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Epizootiology of viral hemorrhagic septicemia virus in Pacific herring from the spawn-on-kelp fishery in Prince William Sound, Alaska, USA

P. K. Hershberger[1,*], R. M. Kocan[1], N. E. Elder[2], T. R. Meyers[3], J. R. Winton[4]

[1]School of Fisheries, Box 355100, University of Washington, Seattle, Washington 98195, USA

[2]Marrowstone Marine Station, Biological Resource Division, U.S. Geological Survey, 616 Marrowstone Point Rd., Nordland, Washington 98358, USA

[3]Juneau Fish Pathology Laboratory, Alaska Department of Fish and Game, PO Box 25526, Juneau, Alaska 98802, USA

[4]Western Fisheries Research Center, Biological Resource Division, U.S. Geological Survey, 6505 NE 65th St., Seattle, Washington 98115, USA

ABSTRACT: Both the prevalence and tissue titer of viral hemorrhagic septicemia virus (VHSV) increased in Pacific herring *Clupea pallasi* following their introduction into net pens (pounds) used in the closed pound spawn-on-kelp (SOK) fishery in Prince William Sound, Alaska. VHSV was also found in water samples from inside and outside the SOK pounds after herring had been confined for several days; however, water samples taken near wild free-ranging, spawning herring either failed to test positive or tested weakly positive for virus. Little or no virus was found in tissue samples from free-ranging, spawning herring captured from the vicinity of the pounds, nor did the prevalence of VHSV increase following spawning as it did in impounded herring. The data indicated that increased prevalences of VHSV were correlated with confinement of herring for the closed pound SOK fishery and that infection was spread within the pounds through waterborne exposure to virus particles originating from impounded fish. In addition, pounds containing predominantly young fish had higher prevalences of VHSV, suggesting that older fish may be partially immune, perhaps as a result of previous infection with the virus. Operation of SOK pounds during spawning seasons in which young herring predominate may amplify the disease and possibly exacerbate the population fluctuations observed in wild herring stocks.

KEY WORDS: VHSV · Virus · Herring · Epizootiology · Disease

INTRODUCTION

Viral hemorrhagic septicemia virus (VHSV), historically a problem of farmed fish in Europe (Castric & de Kinkelin 1980, Jørgensen 1980, Meier & Wahli 1988, Wolf 1988, Schlotfeldt et al. 1991, Meier et al. 1994, Ross et al. 1994), was first detected in North America among returning adult coho *Oncorhynchus kisutch* and chinook salmon *O. tschawytscha* of Washington state, USA (Eaton et al. 1991, Winton et al. 1991, Meyers & Winton 1995). Subsequent studies have shown that VHSV is endemic among a wide range of north Pacific species including Pacific cod *Gadus macrocephalus* (Meyers et al. 1992), Pacific herring *Clupea pallasi* (Meyers et al. 1994), Pacific sandlance *Ammodytes hexapterus*, English sole *Parophrys vetulus* and shiner perch *Cymatogaster aggregata* (Hershberger & Kocan unpubl. data). Detection of VHSV in wild herring from Prince William Sound, AK, following the 1989 'Exxon Valdez' oil spill (Meyers et al. 1994) raised the possibility that VHSV could be highly pathogenic to some North Pacific fishes, which was subsequently confirmed by Kocan et al (1997). Further questions were raised concerning environmental conditions and human activities that might increase the severity of VHSV epizootics among wild herring.

The closed pound spawn-on-kelp (SOK) herring fishery in Prince William Sound is operated to produce

*E-mail: paulh@fish.washington.edu

a high quality specialty product, primarily for Japanese markets. Sexually mature, pre-spawn Pacific herring for this fishery are purse seined, transported to a net pen (closed pound) containing suspended *Macrocystis* blades (Whyte 1979), and confined for 7 to 10 d until eggs are deposited onto the kelp. Post-spawn fish are then released from the pound and presumably rejoin wild stocks. The crude product consisting of kelp blades overlaid with layers of adherent herring eggs is removed from the pound, trimmed of rough edges, and brined. Activities involved in the SOK fishery, such as rapid transport of herring to the closed pounds are reported to cause bruising and scale loss (Shields et al. 1985). High loading densities in the pounds can also result in herring mortalities as great as 33% (Brett & Solmie 1982), '*Vibrio*-like disease' (Hay et al. 1988), or physical damage such as bruising of the opercula and snout, hemorrhage at the base of fins, fin deterioration, and open ulcers on the body and snout (Shields et al. 1985). These signs are similar to the clinical signs of VHSV infection in herring (Meyers et al. 1994). Capture of seemingly healthy, wild *Clupea pallasi* and confinement in laboratory tanks often leads to active VHSV infections after 3 to 7 d (Kocan et al. 1996). Meyers et al. (1994) suggest that stressors such as spawning, capture, nutritional deficiency, or other diseases may contribute to periodic epizootics of viral hemorrhagic septicemia (VHS) in wild populations. This project was designed to determine whether impoundment of herring for the closed pound SOK fishery is correlated with increased prevalence of VHSV and to describe the course of virus infection within the pounds.

METHODS

The locations of SOK pounds and study sites were determined from data on herring biomass and spawning status collected at the beginning of the 1997 and 1998 herring seasons (Fig. 1A,B). Herring age (from scales), weight, and length were recorded from all

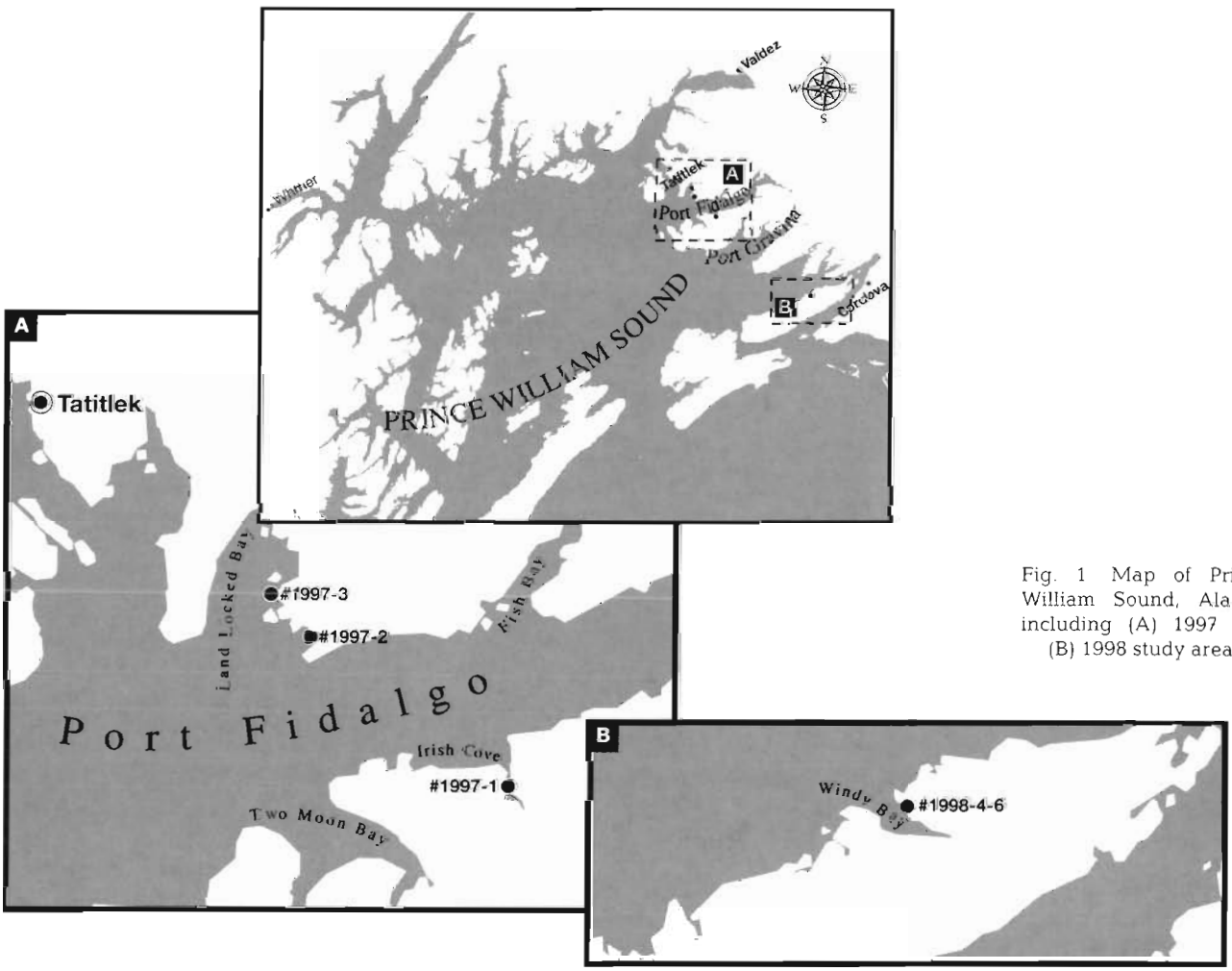

Fig. 1 Map of Prince William Sound, Alaska, including (A) 1997 and (B) 1998 study areas

sampled fish. Water quality measurements were recorded from the middle and 3 m outside of all pounds and included temperature, salinity and dissolved oxygen (DO).

Titers of VHSV in water samples and homogenized herring tissues were determined by plaque assay. Kidney and spleen tissues from each fish were pooled and homogenized in tris-buffered minimum essential medium (1× MEM) containing 100 IU ml^{-1} penicillin, 100 µg ml^{-1} streptomycin, 100 µg ml^{-1} gentamycin, and 2.5 µg ml^{-1} amphotericin B. Serial 10-fold dilutions of the homogenates were plated on monolayer cultures of epithelioma papulosum cyprini (EPC) cells pretreated with polyethylene glycol (Batts & Winton 1989), overlayed with methylcellulose, and incubated at 15°C for 7 d. Virus titers were expressed as plaque-forming units (pfu) g^{-1} of tissue. Virus from infected fish was identified as VHSV using the polymerase chain reaction and VHSV-specific primers (Einer-Jensen et al. 1995).

Water samples (2 ml) were diluted 1:1 in MEM containing 10% fetal bovine serum (MEM-10) in 1997 and 2× MEM containing 40% fetal bovine serum (2× MEM-20) in 1998 and returned to the laboratory for virus assay. Virus titers were expressed as pfu ml^{-1} water.

All viral prevalences were statistically compared using the *Z*-test for proportions (Zar 1984) and comparisons with $p \leq 0.05$ were considered significant.

1997 studies. All 1997 study pounds were located in the Port Fidalgo region (Fig. 1A) and designated Pounds #1997-1 in Irish Cove, #1997-2 at the mouth of Landlocked Bay, and #1997-3 at the head of Landlocked Bay. Random samples of 40 active herring $pound^{-1}$ d^{-1} were collected on consecutive days, beginning when the pounds were loaded until the fish were released 6 to 8 d later. All herring in Pound #1997-1 (4 to 5 metric tons) were caught at the head of Irish Cove in 1 purse seine set and transported only a few hundred meters to the pound on 11 April. Herring in Pound #1997-2 were captured from Two Moon Bay and transported 2.2 km across Port Fidalgo to the closed pound in 2 loads on 13 and 14 April. The limited number of fish added to this pound from the first load (~2 metric tons) prevented 0 d sampling, so the first fish sample was taken the following day (1 d) after an additional 16 metric tons was added (Table 1). A 40 fish sample of moribund herring swimming listlessly on the surface of Pound #1997-2 was removed on 18 April, corresponding to 5 d of impoundment. All herring in Pound #1997-3 (~0.9 metric tons) were loaded from 1 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 added to the pound prevented 0 d sampling without disturbing the kelp, and subsequently the pound operators were unable to catch more herring. The kelp was later removed, and the pound was abandoned. However, sampling of the fish continued and was facilitated by lifting the sides of the pound to concentrate the herring during sampling events. Spawning herring were also sampled from 2 free-ranging schools of wild fish (40 herring $school^{-1}$) in Landlocked Bay on 18 April.

Table 1 Physical and biological characteristics of the 1997 pounds in Prince William Sound, Alaska

Pound no.	No. of permit holders[a]	Estimated biomass (metric tons)	Pound size (m) (L×W×D)	Fish density (kg m^{-3})
#1997-1	1	4–5	5.5 × 11.6 × 4.6	17.0
#1997-2	3	>18	17.7 × 8.5 × 6.1	19.6
#1997-3	4	0.9	6.1 × 7.3 × 9.1	2.2
#1998-4	3	18	5.5 × 11.6 × 4.6	61.4
#1998-5	4	24	9.7 × 8.5 × 9.1	32.0
#1998-6	4	16	6.1 × 17.1 × 6.1	25.1

[a]Each permit holder is allowed 5.67 metric tons of herring

Herring collected from each pound were placed in static water live tanks and necropsied within 2 to 8 h. Spleen and kidney tissues from each fish were placed in sterile plastic bags, packed on ice, and shipped to the Alaska Department of Fish and Game fish pathology laboratory in Juneau, AK, where samples were frozen at −80°C until assayed for VHSV. Herring tissues identified as VHSV-positive on primary isolation were distinguished from those that became positive following blind passage.

Duplicate water samples for VHSV analysis were collected from both the center and 3 m outside each pound every other day at 1 m below the surface and within 1 h of slack tide. Samples were passed through a 0.45 µm filter, diluted 1:1 in MEM-10, shipped to Cordova, AK, where they were frozen at −80°C, then shipped to the University of Washington and finally to the Marrowstone Marine Station, where they were assayed for virus. The 1997 water samples underwent 3 partial freeze-thaw events prior to assay.

1998 studies. Modifications to the 1997 study design were implemented in 1998 to address possible problems with pre-necropsy handling and virus stabilization techniques which were encountered the previous year. Herring were placed on ice immediately after capture rather than held in static live tanks as was done in 1997. Necropsies were performed on herring within 2 h of capture and tissues were immediately placed in MEM-5. Water samples were passed through a 0.45 µm filter and 2 ml of the filtrate diluted 1:1 in 2 ml of 2× MEM-20 to stabilize the virus. All water sam-

ples were kept on ice (not frozen) until plaque assays were performed less than 2 wk later at the Marrowstone Marine Station.

All 1998 pounds, designated #1998 (4, 5, and 6), were located in Windy Bay (Fig. 1B) and loaded at different crowding densities (Table 1) with herring captured on 16 April less than 1 km away near the head of the bay. Herring in Pounds #1998-5 and #1998-6 were loaded from single purse seine sets while Pound #4 was filled with herring captured in 2 sets, made approximately 3 h apart. Random samples of 40 herring pound^{-1} d^{-1} were removed on consecutive days beginning at Day 0, when the pounds were loaded, and continuing until the fish were released 8 d later. Moribund herring from Pound #4 were sampled daily after their initial appearance on Day 2.

A large natural herring spawn in Port Gravina (Fig. 1) on 11 April 1998 was followed by migration of the post-spawn herring to Two Moon Bay the next day. Wild, 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. Actively spawning, free-ranging herring, presumably members of the same school from which impounded fish were captured, were sampled with a cast net near the head of Windy Bay on 21 April 1998.

All sampled herring were immediately placed on ice and transported to a fishing vessel where necropsies were performed. Spleen and kidney samples were removed within 1.5 h; tissues from each fish were pooled in sterile plastic bags containing MEM-5 and shipped on ice to the Marrowstone Marine Station, where they were frozen at −80°C until assayed for VHSV.

Duplicate daily water samples for VHSV analysis were collected from both the center and 3 m outside each pound at 1 m below the surface and within 1 h of slack tide. Water samples were also taken from the center of 2 actively spawning, free-ranging herring schools, one in Fish Bay (Fig. 1) on 11 April and another from the head of Windy Bay on 21 April. Water was also collected from the middle of 2 closed purse seines of post-spawn herring in Two Moon Bay on 12 April and assayed for VHSV.

RESULTS

1997 studies

The prevalence of VHSV in herring tissues from all pounds increased and peaked following 1 to 4 d of confinement, then returned to low levels after 5 to 6 d. The prevalence of VHSV among herring from Pound #1997-1 was 5% on Day 0 and peaked significantly

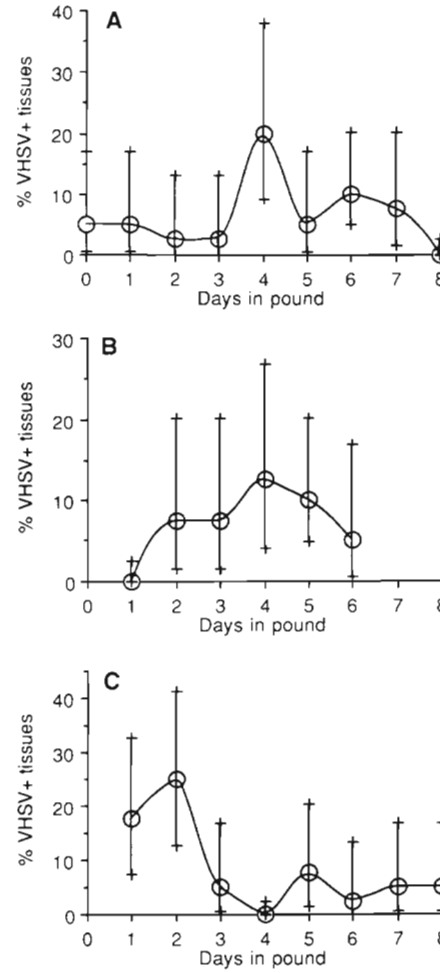

Fig. 2. Viral hemorrhagic septicemia virus in *Clupea pallasi*. Daily VHSV prevalences in herring from (A) Pound #1997-1, (B) Pound #1997-2, (C) Pound #1997-3. Daily 'n' = 40. Error bars indicate 95% confidence limits

higher ($p < 0.025$) at 20% after 4 d (Fig. 2A). Virus prevalence among herring from Pound #1997-2 increased from 0% on Day 1 to a high of 12.5% on Day 4 (Fig. 2B) with significantly greater viral prevalences ($p < 0.05$) on Days 2 to 5. The prevalence of VHSV in both moribund and apparently healthy fish from Pound #1997-2 was similar on Day 5 (12.5 and 10% respectively). No Day 0 samples were taken from Pound #1997-3, but VHSV prevalence peaked at 25% after 2 d of confinement, then dropped to less than 10%, with significantly fewer ($p < 0.05$) VHSV-positive fish on Days 3 to 4 and 6 to 8 of confinement (Fig. 2C). No VHSV was detected in 80 tissue samples of wild, naturally spawning herring collected from Landlocked Bay on 18 April. A slight increase in prevalence (0 to 15%) was noted when tissues were passed blind, reflecting fish with very low titers (<50 pfu g^{-1}) that were probably infected while in holding tanks prior to necropsy;

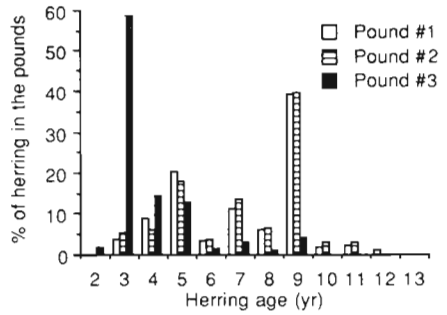

Fig. 3. *Clupea pallasi*. Age composition of herring in each of the 1997 pounds (from scales)

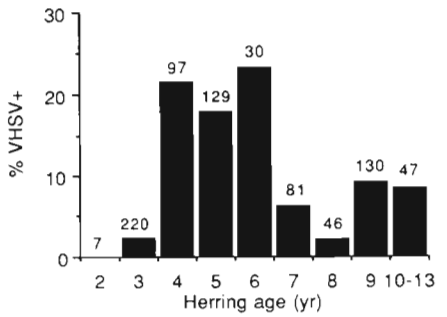

Fig. 4. Viral hemorrhagic septicemia virus in *Clupea pallasi*. Age-related VHSV prevalence among 1997 impounded herring. Numbers represent 'n'

however, prevalence patterns following primary isolation and secondary passage were similar.

Herring age distributions in Pounds #1997-1 and #1997-2 were nearly identical, consisting primarily of 9 yr olds (40%), while Pound #1997-3 contained primarily 3 yr olds (60%) with few (5%) 9 yr olds (Fig. 3). Virus prevalence was associated primarily with the 4 to 6 yr olds (~20% VHSV-positive in each year class) and decreased with age (Fig. 4). Prevalence of VHSV was significantly greater in females (11.8%, 51/431) than in males (7.8%, 48/614; $p < 0.05$) from these groups.

None of the water samples taken inside or outside the pounds during the 1997 season assayed positive for VHSV. DO remained at or above 12 mg l^{-1} throughout the 1997 sampling period, but was about 1 mg l^{-1} lower inside each pound than outside.

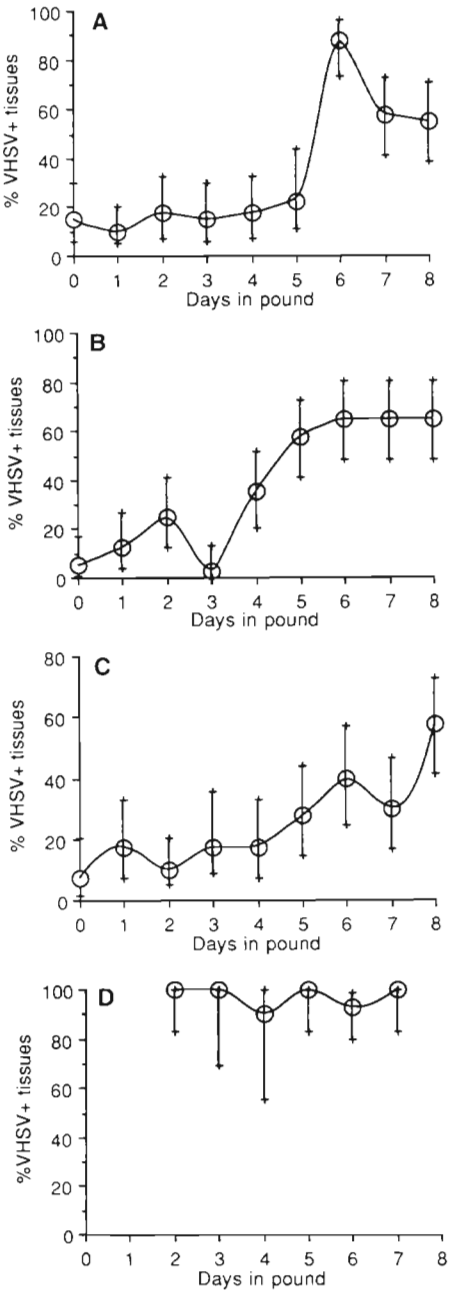

Fig. 5. Viral hemorrhagic septicemia virus in *Clupea pallasi*. Prevalence of VHSV among herring tissues from (A) Pound #1998-4, (B) Pound #1998-5, (C) Pound #1998-6. Daily 'n' = 40. (D) Prevalence of VHSV among tissues from Pound #1998-4 moribund herring. Error bars indicate 95% confidence limits

1998 studies

Problems encountered with the 1997 study design were corrected in 1998, resulting in fewer variables and easier data interpretation. The prevalences of VHSV among herring from each of the 3 study pounds followed similar kinetic patterns and were initially low, followed by peaks between 57 and 87% after 6 to 8 d of confinement (Fig. 5A–C). Only 10 to 22.5% of the herring from Pound #1998-4 tested positive for VHSV from Day 0 through Day 5, which increased significantly ($p < 0.001$) on Days 6–8 to 55–87.5% (Fig. 5A). Virus prevalence among herring tissues from Pound #1998-5 was significantly greater ($p < 0.02$) after Days 2 and 4 to 8 of confinement. A bimodal pattern of VHSV prevalence was indicated by the significant

increases ($p < 0.02$) in virus prevalence on Days 2 and 6 (Fig. 5B). Prevalence of VHSV among herring from Pound #1998-6 steadily increased from 7.5% on Day 0 to 57.5% on Day 8, with significant differences ($p < 0.02$) from Day 0 occurring on Days 5 to 8 (Fig. 5C).

Nearly all moribund herring (90 to 100%) sampled from the surface of Pound #1998-4 had detectable levels of VHSV (Fig. 5D). No moribund herring were sampled from the pound on Day 0 and 1 because so few were present, and none were sampled on Day 8, when the pounds were emptied.

The prevalence of VHSV among free-ranging, post-spawn herring from Two Moon Bay decreased from 17.5% 1 d after the major spawning event to 7.5% 4 d later, but the decrease was not significant ($p > 0.10$). No pre-spawn wild herring were sampled from this school on Day 0 because it was not anticipated that the post-spawn herring would remain in the area. The sample of free-ranging, spawning herring from Windy Bay, collected 5 d after capture of fish for the pounds, indicated that only 5% (2/40) of the unimpounded cohorts tested positive for VHSV.

Significantly more herring from the 1998 pounds ($p < 0.02$) had high virus titers (10^4 to 10^8 pfu g^{-1}) than low titers (400 to 9999 pfu g^{-1}) only on Days 7 and 8, while significantly more moribund herring in Pound #1998-4 had high virus titers than low on each sampling day ($p < 0.001$). There were no differences between the percentages of fish with high and low virus titers in the free-ranging, post-spawn herring from Two Moon Bay. The 2 virus-positive herring of the 40 fish sampled from the actively spawning school in Windy Bay had low titers.

DO remained at or above 8.2 mg l^{-1} inside the pounds but was generally 1 mg l^{-1} higher outside the pounds.

No VHSV was detected in water from inside the pounds prior to introduction of herring on Day 0, but virus was found inside each pound as early as 1 d and outside the pounds as early as 2 d after herring were introduced. Daily concentrations of waterborne VHSV inside each pound followed a bimodal pattern, with the initial, smaller peak occurring after 1 to 4 d, and the second, higher peak occurring just prior to release of the fish from the pounds on Day 8 (Fig. 6). Concentrations of VHSV in the water continued to increase through the final sampling date inside each pound, reaching levels as high as 700 pfu ml^{-1} in Pound #1998-4 after 8 d of confinement. Waterborne virus concentrations 3 m outside Pound #1998-4 increased to over 200 pfu ml^{-1} after 8 d of confinement, but remained below 20 pfu ml^{-1} outside Pounds #1998-5 and #1998-6 through the final sampling day (Fig. 6).

Low titers of VHSV were found in 2 water samples 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 1 d post-spawn herring tested positive for VHSV, with 15 pfu ml^{-1}, but no VHSV was found in water collected 30 min later from a second closed purse seine in the same area. Additionally, water from the middle of a wild herring spawn in Windy Bay tested positive for VHSV with a concentration of 5 pfu ml^{-1}, but no VHSV was recovered from water sampled in the middle of another wild herring spawn in Fish Bay.

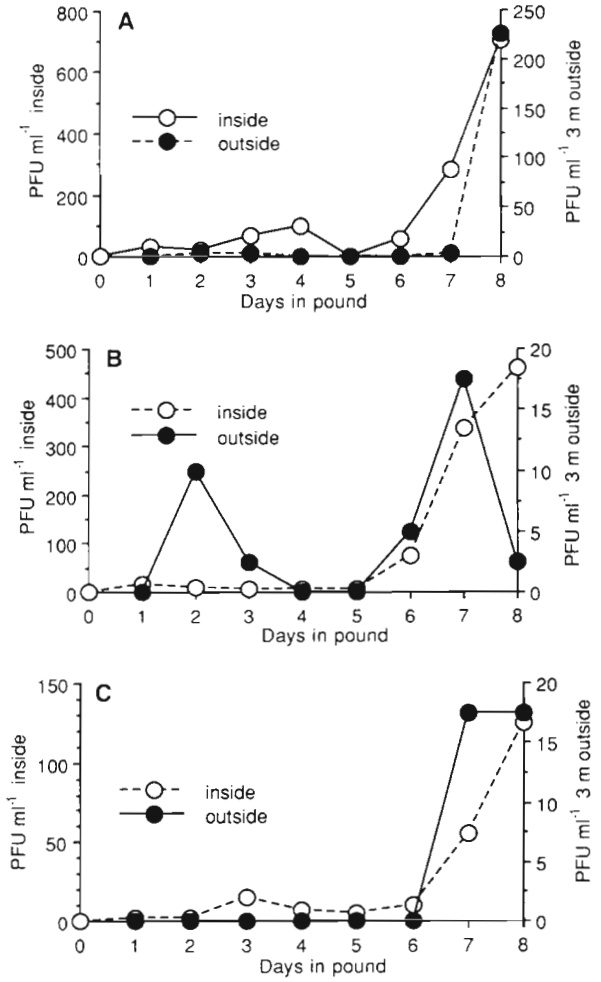

Fig. 6. Daily viral hemorrhagic septicemia virus concentrations in the water inside and 3 m outside (A) Pound #1998-4, (B) Pound #1998-5, (C) Pound #1998-6. Daily concentrations are reported as duplicate means

Nearly identical herring age distributions were loaded into each of the 3 pounds, dominated by 3 yr olds (65 to 68%) and a few 7+ yr olds (Fig. 7). Prevalence of VHSV was highest (60%) in the 1 to 2 yr olds and steadily decreased with age to 12.2% in the 7 to 10 yr olds (Fig. 8). Prevalence of VHSV among males (36.4%, 246/675) and females (33.7%, 247/733) was not significantly different ($p > 0.20$).

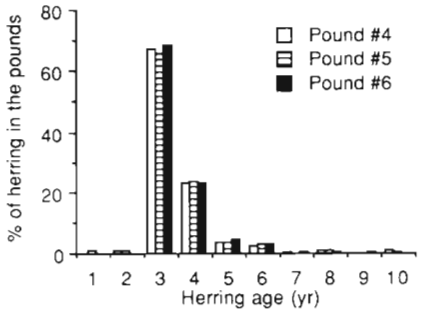

Fig. 7 *Clupea pallasi*. Age structure of herring in each of the 1998 pounds (from scales)

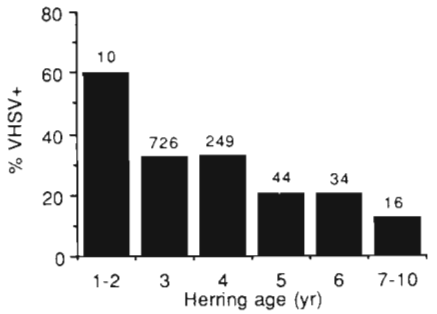

Fig. 8. Viral hemorrhagic septicemia virus in *Clupea pallasi*. Age-related VHSV prevalence among 1998 impounded herring. Numbers represent 'n'

DISCUSSION

The prevalence of VHSV among Pacific herring in SOK pounds consistently increased with confinement time in the pounds, a phenomenon not seen in free-ranging herring from the same stocks. However, the magnitude, temporal occurrence, and duration of the peaks in prevalence varied from year to year. The higher peaks of VHSV prevalence in 1998 (60 to 85.5% after 6 to 8 d) than in 1997 (12.5 to 25% after 1 to 4 d) is believed to be due to a greater susceptibility to VHSV among the younger age classes present in 1998. Inter-annual procedural differences in handling of the tissue samples are not believed to account entirely for the differences in peak viral prevalences because preliminary data from disease monitoring studies in Prince William Sound (PWS) also indicate increased VHSV prevalence among free-ranging herring in 1998. Additionally, unpublished data from our laboratories suggest that increased prevalence of VHSV among impounded herring is not correlated with loading density. Viral prevalence in 1997 was greatest (25%) among herring taken from Pound #1997-3, which contained the lowest biomass (2.2 kg m^{-3}), but which had the highest proportion of newly recruited 3 yr old fish (Fig. 3).

The highest prevalences of VHSV typically occurred in the youngest herring and steadily decreased with fish age (Figs. 4 & 8). Only 2/16 of the impounded herring in the 7 to 10 yr age classes tested positive for VHSV in 1998 and both were found near the end of the study (Days 6 and 7), when the virus prevalence peaked among the impounded fish. Similarly, only 4 herring older than 10 yr tested positive for VHSV in 1997, 3 of which had low level infections that were detected only after secondary passage, and the fourth came from Pound #1997-1 on Day 4, when the viral prevalence peaked at 30% (Fig. 2A). Such a pattern of decreasing VHSV prevalence with increasing age is indicative of an increased immunity among older herring, permitting the older fish to clear the virus before significant viral replication occurs. Total mortality rates among the impounded herring were not recorded, but nearly all moribund fish sampled from the surface of Pound #1998-4 tested positive for VHSV with high viral tissue titers, indicating that mortality was associated with VHSV

Differences in peak viral prevalences among impounded herring from the same SOK season were detected but were not as pronounced as differences between years. Prevalence of VHSV among fish from Pound #1997-3 peaked at 25% (Fig. 2C), higher than any other 1997 pound, presumably due to the predominance of 3 yr old herring (Fig. 3). The high prevalence of VHSV found in these herring after only 1 d was also observed among impounded herring in Puget Sound, WA, where none tested positive for VHSV on Day 0, but the prevalence increased to 12.2% after only 1 d (Hershberger 1999).

Increased prevalences of VHSV among impounded herring (Figs. 2 & 5A–C) appeared to be correlated with activities associated with operation of the closed pound SOK fishery, because virus prevalence among wild, free-ranging herring failed to increase, and possibly decreased, after the 1998 spawn. Wild, actively spawning herring captured from the head of Windy Bay in 1998 were taken from the same vicinity and were of the same age structure as those loaded into all pounds on 16 April, suggesting that all impounded and free-ranging fish came from the same school. Differences in viral prevalence between confined (Fig. 5A–C) and free-ranging herring from the same school indicate that increased viral prevalence was correlated with impoundment.

A greater prevalence of VHSV in females from 1997 pounds was consistent with a similar trend reported among wild herring from the Montague area of PWS in 1997 (Marty et al. 1998). In contrast, no difference in prevalence was correlated with gender among 1998 herring.

In 1998, waterborne VHSV both inside and outside pounds rapidly increased during confinement (Fig. 6) to concentrations capable of producing lethal infections among juvenile laboratory-reared, specific pathogen-free herring (Kocan et al. 1997). The lower concentrations in water samples collected from outside Pounds #1998-5 and #1998-6 may have resulted from samples being taken on the upstream side of slight currents circulating through the pounds during tide changes. Nevertheless, VHSV concentrations in water samples collected within 1998 pounds near the end of the confinement period were substantially higher than natural background levels associated with wild herring, where concentrations never exceeding 15 pfu ml^{-1} were found in only 1 of 4 water samples from wild spawning schools and in 1 of 2 samples from purse seines. The peaks in waterborne VHSV concentrations did not coincide with herring spawning actively within the pounds; rather, they corresponded with increased titers of VHSV in tissues sampled from impounded herring. High concentrations of waterborne virus in and around the pounds provided the most probable exposure route for uninfected impounded herring and for free-ranging fish which were attracted to the spawn emanating from the pounds (pers. obs.). Post-spawn, herring released from the pounds back into the wild while still shedding virus would constitute a further source of infection for wild stocks. Transfer of virus by direct contact between fish in the pounds is improbable because only small percentages of the herring in each pound had visible subdermal hemorrhages and ulcers similar to those previously associated with VHSV infections (Meyers et al. 1994).

Whether significant levels of virus were actually present in the water within or outside the pounds in 1997 is not known because the samples collected that year underwent several freeze-thaw cycles prior to being assayed, and, with the methods employed, each freeze-thaw event resulted in a reduction in recoverable VHSV of as much as 90%. The method of transporting virus in 1998 proved to be superior because there was little loss of titer during transport.

It is unlikely that all VHSV-positive herring entered the pounds as latent carriers of infection. Hershberger (1999) showed that VHSV prevalence (17.5%) among *Clupea pallasi* confined into individual laboratory aquaria was significantly less ($p < 0.001$) than the prevalence among the same lot of herring grouped in a community tank (>77%), demonstrating that transmission was responsible for increased viral prevalences after confinement. Additionally, detectable levels of waterborne virus were found in all 1998 pounds as early as 1 d after introduction of the herring, representing the most probable source of infection for susceptible fish.

Based on the available evidence, we propose the following explanation for the epizootics of VHS which occur among impounded herring. A small percentage of pre-spawn, wild herring carry and shed VHSV at the time they are introduced to the SOK pounds. These early virus-positive fish constitute the first prevalence peak and either die from VHS after 2 d of impoundment, as observed in 1998, or recover from infection, as occurred in 1997 (Fig. 2). Relatively few additional fish are infected immediately after impoundment because waterborne VHSV titers are initially low. Impoundment of herring results in prolonged crowding of fish, thereby increasing both the stress and the probability of exposure to waterborne virus. VHSV shed during the first prevalence peak provided a waterborne source of infection for the remaining susceptible fish. These fish then undergo infections and shed higher levels of VHSV, which explains the increasing viral titers in the surrounding water. The age structure of impounded fish also influences infection rates, because older fish are more likely to have developed non-specific resistance and/or specific humoral immunity from prior exposures to natural background levels of VHSV. Such immunity would render older fish refractory to infection by VHSV.

The prevalences and tissue titers of VHSV, shedding intensity, and duration of infection all vary depending on the susceptibility of fish in the population. The latter may be a function of age and immunity status. Younger fish will have had less opportunity to encounter VHSV than older fish, and as the population ages, generally to a maximum of 10 yr in PWS, a greater proportion of surviving individuals in each age group will have been exposed to VHSV. This may explain, in part, both the decrease in the numbers of older individuals in the population and their increased resistance to VHSV.

Worldwide, clupeoid stocks have been prone to large, cyclic population fluctuations, with declines attributed to overfishing, predation, food availability, El Niño, and species interactions (Blaxter & Hunter 1982). In addition, pathogenic organisms including the systemic fungus *Ichthyophonus hoferi* (Rahimian & Thulin 1996) and VHSV (Meyers & Winton 1995) have been proposed as factors that influence the population size. Hudson et al. (1998) report cyclic fluctuations in a population of wild birds, the red grouse *Lagopus lagopus scoticus*, which were prevented by removal of the parasitic nematode *Trichostrongylus tenuis*. Thus, it is possible that the cyclic fluctuations observed in *Clupea pallasi* populations may be due, at least in part, to the effects of a natural disease process, and that operation of inherently stressful SOK pounds during spawning years predominated by susceptible fish may amplify the role of VHS in this process.

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