weight of 14kd, and M (matrix protein) with a molecular weight of 24kd (Lorenzen *et al* 1988, Kurath and Higman 1997, Lenoir and deKinkelin 1975). These polypeptides are probably transcribed from the RNA strand sequentially in the order 3' N - P - M - G - NV - L 5' (Bernard and deKinkelin 1985, Wagner and Rose 1996). Other proteins may be encoded within a second open reading frame and present only in the cytoplasm of infected cells, as is the case with the rhabdovirus "vesicular stomatitis virus" (VSV) of invertebrates and mammals (Wagner and Rose 1996).

Peplomers on the surface of VHSV are composed of trimers of the viral G protein (Lenoir and de Kinkelin 1975, Gaudin *et al* 1992) located on the outer surface of the viral envelope (Wagner and Rose 1996) and are influential in attachment of the virion to the host cell (Coll 1995, Rigaut *et al* 1991). The G protein is the major antigenic determinant responsible for type specificity, and also gives rise to and reacts with neutralizing antibody (Bernard *et al* 1983, Lorenzen *et al* 1990). The matrix protein is also associated with the viral envelope but on the inner surface (McAllister and Wagner 1975, Wagner and Rose 1996). Both P and M proteins (formerly M1 and M2), share common structural features with their respective VSV equivalents and are thus likely to exert similar functions (Benmansour *et al* 1994), possibly serving as bridging molecules that attach the nucleocapsid to the plasma membrane of the host cell

(McCreedy and Lyles 1989, Benmansour *et al* 1994). The P and M proteins are also thought to play a role in virion assembly and maturation in the cytoplasm of infected cells (Benmansour *et al* 1994). The NV gene encodes a non-virion protein that is expressed in infected cells but is not present in purified virions (Kurath and Higman 1997, Schuetze *et al* 1996, Kurath and Leong 1985). The nucleoprotein N wraps the viral RNA and the L protein works with P and N to transcribe mRNA (Emerson and Yu 1975).

Detection and Diagnosis

Several methods for detection and identification of VHSV have been used, but historically the most sensitive method has been virus isolation in cell culture followed by 50% plaque neutralization (Jorgensen 1992, Jorgensen *et al* 1991, Hoffman *et al* 1981, Evensen *et al* 1994). Pretreatment of cell monolayers with polyethylene glycol prior to virus introduction results in 8 - fold increases in VHSV detection levels and larger plaques (Batts and Winton 1989). Fish cell lines exhibit varying degrees of susceptibility to VHSV, with bluegill fry (BF-2) being more sensitive than *epithelioma papulosum cyprini* (EPC) or chinook salmon embryo (CHSE-214) (Olesen and Jorgensen 1992). Although cell culture is the most widely used technique for virus isolation, some viral strains exist which are not neutralized using rabbit antiserum made against European reference strain F1 (Ahne *et al* 1986). In cases such as this, researchers have turned to alternative detection methods such as immunofluorescence, immunoblot, or immunohistochemistry. Ortega *et al* (1992) report slightly greater detection sensitivity using the fluorescent antibody technique rather than cell culture; however, Evensen *et al* (1994) concluded the

opposite. The fluorescent antibody technique does maintain the advantage of rapid detection, requiring only 1d compared to 7-20d for the plaque assay (Ortega *et al* 1992). An advantage of the immunoblot technique is that inactivated virus can be used as a positive control (Mc Allister and Owens 1987). An enzyme-linked immunosorbent assay (ELISA) has been developed for VHSV that offers the advantage of rapid diagnosis, but it tends to be slightly less sensitive than cell culture (Olesen and Jorgensen 1991). Increased sensitivity of the ELISA can be obtained using rabbit antisera with high binding affinity (Way and Dixon 1988) or by using a high sodium chloride concentration and two non-competitive monoclonal antibodies against the nucleoprotein N (Sanz and Coll 1992). Modifications to both the ELISA and immunofluorescence techniques may render these methods more sensitive than conventional cell culture (Olesen *et al* 1991). New DNA probes and the polymerase chain reaction (PCR) offer the advantage of differentiating between the North American and European strains of VHSV (Einer-Jensen et al 1995), but provision of quantifiable titers is yet unobtainable with these methods (Batts *et al* 1993).

Geographic Range and Isolates

VHSV has historically been a problem of farmed fish in Europe (Wolf 1988). At least 3 serotypes of VHSV have been described with varying reactivity toward neutralizing antibodies (Oleson *et al* 1993). Varying levels of pathogenicity occur among at least 8 different European isolates depending upon the infected host (Jorgensen 1980). Additionally, the virulence of an individual VHSV strain changes with passage in cell culture and a non-virulent

thermo-resistant strain can be selected by careful temperature manipulation (de Kinkelin *et al* 1980).

Following the discovery of VHSV in fishes from the Pacific coast of North America (Meyers and Winton 1995, Meyers *et al* 1994, Meyers *et al* 1992, Eaton *et al* 1991, Eaton and Hulett 1990, Stewart *et al* 1990, Brunson *et al* 1989, Hopper 1989), the North American strain of VHSV has been determined to be serologically similar to European strains, but of low pathogenicity to salmonids (Winton *et al* 1989, 1991). Strains of VHSV from the two continents, although being serologically similar, compose two different RNA fingerprint groups (Oshima *et al* 1993) and the sequence of the nucleoprotein gene from virus isolates on either continent diverges by 13% (Bernard *et al* 1992, Bernard *et al* 1990). Thus, the North American strain is believed to have a non-European origin and is not the result of recent importation (Bernard *et al* 1992, Bernard *et al* 1991, Batts *et al* 1993). Care should be taken in such statements of viral origin since RNA viruses, such as VHSV, generally exhibit a high mutation frequency due to a lack of RNA proofreading enzymes (Holland *et al* 1982).

Host Range

VHSV has a broad host range (Wolf 1988). The disease has historically been a problem of hatchery fish including both freshwater (Bellet 1965, Christensen 1965) and saltwater (Castric and de Kinkelin 1980) reared rainbow trout (*Oncorhynchus mykiss*), pike (*Esox lucius*) (Meier and Jorgensen 1980), turbot (*Scophthalmus maximus*) (Ross *et al* 1994, Schlofeldt *et al* 1991), and grayling (*Thymallus thymallus*) (Meier and Wahli 1988). The virus has been

recovered from wild or feral fishes including rainbow trout (*O. mykiss*) (Ghittino 1965, Ahne and Jorgensen 1993), white fish (*Coregonus sp*) (Ahne and Thomsen 1985, Enzmann and Konrad 1990, Meier *et al* 1986), elvers (*Anguilla anguilla*) (Jorgensen *et al* 1994, Castric *et al* 1992), chinook salmon (*O. tschawytscha*) (Hopper 1989), coho salmon (*O. kisutch*) (Eaton *et al* 1991, Brunson *et al* 1989), brown trout (*Salmo trutta*) (Enzmann *et al* 1992), Atlantic cod (*Gadus morhua*) (Jensen and Larsen 1979, Jensen *et al* 1979, Jorgensen and Olesen 1987), Pacific cod (*Gadus macrocephalus*) (Meyers *et al* 1992), Atlantic herring (*Clupea harengus*) (Dixon *et al* 1997), and Pacific herring (*Clupea harengus pallasi*) (Meyers *et al* 1994, Traxler and Kieser 1994). Experimental susceptibility of other species such as sea bass (*Dicentrarchus labrax*) (Castric and de Kinkelin 1984), rainbow trout x coho salmon hybrids (Ord *et al* 1976), Atlantic salmon (*S. salar*) (de Kinkelin and Castric 1982), golden trout (*S. aguabonita*) (Ahne *et al* 1976), and brook trout (*Salvelinus fontinalis*) (Rasmussen 1965) indicates that other species are susceptible. Physiological differences among strains of the same fish species may affect susceptibility to VHSV (Kaastrup *et al* 1991) and several fish species initially reported to be resistant were later found to be susceptible (de Kinkelin *et al* 1974, Ord 1974).

Clinical signs of infection and pathological changes

Clinical signs and pathological changes associated with VHS vary depending on host species, size, nutritional status, as well as water temperature, crowding, and virus strain. Ghittino (1965) defined 3 stages or "forms" of VHS including the acute, chronic, and nervous stages, which were

later renamed by Wolf (1988) as acute-hyperactive, chronic-subacute, and latent. The first signs of the disease in cultured rainbow trout involve dark coloration or melanosis, followed by possible exophthalmia, lethargy, and listless floating near the surface of containment ponds. Appearance of colorless and pale gills is followed by a distended abdomen and hemorrhages at the base of the pectoral fins and lateral line. Necropsy reveals ascites, swollen stomach, and intestines containing a yellow serous fluid. The liver becomes discolored, hypertrophied, and may contain petechiae, as may the heart (Bellet 1965). The most specific lesions are scattered hemorrhages in the periocular connective tissues, skeletal muscles, perivisceral adipose tissue, swim bladder, and heart (Ghittino 1965). Infected fish exhibit altered renal function, with oligurea and decreases in urine flow rate, glomerular flow rate, tubular fluid reabsorption, filtered electrolytes, and plasma protein (Elger *et al* 1986). These renal effects may, in part, be caused by heavy deposits in the subendothelial-mesangial region and mononuclear cells in the capillaries of the glomerulus (Elger and Hentschel 1983).

Other fish species exhibit similar, but not identical signs. Clinical signs among affected pike, sea bass, turbot, whitefish, brown trout, and grayling begin with listless floating at the surface of the water. Exophthalmia is present, the gills become pale, the abdomen is swollen, and hemorrhages are present within the muscles, on the swim bladder, and around the kidney (Meier *et al* 1986, Schlotfeldt and Ahne 1988, Castric and de Kinkelin 1984, Ross *et al* 1994, Schlotfeldt *et al* 1991, Ahne and Thomsen 1985). Histologically, total necrosis may be detected in the liver, spleen, and intestinal mucosa (Meier and

Wahli 1988). Skin lesions including ulcers, papules, and erosions have been reported from infected cod (*Gadus sp.*) (Jensen and Larsen 1979, Meyers *et al* 1992). Skin lesions as well as severe skin hemorrhaging may accompany VHSV infection in Pacific herring (Meyers *et al* 1994). Infected fish die from hemorrhages caused by infection of the endothelium of blood capillaries, anemia from lesions of the hematopoietic tissues, and impairment of fluid balance due to the destruction of the excretory structures of the kidney (deKinkelin *et al* 1980). VHSV related mortality may be decreased following removal of lymphoid cells from infected hosts (Chilmonczyk and Oui 1988).

The course of VHSV infection in the susceptible host remains uncertain, but mechanisms have been proposed. Virions of VHSV are generally believed to enter the host through the gills (Evensen *et al* 1994) because VHSV can be isolated only from the pillar cells in the secondary lamellae immediately following infection (Chilmonczyk 1980, Neukirch 1984). Additionally, VHSV antigen occurs only in the epithelial cells of the secondary lamella 24h post infection (Konrad *et al* 1989). Although it is generally believed that uptake occurs through the respiratory organs, electron microscopy has thus far failed to demonstrate direct penetration into the gills (Chilmonczyk 1980).

The primary host target cells for VHSV, once through the gills, remains uncertain but primary multiplication does not occur in the gills (Kruse and Neukirch 1990, Yamamoto et al 1992). The first site of viral replication may be in the endothelial cells of the vascular system, possibly the kidney and spleen (Neukirch 1984). However initial propagation of the virus may occur in the

blood vessels of the liver, posterior kidney, and brain because viral antigen occurs in the basement membranes of the arterioles of these organs 2 d post infection (p.i.) (Konrad *et al* 1989). Similarly, cells for primary VHSV replication may be in the spleen and/or kidney because the virus can be found in these organs only 18 h p.i. (Bernard *et al* 1985) and kidney macrophages can support replication of VHSV *in vitro* (Estepa *et al* 1992). Trout leukocytes are stimulated by purified glycoprotein G and nucleoprotein N antigens of VHSV (Estepa *et al* 1991a) and leukocyte lysis occurs after exposure to the virus (Estepa and Coll 1991, Estepa *et al* 1991b), indicating that leukocytes may be target cells for VHSV. Following initial propagation of virus (ca. 3d), the infection spreads to the parenchyma of infected organs and continues to diffuse to other organs, including the brain (Konrad *et al* 1989), skin, and fins (Dorson and Torhy 1993).

Transmission of infection to other susceptible hosts may occur by dislodging of VHSV-positive epithelial cells from the excretory kidney of infected fish (Kruse and Neukirch 1989) and subsequent excretion with the urine (Neukirch and Glass 1984, Neukirch 1985). Cells of the kidney, and not the gastrointestinal tract, are involved in virus shedding because VHSV is found in the urine 2d p.i. and no virus is detected from the feces through 17d. VHSV is then passed to other susceptible fish through the water and taken up through the gills (Jorgensen 1973b, Rasmussen 1965, Ghittino 1965). Transmission of VHSV through the feces of gulls and herons does not occur (Eskildsen and Jorgensen 1973 and Peters and Neukirch 1986). Fish surviving a VHS outbreak can become carriers (Meier *et al* 1994) and the brain may be a target organ for persistence of the virus (Neukirch 1986).

The cycle of infection in a host cell has not yet been fully described for VHSV, but is well understood for the prototype rhabdovirus, VSV (Wagner and Rose 1996), where a series of events including adsorption, penetration, uncoating, transcription, translation, processing, replication, and budding have been described. Phospholipids associated with the host cell plasmalemma are important for binding the G protein peplomers of VHSV to the host cell and subsequent adsorption (Estepa and Coll 1996). Detection of VHSV antigen in cells and cytoplasmic granulo-filamentous structures (presumably newly synthesized viral nucleoproteins) have been reported only 8h p.i., followed by proliferation of virus particles budding from the cell membrane 24h p.i. (Baroni *et al* 1982). Other researchers report VHSV budding from infected cells at 7h p.i., followed by exponential growth for 5-6h, and finally a plateau of virus growth around 24h p.i. (Wolf 1988). The cytoplasm of infected cells generally maintains normal features during the period of exponential growth (Baroni *et al* 1982).

Immunity

Attempts to develop a vaccine for VHSV have thus far met with limited success (Lorenzen and Olesen 1997). Low levels of neutralizing antibodies (NAb) have been found in free-living and cultured fish previously exposed to VHSV (Enzmann *et al* 1987, Ahne and Jorgensen 1993, deKinkelin and Le Berre 1977, and deKinkelin *et al* 1977) and the level of immunity to VHSV is proportional to NAb titers (Bernard *et al* 1985). Antibody in the serum appears relatively late after virus exposure (Jorgensen 1971). Initial protection against VHSV may result from interferon production within exposed fish (Rogel-Gaillard

et al 1993, deKinkelin and Dorson 1973, deKinkelin and Dorson 1982). Elevated immunoglobin (IgM) levels are eventually produced in VHSV-exposed fish and persist for an extended time (Olesen and Jorgensen 1986a).

Vaccine development has thus far focused on both viral proteins and attenuated forms of the virus. Inactivated virus is capable of inducing significant protection only following i.p. injection, and not bath immersion (de Kinkelin and LeBerre 1977). The nucleoprotein N may be necessary in an effective vaccine (Estepa and Coll 1992), but attempted bath immunization of rainbow trout with a peptide of the nucleoprotein N not only failed to produce protection against VHSV challenge, but increased mortality compared to the non-immunized controls (Estepa and Coll 1993). A thermo-resistant VHSV variant lacking the G protein (Bernard *et al* 1983) has been developed through serial passage of virus at increasing temperatures (deKinkelin *et al* 1980) which induces partial protection against a wild VHSV strain when used to immunize rainbow trout (de Kinkelin and Bearzotti 1981). However, others contend that neutralizing antibody to VHSV shows "exclusive specificity for the glycoprotein G" (Lorenzen *et al* 1990) and immunization with a purified and renatured form of this protein induced partial, complement-dependent neutralizing activity in rainbow trout (Lorenzen *et al* 1993). Intramuscular injection of a new DNA vaccine incorporating the N and/or G proteins of VHSV with a cytomegalovirus promoter induces protective immunity against VHS for rainbow trout (Lorenzen et al 1998). Enzmann and Konrad (1984) report stress-related VHS outbreaks in rainbow trout culture and suggest that, rather than focus on vaccine

development, aquaculturists should direct attention towards good hygiene and avoid stressful conditions in the culture ponds.

Project Justification

Activities associated with the closed pound SOK fishery such as rapid transport of herring to the closed pound reportedly cause bruising and scale loss (Shields *et al* 1985). Similarly, high loading densities in the pounds can result in herring mortalities as high as 33% (Brett and Solmie 1982) or physical damage such as opercula and snout bruising, fin-base hemorrhage, fin deterioration, and open ulcers on the body and snout (Shields *et al* 1985). A "*Vibrio* - like disease" with clinical signs of subcutaneous hemorrhage was noted by Hay et al (1988), yet attempted bacterial isolation was unsuccessful. These signs are similar to the clinical signs of VHSV infection in herring (Meyers *et al* 1994). Stressors such as spawning, capture, nutritional deficiency, or other diseases may contribute to periodic VHS epizootics in wild herring populations (Meyers *et al* 1994) and similar stressors have been associated with VHSV outbreaks among cultured rainbow trout in Europe (Enzmann and Konrad 1984). Within SOK pounds, high herring densities are often desired in order to obtain a high quality product containing multiple egg layers on the surface of kelp blades. Laboratory and field studies reported in this dissertation were undertaken to determine whether activities associated with the closed pound SOK fishery were correlated with increased VHSV prevalences among impounded herring and to describe the course of virus infection within the pounds.

Chapter 2: Field Experiments with Spawn-on-Kelp (SOK) Pounds

Prevalence of VHSV was monitored both before and during herring confinement within SOK pounds located in Puget Sound, WA (1996, 1997, and 1998) and Prince William Sound, AK (1997 and 1998). Tissues from each sampled fish were homogenized in tris buffered minimum essential medium (MEM), containing 1% penicillin - streptomycin, 0.2% gentamycin, and 1% amphotericin B, for titration of tissue virus. Virus titers were determined by plating serial 10-fold dilutions of tissue homogenates onto polyethylene-glycol-pretreated EPC cells and incubating the cultures at 15°C for 7d (Batts and Winton 1989). Virus titers were expressed as plaque-forming units (pfu) · g^{-1} of tissue. Tissues from the 1997 Alaska SOK pound study which failed to test positive for VHSV during primary isolation were re-assayed through a secondary, blind passage on EPC cells to detect low levels (< 50pfu · g^{-1}) of infection (Meyers *et al* 1992). All viral prevalences were statistically compared using the Z test for proportions (Zar 1984), and comparisons with $p \leq 0.05$ were considered significant. Gonad fullness, when determined upon necropsy, was recorded as spent (≤ 25% full) or not completely spawned (>25% full).

1996 Puget Sound SOK Fishery

The 1996 closed pound SOK fishery in WA consisted of five pound structures located in the northern region of Puget Sound, near Neptune Beach. For the purpose of this project, daily VHSV prevalences in herring were monitored from one of these pounds. Approximately 0.9 metric tons of sexually mature, pre-spawn herring were captured, transported, and loaded into the pound on 22 May (d 0). Biomass of impounded herring remained low through

the following day when an additional 1.4 metric tons of fish were captured and loaded into the pound. Herring additions to the pound ceased after d 2 (May 24), when 9.8 metric tons of pre-spawn herring were purse seined and loaded into the pound (Figure 1). A random sample of 30 impounded herring was removed shortly after the last load of herring was added (d 2). An additional sample of 41 impounded herring was removed the following day (d 3) prior to release of the fish from the pound and product harvest. Gonad fullness from each sampled fish was recorded upon necropsy and tissues from each fish, including heart, liver, kidney, and spleen, were pooled and later assayed for VHSV.

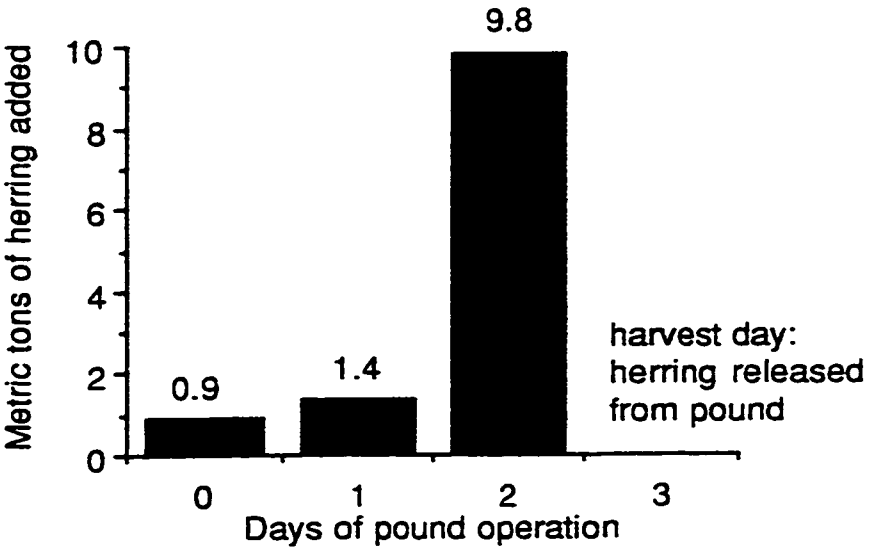

Figure 1. Herring loadings into the 1996 Puget Sound SOK pound.

Active spawning by impounded herring began shortly after the pound was filled on d 2 of operation. Only 3.3% of the impounded herring had spent gonads at the time the pound was filled; however, a milt cloud was observed

emanating from the pound during the afternoon of d 2. Nearly 50% of the impounded fish had spent gonads the following day (Figure 2).

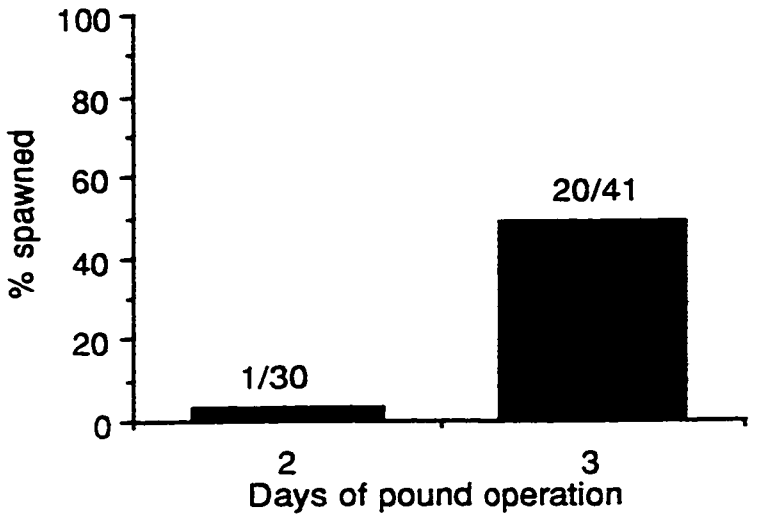

Figure 2. Spawning status of herring in the 1996 Puget Sound SOK pound.

None of the herring (0/30) sampled from the full pound tested positive for VHSV on d 2 of pound operation (May 24). However, the viral prevalence increased significantly ($p < 0.05$) to 12.2% (5/41) prior to release of the herring only 1d later (Figure 3). Most (4/5) of the herring which tested positive for VHSV had high titers ($> 10^4$pfu $\cdot g^{-1}$) in the pooled tissue homogenates. Three of the fish had spent gonads, and one was a juvenile (< 2yr old).

Most of the herring (81%) were confined within the pound for less than 24h and the longest any fish was in the pen was 3d (Figure 1). Although the infection status of the herring at the time of introduction to the pound on d 0 and d 1 was not determined, all impounded herring were captured from the same

geographic area near Neptune Beach and presumably were members of the same school. Thus, it is reasonable to assume that the few fish (19% of the total biomass) added to the pound prior to the sampling date were of the same viral status as those added on d 2. Additionally, because the sample was removed from the pound containing the entire lot of fish, it is reasonable to assume that sample taken on d 2 included herring which were added to the pound on d 0 and 1 (Figure 1) .

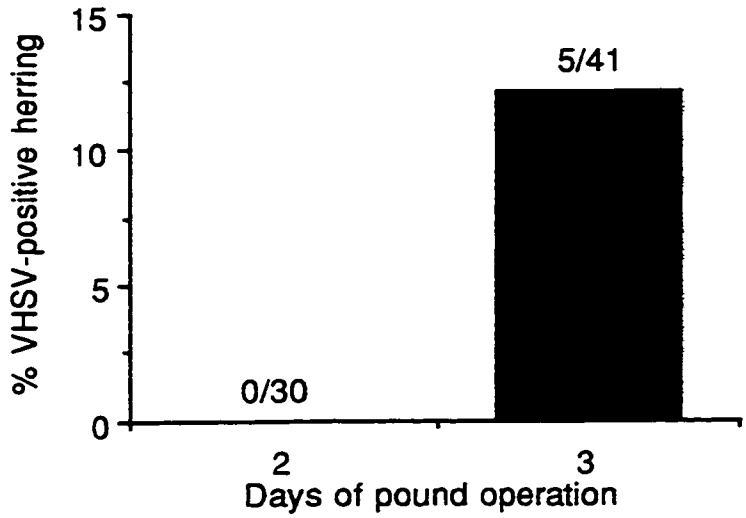

Figure 3. VHSV-positive herring in the 1996 Puget Sound SOK pound.

Results from this pilot study indicated that VHSV infections in herring from SOK pounds increased with confinement time. The increase in both VHSV titer and prevalence among confined herring may have occurred as quickly as 1d post impoundment (Figure 3) because 81% of the herring were confined for only 1 d. Alternatively, the increased prevalence of VHSV on d 3 of pound operation may have represented infections only from the herring which were

added on d 0-1 of pound operation. Regardless, onset of VHSV infection occurred within 1-3d of impoundment.

Gonad fullness among the impounded herring was inversely related to VHSV prevalence; however, further studies are needed in order to determine whether gamete release by free-ranging and impounded herring results in elevated VHSV infections. Unfortunately, logistical complications such as distance from the fishing grounds and uncertainty in predicted herring catch, combined with an unusually early product harvest resulted in fewer sampling days than ideal for a complete study. Regardless, impoundment of herring was correlated with increased VHSV infection among associated fish.

Directions for further research were indicated by several observations made during the 1996 Puget Sound herring SOK season. First, there is a question as to whether other fishes are at risk, due to potential spread of infection to species such as dogfish (*Squalus acanthias).* Second, unconfined herring were apparently attracted to the large milt cloud that emanated from the pound during the period of active spawn. The herring positioned themselves in the downstream flow of water which circulated through the pound (personal observation), thereby potentially exposing themselves to waterborne VHSV. Finally, further studies are needed course of infection among impounded herring.

1997 Puget Sound SOK Replication

Continued studies on confinement of Puget Sound herring in closed SOK pounds have been curtailed since 1996 due to a population decline among the largest herring stock in Washington state at Cherry Point and subsequent closure of the fishery. As an alternative, net pen located in Mats Mats Bay (near Port Ludlow, WA), was filled with herring from a healthy stock. Herring are generally held in the pen for several days to clear their stomachs, after which they are harvested, flash frozen, and sold as bait to sportfishers. The herring bait pen was nearly identical to a closed SOK pound with the exception that no kelp was strung inside the pound. Sampling difficulties encountered during the 1996 SOK study such as daily additions of small herring biomasses to the pound, concerns of kelp decomposition, and inaccessibility to the pound were eliminated through the use of the herring bait pen.

Sexually mature, pre-spawn herring (0.9 metric tons) were purse seined from Port Gamble Bay, WA on 23 February, 1997, brailed into a large flow-through holding tank on board the F/V *Jolly Rodger*, and transported to Mats Mats Bay, where they were wet brailed into the 6.1 x 19.8 x 4.6m net pen at a calculated loading density of 1.6kg · m^{-3}. Herring were confined in the net pen for 8d prior to harvest. Active spawning occurred throughout this time and eggs were deposited on the webbing of the pen.

Prevalences of VHSV among impounded herring were monitored by sampling 100 fish every other day starting on the day herring were loaded into

the pound (d 0) and continuing through 8 d of confinement. Serial 10-fold dilutions of spleen, liver, and kidney homogenates from each sampled herring were later assayed for VHSV. Gender and gonad fullness were recorded from all necropsied fish.

Water samples (2ml) were taken from within the pound, around the outside edge, and 2m outside the pound at slack on selected sampling days. The samples were diluted 1:1 in Tris buffered minimum essential medium containing 5% fetal bovine serum (MEM-5) and frozen at -80°C until analyzed for VHSV using a plaque assay.

Nearly all (96/100) of the herring loaded into the pound on d 0 had full gonads. Active spawning within the pound occurred less than 1d later, after which spawning ceased and ≈55% of the impounded herring were determined to be spent (Figure 4).

None of the herring (0/300) tested positive for VHSV from the time of capture through 3d of confinement. Prevalence of VHSV increased to 1% (1/100) after 5d to 17% (17/100) by the end of the study on d 8 (Figure 5). Tissue titers of VHSV were low (< 10,000pfu · g^{-1}) in all but one virus-positive herring. Most of the herring (10/17) which tested positive for VHSV on d 8 had titers approaching the minimum plaque assay detection limit of 400pfu · g^{-1}. The one VHSV-positive fish on d 5 had not yet spermiated, but 71% (12/17) of the VHSV-positive herring from d 8 had spent gonads.

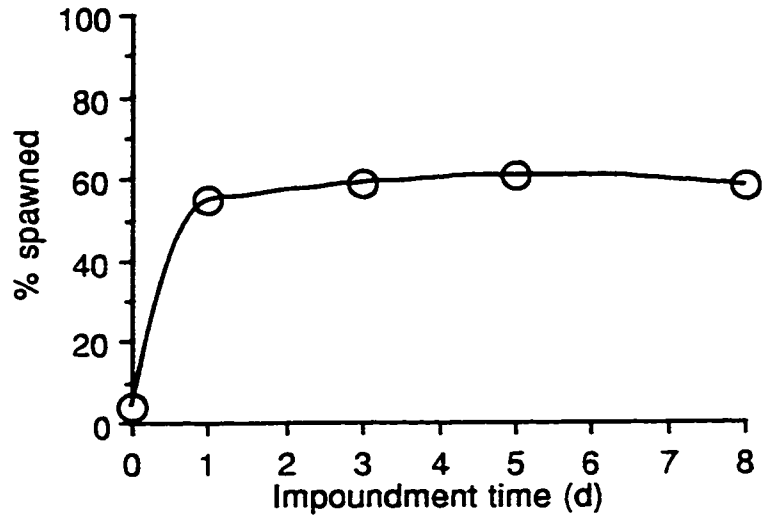

Figure 4. Spawning status of herring in the 1996 Puget Sound SOK pound.

Daily "n"= 100.

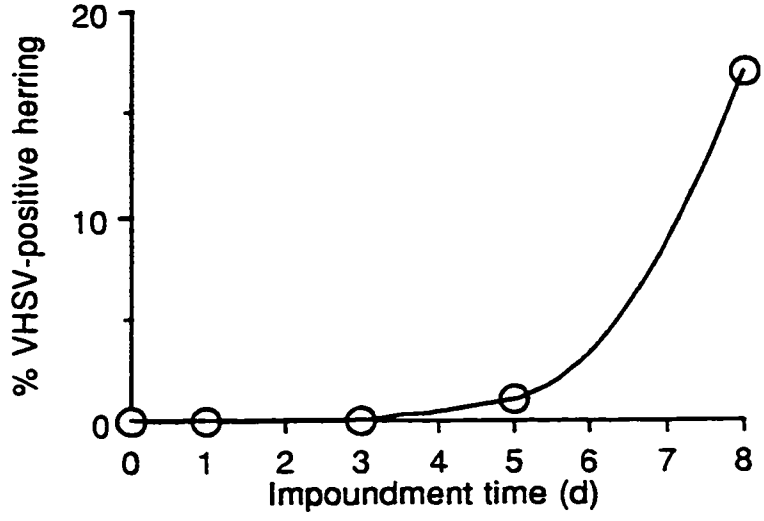

Figure 5. Prevalence of VHSV in impounded herring from Puget Sound, 1997.

Daily "n"= 100.

One water sample, taken from the outside edge of the pound on the last day of the study, tested positive for VHSV at 20pfu · ml^{-1} (Table 1). None of the other water samples tested positive for VHSV.

Table 1. VHSV in water from the 1997 Puget Sound pound.

Day #	Inside Pound	Outside Edge	Outside Pound
1	-	-	-
3	-	-	-
8	-	+ (20pfu · ml^{-1})	-

Prevalence of VHSV among impounded herring increased with the duration of impoundment during both the 1996 and 1997 SOK studies. However, the temporal occurrence of VHSV infection differed, with 17% of the herring becoming VHSV-positive after 8d of impoundment in 1997 (Figure 5) as compared to 12% after only 1-3d in 1996 (Figure 3). This temporal difference may have resulted from the fact that different stocks of herring were used. Spawning recruitment data from Washington state indicate that the Cherry Point herring stock used for the 1996 study experienced a significant population decline. The reason for this population decline remains unknown, but studies are ongoing to investigate whether herring eggs and larvae were exposed to oil, conceivably originating from petroleum refineries in the region. Petroleum has been reported to contribute to increased VHSV susceptibility among herring (Carls *et al* 1995); however, other researchers have found no such correlation (Kocan *et al* 1996a). Alternatively, the observed temporal difference may have resulted from slightly different handling techniques employed. Herring were captured at night, brailed into a large flow-through holding tank on

board the seining vessel, transported to the net pen, and wet brailed into the pound during the 1997 study. More stressful handling techniques were employed in the 1996 study including capture during the day and transport of the fish in the seine to the pound. The additional stress may have resulted in immunosuppression followed by decreased disease resistance and an earlier peak in viral prevalence. Differences in loading densities may also have existed between the 1996 and 1997 studies, however, this question is unanswered because the dimensions of the 1996 net pen were not determined.

Unlike the 1996 study, where high titers were found in 4/5 of the VHSV-positive herring, titers among the 1997 herring remained low throughout study, possibly as a result of lower stress or the delayed peak in viral prevalence. Had the 1997 experiment been continued for a longer time period, it is possible that viral titers may have either increased as a result of the low level infections manifesting into overt disease, or returned to near-0 levels due to an increased immune response resulting in cleared infections.

No waterborne VHSV was found initially from either the center of the pound or 3m outside the 1997 pound. Low levels of virus (20pfu · ml^{-1}) were detected in samples collected from the outside edge of the pound on d 8 (Table 1). Neither waterborne VHSV nor viral prevalence among impound herring (Figure 5) was correlated with active herring spawn (Figure 4). The low levels of waterborne VHSV were not surprising given the delayed peak in active infection and the relatively low VHSV titers (Table 1) found among the sampled

fish. Other factors contributing to the low levels of water-borne virus from this study include the following possibilities:

1) Low levels of VHSV were being shed when the first fish started to test positive for virus. These levels were sufficient to infect other fish within the pound by d 8.
2) The VHSV-positive herring in this study represented latent carriers of the virus. When they relapsed, little waterborne virus was shed.
3) Waterborne VHSV was shed at specific hours of the day when sampling of the water did not occur.
4) VHSV was shed throughout the study but was undetectable due to inadequate isolation techniques. Water sample from the 1997 study were immediately placed in MEM-5 and then frozen until assayed. Subsequent studies have shown that a 90% decrease in the amount of recoverable VHSV results following a freeze-thaw episode. Subsequent studies have also shown that larger serum concentrations in the medium are necessary to stabilize the virus (unpublished data).

1998 Puget Sound SOK Replication

The 1997 Puget Sound study was repeated in 1998 using the same herring bait pen.

Sexually mature, pre-spawn herring (0.9 metric tons) were captured approximately 1km north of Hood Canal in 2 purse seine sets from 20:00 - 22:30 hrs on 15 February, 1998 and brailed into a flow-through live tank on-

board the F/V *Jolly Rodger*. Herring samples for d 0 VHSV prevalences were taken from the live tank after all of the fish had been added. Herring were transported to the net pen in Mats Mats Bay at 00:30 hrs on 16 February and water samples for VHSV assay were taken from the on-board herring transport tanks. Herring were then wet brailed into the pen at a loading density of 1.6kg · m^{-3} and 30 fish · d^{-1} were sampled every other day through 10d. Kidney and spleen homogenates from all sampled herring were later assayed for VHSV.

Water samples (2ml) from both the center and 3m outside the pen were taken every other day, diluted 1:1 MEM containing 10% fetal bovine serum (MEM-10) and analyzed for the presence of VHSV using a plaque assay. Water was similarly sampled from the transport tanks on board the FV *Jolly Rodger* when the live herring were wet brailed into the net pen.

No VHSV was found in any of the herring tissue samples through 10d of confinement, even though nearly identical herring loading densities (1.6kg · m^{-3}) were added to the net pen during the 1997 and 1998 studies. Similarly, no waterborne VHSV was found in any of the water samples through 10d of confinement.

The lack of a detectable increase of VHSV in herring tissues during the 1998 study may have resulted from impoundment of a population of fish that was immune to the virus. The herring stock may have recovered from VHSV infections at an earlier age and developed specific immunity to later viral exposure. Alternatively, it is possible that no VHSV-positive herring were

introduced to the pounds; thus, there was no source of the virus. This speculation is supported by the absence of any detectable waterborne virus in both the transport tank and the pound throughout the study. However, it is contradicted by the fact that tissue samples collected from the net pen on d 10 tested positive for VHSV using a polymerase chain reaction (preliminary data), thereby indicating that a reservoir of virus was present within at least a small percentage of impounded herring. It is believed that the combination of few, if any herring carrying active infection, low or non-existent waterborne virus titers, and an immune population of impounded herring contributed to the lack of any detectable VHSV among this group of herring.

1997 Prince William Sound SOK Fishery

Viral hemorrhagic septicemia has been suggested to be a possible cause of a herring population decline, that began in 1992 in Prince William Sound (PWS), Alaska. Herring returns for spawning year 1993 were forecast at 130,000t, but only 30,000t returned to PWS. While other herring sac-roe fisheries in Alaska caught 100% of their projected catch during 1993, the catch in PWS was < 6% of projected estimates. Of those fish that did return, many failed to spawn and had visible hemorrhagic lesions. The population continued to decline in 1994 and 1995 and VHSV was isolated from 5% (12/233) of the herring (Marty *et al* 1998). As a result of this decline, the PWS herring fishery was closed in 1993 and remained closed through 1996. The fishery was re-opened in 1997 when the stock appeared to be recovering. A portion of the fish examined throughout the period of decline were found to carry VHSV, the systemic fungus *Ichthyophonus hoferi*, herring worms (Anisakidae), coccidians

of the liver and intestines, and myxosporidians in the kidney and gall bladder (Marty *et al* 1995). Of these pathogens, only VHSV and *I. hoferi* are known to cause mortality and significant pathological changes to laboratory-reared, specific pathogen-free herring (Kocan *et al* in press, Kocan *et al* 1997).

Results of the aforementioned Puget Sound studies combined with the herring population decline in PWS prompted investigation of the disease status of PWS herring. This project was designed to determine whether activities involved with the PWS closed pound SOK fishery were associated with increased VHSV infections among the captured and confined herring. An attempt was also made to determine the potential for spread of VHSV infections to free-ranging fish.

All closed pounds sampled during 1997 were located in the Port Fidalgo region (Figure 6) and designated Pound #1997-1 in Irish Cove, #2 at the mouth of Landlocked Bay, and #3 at the head of Landlocked Bay. Random samples (40 active herring · pound^{-1} · d^{-1}) were collected from each pound on consecutive days, beginning when the pounds were loaded and continuing until the fish were released 6-8d later. All herring in Pound #1 (4-5 metric tons) were caught at the head of Irish Cove in a single purse seine set and transported only a few hundred meters to the pound on 11 April (Tables 2 and 3). Herring in Pound #2 were captured from Two Moon Bay and transported 2.2 km across Port Fidalgo to the closed pound in two loads (04:00 hr on 13 April and 00:00 hr on 14 April). The limited number of fish added to this pound during the first load (≈2 metric tons) prevented d 0 sampling, so the first fish

sample was taken the following day (d 1) after an additional 16 metric tons were added. A 40 fish sample of moribund herring swimming listlessly on the surface of Pound #2 was removed on 18 April, corresponding to d 5 of impoundment. All herring in Pound #3 (≈ 0.9 metric tons) were loaded from one purse seine set at the head of Landlocked Bay on 11 April and transported only a few hundred meters to the pound. The limited number of fish in the pound prevented d 0 sampling without disturbing the kelp and the pound operators were subsequently unable to catch more herring. The kelp was later removed, pound operation ceased, and sampling of the fish was facilitated by lifting the sides of the pound to concentrate the herring. Tissues were also sampled from 2 free-ranging schools of spawning herring (40 herring · school^{-1}) in Landlocked Bay on 18 April.

Table 2. Sampling dates from the 1997 PWS pounds.

	Date (1997)								
	4/11	4/12	4/13	4/14	4/15	4/16	4/17	4/18	4/19
Pound 1	d 0	d 1	d 2	d 3	d 4	d 5	d 6	d 7	d 8
Pound 2			NS	d 1	d 2	d 3	d 4	d 5	d 6
Pound 3	NS	d 1	d 2	d 3	d 4	d 5	d 6	d 7	d 8

*NS= no sample

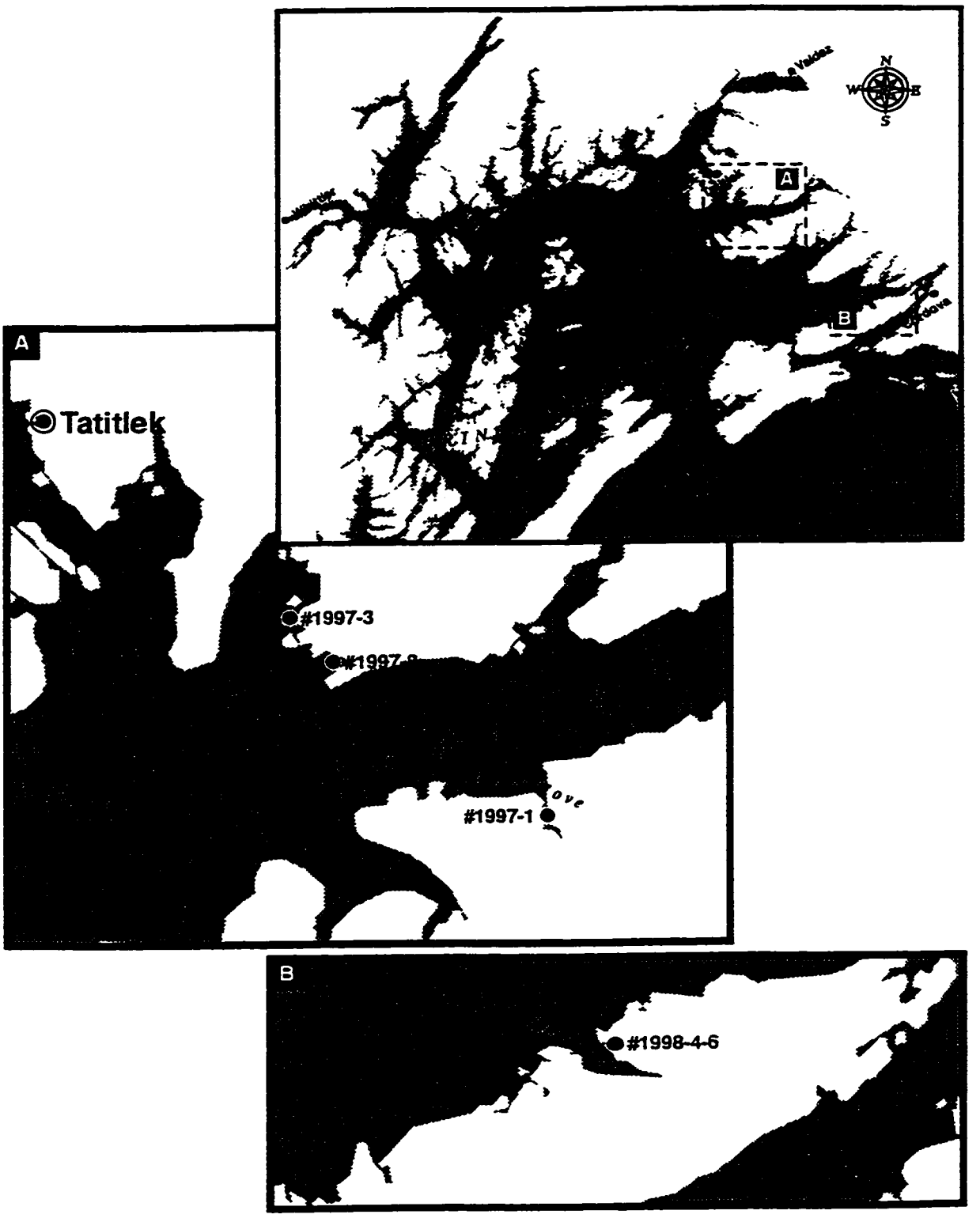

Figure 6. Prince William Sound including 1997 (A) and 1998 (B) study areas.

Herring sampled from each pound were placed in static water live-tanks and held for 2 to 8h prior to necropsy. Data collected from each fish included gender, age (from scales), weight, length, and VHSV presence. Spleen and kidney tissues were removed, placed in sealed plastic bags, packed on ice, and shipped to the Alaska Department of Fish and Game pathology laboratory in Juneau, AK, where samples were frozen at -80°C until assayed for VHSV (Meyers *et al* 1994). Herring tissues identified as VHSV-positive on primary isolation were separated from those that converted to positive following blind passage on EPC cells for an additional 7d.

Duplicate water samples for VHSV analyses were collected 1 m below the surface from both the center and 3m outside each pound every other day within 1h of slack tide. Samples were diluted 1:1 in MEM-10, shipped to Cordova, AK where they were frozen at -80 °C, then shipped to the University of Washington (Seattle, WA) and finally to the Marrowstone Island Field Station where they were assayed for virus. The 1997 water samples underwent 3 partial freeze-thaw events prior to assay. Daily water quality data including dissolved oxygen and water temperature were recorded from inside and outside the pounds at slack tide (± 1h).

All three pounds were of different dimensions and were loaded at various densities (Table 3). Pound #2 was the most crowded, contained the most fish (>18 metric tons) and lacked corner weights to hold the sides of the pound down. Thus, the sides floated up and the herring were crowded to the surface. Predators including kittiwakes, eagles, and sea lions captured herring from this

pound throughout the study period. Pound #3 was the deepest pound, and contained the fewest (0.9 metric tons) and youngest herring (mean age 4yr).

Table 3. Physical and biological characteristics of the 1997 PWS pounds.

	# permit holders*	Mean herring age (yr)	Estimated biomass (metric ton)	Pound size (m) (L · W · D)	Fish density ($kg \cdot m^{-3}$)
Pound #1	1	7	4-5	5.5 · 11.6 · 4.6	17.0
Pound #2	3	7	>18	17.7 · 8.5 · 6.1	19.6
Pound #3	4	4	0.9	6.1 · 7.3 · 9.1	2.2

* each permit holder is allowed 5.67 metric tons of herring

Active spawning inside the pounds continued for the first 1-4d of confinement (Figure 7). Although no d 0 sample was removed from Pounds #2 and #3, it is believed that most of the herring entering the pound had full gonads. Pound operators filled the pounds only with pre-spawn herring and a milt cloud was observed emanating from Pound #3 shortly after the fish were introduced on d 0.

Prevalence of VHSV in herring from all pounds peaked after 1-4d of confinement and declined to low levels after 5-6d. The prevalence of VHSV among herring from Pound #1 was 5% on d 0 and peaked significantly higher, at 20% ($p < 0.025$) on d 4 (Figure 8a). Significantly greater prevalences of virus ($p < 0.05$) from d 1 in Pound #2 were found on d 2-5 (Figure 8 b). Prevalences of VHSV in moribund and apparently healthy fish from Pound #2 were similar on d 5 (12.5% and 10% respectively). No day-0 samples were taken from Pound #3, but VHSV prevalence peaked at 25% after 2d of confinement (Figure

8c), then dropped significantly on d 3-4 and 6-8 ($p < 0.05$). No VHSV was detected in 80 tissue samples collected from free-ranging, actively spawning herring in Landlocked Bay on 18 April. A slight increase in prevalence (0-15%) was noted in tissues that were passed blind, probably reflecting fish with very low titers (< 50 pfu · g^{-1}) that were infected while in holding tanks prior to necropsy.

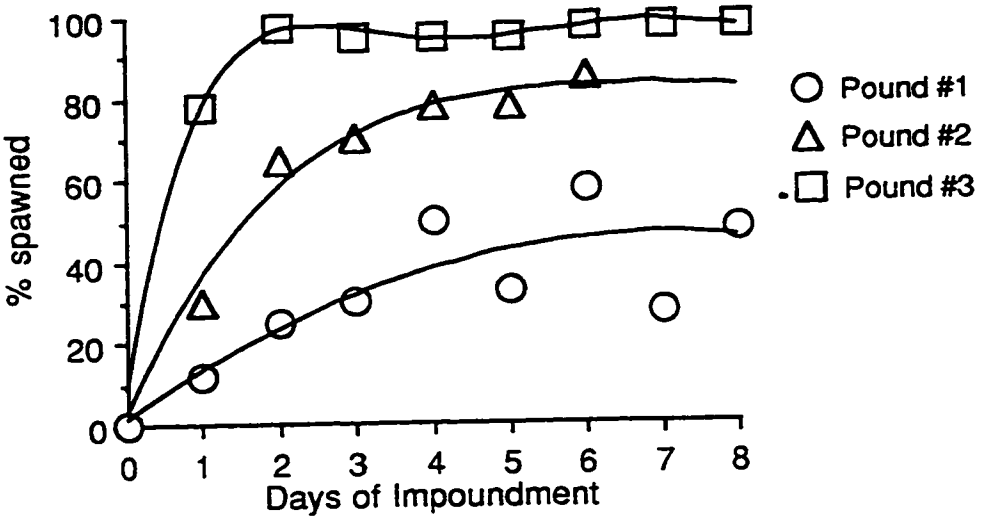

Figure 7. Spawning status of the impounded herring from PWS, 1997. Daily "n" = 40 for each pound. Lines represent 2, 4, and 5 (respectively) order polynomial regressions.

Herring age distributions in Pounds #1 and 2 were nearly identical, consisting primarily of 9yr-olds (40%), while Pound #3 contained primarily 3yr-olds (60%) and only 5% 9yr-olds (Figure 9). VHSV prevalence was associated primarily with the 4 to 6yr-olds (≈20% in each yr class) and decreased with age

(Figure 10). VHSV prevalence was significantly greater in females (11.8%, 51/431) than in males (7.8%, 48/614; $p < 0.05$).

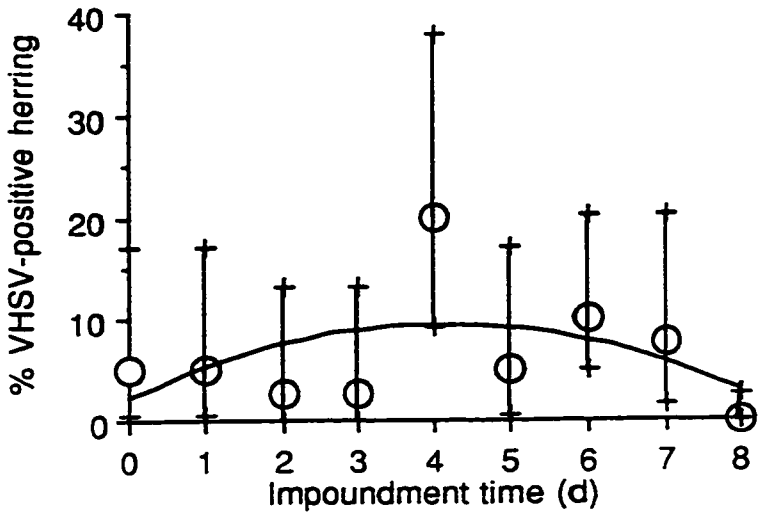

Figure 8a. Pound #1997-1.

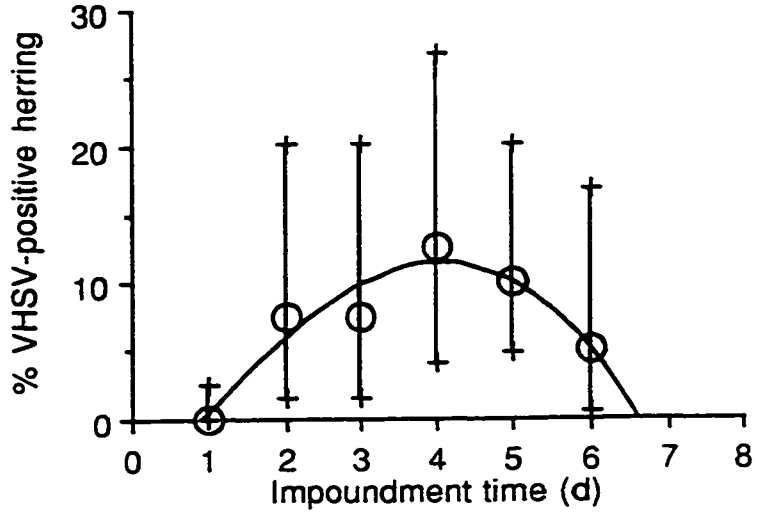

Figure 8b. Pound #1997-2.

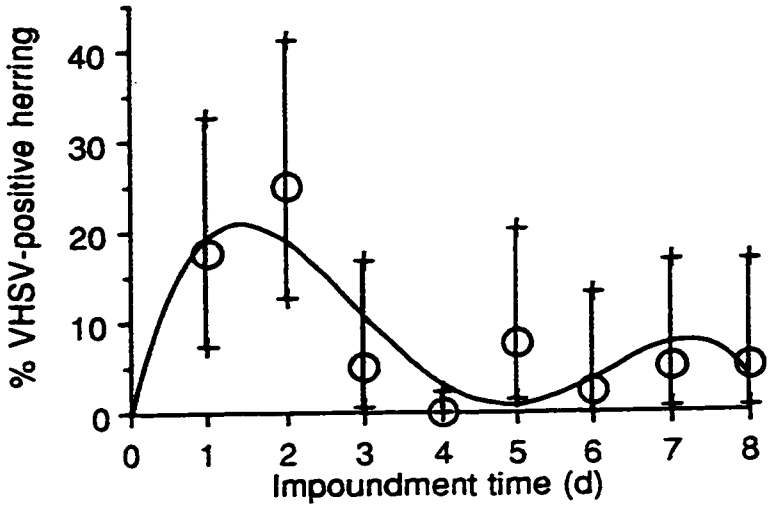

Figure 8c. Pound #1997-3.

Figures 8a-c. Prevalence of VHSV in herring from PWS Pounds #1997-1-3. Lines represent 2, 3, and 4 (respectively) order polynomial regressions. Error bars indicate 95% confidence limits with daily "n"=40.

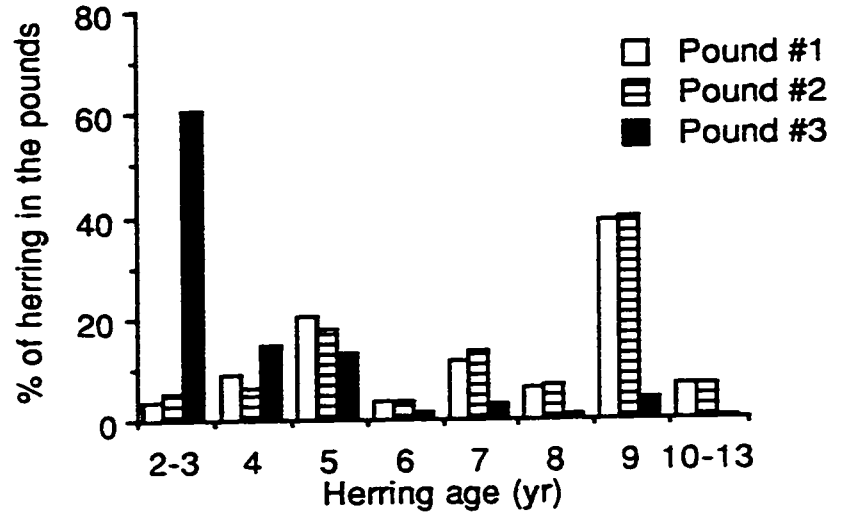

Figure 9. Age structure of impounded herring from PWS, 1997 (from scales).

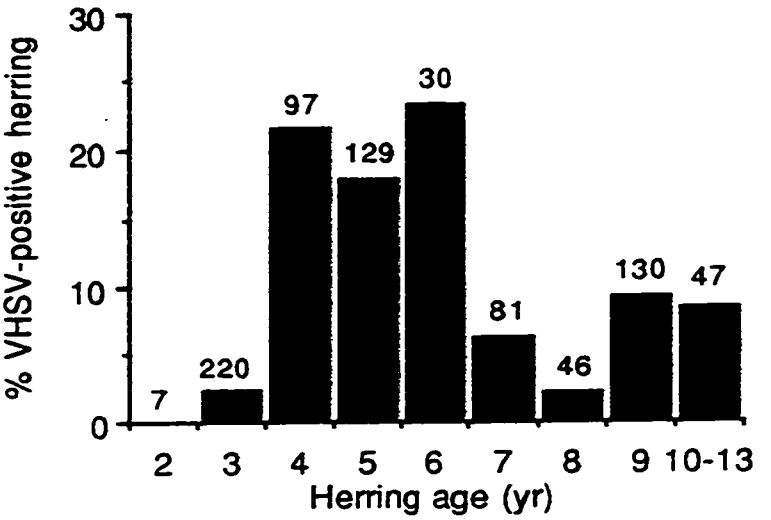

Figure 10. Prevalences of VHSV in herring age classes from the 1997 PWS SOK pounds. Numbers represent "n."

None of the water samples taken during the 1997 season assayed positive for VHSV. Measured DO remained at or above 12mg · L^{-1} throughout the 1997 sampling period in PWS, but was about 1mg · L^{-1} lower inside the pounds than outside (Tables 4-6), and remained at near-optimal levels for adult herring survival throughout the study period.

Table 4. Water quality from PWS Pound #1997-1.

Date	Sampling time (hrs)	DO inside (mg · L^{-1})	DO outside (mg · L^{-1})	Temp. (°C)
4/10 (pre-fish)	08:00	14.00	-	4.6-4.9
4/12 (d 1)	11:40	13.98	15.09	-
4/14 (d 3)	14:15	14.95	15.25	5.7
4/16 (d 5)	10:10	14.50	14.80	5.7
4/18 (d 7)	11:45	12.21	13.65	5.2

Table 5. Water quality from PWS Pound #1997-2.

Date	Sampling time (hrs)	DO inside ($mg \cdot L^{-1}$)	DO outside ($mg \cdot L^{-1}$)	Temp. (°C)
4/10 (pre-fish)	08:00	14.05	-	4.9-5.1
4/13 (d 0)	13:45	15.20	14.97	6.1
4/15 (d 2)	13:50	14.74	14.89	6.0
4/17 (d 4)	10:50	13.93	14.60	5.1
4/19 (d 6)	06:35	12.94	13.16	5.8

Table 6. Water quality from PWS Pound #1997-3.

Date	Sampling time (hrs)	DO inside ($mg \cdot L^{-1}$)	DO outside ($mg \cdot L^{-1}$)	Temp. (°C)
4/10 (pre-fish)	07:30	13.43	-	4.7
4/12 (d 1)	18:00	15.65	15.45	-
4/14 (d 3)	10:15	15.14	15.25	6.1
4/16 (d 5)	06:30	14.50	14.85	5.8
4/18 (d 7)	16:25	13.84	13.75	6.5

VHSV prevalence data from this study indicated that impounded *C. pallasi* underwent epizootics during confinement. Infection rates increased in herring after introduction to the pounds, peaked, and then decreased to near background levels by d 6 to 8. Temporal occurrence and magnitude of the VHSV prevalence peaks varied among the pounds and were possibly correlated with differences in the age classes of impounded herring. The highest (17.5-25%) and earliest (1-2d) prevalence peak occurred in Pound #3, which contained the youngest age classes of herring, primarily 4yr olds (Figure 9). Younger herring may be

more susceptible to VHSV infections than older herring (Figure 10), possibly as a result of increased immune response among older fish. Additionally, impoundment was believed to contribute to active VHSV infections because none of the free-ranging spawning herring (0/80), presumably captured from the same school as the fish in Pound #3, tested positive for VHSV on the same day that 5% (2/40) of the impounded herring tested positive for VHSV.

A large number of dead herring had accumulated in the bottom of Pound #2 by the end of the SOK season. Thereby requiring that the webbing be cut to dump the dead fish. Not all of this mortality was attributable to VHS, since viral prevalence in moribund herring was only 15% (6/40) on d 5, similar to the viral prevalence of 12.5% (5/40) detected among live herring from the pound on the same day. The high loading density in Pound #2 (Table 3) may have contributed to the elevated mortality rate among fish in that pound, but was not correlated with VHSV prevalence. The herring density in Pound #2 was 10x greater than Pound # 3, yet peak viral prevalence was greater in the latter pound. Direct comparison of these two pounds is limited due to the different age class structures of herring loaded into each.

No indication of poor water quality was found in any of the pounds (Tables 4-6). Dissolved oxygen concentrations remained above 12mg $\cdot$ L^{-1} near slack tides, a time when water exchange should have been at a minimum. These levels of DO are more than adequate for adult herring survival.

Although no waterborne virus was detected around the pounds, some samples testing VHSV-negative may have contained virus. Laboratory studies have demonstrated an approximate 90% decrease in VHSV titers in water following freeze-thaw cycles (unpublished data). Due to the remote location of the study site, water samples from the 1997 PWS study underwent 3 partial freeze-thaw cycles prior to assay. These fluctuations probably resulted in a 1,000-fold decrease in the number of recoverable virus particles in the samples and may explain why no VHSV was detected in the water. Additionally, water samples were diluted 1:1 in MEM-10 and subsequent studies have shown that higher levels of fetal bovine serum are needed to stabilize the virus (unpublished data).

Female herring may be more susceptible to active VHSV infections than males. Greater prevalence of VHSV in impounded females than males ($p < 0.05$) was consistent with a similar finding from free-ranging herring in the Montague Island region of PWS during the same period (Marty et al 1998). Also, younger herring (4-6 r olds) were more prone to VHSV infections than older herring (Figure 10), a finding also consistent with observations from free-ranging herring in the Montague area.

1998 Prince William Sound SOK Fishery

A second closed pound SOK study was conducted in 1998 in order to clarify results from the previous year and to investigate possible routes of VHSV transmission among the impounded fish. The epizootiological course of viral infection within the SOK pounds may start with a small percentage of captured

herring carrying and shedding low-levels of virus. Increased prevalence of viral infection may result from exposure of susceptible fish to unnaturally high waterborne VHSV titers inside the pounds. This hypothesis would be supported by the detection of waterborne VHSV near the pounds, yet water samples from the 1997 study failed to test positive.

Modifications to the 1997 study design were implemented for the 1998 study in order to address problems encountered the previous year. Herring were placed on ice immediately after capture rather than held in static live tanks as was done in 1997. In addition, herring were necropsied within 2h of capture and tissues were immediately placed in culture medium (MEM-5). Pilot laboratory studies demonstrated that VHSV titers remained unchanged for 2wk in MEM containing 20% fetal bovine serum (MEM-20) at 4 °C. Consequently, water samples were diluted 1:1 in 2X MEM-20. Additionally, water samples were stored on ice but not frozen prior to assay.

A low herring biomass and poor weather in Port Fidalgo delayed the opening of the 1998 closed pound SOK season until after the primary herring spawn had occurred in the region. Fishery participants subsequently moved east to Windy Bay, on the north side of Hawkins Island, where a large school of pre-spawn herring was detected by spotting planes and a gill net test fisher. All pounds used in the 1998 study, designated #1998 (4, 5, and 6), were operated in Windy Bay (Figure 6) and were loaded with herring captured less than 1km away, near the head of the bay, on 16 April. Herring in Pounds #5 and #6 were loaded from single purse seine sets while Pound #4 was filled with herring

captured in 2 sets, made approximately 3 hours apart. Random samples of 40 herring · pound^{-1} · d^{-1} were removed on consecutive days, beginning when the pounds were loaded, and continuing until the fish were released 8 d later (Table 7). Moribund herring from Pound #4 were sampled daily after their appearance on d 2.

Table 7. Sampling dates from the 1998 PWS pounds.

	Date (1998)								
	4/16	4/17	4/18	4/19	4/20	4/21	4/22	4/23	4/24
Pounds #4-6	d 0	d 1	d 2	d 3	d 4	d 5	d 6	d 7	d 8

A large natural herring spawn occurred in Port Gravina (Fig. 1) on 11 April, 1998 and was followed by migration of the post-spawn herring to Two Moon Bay the next day. Free-ranging, post-spawn herring (40 fish · d^{-1}) were sampled with either a cast net or purse seine on consecutive days following the major spawning event. Another natural spawning event was observed in the head of Windy Bay on 21 April, 1998, corresponding to d 5 of the impoundment period, and 40 herring were sampled from the actively spawning school with a cast net.

All sampled herring were immediately placed on ice and transported to the F/V *Miss Emily* where necropsies were performed. Spleen and kidney tissues were removed within 1.5h of capture, placed in sealed plastic bags containing MEM-5, then shipped on ice to Seattle, WA where they were frozen at -80 °C until being assayed for VHSV at the Marrowstone Island Marine Station. Data collected from each fish included gender, age (from scales),

gonad fullness (Marty *et al* 1998), weight, length, and VHSV titer in spleen and kidney pools.

Duplicate daily water samples for VHSV analyses were collected 1 m below the surface from both the center and 3m outside each pound within 1h of slack tide. Water for VHSV isolation was also sampled from the center of two actively spawning free-ranging herring schools, including one in Fish Bay (Figure 6) on 11 April and another from the head of Windy Bay on 21 April. Water for VHSV assay was collected from the middle of two closed purse seines containing post-spawn herring in Two Moon Bay on 12 April. All water samples were passed through a 0.45µm filter, a 2ml aliquot of the filtrate was diluted 1:1 in 2 x MEM-20, and stabilized samples were stored on ice until plaque assays were performed less than 2 weeks later at the Marrowstone Island Marine Research Station. Daily water quality measurements collected from inside and outside each pound included temperature, salinity (refractometer), and dissolved oxygen.

Active herring spawn inside Pounds #4 and 5 progressed with confinement time until d 5, when 67.5% of the fish had spent gonads (Figure 11). No major spawning occurred in Pound #6 until d 8 when 47.5% had spent gonads. On day 2, 70% of the moribund herring sampled from the surface of Pound #4 had spent gonads , when only 27.5% of the live fish from the same pound had spawned. The gradually increasing spawning profile of impounded 1998 PWS herring contrasted with the spawning behavior of free-ranging herring (Figure 12), impounded Puget Sound herring (Figures 2 and 4), and

herring from the 1997 PWS pounds (Figure 7) where most of the gamete release was completed within 1-2d.

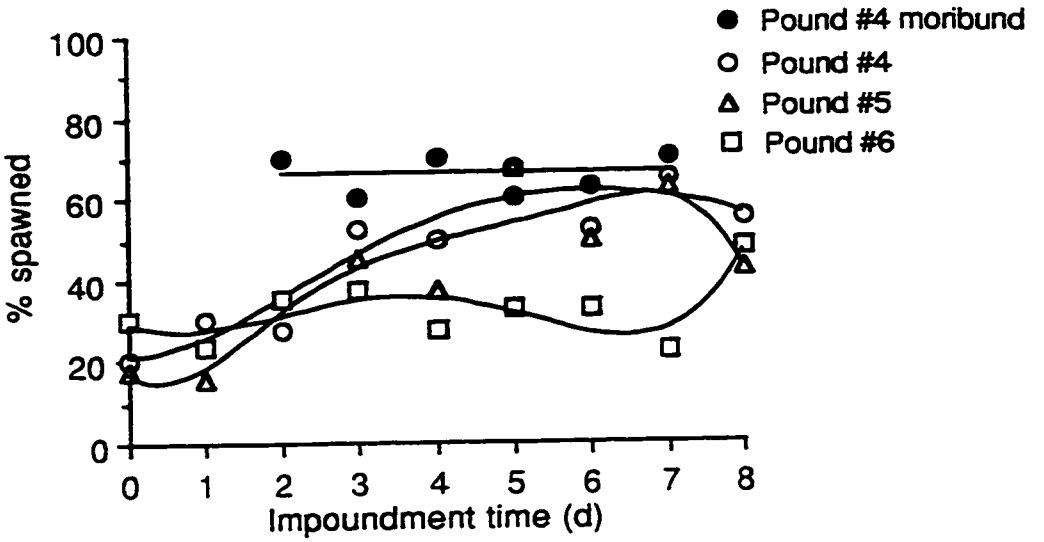

Figure 11. Spawning status of the impounded herring from PWS, 1998. Daily "n" for active fish from each pound = 40; n's for daily moribund herring samples are as follows: d 2 (20), d 3 (10), d 4 (10), d 5 (20), d 6 (40), d 7 (20). Curves represent 5th order polynomial regressions.

Scavengers and predators including kittiwakes, murrs, murrletts, eagles, and sea lions frequented Pound #4 continuously throughout the daylight hours, presumably attracted to the pound by the high density (61.4kg · m^{-1}). Pound #6 contained the lowest density (25.1kg · m^{-3}) and produced the least product yield (836kg).

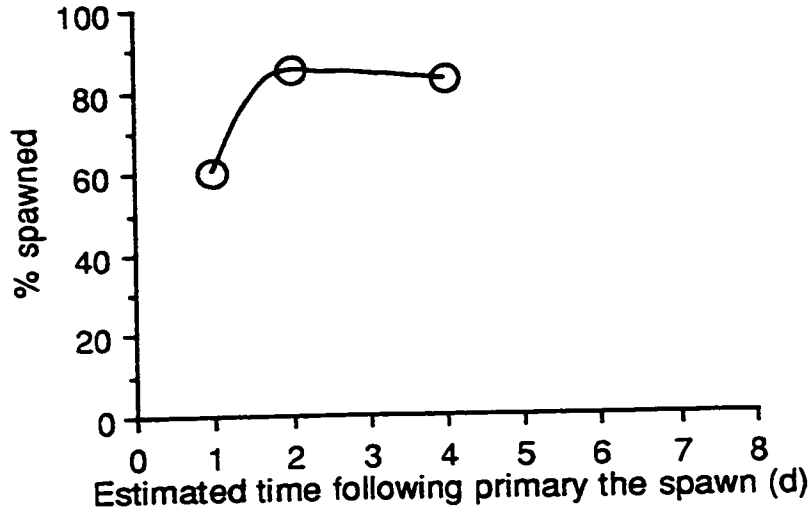

Figure 12. Spawning status of the free-ranging herring from PWS, 1998.

Table 8. Physical and biological characteristics of the 1998 PWS pounds.

	# permit holders*	Estimated biomass (metric tons)	Pound size (m) (L · W · D)	Herring density ($kg \cdot m^{-3}$)	Product weight (kg)
Pound #4	3	18	5.5 · 11.6 · 4.6	61.4	1102
Pound #5	4	24	9.7 · 8.5 · 9.1	32.0	1448
Pound #6	4	16	6.1 · 17.1 · 6.1	25.1	836

* each permit holder is allowed 5.67 metric tons of herring

Herring of nearly identical age distribution were loaded into each of the 1998 PWS pounds. Age classes were dominated by 3yr olds (65-68%) with few 7+yr olds present (Figure 13). Prevalence of VHSV was highest (60%) in the 1-2yr olds and steadily decreased with age to 12.2% in the 7-10yr olds (Figure 14). Only 2/16 herring in the 7-10yr age classes tested positive for VHSV and both were sampled near the end of the study (d 6 and 7), when VHSV

prevalence within the pounds peaked. Unlike impounded herring from the 1997 study, prevalence of VHSV in males (36.4%, 246/675) and females (33.7%, 247/733) was not significantly different ($p > 0.20$).

Figure 13. Age structure of impounded herring from PWS, 1998.

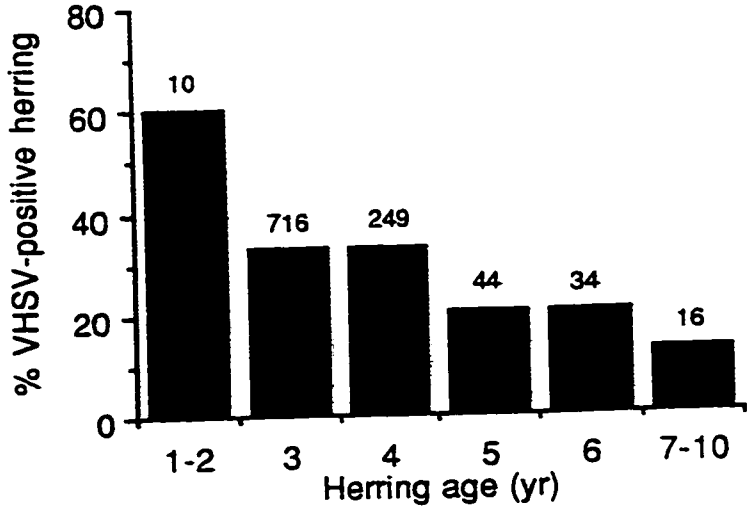

Figure 14. VHSV prevalence with herring age in the 1998 PWS SOK pounds (from scales). Numbers represent "n."

Initially low prevalences of VHSV were followed by peaks between 57-87% after 6-8d of confinement in all three study pounds (Figures 15a-c). Prevalence of VHSV in Pound #4 was low (10-22.5%) from d 0-5, but increased significantly ($p < 0.001$) to 55-87.5% on d 6-8 (Figure 15a). Viral prevalence in herring from Pound #5 increased significantly ($p < 0.02$) after d 2 and 4-8 of confinement. A bimodal VHSV prevalence pattern was indicated by the significant increases ($p < 0.02$) on d 2 and 6 (Figure 15b). Prevalence of VHSV in herring from Pound #6 increased from 7.5% on d 0 to 57.5% on d 8, with significant elevations ($p < 0.02$) occurring from d 5-8 (Figure 15c).

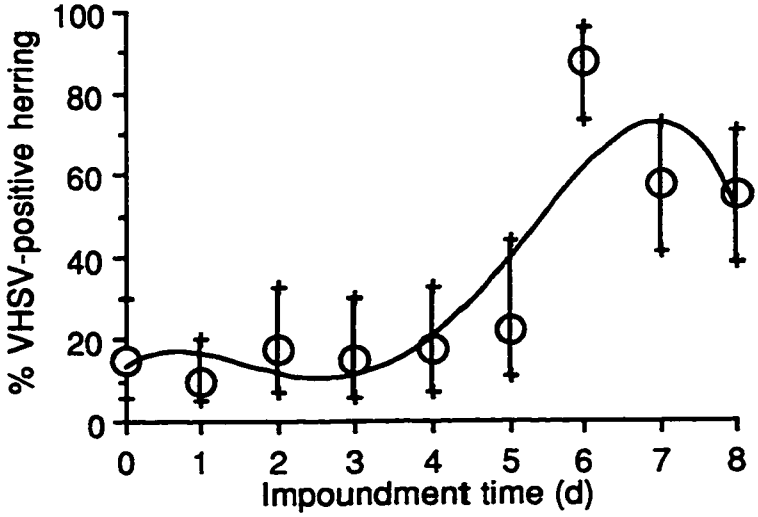

Figure 15a. Pound #1998-4.

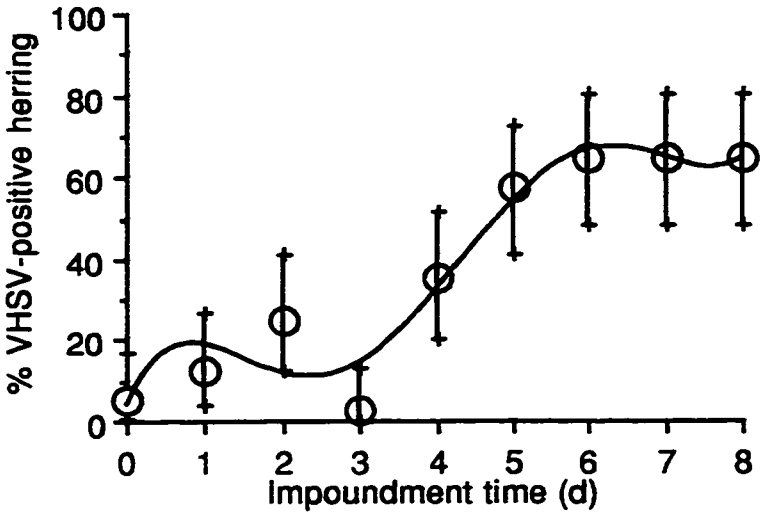

Figure 15b. Pound #1998-5.

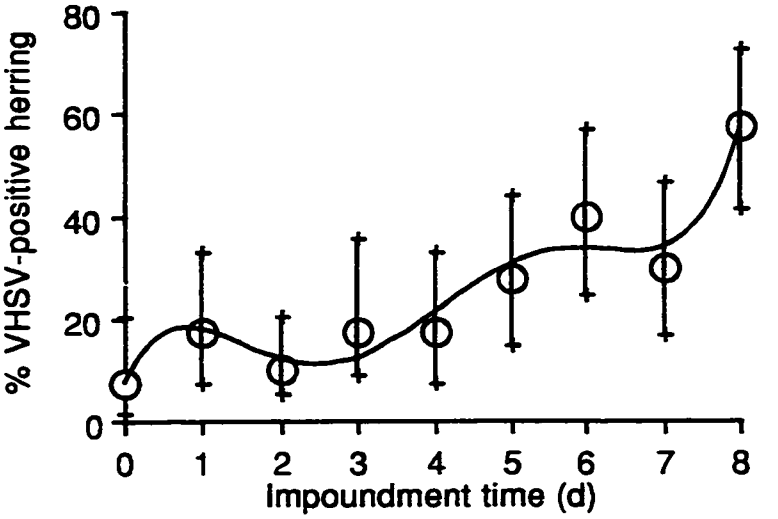

Figure 15c. Pound #1998-6.

Figures 15(a-c). Prevalence of VHSV in impounded herring . Curves represent 5th order polynomial regressions. Error bars indicate 95% confidence limits with daily "n" = 40.

Nearly all moribund herring (90-100%) sampled from the surface of Pound #4 had detectable levels of VHSV(Fig. 16). No moribund herring were sampled from the pound on d 0 and 1 because so few were present, and none were sampled on d 8 when the pounds were emptied.

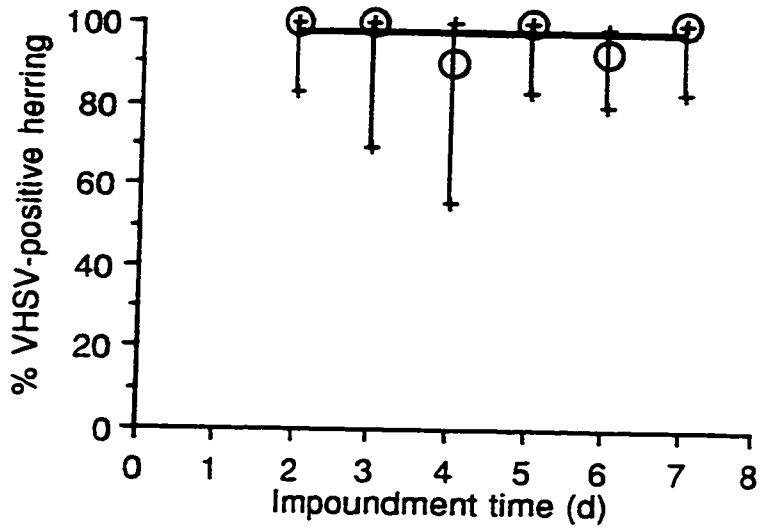

Figure 16. Prevalence of VHSV in moribund herring from PWS Pound #1998-4. Error bars indicate 95% confidence limits.

Prevalence of VHSV in free-ranging, post-spawn herring from Two Moon Bay decreased from 17.5% 1 d after the major spawning event to 7.5% after 4d (Figure 17) but the change was not significant ($p > 0.10$). No pre-spawn, free-ranging herring were sampled from this school on d 0 because the spawning event was not anticipated. However, a sample of free-ranging, spawning herring from Windy Bay indicated that only 5% (2/40) tested positive for VHSV. No free-ranging herring were sampled from Two Moon Bay 3 d post spawn due

to inclement weather and samples were not taken after d 4 because sampling activity was moved to the SOK fishery located in Windy Bay.

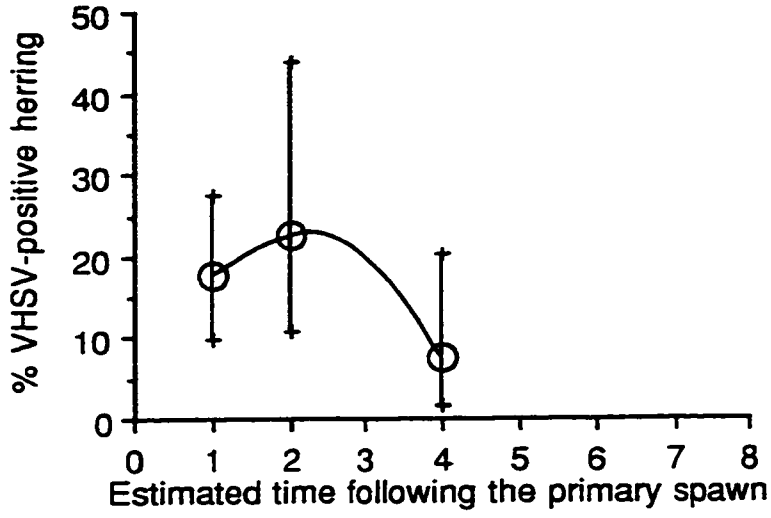

Figure 17. Prevalence of VHSV in free-ranging herring from PWS, 1998. Error bars indicate 95% confidence limits. Daily "n" = 40.

Significantly more impounded herring ($p < 0.02$) had high VHSV titers (> 10,000pfu · g^{-1}) than low titers (400-9,999pfu · g^{-1}) on d 8 in Pounds #4 and #5 and d 7 in Pound #6 (Figures 18a-c). However, significantly more moribund herring ($p < 0.001$) from Pound #4 had high titers than low titers on each sampling day (Figure 19). The percentages of free-ranging post-spawn herring from Two Moon Bay with high and low titers were not significantly different. The two VHSV-positive herring from the actively spawning school in Windy Bay had low titers.

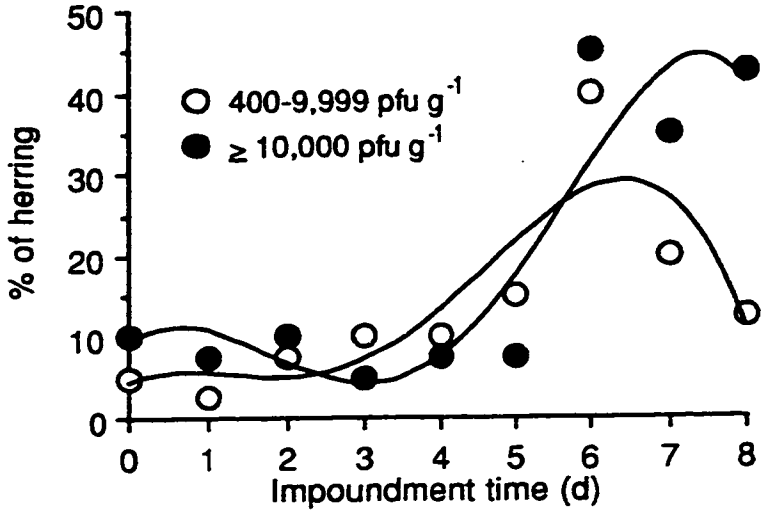

Figure 18a. Pound #1998-4.

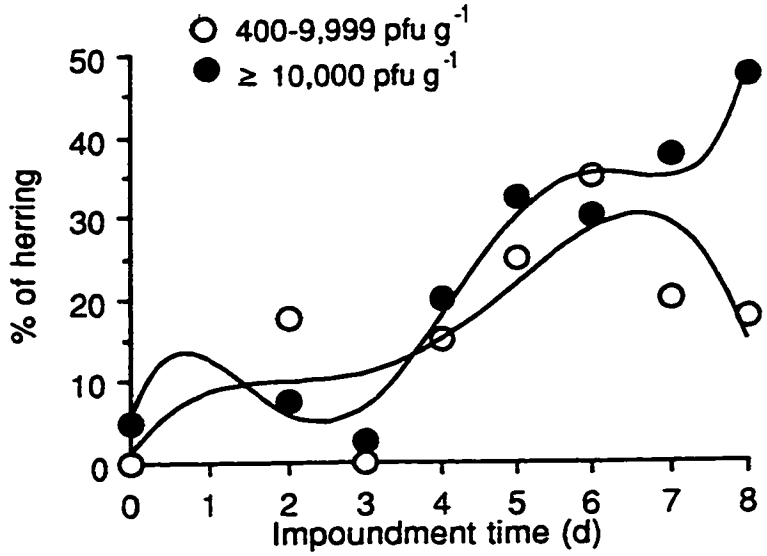

Figure 18b. Pound #1998-5.

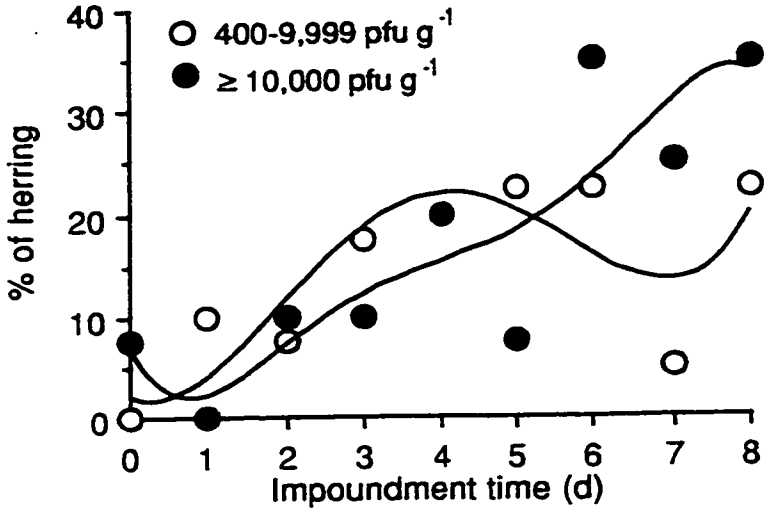

Figure 18c. Pound #1998-6.

Figures 18a-c. VHSV tissue titers in impounded herring, daily n=40. Curves represent 5th order polynomial regressions.

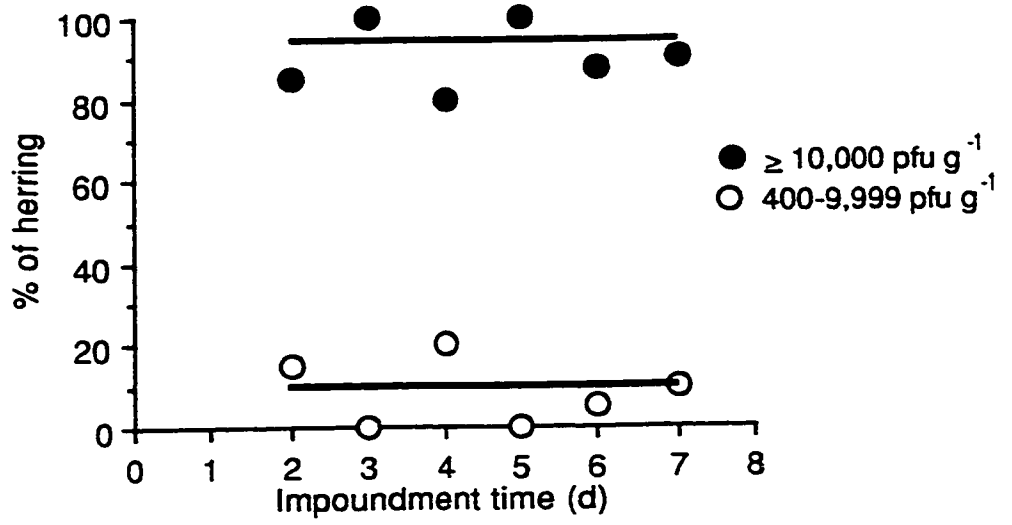

Figure 19. VHSV tissue titers in moribund herring from PWS Pound #1998-4.

Measured DO inside the pounds never fell below 8.2mg · L^{-1}, but was generally 1mg · L^{-1} less than that outside the pounds (Tables 9-11). A trend of increasing DO and water temperature was observed daily both inside and outside the pounds, possibly attributable to photosynthesis from a minor bioluminescent algae bloom that moved through Windy Bay during the study period.

Table 9. Water quality from PWS Pound #1998-4.

Date	Sampling time (hrs)	Slack tide (hrs)	DO inside (mg · L^{-1})	DO outside (mg · L^{-1})	Temp. (°C)
4/17 (d 1)	11:55	11:43	8.3	9.3	5.2
4/18 (d 2)	19:25	-	8.6	9.5	5.2
4/19 (d 3)	13:20	13:34	9.4	9.7	5.5
4/20 (d 4)	14:45	14:45	10.0	10.3	6.1
4/21 (d 5)	16:05	16:00	11.8	13.0	6.4
4/22 (d 6)	17:05	17:03	11.2	13.3	7.4
4/23 (d 7)	18:00	17:57	11.0	13.8	7.7
4/24 (d 8)	06:15	-	12.7	13.4	6.8

Table 10. Water quality from PWS Pound #1998-5.

Date	Sampling time (hrs)	Slack tide (hrs)	DO inside (mg · L^{-1})	DO outside (mg · L^{-1})	Temp. (°C)
4/17 (d 1)	12:20	11:43	9.5	9.6	5.2
4/18 (d 2)	19:40	-	8.5	8.7	5.2
4/19 (d 3)	13:30	13:34	9.2	9.6	5.5
4/20 (d 4)	15:00	14:45	10.3	10.3	6.1
4/21 (d 5)	16:15	16:00	12.4	12.8	6.4
4/22 (d 6)	17:15	17:03	12.9	13.2	7.4
4/23 (d 7)	18:10	17:57	12.9	14.0	7.7
4/24 (d 8)	06:30	-	12.9	13.7	6.8

Table 11. Water quality from PWS Pound #1998-6.

Date	Sampling time (hrs)	Slack tide (hrs)	DO inside ($mg \cdot L^{-1}$)	DO outside ($mg \cdot L^{-1}$)	Temp. (°C)
4/17 (d 1)	12:35	11:43	8.9	9.2	5.2
4/18 (d 2)	19:45	-	8.2	7.9	5.2
4/19 (d 3)	13:40	13:34	9.2	9.8	5.5
4/20 (d 4)	15:10	14:45	9.9	10.3	6.1
4/21 (d 5)	16:25	16:00	12.3	12.7	6.4
4/22 (d 6)	17:25	17:03	12.8	13.8	7.4
4/23 (d 7)	18:20	17:57	12.6	13.7	7.7
4/24 (d 8)	06:50	-	12.9	13.6	6.8

No VHSV was recovered from water samples collected from inside the pounds prior to introduction of herring on d 0. However, low concentrations were found inside each pound as early as 1d and outside the pounds as early as 2d after herring were introduced. Waterborne VHSV concentrations inside each pound followed a bimodal pattern, with an initial, smaller peak occurring within 1-4d, and a second, larger peak occurring just prior to release of the fish from the pounds on d 8 (Figures 20a-c). Concentrations of VHSV in the water continued to increase through the final sampling date inside each pound, reaching levels as high as 700pfu $\cdot$ mL^{-1} in Pound #4 after 8d of confinement.

Waterborne virus concentrations 3m outside Pound #4 increased to over 200pfu $\cdot$ mL^{-1} after 8 d of confinement, but remained below 20pfu $\cdot$ mL^{-1} outside Pounds #5 and 6 through the final sampling day (Figures 21a-c).

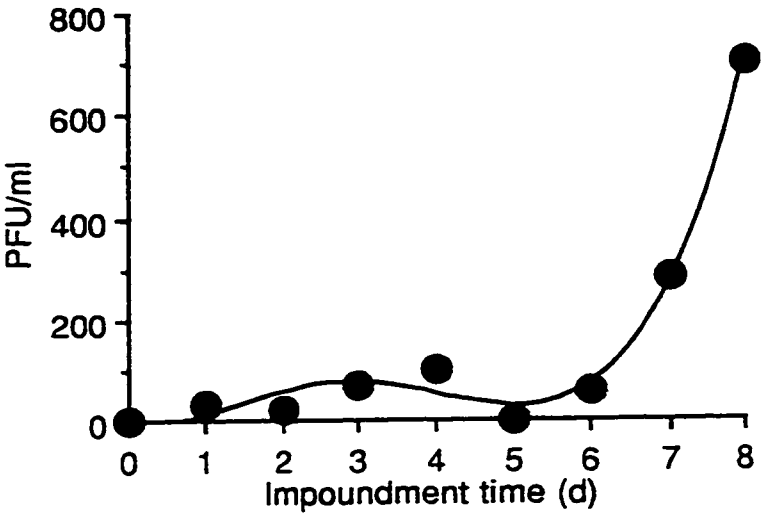

Figure 20a. Pound #4.

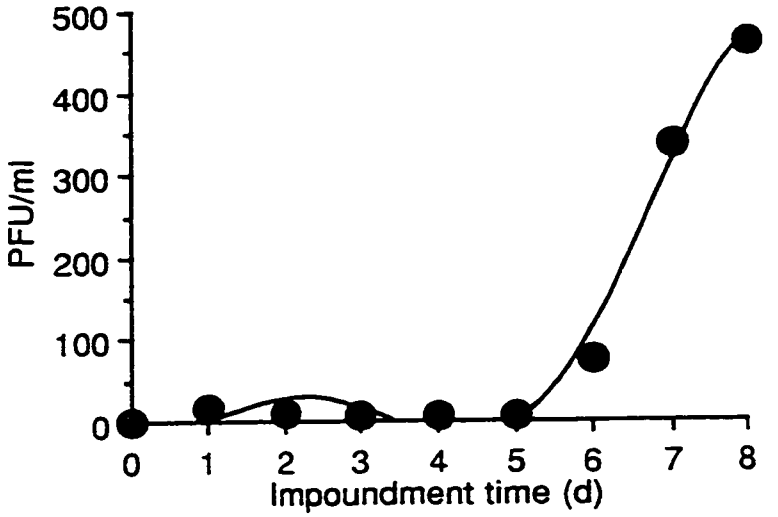

Figure 20b. Pound #5.

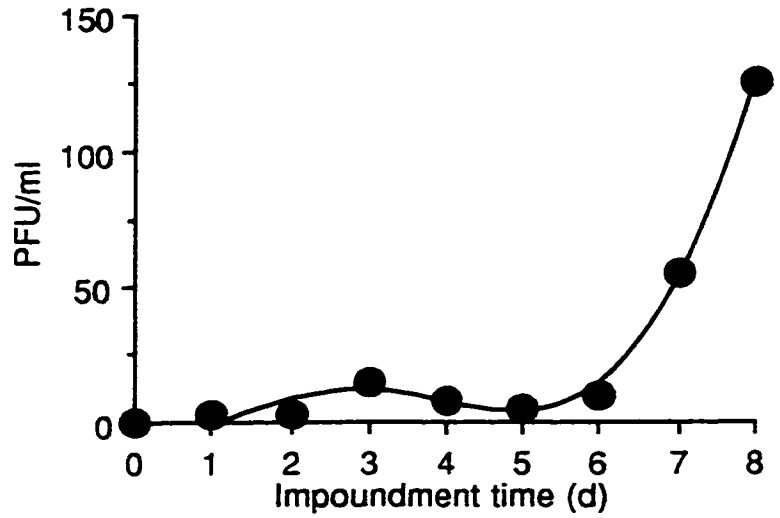

Figure 20c. Pound #6.

Figures 20 (a-c). Waterborne VHSV titers inside 1998 PWS pounds. Daily concentrations are reported as duplicate means. Lines represent 5th order polynomial regressions

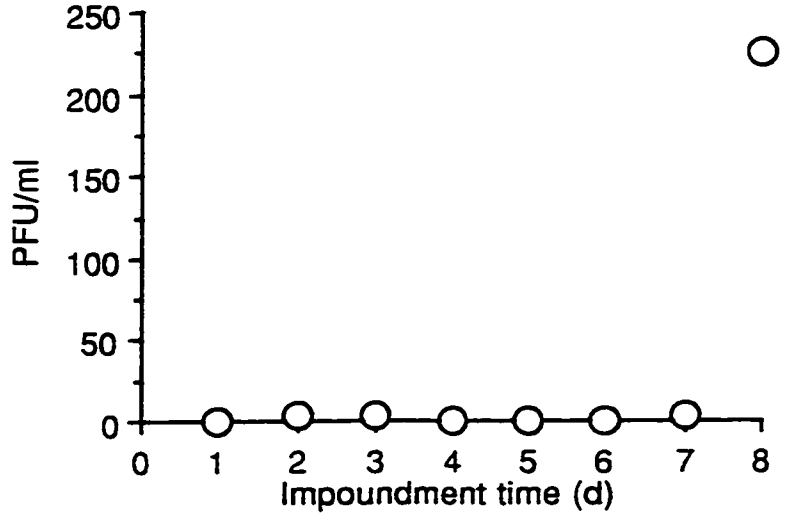

Figure 21a. Pound #4.

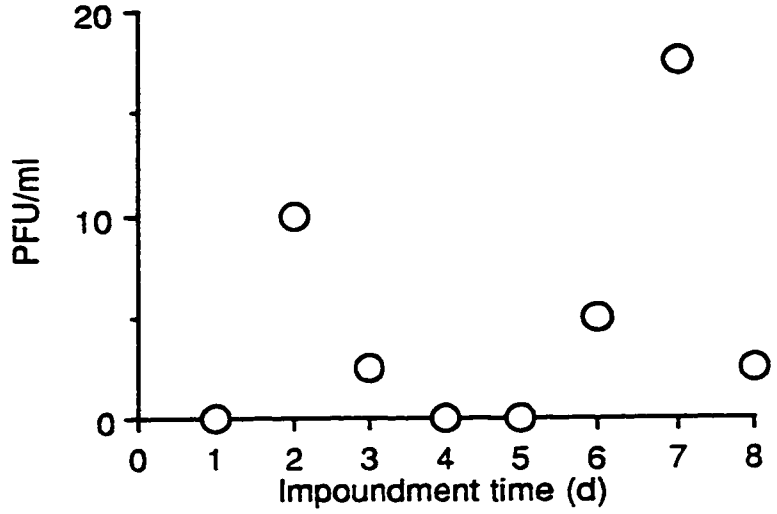

Figure 21b. Pound #5

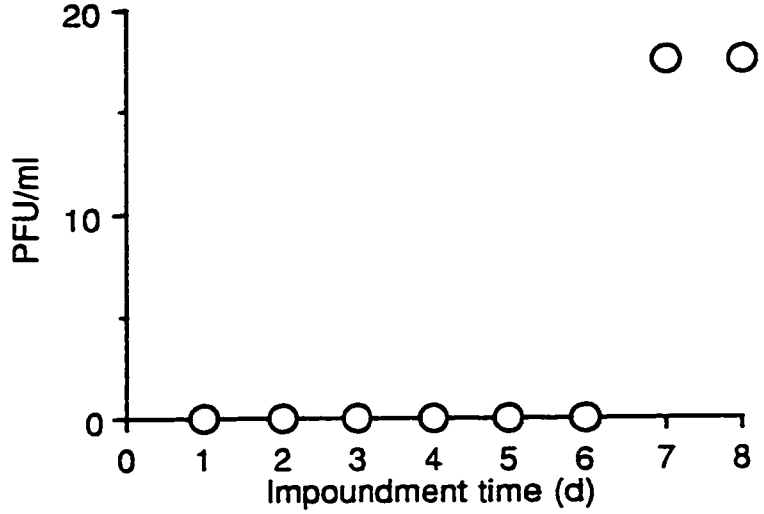

Figure 21c. Pound #6.

Figure 21 (a-c). Waterborne VHSV titers 3 m outside PWS pounds. Daily concentrations are reported as duplicate means.

Low titers of VHSV were found in two water samples collected near free-ranging herring, but virus concentrations never reached levels as high as those detected near the pounds. Water from a closed purse seine in Two Moon Bay containing 1d post-spawn herring tested positive for VHSV with 15pfu · mL^{-1}. However, no VHSV was detected in water collected 30min later from another closed purse seine set in the same area. Water collected from the middle of a school of actively spawning, free-ranging herring in Windy Bay tested positive for VHSV with concentrations of 5pfu · mL^{-1}. However, no VHSV was recovered from water sampled in the middle of another actively spawning, free-ranging herring school in Fish Bay.

VHSV prevalence among impounded herring from all three SOK pounds followed a pattern in which initially low levels increased to as high as 87.5% after 6-8d of confinement (Figures 15a-c). Semblance of a bimodal pattern of virus prevalence was observed in Pound #5, conceivably resulting from the following scenario. A small percentage of herring entering the pounds with low levels of infection (Figure 15b). Once the fish were impounded, the low-level infections increased to a clinical state and diseased herring shed virus which was transmitted via the water to susceptible herring which then become infected, initiating a second peak of prevalence. Among infected herring, some developed high titers while others either recovered from infection or died from lack of adequate immune response. Mortality data provide evidence for the former explanation since moribund herring began swimming listlessly on the surface of Pound #4 after only 2d of confinement with 100% testing positive for VHSV (Figure 16) and a large number having high titers (Figure 19). These

herring must have entered the pound with low level infections which converted to active, fatal disease after 2d. The second increase in viral prevalence probably resulted from exposure of susceptible fish in the pound to waterborne VHSV which was shed by the initial round of low-level infection. Evidence for the second step in the scenario is provided by a parallel bimodal pattern of VHSV concentration in the water, initially peaking between d 3-4 with a second peak occurring at the end of the sampling period on d 8 (Figures 20a-c). VHSV prevalence in herring did not follow a bimodal pattern in Pounds #4 or #6, where only the large peak was present at the end of the study period. Lack of a significant initial peak in these pounds may have been masked by the slightly larger percentage of fish testing positive for VHSV on d 0 (17.5% and 10%) compared to Pound #2 (5%).

VHS was probably the primary cause of mortality within the pounds since nearly all moribund herring sampled tested positive for VHSV (Figure 16) and many had high virus titers (Figure 19). Viral prevalences and tissue titers were higher in moribund fish than apparently healthy herring from the same pound. Few, if any, moribund herring were found on the surface of the pounds prior to 2d of confinement, indicating that the processes of capture and transport of the fish to the pounds did not cause acute mortality from scale loss or other handling stressors. Mortality among impounded herring was directly correlated with loading density. Few moribund fish were observed on the surface of the less crowded Pounds (#5 and #6), but large numbers of moribund herring occurred on the surface of Pound #4 from d 2 through d 7. The majority of the moribund herring sampled from the surface of Pound #4 had already spawned

(Figure 11). VHSV prevalence in dead herring was not nearly as high in 1997 as in 1998, possibly as a result of the younger age classes utilized in 1998 (3yr olds vs. 7yr olds).

Free-ranging spawning herring captured from the head of Windy Bay on 21 April, 1998 were of the same age structure and were captured from the same location as those in the pounds, suggesting that the free-ranging herring were from the same school as the impounded herring captured on 16 April. Thus, the disease status of the impounded herring on d 5 (22.5% - 57% VHSV-positive) could be compared to the disease status of the free-ranging, spawning herring (5% VHSV-positive). Based on such a comparison, it is apparent that the increased prevalence of VHSV observed among captive fish was a result of activities associated with SOK impoundment rather than a natural disease progression which occurs among free-ranging, spawning herring. Similarly, VHSV prevalence in a different school of free-ranging, post-spawn herring appeared to decrease with time (Figure 17) where as VHSV prevalences in impounded herring increased.

The highest VHSV prevalence (60%) occurred in the youngest impounded herring (1-2yr olds) and steadily decreased with age to 12.5% (2/16) in herring over 6 yrs old. Only 2/16 herring in the 7-10yr age classes tested positive for VHSV and both were detected at the end of the study (d 6 and 7) when the prevalence peaked within the pounds. Such a pattern of decreasing VHSV prevalence with age may indicate the presence of an increased number of immunocompetent survivors among older herring,

permitting older fish to clear the infection before a clinical state is reached. Younger fish may not have had sufficient exposure to the virus to produce protective antibodies.

Gamete release by impounded herring (Figure 11) differed from that of free-ranging herring which tended to expel all their gametes in a single spawning event (Figure 12). Impounded herring in Pounds #4 and #5 spawned more slowly and retained more gametes than did free-ranging spawning herring. The percentage of spawn-outs in Pound #6 remained low, around 30%, through d 7 and never increased to more than 50% (Figure 11). Failure of fish in this pound to spawn may have been a combined result of lower quality herring loaded into the pound (30% spawn-outs), fewer herring added (16 metric tons), and lower loading density (25kg $\cdot$ m^{-3}) when compared to the other pounds (Table 8).

High herring densities within the pounds did not have an adverse effect on water quality. Pound # 4 had nearly twice the herring density as Pounds #5 and #6 (Table 8) yet the water quality within all three was similar (Tables 9-11), with DO inside the less crowded Pound #6 being slightly lower. Additionally, water quality inside and outside the pounds was similar, with measured DO's inside the pounds never differing much more than 1mg $\cdot$ L^{-1} from the water 3m outside. Measured DO's and water temperatures inside each of the pounds at slack tides remained near levels considered optimal for herring survival, indicating that occlusion of the pound webbing by herring eggs did not significantly decrease water quality inside the pounds.

Waterborne VHSV was detected inside each of the pounds as early as 1d after herring were added, and titers increased throughout the study period. Peak water-borne VHSV concentrations within all pounds and 3m outside Pound #5 were higher than those reported to produce lethal infections among juvenile laboratory-reared, specific pathogen-free herring with 1hr bath exposures (Kocan *et al* 1997). Detectable peaks in water-borne VHSV concentrations outside Pounds #4 and #6 on d 8 may have been missed by a hydrologic sampling artifacts. Even though an attempt was made to sample water at slack tides, slight currents circulated around the pounds and the water samples from these pounds may have been taken on the upstream side of an eddy. The data indicate the potential for spread of VHSV to fish outside the pounds via water-borne transport. Such a scenario is further supported by observations of free-ranging herring being attracted to the spawn emanating from within the closed pounds.

Detection of waterborne VHSV near schools of free-ranging herring indicates that wild, unimpounded herring naturally shed low levels of virus. Waterborne VHSV in Two Moon Bay was isolated from the inside a partially full purse seine containing 1d post spawn herring. This particular seine was set in shallow water and it hung on the bottom for over an hour before being freed and reeled in. Virus concentrations of 15pfu $\cdot$ ml^{-1} were found in water samples from inside this seine, yet only 15% (6/40) of herring tested positive for VHSV with titers $< 2 \cdot 10^4$pfu $\cdot$ g^{-1}. However, no virus was detected in water sampled 30 min later from another purse seine, set approximately 100m away, even though

viral prevalence among the captured herring was 20% (8/40). Waterborne VHSV ($\approx 5 pfu \cdot ml^{-1}$) was found near free-ranging herring during an active spawning wave which occurred in Windy Bay. Only 5% (2/40) of the herring within this spawning school tested positive for VHSV at very low titers ($< 5 \cdot 10^{3} pfu \cdot g^{-1}$). Conversely, water sampled from free-ranging, spawning herring in Fish Bay failed to test positive for VHSV. These data indicate that waterborne VHSV was present in some aggregations of free-ranging herring exhibiting low level infections.

Chapter 3: Controlled Laboratory Experiments Using Confined Herring

Controlled laboratory experiments were conducted in order to better understand the mechanisms responsible for epizootics which occurred in the net pens. Herring in these studies were captured in Puget Sound and transferred in live tanks to the Marrowstone Island Marine Station (Nordland, WA) where they were placed in flow-through tanks. Variables such as fish density and rate of water flow could be adjusted to address the following questions which arose from the field studies:

1) Do infection patterns of VHSV observed in herring held in net pens also occur in laboratory tanks?
2) Does herring age have an effect on the severity of infection?
3) Does density within the tanks have an effect on the magnitude of VHSV prevalence?
4) Do all VHSV-positive herring develop overt infections after confinement?
5) Is waterborne virus shed from a few VHSV-positive herring carrying low level infections and subsequently spread to other herring?

Susceptibility with Age

Wild herring from three schools of 0yr, 1yr, and 2+yr (spawning) age classes, were purse seined and transported to laboratory tanks where they were sampled at various intervals. Tissues were assayed for VHSV in order to monitor the progression of VHSV infection among confined fish.

Juvenile herring (≈7 months of age) were purse seined on 19 September, 1996, brailed to a live tank on board the F/V *Jolly Rodger*, transported to a net pen, and kept in the pen until a representative subset of approximately 200 herring were removed 2d later and transported to flow-through tanks. Prevalences and titers were monitored daily by sampling 30-40 apparently healthy herring from the tanks on d 2, 7, 8, and 13 of captivity (including time in the net pen). Moribund and dead herring were removed from the tanks and handled in a similar manner to the healthy fish. Daily prevalences and titers of VHSV were determined for all sampled herring by plaque assay of whole body (minus head and tail) homogenates.

Prevalence of VHSV in herring increased from 3.3% 2d after capture to 89.5% after 7d of captivity and decreased to 10% after 10d (Figure 22). Although few herring tested positive for VHSV at 2 d after capture, the transport tanks used to move the fish from Mats Mats Bay to Marrowstone Island contained water that tested positive for VHSV after <1hr transport time. High level s of infection, > 10,000pfu · g^{-1}, predominated in the confined herring on d 7 ($p < 0.001$), but the proportions of VHSV-positive herring with high and low titers were similar on the other sampling dates. A similar pattern of VHSV prevalence was observed among the dead and moribund herring sampled from the tanks; beginning at 0% on d 2-4, increasing to 100% by d 8-10 and returning to 0% after d 14 (Figure 23). Nearly all dead and moribund herring which tested positive for VHSV had high virus titers at each sampling date.

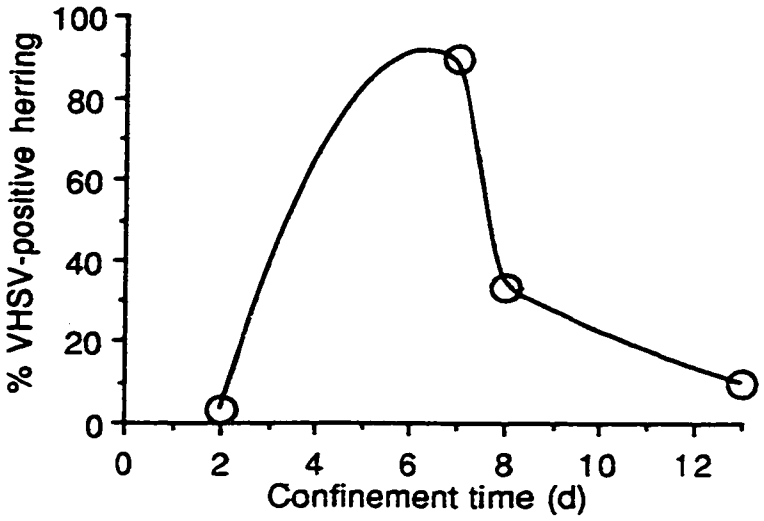

Figure 22. Prevalence of VHSV in 0-yr Puget Sound herring sampled from laboratory tanks. Daily "n" = 30 except d 7, where n = 38.

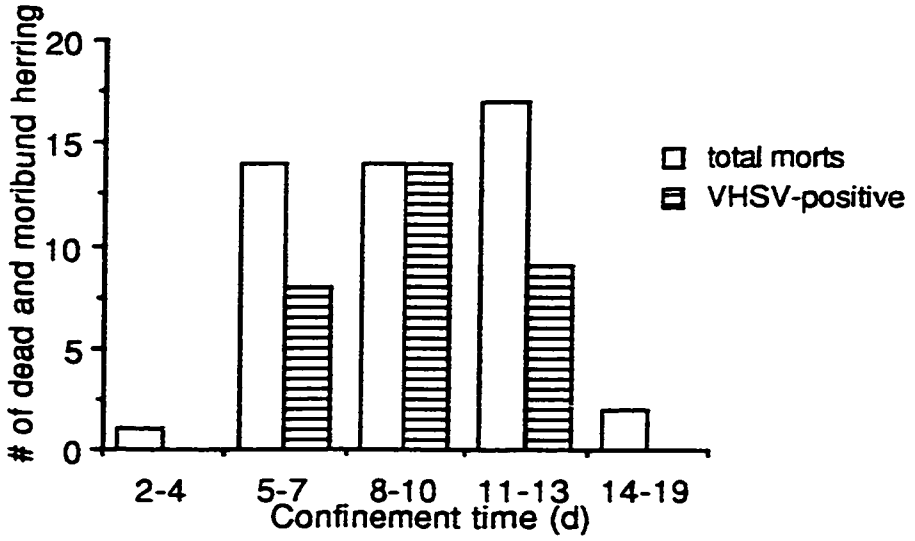

Figure 23. Prevalence of VHSV in dead and moribund 0-yr Puget Sound herring sampled from laboratory tanks.

A second group of herring, predominately 1+yr of age, was purse seined on 12 July, 1996 and handled similarly to the juvenile herring described above

except that the fish were held in the net pen 5d prior to transport to laboratory tanks. Daily VHSV prevalences and titers from the fish in the tanks were monitored by sampling 30 active herring from the tanks at 5d intervals. Moribund and dead herring were removed from the tanks when they appeared and were analyzed similarly to the sampled fish. VHSV prevalence and titers were determined from all sampled herring by plaque assay of viscera homogenates.

Viral prevalence peaked at 20% after 5d of confinement, decreased to 0% after 10d, then increased slightly to 3.3% (1/30 sampled fish) on d 15 and d 20 (Figure 24). All but one of the herring which tested positive for VHSV during the prevalence peak on d 5 had low virus titers. The number of dead and moribund herring collected from the tanks decreased from a high of 16 after 5-9d of confinement to a low of one after 25-29d with the VHSV prevalence in the dead fish decreasing from 68.7% initially to 0% by 25-29d (Figure 25). An increase in the number of dead herring occurred after 30d but was not associated with VHSV infection.

A third group of herring, predominately pre-spawn 2+yr of age, was purse seined on 15 February, 1998, from the same group of herring described in the 1998 Puget Sound SOK replication. An initial sample of 100 fish (d 0) were sampled from the purse seine ≈30min after capture and the remainder were held overnight in a net pen until transported to tanks the following day. Prevalence of VHSV was monitored by sampling 30 live herring from the tanks

every other day for 10d and analyzing viscera homogenates with a plaque assay.

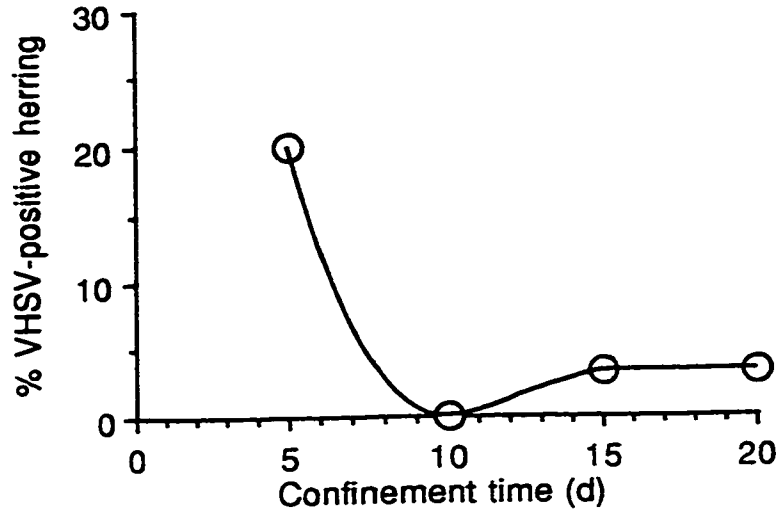

Figure 24. Prevalence of VHSV in 1+yr Puget Sound herring sampled from laboratory tanks. Daily "n" =30.

No herring sampled from the tanks tested positive for VHSV throughout the 10d study period. These results were similar to those obtained from herring held in the net pen for the same duration. Lack of active VHSV infection with this group of herring indicated that the herring were probably immune to VHSV. It is highly improbable that herring of this age had not been previously exposed to VHSV.

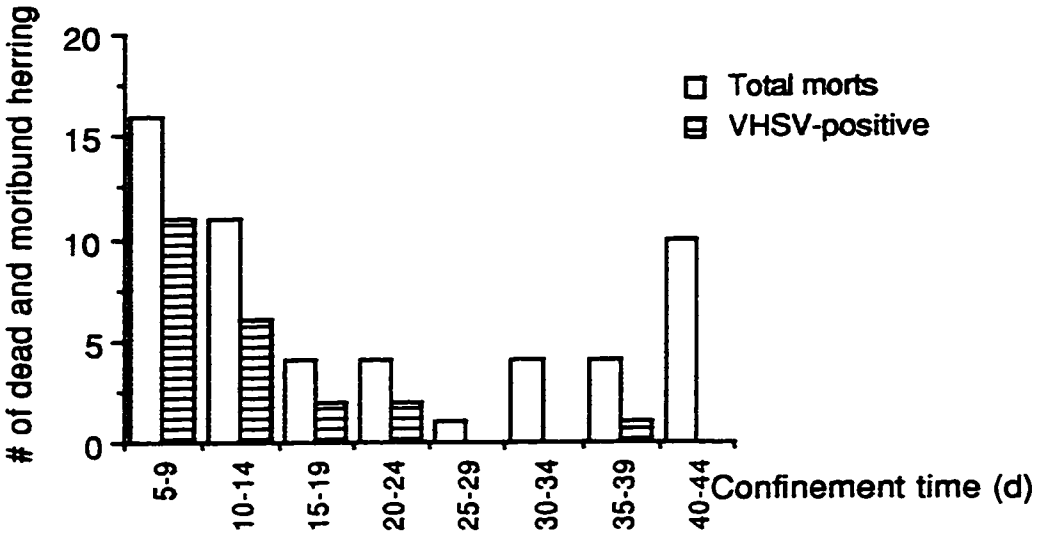

Figure 25. Prevalence of VHSV in dead and moribund 1+yr Puget Sound herring sampled from laboratory tanks.

Data from these confinement studies demonstrated that VHSV epizootics may occur among herring held in laboratory tanks. Additionally, the magnitude, duration, and severity of overt disease was inversely correlated with herring age. Younger herring were more susceptible to VHSV infection with viral prevalence among 0-yr fish approaching 100% and high titers predominating (Figure 22). Viral prevalence decreased with age, peaking at 20% in the 1+yr fish (Figure 24) and 0% in the 2+yr fish. Additionally, a high proportion of the juvenile herring died with high titers (Figure 23), whereas older fish tended to either recover from infection (Figure 19b) or never develop detectable tissue titers. These results suggest the presence of an enhanced immune response among older herring which may have been developed though prior exposure to the virus. An immune population of older herring would have a selective advantage since younger herring, which are unable to produce neutralizing

antibodies to VHSV may die from the disease or sicken and are more easily captured by predators. However, young herring which are able to survive initial infection and clear the virus may then produce antibodies which protect them against recurrent infections later in life.

Crowding

The factors controlling the epizootiology of VHS in captured and confined herring are not completely understood. It is unclear whether stressors associated with confinement, such as capture, transport, and crowding, are sufficient to suppress the immune system and thereby thus increase susceptibility to infection, or whether confinement simply increases the potential for exposure of susceptible herring to a sufficient dose of waterborne virus shed by a small percentage of carriers.

This experiment was designed to determine whether herring densities were correlated with the magnitude of VHSV infections observed among confined herring. Similarly, an attempt was made to determine the source of infection among the confined herring; i.e. whether some or all herring carry VHSV in a latent state or whether active infections develop after exposure of susceptible fish to waterborne virus during confinement.

Immature herring (primarily 1yr of age) were purse seined from Puget Sound on 17 September, 1998, brailed into a live tank on-board the F/V *Jolly Rodger*, transported to Mats Mats Bay, and wet brailed into a net pen. Herring were held in the pen for 2d and were then transported to the Marrowstone

Marine Station on 19 September when an initial sample of 30 fish was taken for VHSV assay. The remaining fish were sorted into four 70gal, flow-through tanks with duplicate densities of 10 and 50 herring · $tank^{-1}$. All tanks were monitored for dead fish, and surviving herring were sacrificed after 7d. Viscera from all fish were assayed for VHSV by plaque assay.

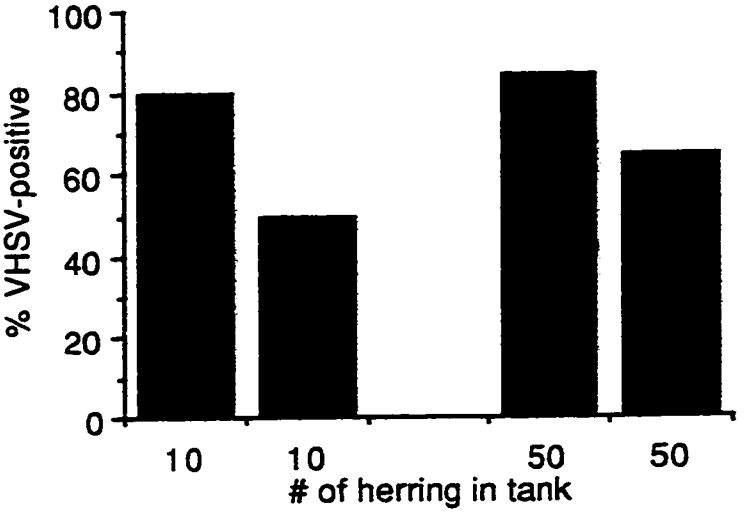

Figure 26. Prevalences of VHSV in Puget Sound herring confined for 7d in laboratory tanks at different densities.

Prevalence of VHSV increased from 3.3% (1/30) on d 0 (prior to introduction to the tanks) to 50-80% in tanks containing 10 herring and 65-84% in tanks containing 50 herring after 7d (Figure 26). Viral prevalences in herring from tanks containing different densities were not significantly different ($p > 0.50$). The data indicated that density had little effect on the magnitude or severity of VHSV infection among confined herring since viral prevalences in both high and low density tanks were similar. It is suggested that other factors

associated with capture and confinement were responsible for increased VHSV prevalences.

Relapsed vs. Newly Acquired Infections

The next experiment was designed to determine whether: 1) a high proportion of wild herring carry inapparent infections of VHSV which are then activated by stressors associated with capture and confinement, or 2) only a small percentage of wild herring carry and shed virus which is then spread through the water to other susceptible herring during capture and/or confinement.

Juvenile herring (0 yr) from a herring ball in Puget Sound were captured using a cast net on 8 August, 1997 and transported in live tanks to the Marrowstone Marine Station where 100 fish were immediately sacrificed. Several hundred fish were placed into a 70gal, flow-through community tank, and 40 were isolated in 10gal, flow-through aquaria (1 fish · $tank^{-1}$). Water from the herring transport tank was sampled for VHSV assay. Dead and moribund herring were collected as they appeared in both the aquaria and the community tank. Survivors in the aquaria were sacrificed after 7d of confinement, as were 60 fish from the community tank. Whole body (minus head and tail) homogenates from all sampled herring were later analyzed for VHSV by plaque assay.

None of the herring sampled immediately after capture (0/100) tested positive for VHSV; however, virus was detected in the transport water, indicating

that a small percentage of the captured, wild herring (< 1%) were VHSV-positive and shedding virus. The viral prevalence in the community tank increased to 77% (46/60) in the active herring and 100% (84/84) in the dead and moribund herring only 7d later (Figure 27). However, only 3% (1/34) of the surviving herring held in individual aquaria tested positive for VHSV after 7d of isolation; all six moribund and dead herring from the aquaria tested positive for VHSV (Figure 28). The total number of isolated herring testing positive for VHSV was only 7/40 (17.5%).

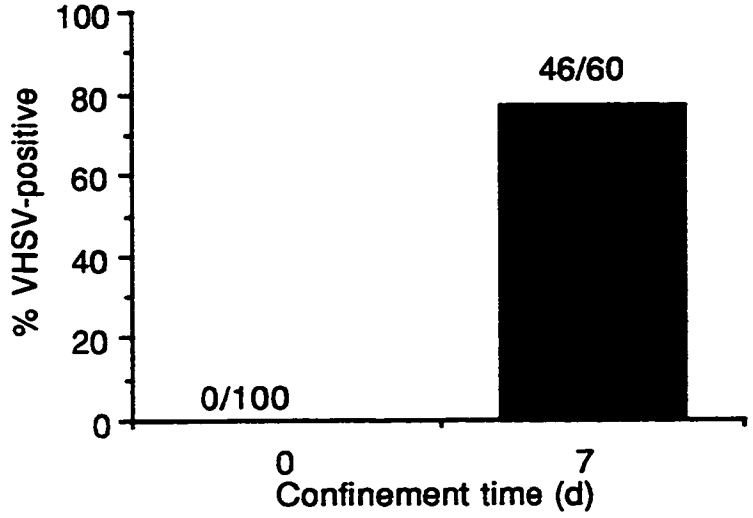

Figure 27. Prevalences of VHSV in Puget Sound herring sampled from a community tank. All the dead and moribund herring (84/84) tested positive for VHSV through 7d.

These data demonstrate that few wild herring carry low-level or latent infections of VHSV because 1) none (0/100) of the fish tested positive for VHSV at the time of capture and 2) significantly fewer ($p < 0.001$) herring tested

positive for VHSV when isolated in individual aquaria than when held in a community tank. Results indicated that only a small percentage (< 1%) of wild juvenile herring carry and shed VHSV because 0/100 of the initial herring tested positive for VHSV, but virus was detected in the transport water. Waterborne exposure to VHSV in the transport tanks probably constituted the route of exposure for the 17.5% of the isolated herring that later tested positive. High densities in the laboratory tanks resulted in exposure of susceptible herring to elevated concentrations of VHSV originating from a small percentage of infected fish. Susceptible herring subsequently became infected and developed active infections. Herring density was of secondary importance in initiating VHSV infections; a source of waterborne virus is prerequisite. Low concentrations of VHSV are shed by free-ranging herring, but the dilution factor of the open sea probably minimizes the waterborne viral exposure to susceptible, wild fish. Free-ranging herring are probably exposed to low level pulse doses of VHSV when herring in the school are startled, form a "herring ball, " and shed virus. It is suggested that such events serve as natural immunization routes and stimulate the immune systems of young herring to produce protective antibodies for VHSV. Thus, it is hypothesized that herring are susceptible to VHS disease epizootics when:

1) Young herring, which are either not yet immuno-competent or have failed to develop protective antibodies to VHSV, are exposed to high waterborne virus titers and cannot clear the infection.
2) Adult herring are exposed to unnaturally high levels of waterborne VHSV through confinement with virus-shedding fish at high density.

3) Herring become immuno-compromised after anthropogenic or natural stressors and are no longer able to suppress low-level infections which are normally cleared in healthy, unstressed fish.

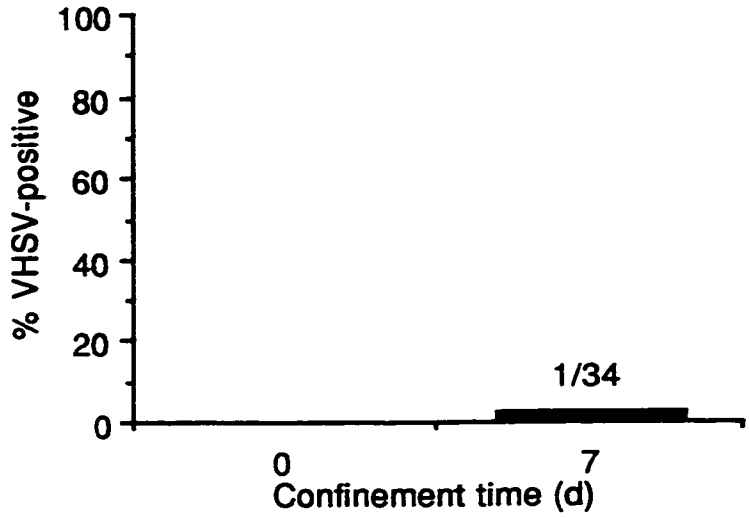

Figure 28. Prevalences of VHSV in Puget Sound herring sampled from individual aquaria. All the dead and moribund herring (6/6) tested positive for VHSV through 7d.

Chapter 4: Conclusions and Closed-Pound SOK Management Options

Confinement of apparently healthy *C. pallasi* into SOK pounds, net pens, or laboratory tanks often leads to increased infections of VHSV which peak relatively soon after capture. Magnitude, duration, and severity of infection depend upon numerous factors including herring age and immune status, shedding intensity, and fish density.

A mechanism to explain how VHS epizootics occur among confined herring can be proposed based upon the available data. Wild herring, a small percentage of which carry and shed low levels of waterborne VHSV are captured and introduced to a confinement structure. Confinement increases the density, thereby increasing both the stress on the herring and the probability of transmission of infectious waterborne VHSV particles. Because relatively few fish initially shed virus and, depending upon the age of the fish, a portion of the population is already immune to low levels of waterborne virus, relatively few fish are infected immediately after impoundment. These early VHSV-positive fish constitute the first prevalence peak and either die from VHS with high viral titers after a brief period of impoundment, as seen in the PWS herring of 1998 (Figures 16 and 19), or recover from infection, as occurred in PWS herring during 1997 (Figures 8a-c). VHSV shed into the water during the first prevalence peak provides a source of infection for the remaining susceptible fish (Kocan *et al* 1997). These fish then became infected and shed more VHSV, which accounts for the second peak of infection and increasing viral titers in the surrounding water.

Although density of impounded herring may influence the magnitude of VHSV infection, as seen in herring from the 1998 PWS SOK pounds (Table 8 and Figures 15a-c), a more factor important is exposure of a susceptible herring population to sufficient levels of waterborne VHSV. A group of susceptible herring confined at high densities will not undergo an epizootic unless a small percentage of the population is VHSV-positive and sheds waterborne virus. Similarly, a group of confined, immune herring will not undergo an epizootic even if exposed to waterborne virus shed by a small percentage of VHSV-positive fish. Exposure of a susceptible group of herring to waterborne VHSV is believed to account for the increased viral prevalences among confined herring, rather than re-activation of latent infections, because isolation of herring into individual aquaria (17.5%) did not result in viral prevalences as high as seen among fish held in a community tank (>77%) (Figures 27 and 28).

Infections of VHSV were less common among older herring then among young herring in the 1997 PWS pound study (Figure 10), the 1998 PWS pound study (Figure 14), and tank studies with Puget Sound herring (Figures 22, and 24), indicating that older herring are less susceptible to infection by VHSV than are younger herring. Older herring are believed to develop immunity to VHSV through exposure to low level waterborne virus at a young age and subsequent recovery from active, but non-lethal, infections. Low levels of waterborne virus were detected near free-ranging herring on several occasions: 1) from a school of free-ranging herring in Prince William Sound, 1998, 2) from a purse seined school of herring from Prince William Sound, 1998, and 3) from transport water containing wild juvenile herring held for less than 2h, when 0/100 of the fish

tested positive for VHSV. These data indicate that exposure of fish to low levels of VHSV does occur in nature and constitutes a likely source of infection for subsequent production of protective antibody. Older year classes that have survived prior exposure to VHSV have a selective advantage. However, a high proportion of young herring whose immune systems are not yet fully developed and which do not produce protective antibodies for VHSV may die from unnaturally high VHSV exposures that follow capture and confinement.

Mitigation techniques designed to maintain the low VHSV prevalences observed among wild herring stocks and to prevent release of large quantities of waterborne virus into the spawn-on-kelp pounds can now be proposed. Use of older returning herring age classes in the closed pound SOK fishery may reduce the likelihood of large epizootics among the impounded herring. Utilization of older herring age classes (primarily 5-9yr olds) during the 1997 SOK fishery resulted in viral prevalence peaks of 12.5-25%, significantly less than the 57-88% which occurred when younger age classes (primarily 3 yr olds) were introduced to the pounds during 1998. Prevalence of VHSV in herring steadily decreased with age class during both SOK study years in PWS. Likewise, VHSV prevalence peaks from herring confinement studies in laboratory tanks were negatively correlated with herring age, reaching levels over 90% when juvenile herring (< 1yr old) were confined, and 0% when 2+yr old spawners were used.

Such a strong correlation of VHSV prevalence with age indicates that management of the closed pound SOK fishery should be based partially on age

of the returning herring. Further, a higher quality product was obtained in 1997 when older herring were impounded. Perhaps managers should consider closure of the closed pound fishery during years dominated by young returning age classes of herring. Impoundment of young herring results in poor product, increased disease potential, and increased potential for removal of these fish from the population by disease-related mortality. Thus, impoundment of young herring is counterproductive to the fishers in at least two ways. First, loss of profit is great due to poor product yield; second, a high potential for removal of the young fish from the population exists through disease-related mortality, which results in the absence of older fish, more suited for a better product in future years.

Closed pound SOK fisheries are gear-intensive, requiring the use of a purse seining vessel, skiff, push pound, closed pound, and spotting plane. Fishers generally form small cooperative units with each fisher contributing his/her own personal gear. This often results in up to four permit holders operating in a single closed pound. A currently existing ADF&G regulation intended to reduce herring density within the pounds states that no more than 5.67 metric tons of herring may be impounded per permit holder. A loop-hole in the regulations exists in that four SOK permit holders are allowed to use a single pound structure. Thus, no limit is currently enforced on the herring density in the SOK pounds. A better management regulation, one which would limit herring densities, would be to authorize a predetermined herring biomass per permit holder as well as a predetermined herring density · enclosure^{-1}.

Cooperative fishers would be required to construct larger pound structures to house the larger herring biomasses within the closed pounds.

A more drastic management option would be to no longer allow closed pound SOK fisheries to operate. Alternative fishing practices such as open pounding are slightly less productive than closed pounding and pose a larger risk. An open pound is constructed similarly to a closed pound, but herring are not captured and confined within webbing. Open pounding involves finding a naturally spawning school of herring, usually with a spotting plane, then pushing the open pound structure containing suspended kelp blades, into the middle of the actively spawning school. The free-ranging herring then naturally spawn on the artificially placed kelp blades. The technique is more risky than closed pounding because herring often spawn in shallow water which necessitates placement of the open pound structure close to shore. Any dirt, sand, or gravel which may cling to the kelp/herring egg product renders the product value-less and unmarketable. Additionally the open pound structures cannot be moved quickly and a large herring spawn may be completed before the open pound can be moved into position.

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Curriculum Vitae

email: paulh@fish.washington.edu
School of Fisheries Box 355100
University of Washington
1140 NE Boat St
Seattle, WA 98195
phone: (206)543-4606

Professional Interests

Fish health and fish diseases
Oil spills and petroleum toxicology
Harmful phytoplankton
Larval fish rearing

Education:

Northland College	B.S. Chemistry	1993
	B.S. Biology	1993
University of Washington	M.S. Fisheries Harmful marine phytoplankton	1995
University of Washington	Ph.D. Fisheries Environmental and anthropogenic conditions prefacing disease epizootics in herring	1998

Honors:

1993 Lakehead Pipeline Scholarship
1993 Deans Hall of Recognition at Northland College
1993 Northland College Science Faculty Award, Biology and Chemistry
1993 Magna Cum Laude Graduate of Northland College
1996 Research award: Richard Van Cleve Memorial Schoarship
1997 Best poster presentation: UW Graduate Student Symposium

Professional Experience:

1995-1998: Research Assistant, University of Washington
Researched environmental and anthropogenic stressors associated with viral and fungal infections of herring. Involved extensive cooperation with Puget Sound herring bait fishers, Prince William Sound herring spawn-on-kelp fishers, WDF&W, ADF&G, and federal government laboratories.

1995-1998: Volunteer, USGS, BRD, Marrowstone Marine Station
Involved in larval herring rearing to specific pathogen free juveniles, tissue culture, viral plaque assays, complement-mediated plaque neutralization tests, PCR, fish immunosuppression studies with petroleum hydrocarbons and synthetic corticosteroids, numerous exposure experiments with viral hemorrhagic septicemia virus and *Ichthyophonus hoferi* in Pacific herring.

1993-1995: Research Assistant, University of Washington
Performed original laboratory and field research studying bloom dynamics of the noxious marine phytoplankton species *Heterosigma carterae*. Described necessary conditions prefacing red tide bloom formation, and corresponding fish mortalities from this alga. Documented first reported occurrence of *Heterosigma-related* mortality to free-ranging fish.

1993: Research Assistant, University of Washington
Developed a semi-continuous culture technique for marine phytoplankton.

1993-1999: Involvement in miscellaneous projects:
- Assessment of Puget Sound herring stock declines at Cherry Point, WA (1998) emphasizing *in situ* hydrocarbon bioaccumulation in mussels and developmental abnormalities in larval herring.
- Researched physiological effects of nitrate and nitrite on salmon and trout eggs for a construction project threatening a trout hatchery near Tacoma, WA.
- Benthic surveillance of Columbia River Salmon Farm (1997).
- Benthic sampling around salmon farms in Puget Sound (1998).
- Water quality assessment of Lake Osoyoos, focussing on anadromous fish passage (1997).
- Biological control of the greenhouse whitefly with the parasitic wasp *Encarcia formosa*.
- Composting 100% of organic cafeteria waste in a low temperature climate.
- Copper concentrations in Odonate naiads and freshwater amphipods.

Teaching Experience:

1994: Teaching Assistant, University of Washington
FISH 430 Biological Problems in Water Pollution
Instructed students in laboratory with computer-generated toxicity models, designed toxicity labs, and provided additional assistance for students with the lectures.

1991-1993:
Teaching Assistant, Northland College
Organic Chemistry
Instructed organic chemistry labs, ensured students were following safe lab procedures, prepared reagents, acted as a moderator between students and the professor, and tutored students in organic chemistry.

1990:
Teaching assistant, Northland College.
Basic Flyfishing Class
Assisted instructor in teaching students basic flyfishing skills including fly casting and fly tying.

Refereed Publications:

Hershberger, P.K., J.E. Rensel, A.L. Matter, and F.B. Taub. 1998. Vertical distribution of the chloromonad flagellate *Heterosigma carterae* in columns: implications for bloom development. Can. J. Fish. Aquat Sci. 54: 2228-2234.

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Hershberger, P.K. 1998. Epizootiology of viral hemorrhagic septicemia virus in confined Pacific herring. Ph.D. dissertation. University of Washington. Seattle, WA.

Kocan, R.M., **P.K. Hershberger**, J.R. Winton. 1998. Investigations of disease factors affecting declines of Pacific herring populations in Prince William Sound: Project II. Controlled Field and Laboratory Studies on VHS & *Ichthyophonus* in Pacific herring. *Exxon Valdez* Oil Spill Restoration Project Annual Report. Restoration Project (97162).

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Hershberger, P.K., J.E. Rensel, J.R. Postel, and F.B. Taub. 1997. *Heterosigma* bloom and associated fish kill. Harmful Algae News. 16: 1-4.

Hershberger, P.K. 1995. Migratory behavior of *Heterosigma carterae*, implications for bloom development. M.S. thesis. University of Washington. Seattle, WA.

Abstracts from Professional Conferences:

1998 UW SOF Graduate Student Symposium (platform)
Co-organizer
Session Moderator
Epizootiology of Viral Hemorrhagic Septicemia Virus in Herring from the Spawn-on-Kelp Fishery.

1998 Western Fish Disease Workshop (platform)
Viral hemorrhagic septicemia virus associated with herring from the 1998 Prince William Sound spawn-on-kelp fishery

1998 EVOS Trustee Council Restoration Workshop (poster)
Viral hemorrhagic septicemia virus in herring from the spawn-on-kelp fisheries

1998 Puget Sound Research (poster)
Viral hemorrhagic septicemia virus in herring from the Puget Sound spawn-on-kelp fishery

1997 PNFHPC: Pathogens and Diseases of Fish in Aquatic Ecosystems (poster)
Viral Hemorrhagic Septicemia Virus (VHSV) in herring (*Clupea pallasi*) from the Puget Sound spawn-on-kelp fishery

1997 UW SOF Graduate Student Symposium (best poster award)
Viral hemorrhagic septicemia virus in the 1997 Prince William Sound Spawn-on-kelp fishery

1996 UW SOF Graduate Student Symposium (poster)
VHSV in Puget Sound Herring

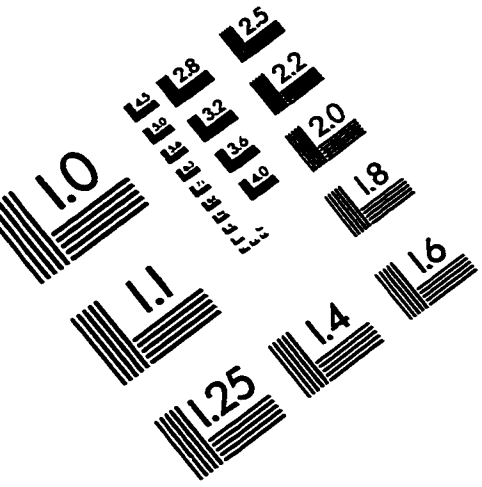
1995 American Society of Limnology and Oceanography (platform)
Vertical distribution of *Heterosigma carterae* in columns: implications for bloom development

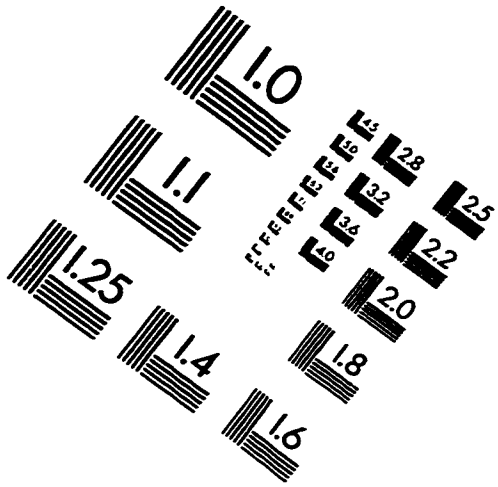
1995 Puget Sound Research (poster)
1994 *Heterosigma* bloom and associated fish kill in Puget Sound

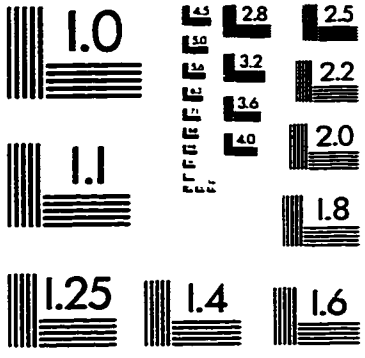
1995 Pacific Ecology Conference (platform)
Vertical distribution of *Heterosigma carterae* in columns: implications for bloom development

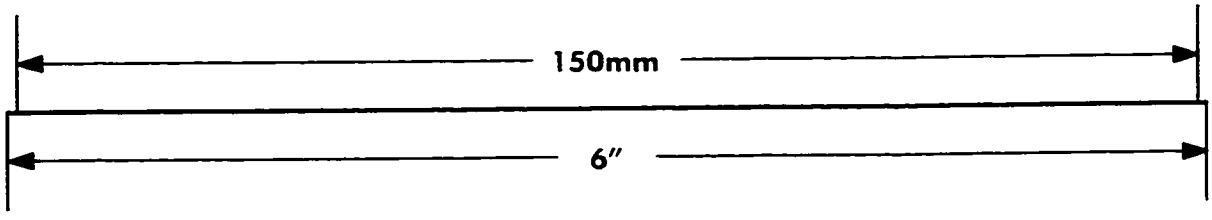
1994 UW SOF Graduate Student Symposium (platform)
Vertical Distribution of the Marine Phytoplankton *Heterosigma sp.*

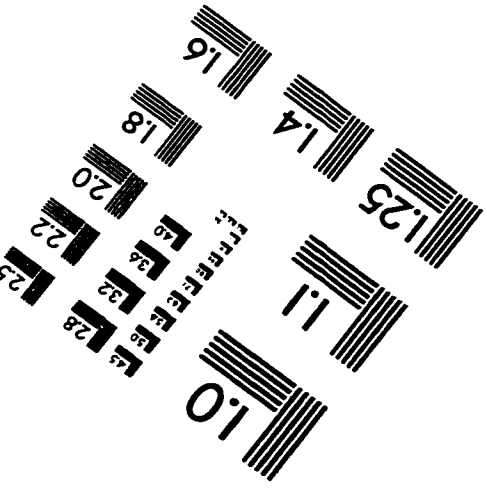
IMAGE EVALUATION
TEST TARGET (QA-3)

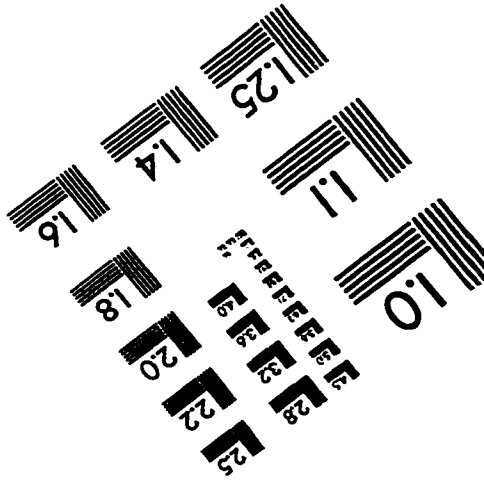












APPLIED IMAGE . Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989