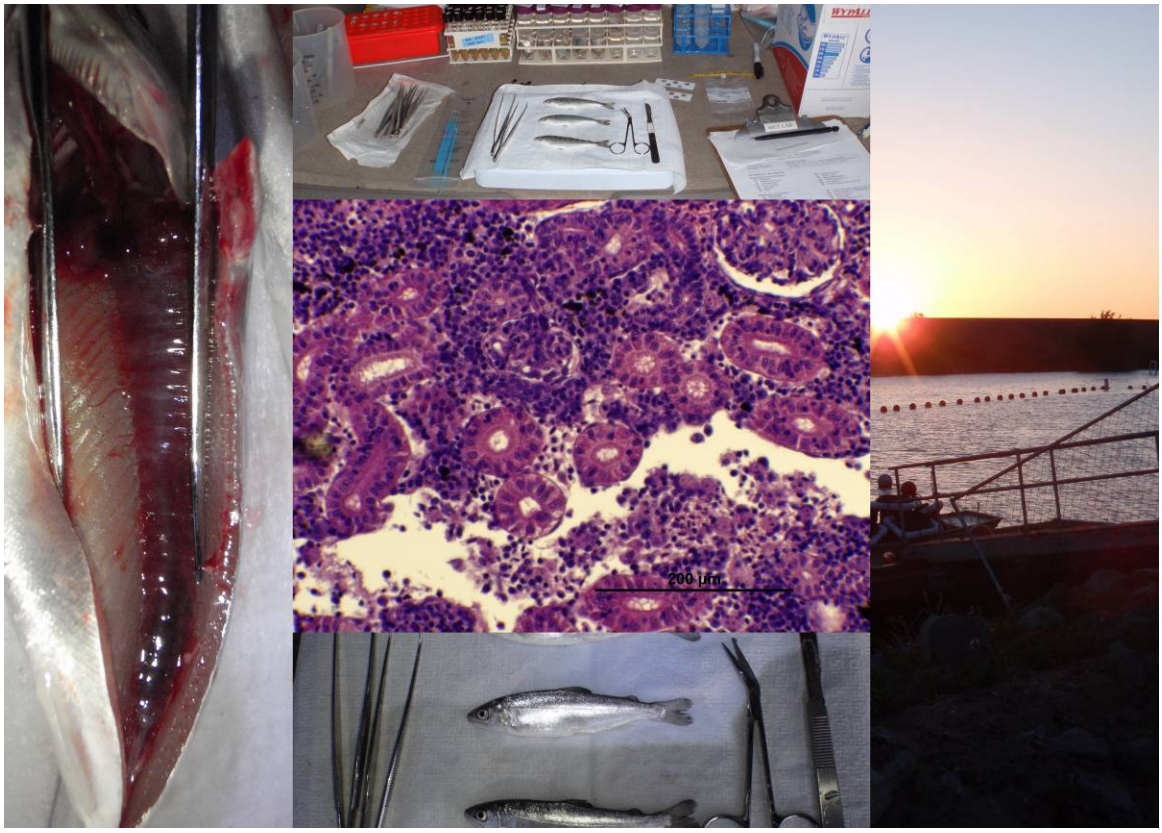
U.S. Fish & Wildlife Service

FY2010 Technical Report:
Health and Physiological Assessment of VAMP Release Groups

Ken Nichols



September 2010

US Fish and Wildlife Service
California-Nevada Fish Health Center
24411 Coleman Fish Hatchery Rd
Anderson, CA 96007
(530) 365-4271 Fax: (530) 365-7150
http://www.fws.gov/canvfhc/

SUMMARY
A general pathogen and physiological screening was conducted on three of the seven 2010 VAMP release (tagged) groups and cohorts of release groups remaining at Merced River Hatchery (MRH). No viral or bacterial pathogens were detected in the release groups. The most significant health problem observed was *Tetracapsuloides bryosalmonae* infection, with majority of salmon examined exhibiting early stages of clinical Proliferative Kidney Disease. No mortality or evidence of physiological impairment was observed either the tagged or MRH groups.

Recommended citation for this report is:
Nichols K. 2010. FY2010 Technical Report: Health and Physiological Assessment of VAMP Release Groups. U.S. Fish & Wildlife Service California-Nevada Fish Health Center, Anderson, CA. Available: http://www.fws.gov/canvfhc/reports.asp.

INTRODUCTION

As a component of the Vernalis Adaptive Management Plan (VAMP) study on reach-specific survival and distribution of migrating Chinook salmon in the San Joaquin River and delta, the CA-NV Fish Health Center conducted a general pathogen screening and smolt physiological assessment. The health and physiological condition of the fish helps explain their performance and survival during the study. Pathogen screening during past VAMP studies has regularly found infection with the myxozoan parasite *Tetracapsuloides bryosalmonae*, the causative agent of Proliferative Kidney Disease (PKD). This parasite has been shown to cause mortality in Merced River Hatchery salmon with increased mortality and faster disease progression in fish at higher water temperatures (Ferguson 1981; Foott, Stone and Nichols 2007). The objectives of this project was 1) survey the juvenile Chinook population used for the VAMP study for specific fish pathogens including *Tetracapsuloides bryosalmonae*, 2) assess smolt development (gill Na^+-K^+ ATPase) and 3) determine if holding and tagging fish in delta water had any detrimental effect on the health.

METHODS

Sample groups – Two groups of fish were examined. The first groups were dummy-tagged fish, held in pens for 48 hours in the San Joaquin River at the Durham Ferry, Old River, and Stockton release sites (tagged). The second group was unmarked cohorts held at the Merced River Hatchery (MRH). Health monitoring was performed during three of the seven 2010 VAMP release periods (Table 1). For tagged groups, 10 fish at each of the 3 release sites were sampled during the 1st, 3rd and 7th releases. For the MRH groups, 30 fish were sampled directly from the hatchery tanks on April 28th and May 5th (1st and 3rd releases).

Table 1. Fish sampled for VAMP 2010 for health and physiological assessment. Groups included dummy tagged fish held for 48 hours at the release sites (Tagged) and unmarked cohorts held at Merced River Hatchery (MRH).

Release	***Dates***	***Tagged***	***MRH***
1st Release	April 28th-29th	30	30
3rd Release	May 5th-6th	30	30
7th Release	May 19th-20th	30	No sample

Sample collection – Fish were euthanized in groups of 3 or 4 fish, any abnormalities were noted, and tissue samples for pathology and physiology assays were collected. Field collection and lab assays are briefly described below:

Bacteriology – A sample of kidney tissue was collected aseptically and inoculated onto brain-heart infusion agar. Bacterial isolates were screened by standard microscopic and biochemical tests (USFWS and AFS-FHS 2007). These screening methods would not detect *Flavobacterium columnare*. *Renibacterium salmoninarum* (the bacteria that

causes bacterial kidney disease) was screened by fluorescent antibody test of kidney imprints.

Virology – Four fish pooled samples of kidney and spleen were inoculated onto EPC and CHSE-214 and incubated for 24 days (including a 14 day blind pass) at 15ºC. (USFWS and AFS-FHS 2007).

Histopathology –The gill, liver, intestine and posterior kidney were rapidly removed from the fish and immediately fixed in Davidson's fixative, processed for 5 μm paraffin sections and stained with hematoxylin and eosin (Humason 1979). All tissues for a given fish were placed on one slide and identified by a unique code number. Each slide was examined under a light microscope. Infections of the myxozoan parasite *T. bryosalmonae* were rated for intensity of parasite infection and associated tissue inflammation. Intensity of infections was rated as None (zero), Low (<10), Moderate (11-30) or High (>30) based on number of *T. bryosalmonae* parasites observed. Kidney inflammation rated as normal, focal, multifocal or diffuse. Data analysis was performed using R version 2.11.1 using Fisher's Exact Test for Count Data.

Gill ATPase - Gill Na^+, K^+-Adenosine Triphosphatase activity (ATPase) was assayed by the method of McCormick and Bern (1989). Gill ATPase activity is correlated with osmoregulatory ability in saltwater and is located in the chloride cells of the lamellae. Data analysis was performed using R version 2.11.1 by Wilcoxon rank sum and Kruskal-Wallis rank sum tests.

RESULTS

Summary results of pathogen testing are presented in Table 1. No obligate viral or bacterial pathogens were detected however *Aeromonas-Pseudomonas* bacteria were isolated in 11% of the bacterial samples. This group of gram-negative bacterial is ubiquitous in soil and water as well as the intestinal tract of fish (Aoki 1999). It is often classified as an opportunistic fish pathogen. No clinical signs of bacterial septicemia were observed in these fish.

Table 1. Summary of pathogen screening of 2010 VAMP study fish. Assays included: virology by tissue culture; bacteriology by culture; fluorescent antibody test for *Renibacterium salmoninarum* (*Rs*-FAT).

Assay	*Samples*	*Total Fish*	*# Pos (%)*	*Pathogen*
Virology	47	150	0	No virus detected
Bacteriology	150	150	0	No obligate bacterial pathogens
			16 (11%)	*Aeromonas/Pseudomonas*
Rs-FAT	60	60	0	None detected

Histopathology – Infections with *T. bryosalmonae* were observed 99% (148/149) of the fish examined. Clinical PKD was observed in both Tagged and MRH fish groups. In tagged sample groups, the incidence of clinical PKD (multifocal or diffuse kidney

inflammation, Table 2) increased from 31% (9/29) during the 1st release, to 40% (12/30) during the 3rd release and 87% (26/30) during the 7th release ($p<0.001$). No difference in incidence of clinical PKD (disease state) was observed between tagged and MRH fish groups in either the 1st ($p=0.125$) or 3rd ($p=0.412$) releases. The intensity of *T. bryosalmonae* infection (number of parasites) was significantly lower in MRH fish compared to tagged fish groups in both the 1st and 3rd releases (Table 3, $p<0.001$ both releases). The intensity of infection increased with later releases in tagged groups ($p<0.001$), but no difference was detected in fish sampled at MRH between the 1st and 3rd release periods ($p=0.072$). An apparent difference in liver glycogen reserves was noted between tagged and MRH fish groups. In histological examination of the liver fish from the MRH groups appeared to have higher hepatic glycogen reserves compared to tagged fish (Figure 1). No significant external parasitic infections or evidence of adverse environmental conditions were identified in any of the gill sections examined.

Table 2. Severity of clinical Proliferative Kidney Disease in Chinook salmon used in the 2010 VAMP studies. Data presented as the number of fish with kidney inflammation rated as normal, focal, multifocal or diffuse in histological examination. Fish were 48-hour dummy tag (Tagged) or Merced River Hatchery (MRH) groups sampled on the 1st, 3rd or 7th releases.

Release	*Group*	*Normal*	*Focal*	*Multifocal*	*Diffuse*
1st Rel	Tagged	1	19	6	3
	MRH	14	12	3	1
3rd Rel	Tagged	1	17	10	2
	MRH	2	20	7	1
7th Rel	Tagged	0	4	13	13

Table 3. Intensity of *T. bryosalmonae* infection in Chinook salmon used in the 2010 VAMP studies. Data presented as the number of fish with zero (None), <10 (Low), 11-30 (Moderate) or >30 (High) *T. bryosalmonae* parasites observed in histological examination of kidney tissue. Fish were 48-hour dummy tag (Tagged) or Merced River Hatchery (MRH) groups sampled on the 1st, 3rd or 7th releases.

Release	*Group*	*None*	*Low*	*Moderate*	*High*
1st Rel	Tagged	0	0	17	12
	MRH	1	13	11	5
3rd Rel	Tagged	0	0	10	20
	MRH	0	6	20	4
7th Rel	Tagged	0	1	1	28

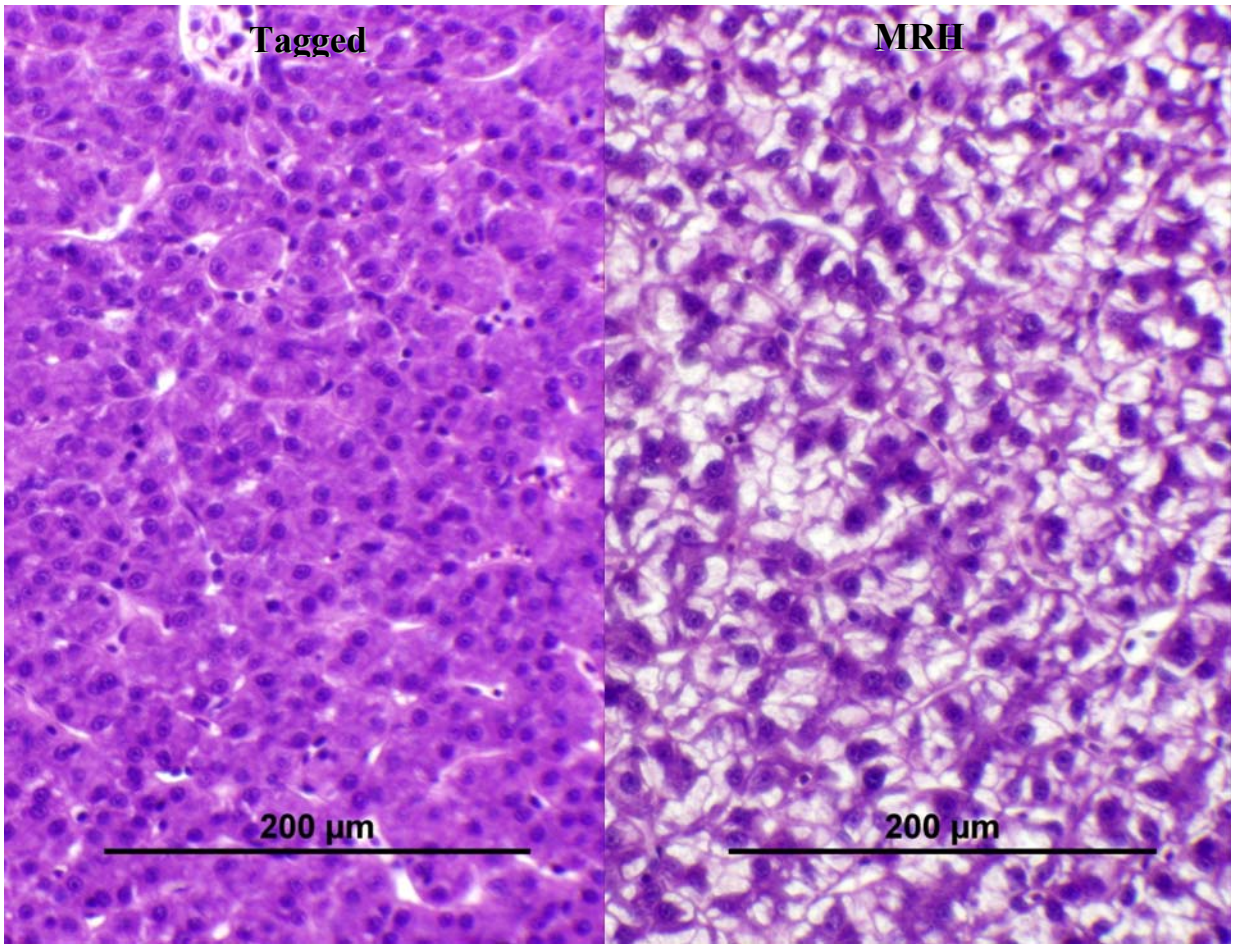


Figure 1. Micrographs of hepatic glycogen reserves (clear white vacuoles) in 48-hour dummy tag (Tagged) and Merced River Hatchery (MRH) salmon livers. Note the dense cytoplasm in smaller hepatocytes of Tagged groups vs. enlarged, vacuolated hepatocytes typical of MRH groups.

Gill ATPase activity values ranged from 2.1 to 14.4 µmol ADP/mg protein/hr. No difference was observed between tagged and MRH fish groups in the 1st and 3rd Releases ($p=0.283$ and $P=0.546$). A slight decline in ATPase activity between releases was observed in tagged groups ($p=0.017$, Figure 2).

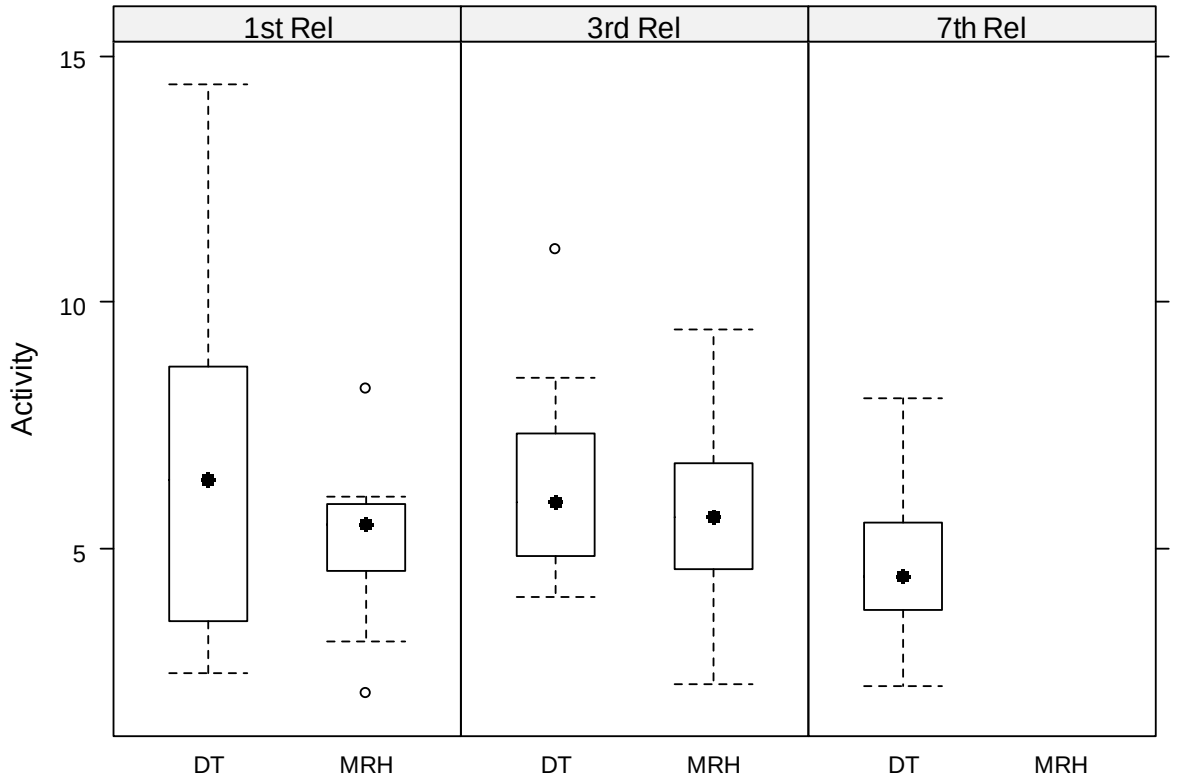


Figure 2. Gill Na+, K+-Adenosine Triphosphatase activity (µmol ADP/mg protein/hour) in Chinook salmon which were dummy tagged and held for 48 hours at the release site (DT) or cohorts of tagged fish held at the Merced River Hatchery (MRH). Sampling was performed on the first (1st Rel), third (3rd Rel) and seventh (7th Rel) of the 7 release periods.

DISCUSSION

Most of the 2010 VAMP study fish were in early stage PKD. High incidences of *T. bryosalmonae* infection are not unusual in juvenile Chinook from MRH. The onset of clinical disease in these fish normally occurs after the VAMP studies have concluded (Foott, Stone and Nichols 2007; Foott and Stone 2008). In 2010, clinical PKD was observed in 31% of the tagged fish from 1st release and by the 7th release 87% of these fish had clinical infections. In 2005 and 2008, VAMP study fish were held in the CA-NV Fish Health Center wet lab and observed through the typical PKD period, and total mortality due to the disease was low at 20%-27% (Foott, Stone and Nichols 2007; Foott and Stone 2008). These studies also found that fish with clinical PKD continued to perform well until late into the disease. No mortality was observed in the tagged fish groups sampled in this study suggesting VAMP study fish had not entered late stage disease by the time fish were released. Proliferative Kidney Disease is progressive and some of the study fish would eventually become impaired due to the disease; however, this study did not follow fish condition or mortality after release. In the future, it would

be possible to estimate performance of study fish after release by tracking mortality in cohorts of the tagged fish held in tanks for the expected study period.

Compared to MRH cohorts, tagged fish groups had higher parasite intensity and lower hepatic glycogen reserves. While there was a higher intensity of *T. bryosalmonae* infections in tagged groups compared to cohorts at held at MRH, no significant difference in disease severity was detected. Replication of this parasite within the fish host is temperature dependent (Ferguson 1981). It was expected that PKD progression would follow the same pattern as parasite intensity. The histological rating system used may not have been sensitive enough to detect the change. A rating system based on several tissues may better summarize overall disease state. A difference in hepatic glycogen reserves between tagged and MRH fish groups was also noted. While it was not quantified the difference between the groups was readily apparent in histological examination of the liver (Figure 1). It is not unusual to find high hepatic glycogen in hatchery fish which are fed a high energy diet. The lower glycogen stores in tagged groups was a possible indicator of short term starvation or stress (Phillips 1969; Barton, Morgan and Vijayan 2002) and was observed in all tagged fish groups. In the future it would be of interest to monitor energy storage to determine if any release groups were at a disadvantage.

Gill ATPase activity in salmonds typically increases and peaks near the time of most active migratory behavior (Duston, Saunders and Knox 1991; Ewing, Ewing and Satterthwaite 2001; Wedemeyer 1996). Median activity levels measured in this study (5.8 μmol ADP/mg protein/hr) were lower than activity levels measured in the 2009 VAMP study (7.3-10.4 μmol ADP/mg protein/hr, Nichols and Foott 2009). Due to differences in sampling conditions and assay conditions between years these values will have some variability. However, the data also suggests activity levels were declining in the later release groups. It is possible 2010 VAMP release groups had already reach peak smolt status and were beginning parr-reversion. Decreases in ATPase activity can also occur due to increases in water temperature (Duston et al. 1991). Once fish reach salt water gill ATPase activity levels can rapidly increase.

Acknowledgments
Biologist with the USFWS Stockton FWO were great help in the logistics of study fish and live cages. Scott Foott with the California-Nevada Fish Health Center assisted with reading of histology samples and the ATPase assay.

REFERENCES

Aoki T. 1999. Motile Aeromonads. Chapter 11 In: Fish Diseases and Disorders, Vol. 3: Viral, Bacterial and Fungal Infections, Woo P T K and Bruno D W, editors, CABI Pub. New York.

Barton, B A, J D Morgan and M M Vijayan. 2002. Physiological and condition-related indicators of environmental stress in fish. In: Adams S M, editor. Biological Indicators of Aquatic Ecosystem Stress. American Fisheries Society, Bethesda, Maryland. 111-148

Duston J, R L Saunders and D E Knox. 1991. Effects of increases in freshwater temperature on loss of smolt characteristics in Atlantic salmon (Salmo salar). Canadian Journal of Aquatic Animal Sciences 48: 164-169.

Ewing R D, G S Ewing and T D Satterthwaite. 2001. Changes in gill Na^+, K^+-ATPase specific activity during seaward migration of wild juvenile Chinook salmon. Journal of Fish Biology 58: 1414-1426.

Foott J S and R Stone. 2008. FY 2008 investigational report: Evaluation of sonic tagged Chinook juveniles used in the 2008 VAMP study for delayed mortality and saltwater survival – effects of Proliferative Kidney Disease. US Fish and Wildlife Service, California-Nevada Fish Health Center, Anderson, CA. Available: http://www.fws.gov/canvfhc/reports.asp (September 2010).

Foott J S, R Stone and K Nichols. 2007. Proliferative Kidney Disease (*Tetracapsuloides bryosalmonae*) in Merced River Hatchery juvenile Chinook salmon: mortality and performance impairment in 2005 smolts. California Fish and Game 93: 57-76.

Ferguson, H W. 1981. The effects of water temperature on the development of Proliferative Kidney Disease in rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Disease 4: 175-177.

Humason G L. 1979. Animal Tissue Techniques, 4th edition. W H Freeman and Co., San Francisco.

McCormick S D and H A Bern. 1989. In vitro stimulation of Na+-K+-ATPase activity and ouabain binding by cortisol in coho salmon gill. American Journal of Physiology. 256: R707-R715.

Nichols K. and J S Foott. 2009. FY2009 technical report: health and physiological assessment of VAMP release groups. U.S. Fish & Wildlife Service California-Nevada Fish Health Center, Anderson, CA. Available: http://www.fws.gov/canvfhc/reports.asp (September 2010).

Phillips A M. 1969. Nutrition, digestion and energy utilization. In: Hoar W S and Randall D J, editors. Fish Physiology. Vol I. Academic Press, San Diego. p. 391-432

Wedemeyer G A. 1996. Physiology of Fish in Intensive Culture Systems. Chapman & Hall, New York.

USFWS and AFS-FHS (U.S. Fish and Wildlife Service and American Fisheries Society-Fish Health Section). 2007. Standard procedures for aquatic animal health inspections. *In* AFS-FHS. FHS blue book: suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2007 edition. AFS-FHS, Bethesda, Maryland.