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Pacific Northwest
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Evaluation of Using Caged Clams to Monitor Contaminated Groundwater Exposure in the Near-Shore Environment of the Hanford Site 300 Area

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Prepared for the U.S. Department of Energy
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Summary

Past operations at the Hanford Site 300 Area have resulted in the release of radiological and chemical contaminants to the soil and groundwater and eventual transport to the Columbia River. Contaminants of concern at the 300 Area include uranium, trichloroethene, and cis-1,2-dichloroethene. The primary contaminant of concern is uranium, which remains above drinking water standards in much of the shallow groundwater beneath the 300 Area. As part of efforts to assess the risk that contaminated groundwater may pose to ecological receptors, the U.S. Department of Energy (DOE) has funded investigations of aquatic organisms living in the near-shore river environment where contaminated shallow groundwater upwelling may occur. One group potentially at risk is benthic organisms that live within the unconsolidated portion of the riverbed and are in contact with groundwater upwelling.

The Asiatic clam (*Corbicula fluminea*) is a benthic species that has been identified as an indicator species for locating and monitoring contaminated groundwater in the Columbia River. Pacific Northwest National Laboratory conducted a field study to explore the use of caged Asiatic clams to monitor contaminated groundwater upwelling in the 300 Area near-shore environment and assess seasonal differences in uranium uptake in relation to seasonal flow regimes of the Columbia River. Additional objectives included examining the potential effects of uranium accumulation on growth, survival, and tissue condition of the clams.

The study was conducted between November 2003 and February 2005 at two near-shore locations. One location was a site of known contaminated groundwater upwelling near the Hanford Site 300 Area, and the other was an upstream reference location near the State Highway 240 Vernita Bridge. Clams used in the study were collected near the upstream reference location, divided into two groups, and then deployed in mesh containers at the two field sites for four seasonal periods (two winters, one spring, one summer). Two mesh container designs were tested in the study. Tissue concentrations of uranium and tissue condition were measured in the spring, summer, and second winter deployments. Growth and survival were measured in all deployments. U.S. Army Corps of Engineers discharge data was used to monitor river discharge, and screened-piezometer water quality data loggers were used to monitor water depth, specific conductance, and temperature throughout the study in an effort to assess the presence or absence of groundwater where clams were deployed.

Uranium concentrations in clam soft tissues varied between seasonal periods; the highest levels occurred in clam samples from the spring and summer deployments, and the lowest level occurred in samples from the winter. Hydrologic data indicated that periods of higher accumulation corresponded to periods of predominantly lower flows (<100 kcfs). Water chemistry data showed an increase in groundwater presence during these periods as well. Measures of growth, survival, and tissue condition did not show signs of a toxicological effect from uranium exposure. Factors that may have affected the results of the study but which were outside the project scope include indirect effects of abiotic parameters such as substrate, temperature, water velocity and depth on clam physiology; and the variability in contaminant uptake and depuration within each deployment.

Growth coincided in a general sense with other seasonal and age-related trends that have been reported for Asiatic clams and other bivalves but was much lower overall than indicated in other growth studies. Low growth may have been due to reductions in shell length that occurred throughout the study,

which we believe were caused by physical wear on the shell from contact with the mesh tubing in which clams were contained. Because of this, the particular clam containment designs used in this study may have limited utility for assessing physiological effects in Asiatic clams that might be associated with contaminant accumulation. The design was adequate for obtaining a representation of seasonal and site-specific differences in contaminant accumulation, but actual levels of accumulation and rates of growth and survival under natural conditions may be different than those observed in this study.

Additional testing of other clam containment designs that better simulate a clam's natural environment may yield a design that is more useful for conducting caged clam environmental monitoring studies on the Hanford Reach. Other research suggested includes characterizing the variability in contaminant uptake that may be associated with daily changes in groundwater exposure in the near-shore area. Also, more information is needed regarding the effects on clam ecology of abiotic parameters such as substrate, temperature, water velocity, and depth, and how they may influence contaminant uptake.

Acknowledgments

The authors thank Dana Ward, DOE Richland Operations Office, for his support of better understanding the complex interactions of Hanford contaminants in the environment. We would also like to thank Brian Miller, James Bernhard, Gerald Turner, Jennifer Panther, Jacklyn Newell, Rhett Zufelt, and Robin Durham for their help in the field and in collecting samples. Jill Brandenberger at the Battelle Marine Sciences Laboratory in Sequim, Washington, conducted the uranium analyses. Ralph Elston of AquaTechnics and the Pacific Shellfish Institute, Sequim, performed the histological assays. We also thank Craig McKinstry for conducting the statistical analyses. Evan Arntzen, Brad Fritz, Bob Peterson, and Donny Mendoza provided valuable insight on hyporheic interactions at the 300 Area and interpretation of water quality data. Janelle Downs and Amanda Stegen provided additional technical review.

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1.0 Introduction

Several contaminants of potential ecological concern, including chromium, uranium, and strontium-90, have been identified in the soil, sediment, groundwater, wildlife, and Columbia River on the U.S. Department of Energy (DOE) Hanford Site in south-central Washington (Patton et al. 2003a; Poston et al. 2004; Weiss 2005; Fluor Hanford, Inc. 2005). In certain areas, these contaminants are mobilized and transported by groundwater, eventually entering the river as either bank seepage (McCormack and Carlile 1984; Dirkes 1990; Peterson and Johnson 1992) or upwelling through the riverbed (Campbell 1994; Lee et al. 1997; Peterson and Connelly 2001; Patton et al. 2003b). As a result, aquatic biota can be exposed to these contaminants, particularly in the near-shore areas where groundwater first enters the river.

In 2002 and 2003, above-background levels of Hanford-derived contaminants were found in benthic organisms (organisms that spend the majority of their life cycle on the bottom of marine or freshwater environments) living along the 300 Area shoreline (Patton et al. 2003b; Tiller 2004). Some of the highest levels of these contaminants, particularly uranium, were found in Asiatic clams (*Corbicula fluminea*). In response to these findings, Pacific Northwest National Laboratory (PNNL) conducted a field study to examine the use of caged Asiatic clams to conduct environmental monitoring in near-shore areas of the Hanford Reach and assess seasonal differences in uranium uptake in Asiatic clams.

The Asiatic clam was introduced to North America in the 1930s and has since become one of the most abundant mollusks in the Columbia River (Britton and Morton 1979; McMahon 1982). It can be found in a variety of benthic habitats on the Hanford Reach, including those in near-shore areas where groundwater upwelling occurs. Its elevational distribution in near-shore areas is limited by the amount of time the shoreline is submerged (Turner 2004), which varies due to changes in discharge at Priest Rapids Dam. Asiatic clams are found primarily at elevations that are inundated greater than 95% of the year and are characterized by the presence of persistent periphyton growth, hereafter referred to as the *periphyton line* (Turner 2004). The highest densities of Asiatic clams on the Reach are found in cobble substrates (51–200 mm in diameter) with low embeddedness, where mean densities are approximately 500 clams per square meter (Turner 2004).

The local abundance of Asiatic clams in the near-shore environment of the Hanford Reach allows this species to be utilized as a practical bioindicator for monitoring contaminated groundwater entry in the Columbia River (Patton et al. 2003b; Tiller 2004; DOE-RL 2005). Because the species commonly can be found in the near-shore region of the river, it is relatively easy to collect from point sources of contamination and areas exposed to contaminant upwelling. In addition, this species has certain life history characteristics (e.g., relatively sessile, restricted to benthic habitat, filter-feeder) that make it a favorable bioindicator species for monitoring aquatic contamination. These characteristics have been documented in a number of studies (Doherty 1990; McCloskey et al. 1995; Boltovskoy et al. 1997; Johnson 1999; Labrot et al. 1999; Cataldo et al. 2001) that have utilized this species for monitoring environmental contamination in freshwater ecosystems.

Although Asiatic clams are believed to be practical bioindicators on the Hanford Reach, there is a lack of information regarding how rates of contaminant accumulation and depuration, and subsequent effects on clam health, may be affected by seasonal differences in river stage and temperature. Previous

and ongoing studies have shown that the presence of groundwater in the near-shore environment is variable due to fluctuations in subsurface hydraulic gradients that result from changes in river stage (Peterson and Connelly 2001). Given this relationship, we suspected that tissue concentrations of contaminants in Asiatic clams and the subsequent potential effects on their health may vary throughout the year as well. To examine this relationship, PNNL conducted a small-scale field study between November 2003 and February 2005 under the DOE-funded Public Safety and Resource Protection Program (PSRPP). The purpose of this study was to explore the use of caged Asiatic clams for conducting near-shore environmental monitoring and to assess seasonal differences in contaminant uptake in clams with respect to seasonal flow regimes of the Columbia River.

This report describes the use of caged Asiatic clams to assess seasonal differences in uranium accumulation in a near-shore environment adjacent to the 300 Area. Potential effects on clam growth, survival, and tissue condition are discussed with respect to both uranium accumulation and possible cage-related sources of variation. The methods used to obtain and analyze these data are described in Section 2, followed by the results in Section 3 and an interpretive discussion of the results in Section 4. Section 5 provides concluding remarks about the study findings.

2.0 Materials and Methods

Asiatic clams collected at an area upstream of current and historic Hanford operations were placed in mesh containers and deployed at a known contaminated groundwater site and an upstream reference location during four seasonal periods. The following sections document the materials and methods used to collect clams, conduct the field exposures, and analyze contaminant concentrations, changes in growth, survival, and tissue condition. The study area and components of the field design, including the mesh container design, collection and deployment procedures, and ancillary data collection related to hydrologic conditions and water chemistry, are described in Section 2.1. The laboratory procedures, which include measurements of growth and survival, tissue concentrations of uranium, and the assessment of tissue condition (i.e., histology), are documented in Section 2.2. The statistical analysis of growth data is described in Section 2.3.

2.1 Field Conditions and Procedures

This section outlines the field conditions and procedures for all field-based tasks of the study. Section 2.1.1 provides a brief description of the study area and individual study sites. Sections 2.1.2 through 2.1.4 describe the field components of the study design, including the design of mesh containers used to contain clams, the collection and deployment techniques for conducting the exposures, and the collection of hydrologic and water chemistry data used to assess seasonal patterns of groundwater exposure.

2.1.1 Study Area

The study was conducted on the DOE Hanford Site, which occupies approximately 1,517 km^2 (586 mi^2) of southeastern Washington just north of the city of Richland. The site also encompasses approximately 78 km (48 mi) of the only non-impounded portion of the Columbia River upstream of Bonneville Dam known as the Hanford Reach. The site originally was acquired by the federal government in 1943 and until the 1980s was dedicated primarily to the production of plutonium for national defense and the management of resulting waste. Today, the primary DOE mission for the Hanford Site is waste cleanup.

Part of this cleanup mission is to address the risks posed by contaminants that are transported by groundwater to the Columbia River. This contaminated groundwater enters the river in the form of either bank seepage (McCormack and Carlile 1984; Dirkes 1990; Peterson and Johnson 1992) or upwelling through the riverbed (Campbell 1994; Lee et al. 1997; Peterson and Connelly 2001; Patton et al. 2003b). As a result, aquatic biota may potentially be exposed to these contaminants, particularly in the near-shore areas where groundwater first enters the river. One such area is the Hanford Site 300 Area shoreline, which was the location of one of our two study sites (Figure 2.1). Past operations at the 300 Area have resulted in the release of several radiological and chemical contaminants to the soil and groundwater, including uranium, which was used in fabrication of nuclear fuel (Gerber 1992; Deford et al. 1994; Peterson et al. 2007). Our first study site was located in the near-shore area in front of a riverbank spring referred to as 300 Area Spring 9.[1] This site is a known location for contaminated groundwater seepage

[1] This site name was derived from Patton et al. (2003b), which refers to it as the name commonly used in other reports describing the Hanford Site under the Comprehensive Environmental Response, Compensation, and Liability Act. This site is referred to also as DR42-2 under the Surface Environmental Surveillance Project.

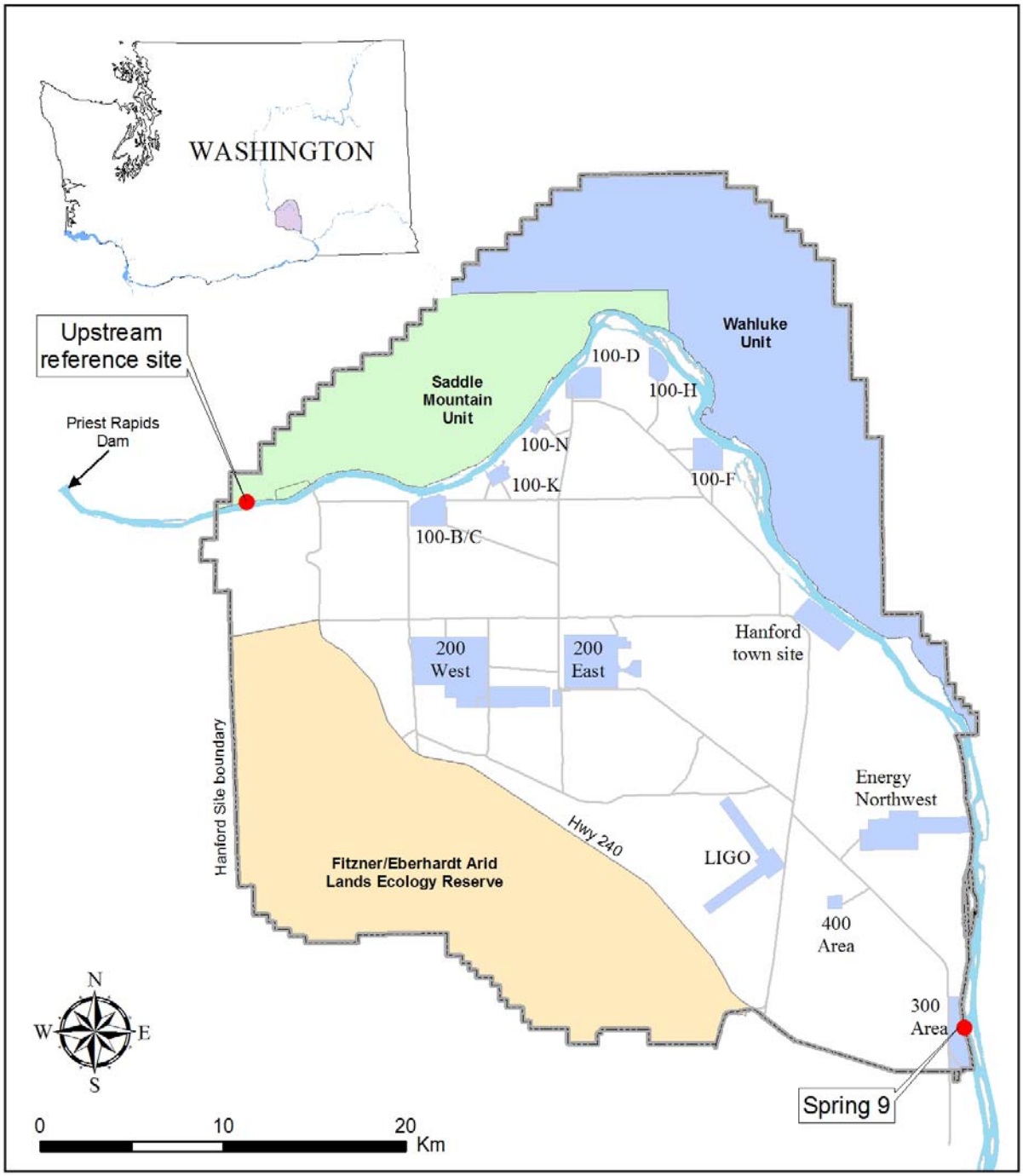

Figure 2.1. Caged Clam Study Site Locations

and upwelling (Patton et al. 2003b). Our second study site was located upstream of current and historic Hanford Site operations near the State Highway 240 Vernita Bridge (Figure 2.1). This site served as a reference site because it is outside the area of Hanford groundwater influence.

2.1.2 Design of Clam Containers

Two different container designs were used to contain clams—mesh tubes suspended within polyvinyl chloride (PVC) pipe frames (hereafter referred to as PVC cages) and mesh tubes staked individually to the river substrate (referred to as individual tubes from here on). The PVC cages consisted of a rectangular PVC pipe (2.54-cm-diameter) frame that had six sections of nylon mesh (4-mm) tubing (except for the first cage deployed at Spring 9, which consisted of four tubes) stretched across the frame. Each section of tubing contained 25 clams that were segmented into individual compartments by cinching off the tube with plastic cable ties. The PVC frames were also covered by 1.27-cm Vexar mesh (E. I. du Pont de Nemours and Company, Wilmington, Delaware) to protect the mesh tubes from being torn by debris. This design is similar to that described by Salazar and Salazar (1995), who originally developed it for suspending bivalves vertically in the water column of marine environments. For this study, however, we attached cement cinder blocks to both ends of the frame and anchored it so that it lay flat on the bottom of the river, with the mesh tubes lying (from top to bottom) parallel to the direction of flow.

The individual tubes were employed in addition to the PVC cages halfway through the study and were intended to better simulate the natural conditions of a clam living in the river cobble. The tubes themselves were essentially the same nylon mesh tubes used in the PVC cages (25 clams per tube), but instead of being suspended within PVC frames, they were laid directly over the river cobble. Each tube was placed perpendicular to the direction of flow and spaced at approximately 0.5-m intervals either upstream or downstream of the other tubes. Twelve-inch stainless steel stakes were used to anchor both ends of the tube to the substrate. This design allowed for better water flow over the clams and for the tubes to conform more closely to the substrate. Although this design is referred to as *individual tube* throughout this report, often we refer to both container designs in the cumulative sense as *cage* or *cages*.

2.1.3 Collection and Deployment of Caged Clams

Clams used in this study were collected upstream of current and historic Hanford Site operations (approximately 3.5 km upstream of the State Highway 240 Vernita Bridge). Although we did not analyze clams for uranium prior to each deployment, we assumed their initial tissue concentration of uranium was at baseline level at the beginning of each exposure. This assumption is based on both weekly and monthly monitoring of Columbia River water at Priest Rapids Dam and the Vernita Bridge from 2000 through 2005, which indicates that uranium concentrations have remained relatively stable (~0.40 pCi/L) for the past 6 years (Poston et al. 2006). Clams were collected at slightly greater water depths (i.e., 0.2–0.5 m deeper) than the uppermost elevation of persistent periphyton growth. This depth represents the lower limit for river elevation typically allowed by Priest Rapids Dam; thus, organisms living below this depth are said to be inundated 100% of the time. Upon collection, clams were placed into a cooler of fresh river water until they were measured for initial size and weight. Size was measured as the shell length (greatest anterior-posterior distance to the nearest 0.01 mm) using a pair of digital Vernier calipers. Weight was measured as the whole-animal wet weight (WAWW) to the nearest 0.01 g. Clams were then grouped by size classes (small [11–22 mm] and large [>22 mm]) and placed into cages.

Cages were placed just below the persistent periphyton growth line to ensure that clams at both sites remained underwater at all times. Deployments were intended to last 90 days; however, high river flows often made it impossible to recover clams at exactly 90 days. Deployment periods (DPs) were as follows:

- November 2003–February 2004 (DP-1)
- February–June 2004 (DP-2)
- June–October 2004 (DP-3)
- November 2004–February 2005 (DP-4).

The number of cages and clams remained the same between sites throughout most deployments (Table 2.1). At the end of each DP, clams were recovered and brought back to the laboratory for uranium tissue concentration analysis, growth measurements, determination of survival, and histological assessment (i.e., assessment of tissue condition). Clams were transported in coolers of fresh river water from the field to the laboratory.

Table 2.1. Number of Clams in PVC Cages and Individual Tubes per Deployment

			Deployment Period[(a)]				
			DP-1	DP-2	DP-3	DP-4	Total
PVC Cages	Spring 9	Number of cages	1	1	1	1	4
		Total number of clams	100	150	150	150	550
		Max number of days	92	99	80	93	– –
	Vernita	Number of cages	1	1	1	1	4
		Total number of clams	150	150	150	150	600
		Max number of days	89	103	98	116	– –
Individual Tubes	Spring 9	Number of tubes	– –	– –	6	6	12
		Total number of clams	– –	– –	150	150	300
		Max number of days	– –	– –	68	93	– –
	Vernita	Number of tubes	– –	– –	6	6	12
		Total number of clams	– –	– –	150	150	300
		Max number of days	– –	– –	71	116	– –

(a) DP-1 = November 2003–February 2004, DP-2 = February–June 2004, DP-3 = June–October 2004, DP-4 = November 2004–February 2005.

2.1.4 Collection of Hydrologic and Water Quality Data

River discharge (as measured at Priest Rapids Dam) and water depth were monitored throughout the study to identify physical conditions that affected groundwater intrusion. Hourly discharge data were obtained online from the University of Washington School of Aquatic & Fishery Sciences Internet database, Columbia River Data Access in Real Time (DART).[1] Water depth was measured hourly at each site using Solinst data loggers (LTC Levelogger, Model 3001 LTC, Solinst Canada Ltd., Georgetown, Ontario, Canada) that recorded total pressure. Barometric pressure data from another

[1] http://www.cbr.washington.edu/dart/dart.html.

Solinst logger installed onshore near the White Bluffs were used to correct total pressure data to derive water depths. The data loggers at each site were installed in piezometer tubes (5.08-cm-diameter tube with 0.145-mm pore diameter) with 30-cm screens driven into the riverbed so that the screened portion was 3 to 33 cm below the riverbed. Asiatic clams are typically found in the first 10 cm of the riverbed on the Hanford Reach (Turner 2004). The Solinst loggers also recorded specific conductance (millisiemens per centimeter, mS/cm) and temperature (degrees centigrade, °C) on an hourly basis, which were used to evaluate the presence/absence of groundwater. Specific conductance was used as a measure of groundwater presence because Hanford groundwater has a higher ion composition than the Columbia River (Zachara et al. 2005). The specific conductance of shallow groundwater on the Hanford Site usually ranges between 0.350 and 0.450 mS/cm, whereas near-shore river water typically ranges from 0.130 to 0.150 mS/cm (Peterson and Connelly 2001). These differences make it possible to locate groundwater discharge by taking conductance measurements near the riverbed surface (Lee et al. 1997). Temperature was used as a secondary indicator of groundwater presence because the temperature of Hanford groundwater is warmer than that of the river during winter and spring months (Campbell 1994).

2.2 Laboratory Procedures

This section describes the methods and procedures used in the laboratory-based portions of this study, including procedures for measuring growth and survival, measuring uranium concentrations in clam soft tissue, and assessing histological conditions.

2.2.1 Measurements of Growth and Survival

Upon recovery from the field, clams were placed in coolers of fresh river water and immediately transported back to the laboratory. Clams were then removed from cages for size measurements and survival determination. Shell length was measured in the same fashion as it was at the beginning of the deployment (i.e., greatest anterior-posterior distance to the nearest 0.01 mm) using the same pair of Vernier calipers. Weight was also measured using the same method as at the beginning of the deployment (WAWW to the nearest 0.01 g) using the same scale. Survival was determined by visual observation of activity (e.g., filtering or foot extrusion) or light prying on the shell to elicit a closure response. After measures of growth and survival were complete, clams were separated into groups for either uranium analysis or histology.

2.2.2 Concentration of Uranium in Soft Tissues

Whole-body soft tissue samples were collected in DP-2, DP-3, and DP-4 for uranium analysis. DP-1 tissue samples were not collected because it was the initial proof-of-principle deployment. Tissues were extracted by flash-steaming clams (deionized water and a stainless steel cauldron were used for steaming), which allowed for easy removal with forceps. Tissue samples were then placed into pre-cleaned plastic containers and frozen until the time of shipment for analysis. To obtain enough tissue mass for each sample, multiple individuals were combined into composite samples. In general, only individuals from the same mesh tube (either from within a PVC cage or within an individual tube) were combined, except in DP-4 when multiple tubes of the same size class were combined for PVC cages; i.e., all three tubes of small clams (11–22 mm) from a PVC cage were combined, and all three tubes of large clams (>22 mm) from a PVC cage were combined (Table 2.2). Samples were then shipped to the Battelle Marine Sciences Laboratory in Sequim, Washington, for analysis.

Table 2.2. Number of Soft Tissue Samples Submitted for Uranium Analysis

		Deployment Period			
		DP-2	DP-3	DP-4	Total[(b)]
Spring 9	PVC cages	6 (1)[(a)]	6 (1)	2	16
	Individual tubes	– –	6	6 (2)	14
Total[(b)]		**7**	**13**	**10**	**30**
Vernita	PVC cages	6 (1)	6	2	15
	Individual tubes	– –	6	6	12
Total[(b)]		**7**	**12**	**8**	**27**

(a) Number of duplicate samples are shown in parentheses ( ).
(b) Total includes duplicate samples taken after tissue was homogenized.

Before they were analyzed, samples were freeze-dried and homogenized using a ball-mill according to Battelle Standard Operating Procedure MSL-C-003, *Percent Dry Weight and Homogenizing Dry Sediment, Soil and Tissue*. Tissue samples were then digested according to MSL-I-024, *Mixed Acid Tissue Digestion*. An approximately 500-mg aliquot of each dried, homogeneous sample was combined with nitric and hydrochloric acids (aqua regia) in a Teflon vessel and heated in an oven at 130ºC (±10ºC) for a minimum of 8 hours. After heating and cooling, deionized water was added to the acid-digested tissue to achieve analysis volume. Uranium concentrations in clam tissue were measured using inductively coupled plasma-mass spectrometry (ICP-MS) according to MSL-I-022, *Determination of Elements in Aqueous and Digestate Samples by ICP/MS*. This procedure is based on two methods modified and adapted for analysis of solid sample digestates: EPA Method 1638, *Determination of Trace Elements in Ambient Waters by Inductively Coupled Plasma-Mass Spectrometry* (EPA 1996), and EPA Method 200.8, *Determination of Trace Elements in Water and Wastes by ICP-MS* (EPA 1994).

2.2.3 Histological Assessment of Tissue Condition

Clams selected for histological assessment (i.e., examination of anatomical structures and tissue condition under a microscope) were shipped live overnight to an independent laboratory (AquaTechnics, Sequim, Washington) to be analyzed by a shellfish expert. To keep clams alive during shipment, we placed them in open bags containing moist paper towels and packaged them in a cooler with blue ice to keep them cool. This method, recommended by the shellfish expert, was successful in keeping clams alive during shipping and holding.

The histological assessment consisted of an evaluation of 19 different tissue conditions in the following organ systems: digestive, reproductive, respiratory, skeletomuscular, nervous, and excretory (Table 2.3). We selected tissue conditions that represent a wide range of possible physiological responses to contaminant exposure because little is known about the effects of uranium exposure on clam biology. Sex of an animal was recorded. However, it was not used as an indicator of environmental stress because Asiatic clams may be either simultaneous or protandric (sex-switching) hermaphrodites, and the chronology of spawning activity in the Columbia River is not known for this species. Most organs or tissues were given a condition score using a predetermined index rating, except for those in which the total number of lesions was reported (i.e., follicular cysts, degenerative follicles, and reproductive ducts with necrotic cells).

Table 2.3. Tissue Conditions Evaluated Histologically

System	Organ/Tissue/Cell Type	Condition Evaluated
Digestive	Conducting tubule epithelial cells	Cell height
		Amount of shedding
		Inflammation
	Adsorptive epithelial cells	Vacuolation
Reproductive	Gonads	Sex
	Follicles/spermatic tubules	Stage of development
		Number of follicular cysts
		Number of hyaline degenerative follicles
	Ova	Normal/abnormal
	Reproductive ducts	Number of necrotic cells
Respiratory	Gills - Epithelial cells - Larvae	Inflammation
		Amount of shedding
		Presence/absence of brooding larvae
Skeletomuscular	Connective tissue	Inflammation
	Foot musculature and epithelium	Presence/absence of lesions
	Adductor muscle	Presence/absence of lesions
	Mantle epithelium	Presence/absence of necrotic cells
Nervous	Nerves/ganglia	Presence/absence of lesions
Excretory	Kidney epithelial cells	Presence/absence of necrotic cells

2.3 Statistical Analyses

A two-step analysis of variance (ANOVA) process was used to determine how the following independent factors might explain changes in shell length (ΔL) and whole-animal wet weight (ΔW): site (*S*), deployment period (*DP*), deployment type (*DT*), and size class (*SC*). Levels for each of these factors are given in Table 2.4. The strength of the relationship between the independent factors and response variables (ΔL and ΔW) was measured by the amount of variance explained by the ANOVA process.

Table 2.4. Description of Factors Used in ANOVA Models

Factor	Factor Variables				Levels	Factor Type
Site	Spring 9		Vernita		2	Fixed
Size class[(a)]	Small		Large		2	Fixed
Deployment period[(b)]	1	2	3	4	4	Fixed
Deployment type	PVC cage		Individual tube		2	Fixed

(a) Small = 11–22 mm, Large = >22 mm.
(b) 1 = Nov 2003–Feb 2004, 2 = Feb–Jun 2004, 3 = Jul–Sep 2004, 4 = Nov 2004–Feb 2005.

The first step in the ANOVA process was to test each independent factor in two separate but parallel univariate ANOVAs (i.e., one for each response variable). In the second step, independent factors were entered sequentially into a linear ANOVA model (one for each response variable) that performed tests of

significance for improved model fit as each factor was added. Factors were added in descending order based on the amount of variance attributed to each factor in the first step. It can be visualized as fitting a sequence of models as follows:

$$
\begin{aligned}
Y_{\Delta L} &= DP \\
&= DP + SC \\
&= DP + SC + DT \\
&= DP + SC + DT + S
\end{aligned}
$$

This "improved-fit" method was chosen because it effectively controls for colinearity between independent factor variables. It also allowed us to identify whether specific combinations of factors significantly influenced ΔL or ΔW. An example of this would be determining whether the variance in ΔL was significantly different between small and large clams in each deployment period (i.e., $Y_{\Delta L} = DP + SC$). Because the ANOVA model assessed only variance, we had to calculate mean growth rates in a separate step to have a growth metric that was comparable to other studies. This was done based on each factor combination used in the model. Therefore, each mean growth rate is representative of the model results.

3.0 Results

Caged Asiatic clams were placed in the near-shore area at a known contaminated groundwater site and an upstream reference location during four seasonal periods. Uranium accumulation, growth, survival, and tissue conditions were compared between sites. Qualitative data on hydrologic and water quality conditions also were collected to characterize the presence or absence of groundwater. This section summarizes those results, beginning with seasonal patterns of uranium accumulation and a discussion about seasonal hydrologic factors that may have influenced accumulation. Potential effects on growth, survival, and tissue condition are presented thereafter.

3.1 Soft Tissue Concentrations of Uranium

Mean soft tissue concentrations of uranium in caged clams at Spring 9 varied between deployment periods, indicating there were seasonal differences in uranium accumulation for clams at that site. Tissue concentrations in clams at Spring 9 were greater than in those at Vernita in DP-2 and DP-3 but did not differ from Vernita in DP-4 (Table 3.1). We compared soft tissue concentrations of uranium from our study to those reported by Patton et al. (2003b) for clams collected in situ (in their original place) at Spring 9 and at another Vernita location in 2001 at similar depths (<0.5 m below the periphyton line). Spring 9 concentrations were considerably lower in our study, whereas Vernita concentrations were approximately the same (Figure 3.1).

Table 3.1. Mean Soft Tissue Uranium Concentrations (µg/g dry weight) in Caged Clams

Deployment Period	Site Name	Mean	Standard Error	Standard Deviation	*N*	Minimum	Maximum	Difference Between Site Means[(a)]
DP-2	Spring 9	0.36	0.08	0.21	7	0.24	0.84	0.17
	Vernita	0.19	0.02	0.05	7	0.11	0.28	
DP-3	Spring 9	0.37	0.03	0.12	13	0.23	0.57	0.20
	Vernita	0.17	0.01	0.04	12	0.13	0.24	
DP-4	Spring 9	0.14	0.01	0.04	10	0.09	0.21	0.02
	Vernita	0.12	0.01	0.03	8	0.09	0.17	

(a) Calculated by subtracting Spring 9 mean from Vernita mean.

3.2 Hydrologic Conditions and Water Quality

River discharge varied at both daily and seasonal scales, resulting in variations in water depth at both sites (Figure 3.2). The diel fluctuation of water depth was similar between sites, although the amplitude of change was much less at Spring 9 (Figure 3.2) due to the longer downstream distance from Priest Rapids Dam and the influence of the pool behind McNary Dam. Daily mean water depth at the locations where clams were deployed ranged from 0.58 to 4.23 m at Vernita and 0.5 to 2.0 m at Spring 9. Similar minimum depths measured at sites indicated that placement of clams at the periphyton line was a reliable method for ensuring clams remained inundated at a similar minimum elevation. Diurnal changes in water depth varied the most during the summer (DP-3) and winter (DP-4) periods, although river discharge

trends were noticeably different between the two. The summer period was characterized by a decreasing trend, whereas the winter period was characterized by an increasing trend.

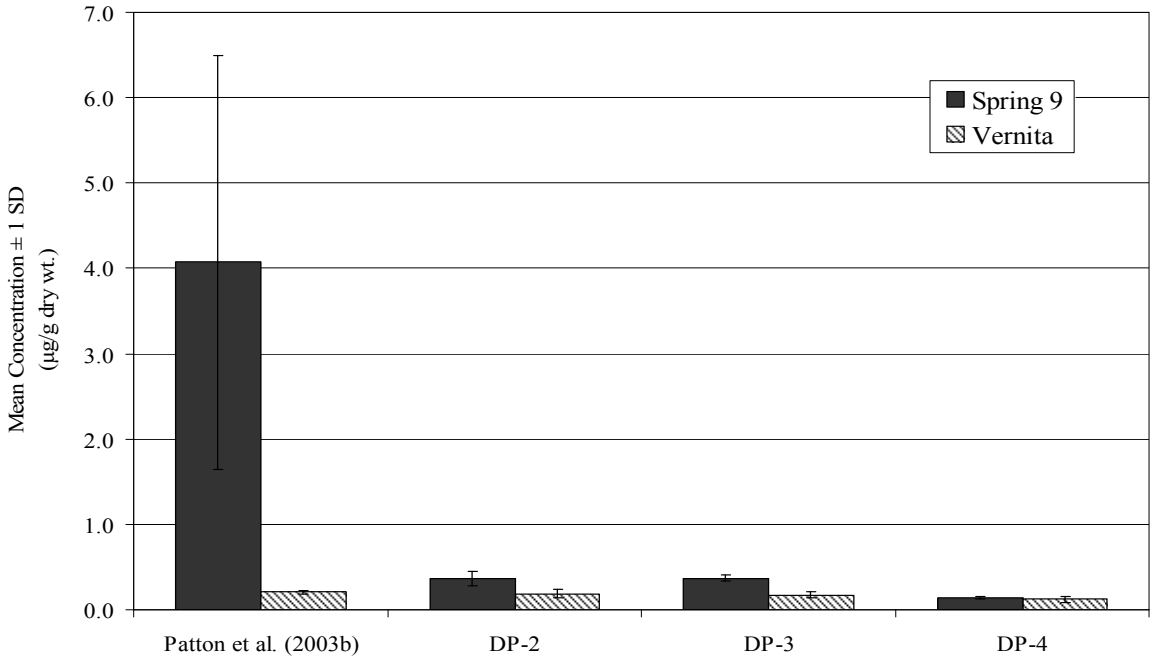

Figure 3.1. Comparison of Mean Soft Tissue Concentrations of Uranium in Caged Clams and In Situ Clams Analyzed by Patton et al. (2003b)

Specific conductance measurements greater than 0.15 mS/cm (i.e., greater than the upper range for normal background specific conductance of river water) at Spring 9 indicated the presence of groundwater upwelling and generally corresponded to periods of lower river discharge (e.g., <100 kcfs) (Figure 3.3) or water depths less than 1.1–1.2 m (Figure 3.4). The variability of specific conductance measured during these lower flow periods was greater than was observed during higher flows. Higher specific conductance readings at Spring 9 were more common during DP-2 and DP-3 (Figure 3.5), which corresponded to deployment periods in which uranium accumulation was greatest. Daily fluctuations in specific conductance were inversely related to river discharge and water depth throughout the study. Specific conductance measurements greater than 0.15 mS/cm were observed at Vernita as well. However, the highest of these measurements was 2–3 times lower than the highest measurements recorded at Spring 9.

Large fluctuations in water temperature at Spring 9 (maximum change of 6.24°C in 1 hour), which may indicate groundwater upwelling, were observed primarily in the early spring (DP-2) and midsummer (DP-3) (Figure 3.6). Temperature fluctuated the most during periods of lower river discharge (e.g., <100 kcfs) or water depth and periods of high amplitude change in river elevation. An inverse relationship between water temperature and discharge/water depth at Spring 9 did exist at times (Figure 3.6) but was not as evident as the relationship between specific conductance and discharge/water depth.

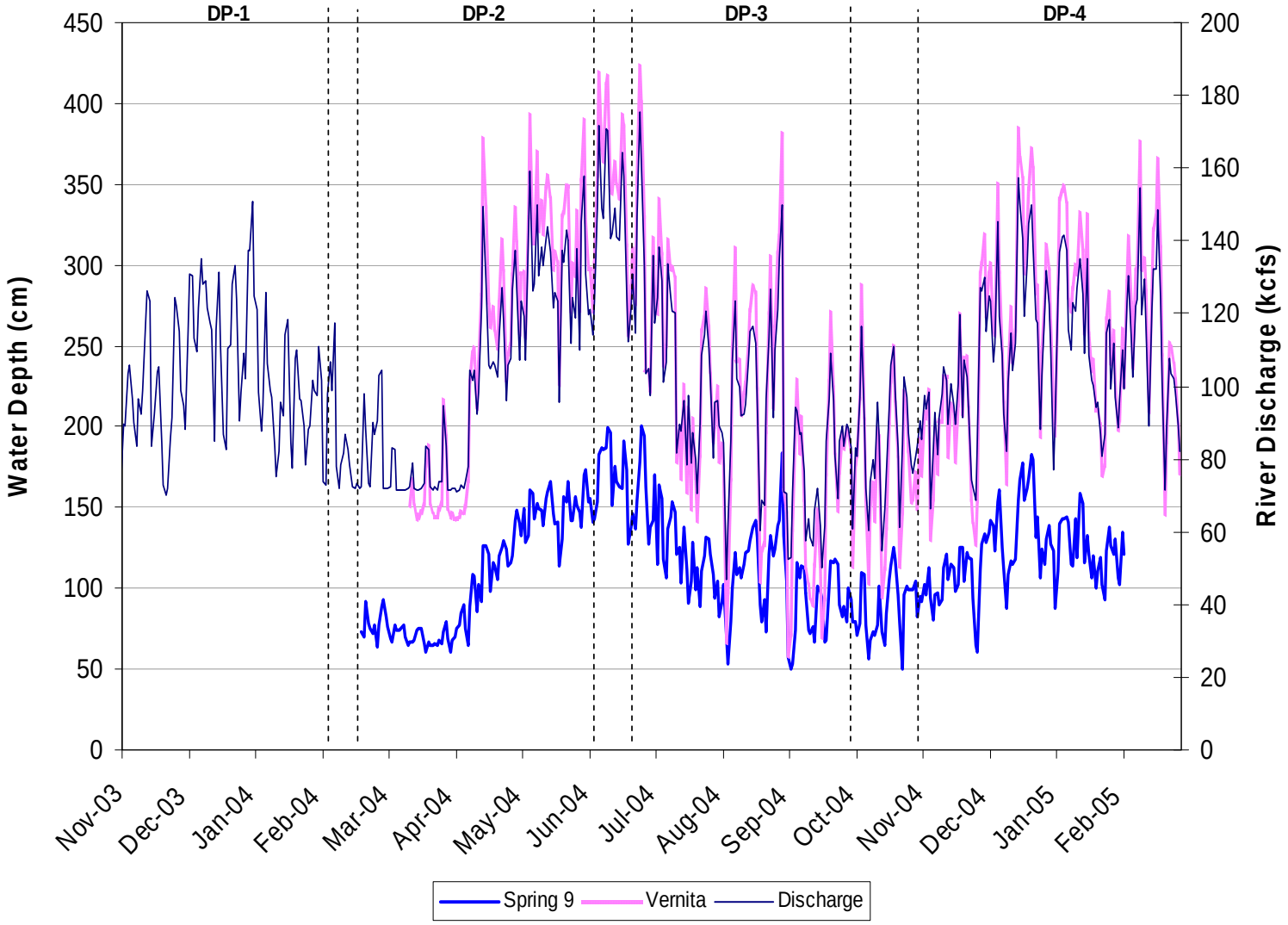

Figure 3.2. Daily Mean Water Depth at Spring 9 and Vernita Study Sites

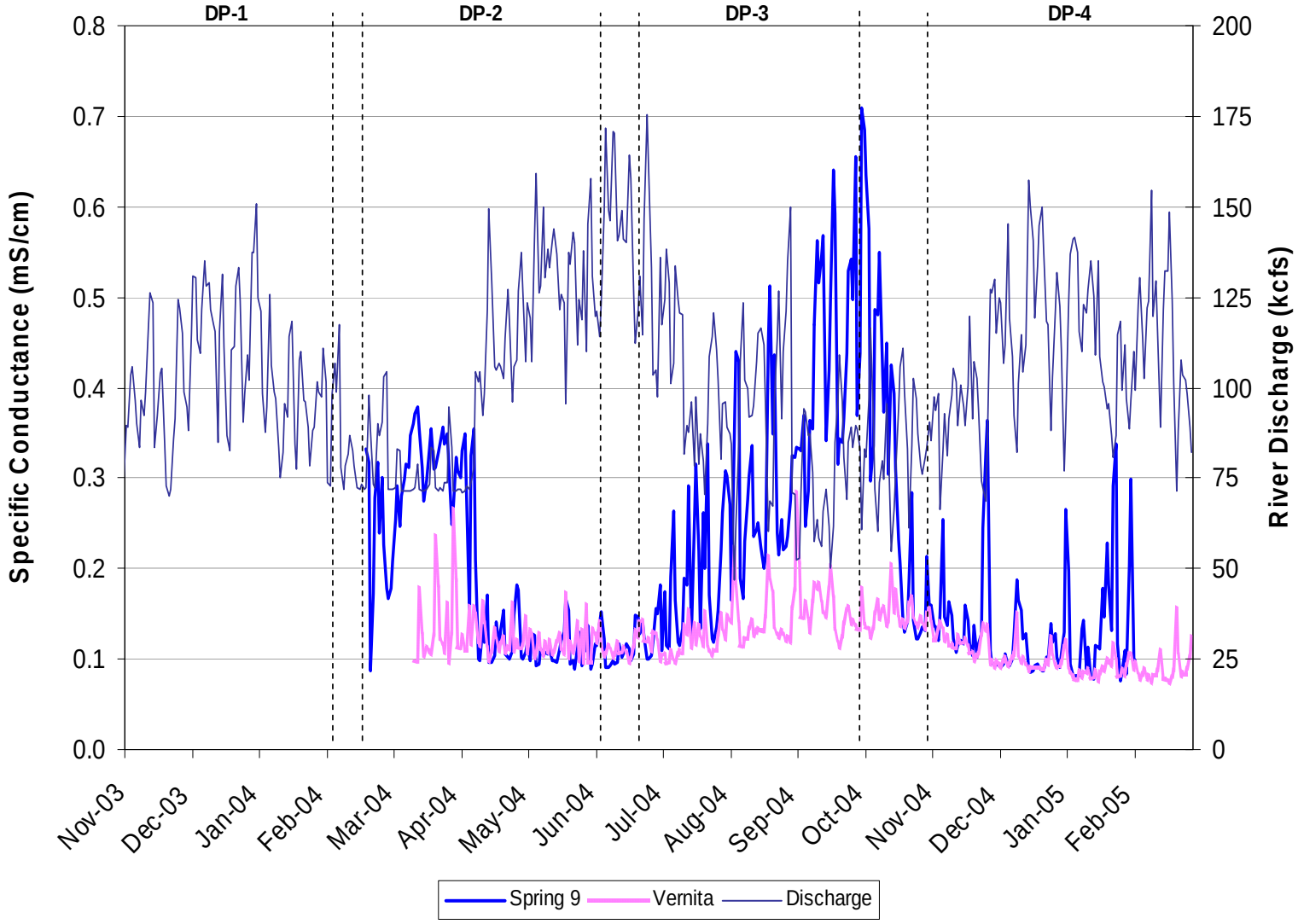

Figure 3.3. Daily Mean Specific Conductance 3–33 cm Below Riverbed Surface

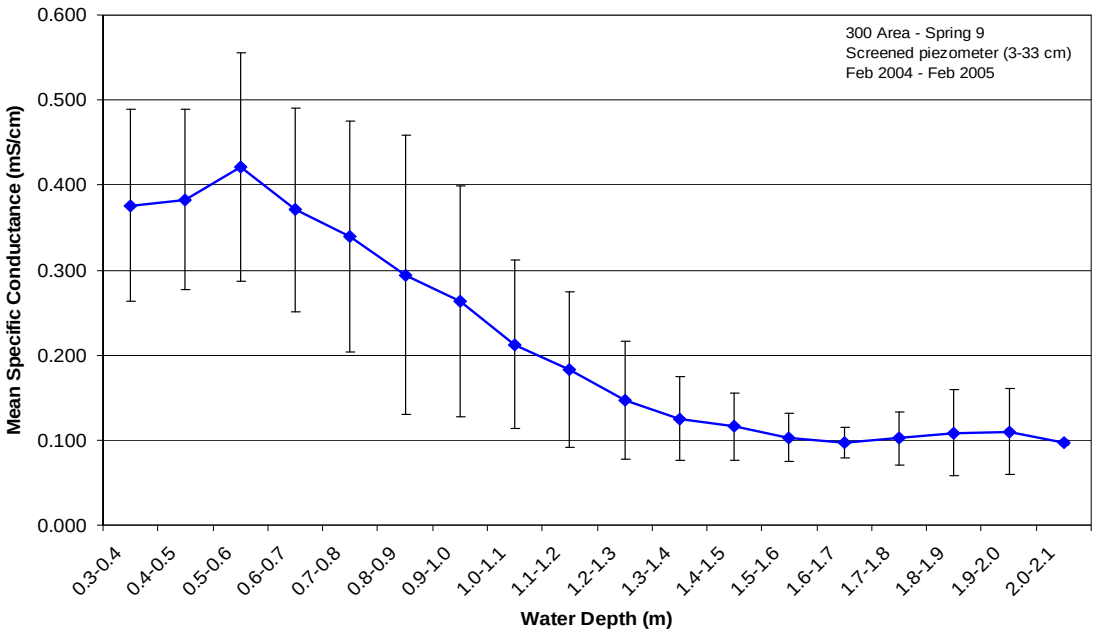

Figure 3.4. Spring 9 Mean Specific Conductance (±1 SD) Based on Water Depth (mean specific conductance based on continuous hourly data collected at Spring 9)

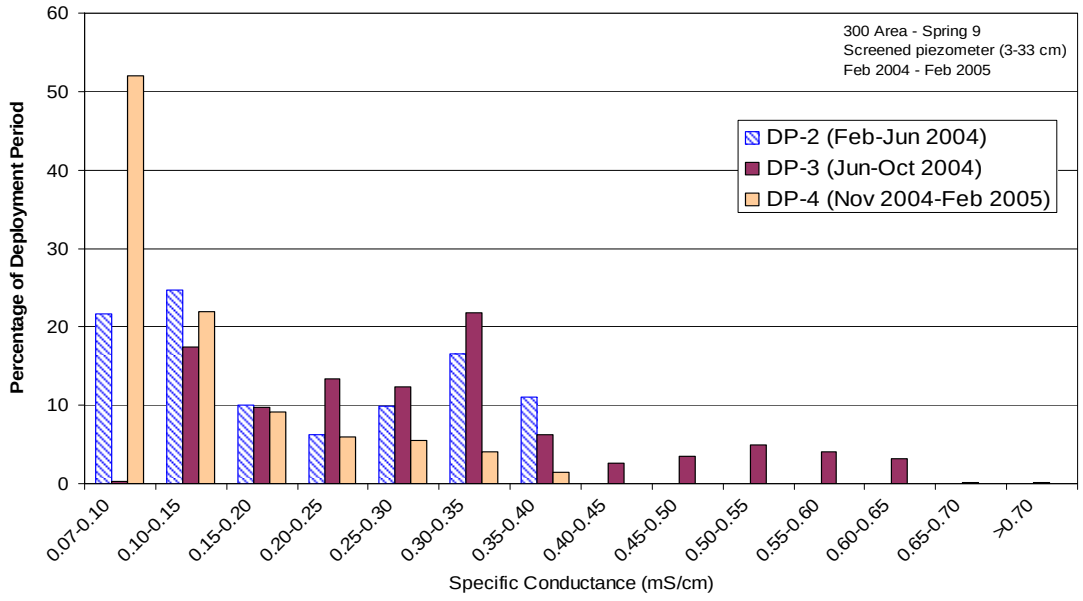

Figure 3.5. Spring 9 Specific Conductance Readings Shown as a Percentage of Occurrence

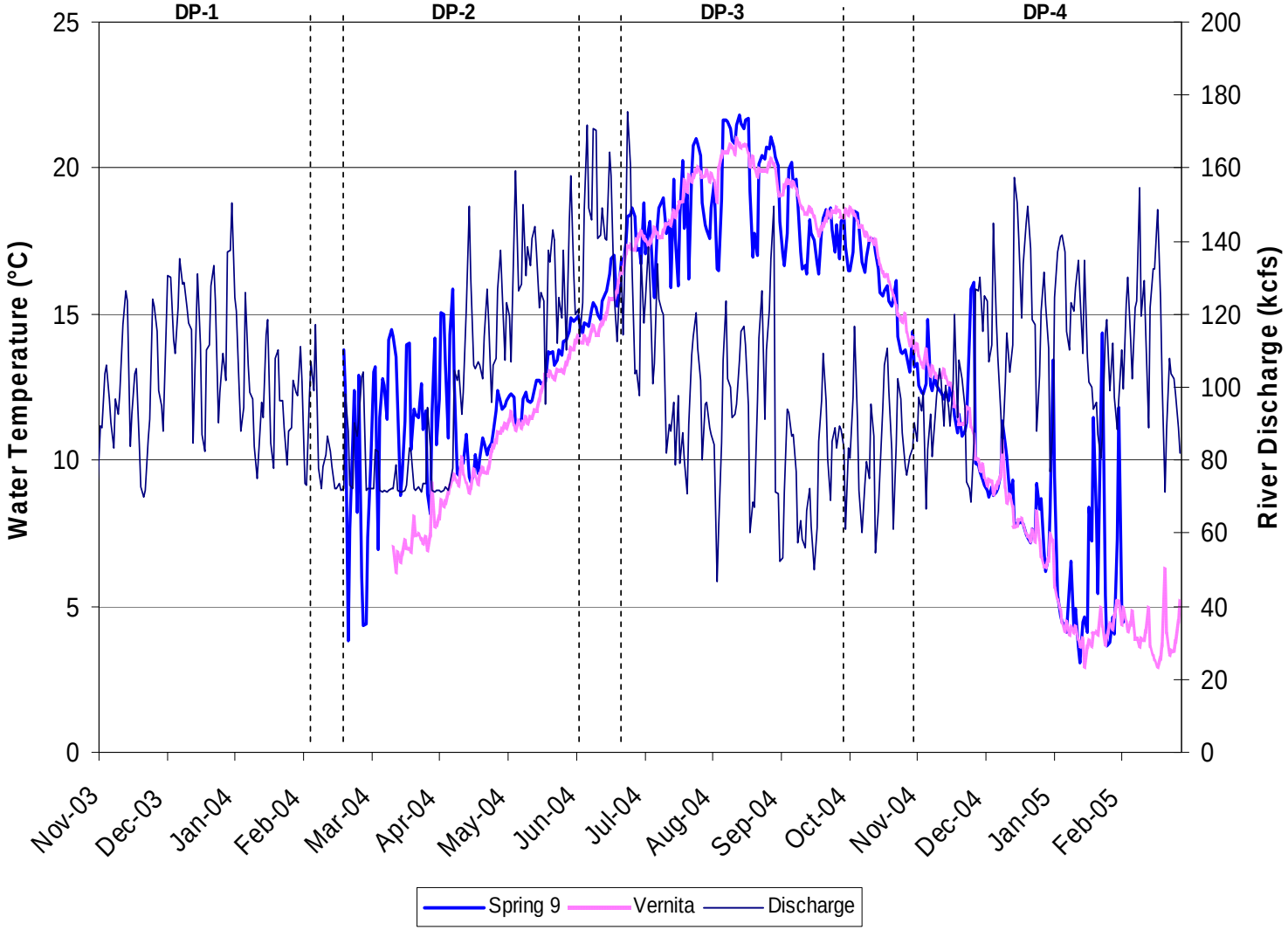

Figure 3.6. Daily Mean Water Temperature 3–33 cm Below Riverbed Surface

3.3 Growth

The effect of deployment period (*DP*), size class (*SC*), deployment type (*DT*), and site (*S*) on change in shell length (ΔL) and change in whole-animal wet weight (ΔW) was evaluated using two "improved-fit" ANOVA tests (one for each response variable). Each test assessed factor effects in an additive fashion by adding one factor at a time based on the amount of variation explained by each factor in a univariate ANOVA (order was from highest to lowest). Results of the univariate ANOVAs for ΔL and ΔW were the same with respect to the ordering of factors. Therefore, the improved-fit tests for ΔL and ΔW were structured the same; that is, Test 1 = *DP*, Test 2 = *DP* + *SC*, Test 3 = *DP* + *SC* + *DT*, and Test 4 = *DP* + *SC* + *DT* + *S* (Table 3.2).

Table 3.2. ANOVA Results for Change in Length (ΔL) and Change in Weight (ΔW)

Dependent Variable	Model	df	Deviance	Residual df	Residual Deviance	*F*	*P*
ΔL	NULL			2000	1623.81		
	DP	3	436.86	1997	1186.95	254.41	<0.001
	DP+SC	1	29.60	1996	1157.35	51.71	<0.001
	DP+SC+DT	1	15.28	1995	1142.07	26.69	<0.001
	DP+SC+DT+S	1	0.73	1994	1141.34	1.27	0.2591
ΔW	NULL			2000	324.32		
	DP	3	44.72	1997	279.60	111.66	<0.001
	DP+SC	1	9.15	1996	270.45	68.52	<0.001
	DP+SC+DT	1	2.09	1995	268.36	15.65	<0.001
	DP+SC+DT+S	1	2.16	1994	266.20	16.18	<0.001

Deployment period, size class, and deployment type were significant factors in the improved-fit test for change in length ($P < 0.001$; Table 3.2). The addition of site as a model factor was not significant ($P = 0.2591$; Table 3.2). The results of the improved-fit test for change in WAWW were essentially identical, with the exception of site being a significant factor ($P < 0.001$; Table 3.2). In both models, deployment period was the most important factor, as indicated by the fact that it explained more variation than the other three factors combined. Size class explained the second-most variation in both models, followed by deployment type and site.

Mean growth rates based on the ANOVA factor combinations (Figures 3.7 and 3.8) provided further clarification of the ANOVA results, illustrating that season and age had a greater relative effect on growth than either deployment type or site. Mean shell and WAWW growth rates were greatest during summer months and in younger individuals. Of the two DPs in which both deployment types were used (DP-3 and DP-4), mean shell and WAWW growth rates were greater for clams held in PVC cages in the summer (DP-3) and the same for both deployment types in the winter (DP-4). Although site was not significant in the change in length test, there was at least one period in which shell growth was different between sites (was lower at Vernita in DP-1). The reason this did not result in site being a significant factor in the change in length test is that the test evaluated how well site explained change in length with respect to the other factors (i.e., site differences were not statistically significant across DPs, size classes, and deployment types). Comparison of WAWW rates by site indicated that WAWW growth was lower at Spring 9

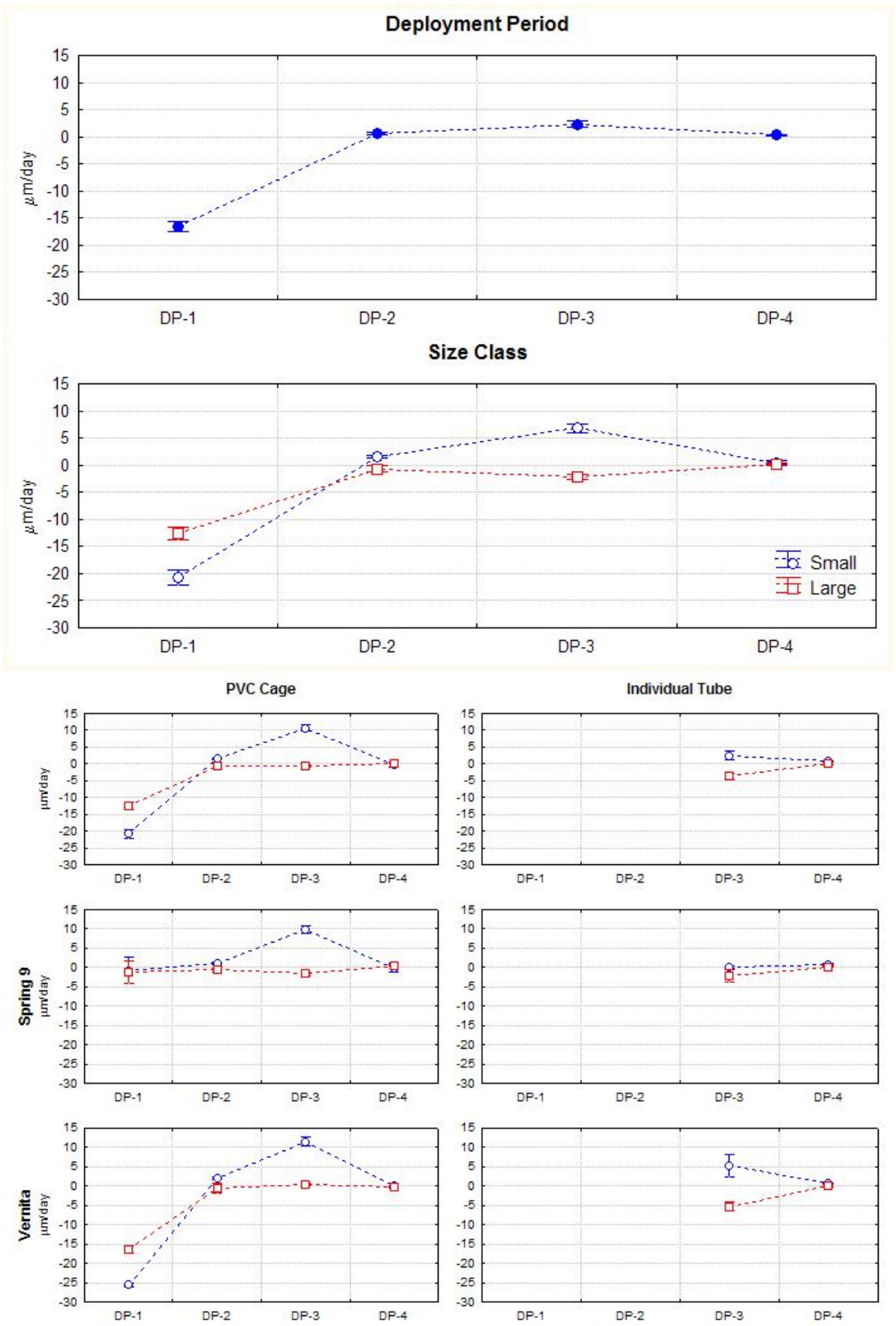

Figure 3.7. Mean Shell Length Growth Rates (±1 SE) Based on ANOVA Factor Combinations

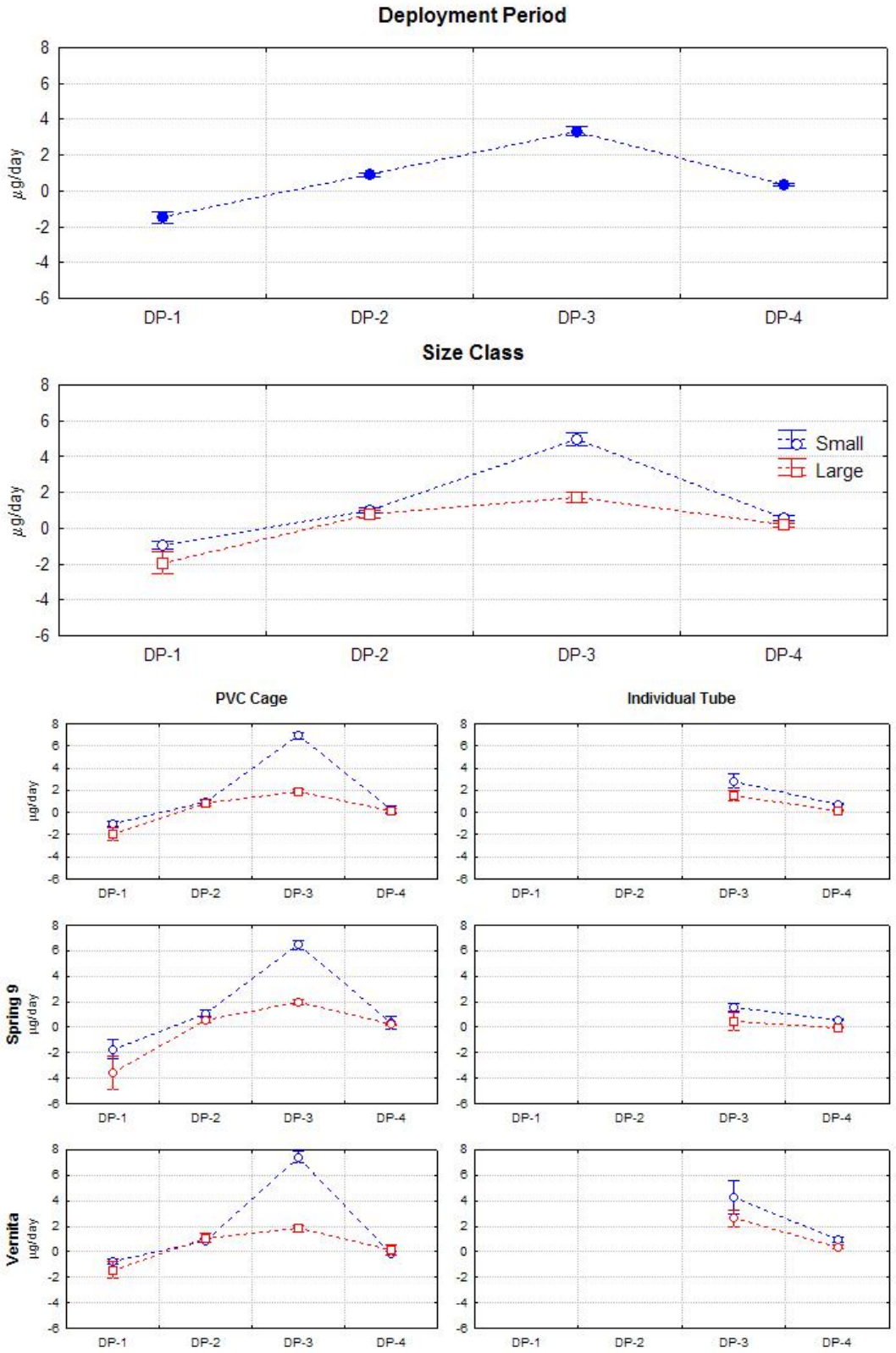

Figure 3.8. Mean WAWW Growth Rates (±1 SE) Based on ANOVA Factor Combinations

in DP-3 for both size classes and deployment types (except for large clams in PVC cages). In the other three deployment periods, WAWW growth rates were similar between sites.

Some of the above differences in growth may have been partially due to a potentially adverse effect of the cage design. This was indicated by negative changes in shell length and WAWW that occurred at varying frequencies in all DPs, regardless of size class, deployment type, or site (Table 3.3). In some cases, negative changes in shell length or WAWW were prevalent enough to have resulted in negative mean growth (Figures 3.7 and 3.8). Negative changes in shell length are thought to also have contributed to negative changes in WAWW because shell mass constitutes part of the WAWW.

Table 3.3. Percentage of Clams (based on improved-fit model data sets) That Experienced a Reduction in Shell Length

Improved-Fit Models	Data Set Description	Deployment Period			
		DP-1	DP-2	DP-3	DP-4
DP	Deployment period only	92.1	33.0	43.6	31.4
DP + SC	Small clams	90.8	23.6	25.1	18.3
	Large clams	93.3	47.5	61.8	43.9
DP + SC + DT	Small clams, PVC cage	90.8	23.6	11.3	24.7
	Large clams, PVC cage	93.3	47.5	61.5	36.2
	Small clams, ind. tube			40.2	14.4
	Large clams, ind. tube			62.0	49.6
DP + SC + DT + S	Small clams, PVC cage, Spring 9	52.9	31.7	10.8	16.1
	Small clams, PVC cage, Vernita	100.0	15.9	11.8	44.4
	Small clams, ind. tube, Spring 9			48.4	17.8
	Small clams, ind. tube, Vernita			31.0	11.0
	Large clams, PVC cage, Spring 9	72.7	57.5	72.9	31.6
	Large clams, PVC cage, Vernita	100.0	37.5	52.1	41.7
	Large clams, ind. tube, Spring 9			58.5	37.0
	Large clams, ind. tube, Vernita			65.6	63.2
Shaded cells represent deployment periods in which individual tubes were not deployed.					

Variability in the amount of water retained in clams (either as tissue water or interstitial water in the gills) and in their body condition also may have affected the WAWW. We assessed this variability for 100 clams that were not part of the field study by comparing dry soft-tissue weights to WAWWs. Dry soft-tissue weights were obtained by drying the soft tissue of each clam (clams were steamed for ~10 minutes to easily remove the soft tissues) in an oven at 60°C until the difference in dry weight between consecutive drying cycles (24-hour cycles) was less than 5%. The comparison of weights showed that soft-tissue weight (i.e., dry weight) was more variable than WAWW as clam size increased (Figure 3.9), indicating that WAWW is not a reliable predictor of soft-tissue mass for all clams; therefore, changes in WAWW observed in this study may not have accurately reflected changes in body mass.

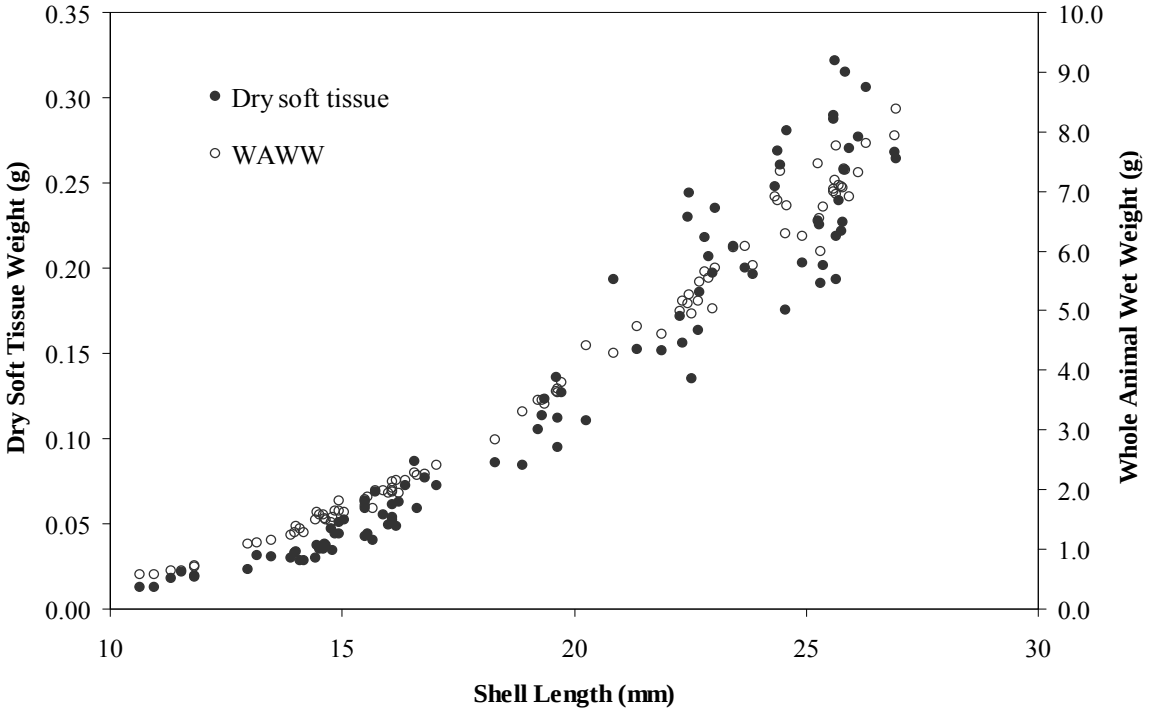

Figure 3.9. Dry Soft-Tissue Weight and Whole-Animal Wet Weight (WAWW) for 100 Non-Caged Clams

3.4 Survival

Survival rates of caged clams (Table 3.4) showed no clear indication that the observed tissue concentrations of uranium had a negative effect on survival. For example, survival at Spring 9 was both higher and lower than the reference site in the two DPs in which uranium accumulation at Spring 9 was elevated (DP-2 and DP-3). Furthermore, differences in survival appeared to vary considerably between sites, size classes, and deployment types, which may indicate the influence of either experimental conditions or other factors not measured in the study.

Table 3.4. Percentage Survival of Caged Clams (standardized to 90 days)

Size Class	Deployment Period	Spring 9		Vernita	
		PVC Cage	Ind. Tube	PVC Cage	Ind. Tube
11–22 mm	DP-1	34	– –	93	– –
	DP-2	82	– –	86	– –
	DP-3	85	82	91	69
	DP-4	83	99	61	98
>22 mm	DP-1	47	– –	92	– –
	DP-2	82	– –	77	– –
	DP-3	76	82	95	86
	DP-4	77	99	72	93

3.5 Histology

Histological examination of caged clam tissues and organs showed no apparent toxicological effect from the observed tissue concentrations of uranium at Spring 9. For example, frequencies of normal appearance in the digestive system (Table 3.5), reproductive system (Table 3.6), gills (Table 3.7), and skeletomuscular, nervous, and excretory system tissues (Table 3.8) were sometimes lower at the reference site, despite elevated tissue concentrations of uranium at Spring 9. In addition, the lowest frequencies of normal appearance for many of the conditions examined (all digestive system conditions, follicular cysts, and gill epithelial cell inflammation) were observed in the second winter deployment (DP-4), when tissue concentrations of uranium were lowest.

Table 3.5. Summary of Histological Examination of Caged Clam Digestive Systems

Site	Deployment Period	Sample Size	Percentage of Clams			
			Normal Epithelial Cell Height	No Epithelial Cell Loss	No Vacuoles	No Inflammation
Spring 9	DP-2	30	100	92.3	96.2	61.5
	DP-3	61	61.4	45.6	38.6	7.0
	DP-4	23	77.3	77.3	22.7	22.7
Vernita	DP-2	30	96.6	93.1	96.6	51.7
	DP-3	60	54.2	57.6	23.7	8.5
	DP-4	43	90.5	83.3	19.0	7.1

Table 3.6. Summary of Histological Examination of Caged Clam Reproductive Systems

Site	Deployment Period	Sample Size	Percentage of Clams		Average Number per Clam ± 1 SD		
			Signs of Reproductive Development	Normal Ova	Follicular Cysts	Necrotic Cells	Degenerative Follicles
Spring 9	DP-2	30	100	46.2	2.0 ± 3.0	0.8 ± 1.5	0.6 ± 1.0
	DP-3	61	96.5	25.5	6.3 ± 6.0	1.5 ± 2.0	1.9 ± 2.7
	DP-4	23	95.5	28.6	7.1 ± 7.7	1.7 ± 2.2	1.6 ± 3.1
Vernita	DP-2	30	93.1	33.3	2.3 ± 2.4	0.7 ± 1.6	0.6 ± 1.3
	DP-3	60	95.0	26.3	4.2 ± 4.9	1.1 ± 1.9	0.8 ± 1.6
	DP-4	43	37.2	6.3	6.3 ± 5.6	1.8 ± 2.2	0.8 ± 0.9

Table 3.7. Summary of Histological Examination of Caged Clams Gills

General Location	Deployment Period	Sample Size	Percentage of Clams		
			No Inflammation	No Epithelial Cell Loss	Larvae Present
Spring 9	DP-2	30	61.5	46.2	0.0
	DP-3	61	84.2	63.2	29.8
	DP-4	23	77.3	68.2	0.0
Vernita	DP-2	30	73.3	55.2	0.0
	DP-3	60	83.3	63.3	30.0
	DP-4	43	86.0	34.9	0.0

Table 3.8. Summary of Histological Examination of Caged Clam Skeletomuscular, Nervous, and Excretory Tissues

General Location	Deployment Period	N	Percentage of Clams					
			No Lesions in Connective Tissue	No Lesions in Kidney	No Lesions in Nerve/ Ganglia	No Lesions in Adductor Muscle	No Lesions in Foot	No Lesions in Mantle
Spring 9	DP-2	30	84.6	100	100	100	100	100
	DP-3	61	94.7	100	100	100	100	100
	DP-4	23	100.0	100	100	100	100	100
Vernita	DP-2	30	79.3	100	100	100	100	93.1
	DP-3	60	96.7	98.3	100	100	100	100
	DP-4	43	97.7	100	100	100	100	100

4.0 Discussion

This section provides an interpretation of results of caged clam deployments conducted between November 2003 and February 2005. Seasonal differences in soft tissue concentrations of uranium in clams are discussed primarily with respect to how surface hydraulic conditions affect groundwater infiltration in the near-shore area. Growth, survival, and histology results are discussed with respect to how they relate to uranium accumulation and other experimental design factors.

4.1 Comparison of Uranium Concentrations in Asiatic Clams Collected at the 300 Area

Differences in the Spring 9 concentrations measured in our study and those measured by Patton et al. (2003b) may be due to a number of reasons. One reason may be that the length of exposure was different, given that clams collected by Patton et al. (2003b) presumably had been there their entire lives (~1–3 years) whereas clams in this study were there for approximately 90 days. However, other PNNL laboratory studies have shown that accumulation and depuration of uranium may be relatively rapid, on the order of days (A. L. Bunn and colleagues, PNNL, unpublished data).[1] Other factors include a difference in the time of year in which clams were collected and possible changes between years in the uranium concentration in groundwater. The similarity in uranium concentrations at Vernita in our study and that of Patton et al. (2003b), and between each of our deployments, indicates that clams deployed at Vernita provided a good representation of baseline conditions for uranium.

4.2 Relationship Between River Discharge, Groundwater Indicators, and Uranium in Clams

Priest Rapids hourly discharge, combined with water depth, specific conductance, and temperature data from each site, was useful in characterizing the presence or absence of groundwater at shallow riverbed depths (3–33 cm) at our sites. Groundwater presence (specific conductance >0.15 mS/cm) at Spring 9 was most apparent when river discharge at Priest Rapids Dam averaged less than 100 kcfs or when average water depths near the cages were less than 1.1–1.2 m. High variability in specific conductance readings during these periods indicated the concentration of groundwater at shallow riverbed depths is influenced by factors other than river stage. Factors may include those that affect bank infiltration, entrainment of river water in the pore space of riverbed sediments, and hydraulic pathways of groundwater (Campbell 1994; Peterson and Connelly 2001).

The variability in specific conductance at Spring 9 indicated that exposure to groundwater (and, subsequently, exposure to contaminants) for clams and other benthic organisms in the near-shore environment, is dynamic and difficult to quantify. Clams were likely exposed to varying concentrations of groundwater on a daily basis throughout each deployment period. Given the high variability in exposure, we feel that our approach for estimating exposure by looking at the percentage occurrence of certain

[1] Bunn AL, RE Durham, BG Fritz, DP Mendoza, AL Miracle, and A Stegen. "Bioaccumulation and biomarker expression in *Corbicula fluminea* from pulsed exposure studies with uranium." Presentation to the Society of Environmental Toxicology and Chemistry North America 28th Annual Meeting, November 11–15, 2007, Milwaukee, Wisconsin.

specific conductance levels was appropriate for explaining seasonal differences in uranium accumulation. However, it did not provide enough information to explain why tissue concentrations at Spring 9 were similar between DP-2 and DP-3, despite what appeared to be greater relative exposure in DP-3. This may be due to a number of factors such as variability in the concentration of contaminants in groundwater (Peterson and Connelly 2001), seasonal variation in contaminant assimilation in clams due to changes in activity and/or physiology (Foster-Smith 1975; Way et al. 1990; Heinonen et al. 2001), the time of day in which clams were recovered from the field, or differences between deployments in the daily fluctuation of river discharge and duration of exposure to contaminated groundwater.

The latter two assertions are based on a recent PNNL laboratory study that examined uranium uptake and depuration for Asiatic clams under a "pulsed" exposure scenario (A. L. Bunn and colleagues, PNNL, unpublished data); i.e., clams were intermittently exposed to uranium and clean water at controlled intervals. This type of exposure scenario is similar to that which occurs at Spring 9 because daily changes in river discharge influence the rate of mixing between groundwater and river water, essentially creating a pulsed exposure environment. The results of the study suggest that changes in uranium concentration in the water for as short as 24 hours can result in significant changes in the soft-tissue uranium concentration in clams. In addition, the study found that the depuration rate for uranium may be slower than the uptake rate; thus, without information about the concentration of uranium in water for more than 24 hours prior to the collection of clams, the concentration in the soft tissue may only provide an indication that the clam was exposed at some point to uranium (i.e., it may not provide any information about long-term exposure). Based on this information, it is reasonable to assume that the soft-tissue concentrations of uranium observed in this study may have been influenced by the level of exposure near the time in which clams were collected or by repetitive exposure to varying concentrations of contaminated groundwater. We suggest that in future studies, researchers should seek to assess how these potential sources of variability affect the uptake and assimilation of contaminants by benthic biota.

4.3 Growth and Survival

Growth appeared to be affected by our cage design to some degree, as indicated by the occurrence of reductions in shell length in all DPs. Although shell-length reductions may have resulted from either measurement or transposition error, we found no evidence of such after conducting extensive efforts to assess the accuracy of our measurement techniques and data analysis. Shell-length reductions have been noted in other field and laboratory studies (Belanger et al. 1986a, 1986b, 1986c, 1987, 1990a; Foe and Knight 1987), all of which have suggested that it may be related to physiological stress caused by reduced siphoning activity (intake of water for food and oxygen) in response to the presence of contaminants or lack of food. Given that shell reductions occurred at our reference site (and often more so than at Spring 9), it is unlikely that they were related to contaminant exposure. We believe a more plausible explanation would be a lack of food due to some aspect of the cage design that may have affected the flow of water and, consequently, the availability of food items (algal and diatoms) suspended in the water. Differences in cage flow-through were apparent in our study, based on the amount of periphyton growth that occurred on each cage type. PVC cages generally had more periphyton growth on them than individual tubes, indicating that water flowed more slowly through them (probably due to the additional Vexar mesh wrapped around them).

Another aspect of the cages that may have affected growth is the compartmentalization of clams in the mesh tubing. In a similar study, Asiatic clams that were allowed to burrow and move freely in mesh

boxes filled with gravel substrate grew significantly more (and with less variability) than "free-floating" clams in a mesh bag (Hull et al. 2004). Hull et al. (2004) attributed this difference to two main aspects of the mesh substrate boxes that may have facilitated the natural feeding behavior of clams better than mesh bags. One aspect was that the rigid structure of the boxes would easily prevent accumulated objects (e.g., cobble, debris) from lying directly on the clams, which may restrict their siphoning activity. The PVC cages in our study may have had a similar benefit due to the semi-rigid Vexar mesh that was wrapped around the frames. Coincidentally, growth was greater in PVC cages than individual tubes in the summer deployment (DP-3); however, we did not see a difference in growth between the two deployment types in the winter (DP-4) and lack data to make comparisons for other seasons. Another aspect of the mesh substrate boxes used by Hull et al. (2004) was that they may have better facilitated pedal-feeding behavior (use of the foot to obtain food directly from substrate), which has been shown to be an important aspect of the ecology of Asiatic clams (Hakenkamp and Palmer 1999) and other bivalves (Yeager and Cherry 1994; Gatenby et al. 1996). Pedal-feeding behavior by clams in our study may have been restricted as a result of confinement in individual mesh compartments.

In addition to the potential effects of our cage design on food availability and feeding behavior, the design may have potentially caused abnormal shell wear, resulting in shortened shell lengths. Based on field observations, we know that shell wear occurs naturally on the dorsal region of clams in our study area (possibly from burrowing in cobble substrate). Therefore, it may be possible that shell wear could have occurred along the valve (shell half) edges where shell length is measured. Because clams lacked any substrate into which to anchor themselves, they may have been subjected to periodic rocking or tumbling within their mesh compartments due to hydraulic turbulence. Although the mesh tubing did not seem abrasive, it may have been sufficiently coarse to cause shell wear over time. In addition, clams may have been prone to shell wear due to poor health caused by lack of food or reduced siphoning activity. This assertion is based in part on a study by Prezant and Chalermwat (1983), who found that the shell microstructure became weakened in Asiatic clams when they were stressed.

Despite the reductions in shell length that occurred in our study, growth still generally coincided with seasonal and age-related growth trends described in other Asiatic clam growth studies (Buttner and Heidinger 1980; Dauble et al. 1985; Belanger et al. 1990b; Hornbach 1992). Seasonal growth rates were greatest during summer months when the primary productivity in the river is high and lower during the winter when productivity is low. Also, growth rates of small clams were greater than those of large clams. When compared to other growth studies (Buttner and Heidinger 1980; Dauble et al. 1985; Fritz and Lutz 1986; Belanger et al. 1990b), the shell growth rates at both sites in this study were considerably lower. For example, Dauble et al. (1985) observed shell growth rates ranging from 18 to 63 µm/day in the summer and 2.0 to 3.6 µm/day in the winter for Asiatic clams kept in a once-through artificial stream system that used Columbia River water (temperatures were maintained at 20°C and 10°C for summer and winter). By comparison, mean summer (DP-3) and winter (DP-4) shell growth rates at both sites in our study were approximately 6 to 7 times lower than the minimum rates observed by Dauble et al. (1985).

Evaluation of survival rates for deployment periods in which soft-tissue concentrations of uranium were measured did not indicate significantly greater mortality in clams exposed to uranium at Spring 9. Survival was both higher and lower at Spring 9 in the periods in which mean tissue concentrations of uranium were greater than those observed at the reference site. Although reduced survival was observed at Spring 9 in the first winter deployment (DP-1), the cause is unclear because we did not collect tissue concentration or water chemistry data at that time. Overall, survival rates were variable among sites, size classes, and deployment types, which may be further indication that confinement affected overall health.

In addition, it has been shown that uranium is relatively nontoxic to Asiatic clams in a laboratory environment (Labrot et al. 1999) at concentrations similar to those typically present in the hyporheic zone at Spring 9 (Fritz et al. 2007).

4.4 Histology

The toxicokinetics (i.e., movement and deposition of contaminants within an organism's body) of uranium in Asiatic clams and the histological interpretation of subsequent effects are not well understood. At environmentally relevant exposures to uranium (100 µg/L) in a laboratory environment, Simon and Garnier-Laplace (2004) found that Asiatic clams accumulated more uranium in the digestive gland. In our histological assessment, we found no indication that the observed tissue concentrations of uranium in caged clams at Spring 9 caused cellular or tissue damage in the digestive gland. This is supported by the fact that frequencies of normal appearance in the digestive gland tissue were often lower at the reference site in the summer deployment (DP-3), even though the mean tissue concentration of uranium was approximately twice as high at Spring 9.

The variability in our histological data seems to indicate that factors other than uranium exposure affected the tissue condition and overall health of caged clams in our study. Aside from the potential effects caused by confinement, it is also likely that seasonal factors such as water temperature played a significant role. Water temperature may have affected the condition of clams and subsequent histological analyses because it affects the timing of reproductive events such as oogenesis, spermatogenesis, gastrulation, and larval development (Kraemer and Galloway 1986). When water temperatures drop below 15°C, spermatogenesis (generation of sperm cells) in Asiatic clams will subside while oogenesis (generation of eggs) continues, resulting in the carrying of unfertilized eggs (Kraemer and Galloway 1986). Most of the reproductive conditions evaluated in this study are generally associated with the resorption of unspawned products such as unfertilized eggs.[1] Therefore, their presence, absence, or condition may have been indicative of the water temperature and time of year in which clams were deployed and collected. The presence of larvae in the gills observed at the end of DP-3 (October) indicates that these clams were reproductively active at the end of the summer and early fall and is consistent with the observations of Kraemer and Galloway (1986). The fluctuation in water temperature caused by groundwater influx at Spring 9 could have compounded this effect even further.

4.5 Other Sources of Environmental Variation

The effects of abiotic factors such as substrate, temperature, water velocity, and depth on Asiatic clam ecology remain poorly understood. Ways in which these variables relate to foraging ecology and subsequent effects on contaminant uptake (particularly for benthic organisms) have been identified as important yet understudied relationships in aquatic toxicology studies (Power et al. 1988; Heinonen et al. 2001; Vaughn and Hakenkamp 2001). For filter-feeders such as Asiatic clams, uptake is generally considered to be related directly to their filtration rate because exposure increases as a greater volume of water is passed through the organism (Graney et al. 1984; Bucci 2006). However, to our knowledge, nothing is known about how abiotic factors like substrate, temperature, water velocity, and depth influence filtration rates of Asiatic clams.

[1] Elston, R. 2005. Telephone call to Ralph Elston (Pacific Shellfish Institute) from Kyle Larson (Pacific Northwest National Laboratory), September 21, 2005.

Previous studies have shown that filtration rates of Asiatic clams (Way et al. 1990) as well as other bivalve species (Foster-Smith 1975) generally increase when the concentration of suspended material in water is low and decrease when concentration is high, thereby achieving an "optimal" rate of particle removal. Abiotic factors such as substrate, velocity, and depth influence the concentration of suspended material (which would include contaminants) because they determine how "well-mixed" an organism's environment is with respect to delivery of food and nutrients (Power et al. 1988). These types of physical features also have been shown to dramatically affect productivity and respiration of benthic algal communities (Cardinale et al. 2002), which are a primary food source for clams. Seasonal differences in primary productivity also may affect food availability and filtration rates (Way et al. 1990).

The role of abiotic factors with respect to how they may influence clam ecology likely has a significant effect on results found in this study. Although the substrate at the reference site at Vernita was similar to Spring 9, there were considerable differences between the two sites in flow and water depth. These differences could have affected food availability for clams, thereby causing changes in feeding behavior and overall health. Such effects may need to be considered in interpreting past and present studies on the Hanford Reach. These effects also should be taken into consideration in the design of future studies because the Vernita area is commonly used as a reference location for assessing the relative impact of Hanford groundwater on aquatic biota.

5.0 Conclusions

The use of field-caged clams was successful in assessing general seasonal differences in uranium accumulation in the near-shore environment of Spring 9. This conclusion was supported by the results of continuous monitoring of specific conductance in shallow riverbed water (3–33 cm), which helped to confirm that seasonal mean soft-tissue concentrations of uranium did correspond with relative exposure. However, several potential sources of variation may have affected uranium accumulation in this study. The first was the design of the cages, which may have prevented clams from feeding or behaving normally, thereby affecting filtration rates and subsequent uptake of uranium in the water. The second source of variation was the time in which clams were retrieved from the river at the end of each DP. Results of a recent PNNL laboratory study suggest that uranium soft-tissue concentrations in clams may change significantly in relatively short periods of time (24 hours); thus, it is reasonable to assume, given the daily variation in river discharge on the Hanford Reach, that the tissue concentrations of uranium measured in this study may have been influenced by the concentration of groundwater present in the riverbed at the times in which clams were retrieved. Another source of variation was the potential effects on clam biology of abiotic factors such as substrate, temperature, water velocity, and depth. These factors may play a complicated role in contaminant uptake because they may influence the delivery of food and nutrients to clams, which may subsequently alter their feeding behavior.

Differences in growth, survival, and tissue condition that may have been related to uranium accumulation were not apparent in this study. Growth results were considered largely inconclusive due to a large proportion of clams that experienced a reduction in shell length. These reductions in shell size are believed to be related to the cage designs, which may have affected clam behavior and made them prone to shell erosion. Interpretation of histological data also was limited due to a lack of knowledge about the physiological pathways and effects of uranium in clams.

Despite the limitations discussed above, there may still be some utility in using caged Asiatic clams to conduct environmental monitoring of contaminated groundwater exposure in the Columbia River near-shore environment. Their widespread distribution and local abundance on the Hanford Reach makes them one of the most practical bioindicators of benthic exposure. Plus, their ability to be deployed at spatially discrete locations allows for potentially greater resolution of contaminant exposure. However, additional testing of alternative cage designs and the relationship between contaminant accumulation and daily variation in exposure should be conducted. Cage designs that incorporate natural substrate and allow clams to move freely within them may provide a more accurate representation of contaminant accumulation. Assessing the relationship between contaminant accumulation in clams and daily variation in exposure in the near-shore environment will allow for more meaningful interpretations of tissue concentrations of contaminants in clams. In addition, we suggest that more information is needed regarding how abiotic factors (e.g., substrate, temperature, water velocity, depth) may influence contaminant uptake and assimilation in benthic organisms. Results of such research may be valuable in evaluating and selecting potential reference locations in future studies, including sites on the upper Hanford Reach (Vernita) and Franklin County side of the Columbia River.

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Appendix

Uranium Results

Appendix

Uranium Results

Table A.1. Uranium Concentrations (μg/g dry weight) in Whole-Body Soft Tissue of Asiatic Clams

Site Name	DP-2		DP-3		DP-4	
	HEIS Sample Number	U	HEIS Sample Number	U	HEIS Sample Number	U
Spring 9	B1CP36	0.251	B1CP48	0.550	B1CP72	0.180
	B1CP36	0.256	B1CP49	0.565	B1CP73	0.111
	B1CP37	0.325	B1CP50	0.230	B1CP73	0.104
	B1CP38	0.842	B1CP51	0.280	B1CP74	0.187
	B1CP39	0.304	B1CP52	0.340	B1CP75	0.115
	B1CP40	0.236	B1CP53	0.391	B1CP75	0.113
	B1CP41	0.326	B1CP54	0.383	B1CP76	0.206
			B1CP55	0.389	B1CP77	0.123
			B1CP56	0.381	B1CP78	0.093
			B1CP57	0.237	B1CP79	0.190
			B1CP57	0.229		
			B1CP58	0.538		
			B1CP59	0.261		
Vernita	B1CP42	0.278	B1CP60	0.238	B1CP86	0.097
	B1CP43	0.174	B1CP61	0.195	B1CP87	0.150
	B1CP44	0.228	B1CP62	0.140	B1CP88	0.100
	B1CP45	0.166	B1CP63	0.145	B1CP89	0.159
	B1CP46	0.185	B1CP64	0.153	B1CP90	0.088
	B1CP46	0.184	B1CP65	0.181	B1CP91	0.165
	B1CP47	0.113	B1CP66	0.235	B1CP92	0.121
			B1CP67	0.136	B1CP93	0.095
			B1CP68	0.183		
			B1CP69	0.126		
			B1CP70	0.197		
			B1CP71	0.161		

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