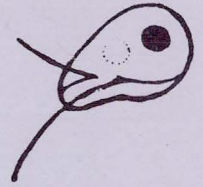


FISH HEALTH SECTION

A S F

NEWS LETTER



Volume 15, Number 1

January 1987

FUTURE OF THE NFHRL BIOLOGICS PROGRAM

G.L. (Pete) Bullock
NFHRL, USFWS
Box 700

Kearneysville, WV 25430

The biologics section was established to provide diagnostic reagents, principally antisera, for use in serological diagnosis of the major bacterial and viral fish pathogens. The rationale for the existence of the section was that there was no commercial source for the diagnostic reagents and the newly developed diagnostic and detection procedures could not be carried out without specific antisera. For the first few years antisera and antigens were prepared for the majority of the bacterial and viral agents. However, because of continued diminishing funding and personnel, production of reagents has been reduced. Although the biologics section will no longer provide the variety of diagnostic antisera, limited amounts of polyclonal and, when available, monoclonal antisera will still be supplied until such time as a private company would begin to market those antisera. The following reagents are supplied in one milliliter amounts with instructions on reconstitution and dilution. Requests for up to 10 ml of a specific antiserum are filled routinely but prior arrangements must be made with Dr. Anderson or Mrs. Dixon for larger amounts.

1. Rabbit anti-*A. salmonicida*. Cross reacts with some strains of *A. hydrophila*.
2. Rabbit anti-*A. salmonicida* FITC. cross reacts with some strains of *A. hydrophila*.
3. Rabbit anti-*Y. ruckeri*. Type I only.
4. Rabbit anti-*Y. ruckeri* FITC. Type I only.
5. Goat anti-*R. salmoninarum*
6. Goat anti-*R. salmoninarum* FITC
7. Rabbit anti-IPN polyvalent. Presently contains six serotypes and will neutralize 95% of all isolates.
8. Monoclonal IHN. A hybridoma cell line producing a binding antibody that cannot be used in a serum neutralization test but will bind all strains tested in the ELISA immunoblot.
9. Monoclonal VHS. A hybridoma cell line producing a binding antibody used in the ELISA immunoblot and will bind all three VHS serotypes.

WHIRLING DISEASE IN OREGON

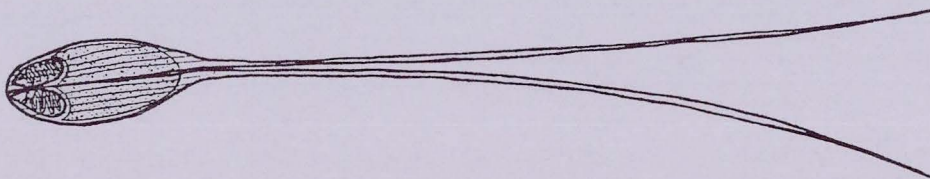
R. A. Holt, A. Amandi, C.R. Banner and T.D. Kreps
Oregon Department of Fish and Wildlife
OSU Department of Microbiology
Corvallis, OR 97331

Myxosoma cerebralis, previously unidentified in Oregon, has been discovered in salmonids in the Lostine River (Grande Ronde River drainage in northeast Oregon). Spores were found in rainbow and brook trout and chinook salmon juveniles collected from several sites over an eight-mile portion of the river. In addition, parasitized fish were identified in a large privately owned lake and in a private trout hatchery located near this river. The disease was discovered when fish with typical signs of whirling disease, i.e., skeletal deformities and whirling behavior, were submitted to our laboratory by the private hatchery owner. Fish from the private hatchery had been shipped to numerous locations in eastern Oregon and to a few sites in western Oregon. We have implemented a large sampling program to determine the extent of this parasite in northeastern Oregon and at all locations where fish had been transported. Results of this sampling will determine if containment of the parasite will be possible in Oregon. No obvious importation of fish from known *M. cerebralis* positive locations has been documented. Adult steelhead trout and chinook salmon which migrate into the Wallowa drainage in large numbers could have been the source, but other possibilities of parasite introduction exist.

NOMINATIONS SOUGHT FOR SNIESZKO AWARD

The Awards Committee is soliciting nominations for the S.F. Snieszko Distinguished Service Award. This is the highest award of the Fish Health Section and is presented for the purpose of honoring individuals for outstanding accomplishment in the field of fish health.

Nominations and supporting information should be sent to John Rohovec, Department of Microbiology, Oregon State University, Corvallis, OR 97331. Nomination dossiers should be received prior to February 28, 1987.



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 Ron Goede, Technical Procedures Committee
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 Others to be announced

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 Ron Hedrick (2 years)
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TO THE EDITORS

Dear Sirs:

The article "Proliferative Kidney Disease (PKD) in Arctic char (*Salvelinus alpinus*) from Newfoundland" by B.D. Hicks and H.W. Ferguson that appears in Volume 14, Number 4 of the newsletter is misleading. Both the title and the last paragraph imply that the Province of Newfoundland is the source of the PKD that the authors observed. Yet if one reads more carefully, the disease outbreak occurred in the author's laboratory in Ontario. They further discuss a number of possible sources of the pathogen including numerous introductions of salmonids to their facilities from areas other than Newfoundland as well as the presence of wild trout in the water supply. To me these sources are every bit as suspect as the parent stock of Newfoundland char, whose habitat is likely far colder than those in which this disease has been documented thus far.

Those of us in regulatory agencies who must maintain a watch against the importation of the PKD organism may have been misled by the article. It is **Ontario** more than Newfoundland which should now be added to the list of regions in which the pathogen is now known to occur.

Sincerely,
 /s/ Peter Walker
 Peter Walker, Fish Pathologist

Colorado Division of Wildlife
 P.O. Box 917
 Fort Morgan, CO 80701

Drs. Hicks and Ferguson Reply:

Dear Dr. Winton:

The fertilized Arctic char eggs from the Fraser River (Labrador) were transported to the laboratory in Newfoundland. The PKD outbreak occurred in the char while they were in the laboratory in Newfoundland. Only formalin fixed samples of the char were submitted for pathological examination to our laboratory (Fish Pathology Laboratory) in Ontario. To the best of our knowledge PKD has not been diagnosed from wild or cultured fish from Ontario stock.

We trust that these comments will clarify any misunderstandings.

Sincerely,
 /s/ Bradley Hicks
 /s/ Hugh Ferguson
 Brad Hicks and Hugh Ferguson

PASSAGES

Dave Ransom has moved from Oregon, where he was employed by Oregon Aqua Foods. He now works for PepsiCo Inc. in research and technical services. His new address is 100 Stevens Avenue, Valhalla, NY 10595. The phone number is 914-742-4880. Dave's help on the editorial board of the Newsletter and his contributions to fish health are recognized and his presence will be missed.

Steve Newman has moved from Argent and now is Research Director and General Manager of Microtek, Research and Development, Ltd. His new address is P.O. Box 2460, Sidney, B.C. Canada V8L3Y3. The phone number is 604-655-1455.

Jim Winton has become a Fed. He has moved from the OSU Marine Science Center in Newport, Oregon to the National Fisheries Research Center, Building 204, Naval Support Activity, Seattle, WA 98115. His new phone number is 206-526-6282.

HOST TISSUE RESPONSE TO *CERATOMYXA SHASTA* TROPHOZOITES IN APPARENT ABSENCE OF SPORES MAY RESULT IN MISDIAGNOSIS

W.T. Yasutake

Seattle National Fishery Research Center
Bldg. 204, Naval Station, Seattle, WA 98115

At the present time, the presence of *Ceratomyxa shasta* spores in the mature stage is used to determine whether fish are infected with this highly infectious myxosporidan. Routine field diagnosis is made by preparing a wet mount from a scraping of the ascending intestine and by looking for spores with a light microscope. A recent histopathological study of returning adult coho salmon indicated varying degrees of host response to the *C. shasta* trophozoite, the immature form of the myxosporidan, although there was an apparent absence of the mature spores. Moderate to extensive histopathological tissue response was observed in the cecal wall of all 28 *C. shasta*-infected coho salmon; however, spores were observed in only 50% of these infected fish. Although we did not do serial sections, our findings suggest that even if spores were not seen, fish could indeed have a myxosporidan infection. Spores can usually be readily observed in wet mounts, while trophozoites, since they resemble tissue cells such as epithelial cells, are not readily seen when the routine scanning technique is employed. We are confident that with a little experience, fish pathologists can learn to detect the amoeboid movement of the trophozoites in wet mount. Because of the significance of *C. shasta* to fish health certification on the West Coast, it would be prudent not to misdiagnose by overlooking the presence of the trophozoites. Since the presence of trophozoites alone, even in absence of spores, is indicative of the myxosporidan infection, it is recommended that the presently used clinical method of determining the presence of *C. shasta* infection be reassessed.

DISTRIBUTION OF *CERATOMYXA SHASTA* IN THE COLUMBIA RIVER BASIN

Jeri Hoffmaster, James Sanders, and Don Stevens
Oregon State University, Corvallis, OR 97331

The distribution of the infectious stage of *Ceratomyxa shasta* in the Columbia River Basin has been investigated in a four year study funded by the Bonneville Power Administration. Previous studies have established the presence of the infectious stages in the Columbia River below its confluence with the Deschutes River. In this study we have extended the known range of the parasite in the Columbia River to its confluence with the Snake River, and in the Snake River to Oxbow Dam, which was the furthest exposure site. Infection levels at most Columbia and Snake River sites were low, 5-6% at McNary Dam and 11% at Little Goose Dam. However, infection levels in fish held at Hell's Canyon and Oxbow Dam on the Snake River were greater than 90% when water temperatures were high (21 C) in July, 1986. Infections were also detected in fish held in the Washougal River and in the east fork of the Lewis River. At this time, no infections have developed in fish exposed in the mainstem of the Columbia River above its confluence with the Snake River or in the following tributaries: the Salmon, N. Lewis, Wind, Clearwater, Elokoman, White Salmon, Klikitat, Grande Ronde, Imnaha, Wallowa, Umatilla, and John Day Rivers, and Looking-glass Creek.

The Fish Health Section Directory is no longer an apparition. Thanks to Rowan Gould and the tireless assistance of Vicky Adriance, you should be receiving your copy soon.

Recruit a new Fish Health Section member today!

ISOLATION OF THE BACTERIUM CAUSING FOCAL NECROSIS (FATAL INFLAMMATORY BACTEREMIA) IN PACIFIC OYSTERS (*CRASSOSTREA GIGAS*)

C.S. Friedman¹, H. Beattie², R. Elston³ and R.P. Hedrick¹,

¹Aquaculture and Fisheries Program, Department of
Medicine, School of Veterinary Medicine,
University of California, Davis, CA

²Department of Aquaculture and Invertebrate Fisheries
University of Washington
Seattle, WA

³Battelle Marine Research Laboratory, Sequim, WA

Focal necrosis of adult Pacific oysters (*Crassostrea gigas*) has been reported to coincide with recurrent mortalities in Matsushima Bay, Japan (Sinderman, 1974, Diseases of Marine Fish and Shellfish). The disease, now designated fatal inflammatory bacteremia (FIB), has also been observed among Pacific oysters in Washington state where it is believed to be a major cause of adult mortalities in late summer to early fall (Elston et al., Aquaculture, submitted). The etiology and pathogenesis, distribution and effects of the disease on the oyster are still poorly understood. We have examined affected oysters from 10 sites in Washington state and two sites in British Columbia in an effort to better characterize the disease and the agent.

Initial investigations by Elston et al. (as above) showed that the principal lesion is a pustule commonly found on the mantle or adductor muscle that consists of host inflammatory cells (amoebocytes) surrounding tufts of bacteria. The lesion is not characterized by necrosis but instead by this inflammatory infiltration of amoebocytes giving it a granulomatous-like appearance.

The bacterium, as observed in tissue sections or smears made from affected oysters, is gram positive, acid-fast, branched and beaded as typical for actinomycetes. We have successfully isolated the agent from diseased oysters collected at two sites in Puget Sound, Washington state and one location in British Columbia. The cultured isolates share all of the characteristics of the bacteria as observed from infected oysters. Thin-layer and gas chromatographic analyses of all of the isolates indicate they share identical mycolic acids that would suggest they belong to the genus *Nocardia*. Further characterization of the cell wall, combined with serological studies of the bacterium to known strains of *Nocardia* should finalize the taxonomic placement of the new bacterium from oysters.

Preliminary studies on the distribution of FIB among oysters in North America indicate the disease is widely spread throughout Washington state and British Columbia and presumably in California (Katkanski and Warner, 1974). The disease is most problematic among adult oysters in warm shallow bays in the mid to late summer and early fall and in certain areas it may be responsible for the phenomenon known as "summer mortality".



PUBLICATIONS AVAILABLE

The European Aquaculture Society has announced the availability of two new publications:

- Realism in Aquaculture, Achievements, Constraints, Perspectives. M. Bilio, H. Rosenthal, and C.J. Sindermann (Editors)
- Pathology in Marine Aquaculture - Pathologie en Aquaculture Marine. C.P. Vivares, J.-R. Bonami, and E. Jaspers (Editors).

Both these publications are available from: EAS, Prinses Elisabethlaan 69, B-8401 Bredene, Belgium.

RENIBACTERIUM SALMONINARUM "BAR FORMS" AND THEIR ASSESSMENT BY QUANTITATIVE FAT

John Cvitanich,
Anadromous, Inc., 500 S.W. Madison
Corvallis, OR 97333

Because of the high cost of erythromycin, we have spent the past 3 years trying to critically evaluate its efficacy against BKD while at the same time attempting to collect meaningful data for the Investigational New Animal Drug (INAD) program. In the process we developed a sensitive, quantitative, direct fluorescent antibody technique (QFAT) and observed *Renibacterium salmoninarum* (Rs) "bar forms" which we believe to represent a direct manifestation of a host response.

In our hands, the QFAT is at least as sensitive as the current ELISA's, and has the additional advantage of being able to assess and quantitate "bar-form" development. The major differences between QFAT and conventional FAT are that (1) entire kidneys are homogenized, (2) approximately 100% cellular confluent smears are prepared so that a 400X or 1000X field can be defined, (3) xylene is used as a pre-staining wash step to improve staining qualities, (4) smears are stained for 1 hour, and (5) up to 300 defined fields are examined using both 400X and 1000 X. The results are expressed as Rs/100 OIF. "Bar form" is the term coined to describe a Rs bacterium that is thought to be host modified, and can be detected only by FAT. Figure 1 is a representation of a FAT typical Rs staining morphology, in which bright green fluorescence is concentrated around the cell periphery. Figures 2 and 3 represent the predominant bar forms typically observed by FAT of kidney smears. Bar forms characteristically demonstrate an intense staining bar at the division plane in the dividing form (Figure 2) and at the end of the cell in divided or separated cells (Figure 3). In the dividing form, the bar actually appears to protrude from the cell. In bar forms, bright green fluorescence is concentrated in the bar, dull green fluorescence is uniformly distributed throughout the cell, and there is no bright peripheral fluorescence. While there can be variability in the size of the bar form, bar location, and the characteristics of the bar itself, they all are variations of the same basic structure.

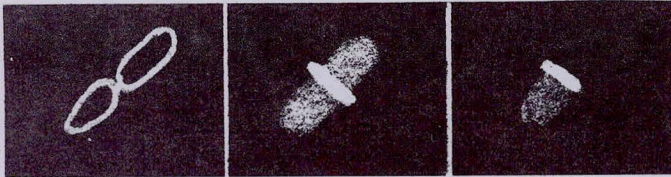


Figure 1: FAT staining morphology of a normal Rs cell.

Figure 2: FAT staining morphology of a typical dividing bar form. Division or separation results in the form seen in Figure 3.

Figure 3: FAT staining morphology of the most frequently encountered bar form.

Commonly associated with bar forms are bright fragments, believed to be products of bar form degeneration and which seem to increase in number as recovery from infection occurs. Later in the host response, variable-sized pockets of condensed fragments and deteriorated bar forms are seen. These "debris sacs", as we have crudely termed them, are usually devoid of any nucleus and are believed to be cytoplasmic remnants of phagocytic cells that have fragmented.

Since our first recorded observation of a bar form in 1983 in a juvenile coho from salt water, we have found them at private, state, and federal (USFWS) facilities in Oregon and Washington. To date they have been observed in coho, spring and fall chinook, and Atlantic salmon. They have been observed in both males and females, in fresh and salt water,

in antibiotic-treated and untreated fish, and at all stages of development from fry to adults. We believe that they are widely distributed and our observations are not ones of a recent phenomenon.

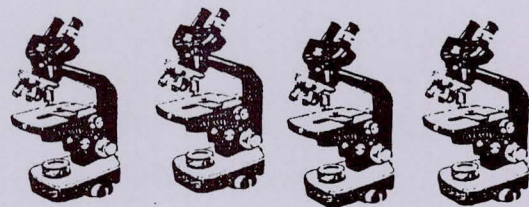
Extensive monitoring of natural situations, as well as special BKD studies with QFAT, have repeatedly put us in the right place at the right time to not only detect bar forms and their associated structures, but have enabled us to piece together the sequence of events we believe occurs. The facts and observations accumulated over the past three years have allowed us to speculate that bar forms, with their associated structures, represent host-affected Rs cells that are rendered non-virulent and non-viable. The process of bacterial degradation may take three months or more. The accumulated data which support this contention are as follows:

1. Fish that die from BKD have either no detectable bar forms or generally less than 0.1%.
2. Repeated attempts to culture bar forms on numerous media formulations have been unsuccessful.
3. General observations over the course of an outbreak indicated that bar forms deteriorate with time: they have a reduction and variability in size, weaker staining properties, what appears to be their conversion to fragments, and their association with debris sacs.
4. Transformation from normal Rs cells to bar forms may occur spontaneously or may be associated with oxytetracycline or erythromycin treatments. In these cases, there is no further progression in the infections and fish appear to recover.

While QFAT is admittedly more time consuming, it has consistently provided useful data which has allowed us to follow fish populations at various times and at each sampling construct what we simply call a KD "severity curve". Plotting both "normal Rs" and "bar form Rs" enables us to assess not only Rs prevalence but, more importantly, (1) severity levels among individual fish, (2) the % bar forms among those same fish which allows determination of the degree of host response, (3) the % of the population which is terminally affected, and (4) what proportion (%) of the population might benefit from any treatment.

Recognizing bar forms and their associated structures, as well as how to use the QFAT system, takes considerable experience. However, being able to qualitatively and quantitatively determine Rs changes directly within the host is invaluable for assessing pathogenesis, effects of diets and treatments, and spontaneous recovery from KD.

Current work, including EM evaluation, is ongoing in an attempt to further elucidate characteristics of bar forms, as well as the mechanism and sequence of events that occur within the host.



The editors of your Newsletter solicit contributions from the membership. Deadline for submission for the next publication is March 15, 1987.

DETECTION OF INFECTIOUS PANCREATIC NECROSIS VIRUS CAN BE ENHANCED BY FRACTIONATING OVARIAN FLUID

P.E. McAllister
U.S. Fish and Wildlife Service,
National Fish Health Research Laboratory
Box 700, Kearneysville, WV 25430

Infectious pancreatic necrosis virus (IPNV) is one of the most ubiquitous of the fish viruses. In young salmonids, high levels of mortality are generally associated with IPN infection; whereas, for fish older than 4 months, mortality is usually negligible. Survivors of infection can remain lifelong, asymptomatic carriers of the virus. These fish serve as the reservoir of infection for contemporary and subsequent generations of fish by shedding infectious virus with urine, feces, and sex products.

The principle nondestructive method for detection of virus carrier adult fish is the assay of sex products collected at spawning. We studied the fluid associated with the eggs (ovarian fluid) comparing the incidence of virus detection and the relative virus titer of fractions of ovarian fluid separated by centrifugation. We found that the pellet fraction, rather than the supernatant fraction, should be used for virus assays.

Ovarian fluid samples were collected from a population of brook trout (*Salvelinus fontinalis*) carrying IPNV. The samples were assayed for infectious virus within 4 h after collection. The ovarian fluids were fractionated by centrifugation at 1500 X g for 20 min at 4°C. The pellet fractions, which contained predominantly lymphocytes, macrophages, smudge cells, and erythrocytes, were resuspended in 0.1 M phosphate buffered saline at one-tenth the original fluid volume and disrupted by sonication for ten 1 s bursts at 100 watts. Infectivity assay showed the normalized virus titer of the pellet fraction was up to 3000-fold greater than that of the supernatant fraction. The incidence of virus detection was consistently greater for the pellet fraction (100%) than for the supernatant fraction (50-60%). Infectious virus in the pellet fraction was detected at a level equivalent to a titer of 5×10^{-1} plaque forming units per milliliter in the original ovarian fluid.

BRIEF REPORTS

Whirling Disease Reclassified. The California Fish and Game Commission downgraded the classification of whirling disease of salmonids from Category 3, catastrophic, to Category 2, serious, at its July 31, 1986 meeting.

This amendment to section 245, Title XIV, CAC, was proposed by the Department of Fish and Game and had the support of the Aquaculture Disease Committee and CAA. Reprinted from: California Aquatic Farming, October 1986.

According to Tore Hastein, head of Norway's National Veterinary Institute, the greatest disease problem in the country's salmon farming industry is hemorrhagic syndrome, the so called "Hitra disease." Since 1977, it has caused more mortalities than any other disease. One of the vaccines for it is being produced by Biomed Research Laboratories in Seattle. Environmental diseases like red tides have caused problems from time to time, and toxic algae blooms seem to be on the increase. Reprinted from Aquaculture Digest, December 1986.

The USFWS has expressed willingness to discuss informally or respond to written requests concerning the issue forwarded by Keith Johnson concerning "tankering" of Atlantic salmon from Norway. Questions should be made to John G. Nickum, USFWS, Division of Program Operations, Division of Fish Hatcheries, Washington, D.C. Telephone: 202-653-8746.

FUTURE MEETINGS

International Aquaculture Conference shall take place during "Aquaculture Europe '87" in Amsterdam, The Netherlands, from June 2-4, 1987. Send inquiries to EAS, Prinses Elisabethlaan 69, B-8401 Bredene, Belgium.

The first International Congress of Aquariology, which was held in Monaco in 1960, has been a milestone in the history of this science, where the bases of modern scientific aquariology were established. An International Congress of Aquariology has not been organized since. The Oceanographic Museum invites you to participate in the

2nd INTERNATIONAL AQUARIOLOGY CONGRESS

which will be held in Monaco from February 22nd to 28th, 1988, under the High Patronage of H.S.H. Prince Rainier III of Monaco.

During this congress, world specialists will gather to evaluate the advances in knowledge and techniques and to discuss problems related to aquariums.

Efforts will be made as to encourage the participation of representatives from tropical countries; they are particularly concerned with the problems of developing their resources while protecting their environment.

If you are interested in receiving further information, please write 2e Congres International D'Aquariologie, Musee Oceanographique, MC 98000 Monaco.

SHORTCOURSE ANNOUNCEMENT

The annual fish disease shortcourse entitled "Introduction to Fish Health," will be presented March 23-27, 1987. This shortcourse is co-sponsored by the Fish and Wildlife Service and the Aquaculture Program of Mt. Hood Community College.

The shortcourse will begin at 8:00 a.m. on March 23 in the Fisheries and Horticulture Building of the Mt. Hood Community campus in Gresham, OR. Resident instructors for this course will be Jim Warren and Steve Leek. The scope and content of the material presented is designed to provide hatchery personnel with up-to-the-minute knowledge of disease problems in salmonid fishes.

Twenty participants can be accommodated on a first-come, first-served basis. When selections have to be made, we seek assistance from agency administrators in identifying those individuals with the highest priority for training. The closing date for receiving applications is February 1, 1987. Selections will be made during the following week and successful applicants will be notified as soon as possible. Motel and transportation information will be provided in mid-February. Additional information is available by calling Jim Warren at (206) 696-7605.

If you want a space to be reserved for the 1987 shortcourse, letters of application (including an approved form SF-182 for U.S. Government applicants) should be sent to the Leetown Fisheries Academy. Applications should be submitted as soon as possible and certainly no later than February 1, 1987. Send applications to:

Fisheries Academy
National Fisheries Center-Leetown
Box 700
Kearneysville, WV 25430

ERRATA

The volume and number of the last Newsletter should read Volume 14, number 4, not 5 as indicated on the first page.

FISH HEALTH NEWSLETTER

The Fish Health Newsletter is a quarterly publication of the Fish Health Section of the American Fisheries Society. Submissions of any length on a topic of interest to fish health specialists are encouraged and should be addressed to one of the editorial staff or to a member of the publication committee.

Editors:

James R. Winton
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FHS NEWSLETTER
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