Geoduck Studies in Hood Canal

Progress on Work Associated with House Bill 1896 Final Report to the 2008 Legislature House Select Committee on Hood Canal

November 30, 2007







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FACT SHEET

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Authors:	Section 1. Geoduck Index Stations by Bob Sizemore, Washington Department of Fish and Wildlife Section 2. Geoduck Age Structure by Juan L. Valero, University of Washington Section 3. Geochemical Analysis by Yongwen Gao Ph.D., Makah Tribe
Lead Agency:	Washington Department of Natural Resources Aquatic Resources Division 1111 Washington Street SE P.O. Box 47027 Olympia, WA 98338
Responsible Official	: Rich Doenges, Manager Aquatic Resources Division Washington Department of Natural Resources 1111 Washington Street SE P.O. Box 47027 Olympia, WA 98338

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STATUTORY REQUIREMENTS

RCW 79.135.050

Study of Hood Canal geoduck population — Report. (Expires July 1, 2008.)

The department shall conduct a study to determine if changes to the geoduck populations in Hood Canal have occurred over time. The department's study shall compare prior population surveys with current surveys conducted as part of this study. The study shall incorporate geoduck beds representative of the northern, central, and southern areas of Hood Canal. No later than January 1, 2006, the department shall submit a report describing the study results to the appropriate committees of the legislature.

RCW 79.135.060

Study of Hood Canal's geoduck population levels and environmental conditions --Report. (Expires July 1, 2008.)

The department shall conduct a study to assess the relationship between the Hood Canal's geoduck population levels and environmental conditions, including dissolved oxygen concentrations. To conduct this study, the department shall establish geoduck index stations near the department of ecology's Hood Canal water sampling stations. The index stations shall include stations representative of the northern, central, and southern areas of Hood Canal. No later than December 1, 2007, the department shall submit a report describing the study results to the appropriate committees of the legislature.

RCW 79.135.070

Study of age and shell oxidation rate of Hood Canal geoduck -- Report. (Expires July 1, 2008.)

The department shall conduct a study to establish an age profile and analyze the shell oxidation rate of Hood Canal geoduck. To conduct this study, the department shall establish sampling stations representative of the northern, central, and southern areas of Hood Canal. No later than December 1, 2007, the department shall submit a report describing the study results to the appropriate committees of the legislature.

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REPORT SYNOPSIS

There have been increasing concerns regarding the source and biological/environmental impacts of low oxygen events in Hood Canal. Geoducks can be a good indicator of environmental change given their longevity, abundance, distribution and data availability. The Washington State Legislature recognized the importance of the low dissolved oxygen problem and the value of studying geoduck clams in Hood Canal. Engrossed Second Substitute House Bill 1896 (hereafter, HB1896) required studies of geoduck clam populations in Hood Canal and was passed by the House on March 11, 2005, the Senate on April 14, 2005 and was approved by the Governor on May 6, 2005.

The attached report provides a progress report on the research mandated under HB1896. The key results/findings from this research are as follows:

1) We have established geoduck index stations in North, Central, and South Hood Canal for the long-term monitoring of geoduck abundance in Hood Canal.

2) Our results show that geoducks in southern Hood Canal are markedly younger than in the rest of Hood Canal. As a follow up to this project, we have initiated research on overall abundance, environmental forcing and catch history data to estimate trends in abundance, population structure, recruitment strength and different sources of mortality.

3) We initiated a study to use geochemical tracers (e.g., stable isotope δ^{18} O and δ^{13} C, trace element Sr, Mg, Fe, Mn, and Cd; and organic carbon and sulphur) to describe environmental conditions that geoduck were exposed to over time. Overall these geochemical tracers appeared to suggest that the low DO events might be related to habitat changes and/or water conditions in Hood Canal.

EXECUTIVE SUMMARY

This second report completes the requirements of HB1896 and RCW 79.135.060 and RCW 79.135.070. This report is divided into three main sections to discuss establishment of geoduck index stations, provide a status report on geoduck age structure, and provide a status report on the geochemical analysis of geoduck shells and seawater from Hood Canal.

Section 1. Geoduck Index Stations: The Washington Department of Fish and Wildlife (WDFW) has established geoduck index stations in North, Central, and South Hood Canal for the long-term monitoring of geoduck abundance in Hood Canal. Dissolved oxygen readings from Washington Department of Ecology (ECOLOGY) water quality monitoring stations are presented to help characterize environmental conditions in the three subregions of Hood Canal. The relative abundance of geoducks at an index station in North Hood Canal is high, compared to Central and South Hood Canal. This index station information supports density information collected during commercial geoduck tract surveys in Hood Canal. The DO conditions in North Hood Canal are favorable to support growth and survival of marine animals, compared to DO

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conditions in southern Hood Canal. In South Hood Canal critical hypoxia (low dissolved oxygen) conditions periodically occur and DO levels are often low enough to affect biological processes of marine animals. This situation has become worse in recent years (since June 1997). As follow-up to the 2005 progress report, a survey of an invasive tunicate was done in 2007 at the Tahuya River delta, and the tunicate population has collapsed from previous introduced levels.

Section 2. Geoduck Age Structure: The University of Washington reports the results on age structure in three sectors of Hood Canal: North (NHC), Central (CHC) and South (SHC). Age frequency distributions were estimated for subtidal geoducks at six depth strata on one unfished location for each sector. Our results show that SHC geoducks are markedly younger than in the rest of Hood Canal. As a follow up to this project, we are currently implementing an age structured model incorporating available age, abundance, environmental forcing and catch history data to estimate trends in abundance, population structure, recruitment strength and different sources of mortality: baseline natural mortality, episodic events (e.g. low oxygen) and fishing mortality.

Section 3. Geochemical Analysis; Dr. Yongwen Gao, a research scientist for the Makah Tribe, first demonstrated how the geochemical approach (e.g., stable isotope δ^{18} O and δ^{13} C, trace element Sr, Mg, Fe, Mn, and Cd; and organic carbon and sulphur) can provide independent chemical records of environmental conditions that an individual geoduck experienced using animals and seawater samples that had been collected from each of the sites in the North, Central, and South Hood Canal.

In general, the δ^{18} O values of Hood Canal geoduck shells ranged from -1.34 to +0.95‰ VPDB, while the δ^{13} C values ranged from -2.19 to +0.35% VPDB. As compared with other carbonate proxies in the region, such as otoliths of yelloweye rockfish (Sebastes ruberrimus), the isotopic compositions of geoduck shells were much lower in δ^{18} O but higher in δ^{13} C for the first 10 years. These isotopic differences indicated that the life history of geoduck was different from that of rockfish, particularly in habitat and behavior.

The δ^{18} O variations from shell carbonate and seawater showed that the samples from Hamma-Hamma (H. Hamma) were largely different from Vinland and Tahuya, indicating significant influence by the H. Hamma River input. However, δ^{13} C variations between the three sampling sites appeared to suggest that the food sources or nutrient loadings of the central and southern Hood Canal were different from those of the northern sub-region.

The Washington Department of Ecology (ECOLOGY) provided a large volume of observation data in Hood Canal from 1990-2005. It appeared that the low DO concentrations in the southern Hood Canal started from 1994 and reached the lowest levels in 1997 or 1998. There were no apparent correlations between DO and water temperature, and salinity. However, comparisons between DO and δ^{18} O, δ^{13} C, and Sr/Ca and Fe/Mn ratios showed consistent variations over the last 16 years, during which significant fish kills occurred in Hood Canal in 200-03. Overall these geochemical tracers appeared to suggest that the low DO events might be related to habitat changes and/or water conditions in Hood Canal.

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1.0 SECTION I. Geoduck Index Stations by Bob Sizemore, WDFW.

A report on RCW **79.135.060** - A study of Hood Canal's geoduck population levels and environmental conditions.

1.1 INTRODUCTION

To assess the relationship between future changes in geoduck clam abundance, and marine water conditions experienced by geoducks in Hood Canal, geoduck index stations were established near existing Washington Department of Ecology (ECOLOGY) water quality sampling stations. Locating geoduck index-of-density stations near these ECOLOGY stations allows comparative analysis of relative geoduck abundance and water quality conditions, over time. By using existing water quality data near the geoduck index stations, there is no need to collect additional water quality information. The precision of the geoduck abundance estimates is high at index sites, and variance is reduced to zero, by taking a complete census of geoducks within a small, defined area. Though, there may be observation error associated with this tagging method (described below). If environmental conditions significantly change (for better or worse) in Hood Canal, then the geoduck index stations may serve as good biological indicators of this change. The changes in geoduck abundance at index stations can also be used to compare and validate inferences made from other methods of estimating recruitment and natural mortality, such as age structure data (see Section II). A disadvantage of establishing new geoduck index stations is the lack of prior historic index data to monitor prior abundance trends. To analyze historic trends, geoduck tract surveys, dating back to 1970, may be a reliable source of information (Sizemore, 2006).

1.2 METHODS TO ESTABLISH INDEX STATIONS

Establishment of geoduck index stations is done by, or under the direction of, Washington Department of Fish and Wildlife (WDFW) scuba divers/biologists, using methods similar to establishing geoduck siphon show plots (Bradbury, 2000). A subtidal area is chosen and delineated with sinking polypropylene line that is staked to the substrate with rebar "staples". The index site is typically set up as a simple rectangle and the surface area of the station can be calculated. This permanent delineation also allows for repeated visits to the same location, for many years, if the station is properly maintained. Thin PVC stakes ("tags") are carefully placed by scuba divers near the visible geoduck siphons (termed "shows" by WDFW biologist Lynn Goodwin, retired) at a pre-specified distance, depth into the substrate, and compass orientation to "tag" the siphon. This allows for consistent and reliable identification of siphon shows with minimal disturbance to geoducks within the plot. Not all geoduck siphons are visible during a tagging dive within the plot, so it is necessary to repeat this process on subsequent days. It generally takes several days to several weeks to tag all geoducks within a plot. Removal and enumeration of all tags, placed next to geoduck siphons, will provide an estimate of the total population of geoducks within the plot.

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This method is close to a census of geoducks, though a low number of animals may be relatively inactive during the tagging process and will persistently not show and not be tagged. This introduces a bias of under-estimating the "true" population. There is also a real possibility in a dynamic marine environment that tags will pull completely out of the substrate. An accounting of tags placed and tags recovered will identify how many tags are lost. But it does present a bias of under-estimating the population if an animal with a lost tag does not "re-show" during the tagging period. No assumption is made that a lost tag is associated with a particular live animal, since tags could be inadvertently dropped or animals that have lost a tag could be re-tagged with a different tag.

1.3 LOCATION OF INDEX STATIONS

Geoduck index stations were established in northern, central and southern sub-regions of Hood Canal (Figure 1.1), as defined by Sizemore (2006). All of these stations were established in subtidal areas that have had no commercial geoduck harvest; this reduces or eliminates effects of fishery mortalities and controls variables affecting total mortality. In northern Hood Canal, the index station named Lofall/Vinland along the eastern shoreline is located in a moderate to high water current area off a point of land at a water depth of -35 feet (adjusted to mean lower low water - MLLW). Prior to this study, a geoduck siphon show plot had been established at Lofall/Vinland at a 33 foot (MLLW) water depth, and results from this earlier survey estimate a high density of geoducks (0.516 geoducks/sq.ft.). A second index station station was established along the western shoreline of central Hood Canal, northerly of Quatsap Point at water depths between -29 to -30 feet (MLLW). One end of the Quatsap station is at -29 feet (MLLW) and the other end is at -30 feet (MLLW). A third index station was established offshore of Musqueti Point in southern Hood Canal at water depths of -18 to -20 feet (MLLW). A significant effort was made to find a location in southern Hood Canal with a sufficient density of geoducks, to establish a reasonably-sized index station in this sub-region. The Musqueti Point index station location was selected after several days of exploratory surveys near the shoreline of Union, the Tahuya River delta, and the Great Bend area.

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Figure 1.1 Geoduck index station locations in Hood Canal.

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1.4 GEODUCK ABUNDANCE AT THREE INDEX STATIONS

There are major differences in estimated geoduck abundance between the three geoduck stations established in Hood Canal (Table 1.1). The highest density of geoducks is found in northern Hood Canal at the Lofall/Vinland index station (the 2006 density was 0.676 geoducks/sq. ft.). An objective when establishing index stations is to have at least 100 geoducks within the station at the conclusion of tagging, in order to detect small differences in changes of abundance. At Lofall/Vinland, the density was sufficiently high enough to establish a station with an area of 450 square feet, and also have a total count of around 300 geoducks within the station. The initial trend at the Lofall/Vinland index station over two years (2004 and 2006) is increasing density, though additional surveys are needed in future years to monitor the geoduck abundance at this site. The high density and apparent stability in 2004 and 2006 indicate that conditions are favorable to successful geoduck recruitment and survival at this site. However, caution should be taken about inferring trends with only two data points and also the possibility for observation error, noted in the methods section above.

At the Quatsap Point site, in Central Hood Canal, the density from the initial tagging of geoducks in 2006 was 0.029 geoducks/sq. ft. or about 4.5% of the abundance found at Lofall/Vinland. It is not possible to estimate a trend at Quatsap Point, since there is only a single data point. Based on exploratory surveys in Central Hood Canal, this site was chosen as an area that had a reasonable potential to be a good index station. Since low geoduck densities have been observed during exploratory surveys in Central Hood Canal, and also at this index station site, the total area of the index station was increased to 4500 square feet in an attempt to meet the objective of having 100+ geoducks within the station. The total number of geoducks tagged at this site in 2006 was 132.

In southern Hood Canal it was a challenge to find a suitable geoduck index site based on low observed abundance within this sub-region. A large (7200 square feet) index station was established in favorable sand substrate offshore of Musqueti Point. The highest abundance of geoducks observed at this site was between the -18 and -20 foot (MLLW) water depth contours, so the index station was established at these depths. In spite of a relatively large index station area, only 63 geoducks/sq. ft. This is about 1.3% of the abundance found at Lofall/Vinland index station (and about 31% of the abundance found at the Quatsap index station).

 Table 1.1 Geoduck density estimates at index stations in northern, central and southern Hood Canal.

Station	Water depth (ft., MLLW)	Year	Station Area (sq. ft.)	Geoduck Count	Geoduck Density (geo./sq. ft.)
Lofall/Vinland	-35	2004	450	290	0.644
Lofall/Vinland	-35	2006	450	304	0.676
Quatsap	-29 to -30	2006	4500	132	0.029
Musqueti Point	-18 to -20	2007	7200	63	0.009

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1.5 RELATIONSHIP TO WATER QUALITY DATA

Most marine animals require dissolved oxygen (DO) for reproduction, recruitment, growth, and survival. A number of physical and chemical factors interact in the marine environment that may affect dissolved oxygen including water temperature, salinity, acidity or alkalinity, and water mixing. Oxygen is produced by biological processes (photosynthesis by plants) and is used by biological and non-biological processes (for example, animal respiration and decomposition of dead organic matter). Animals with limited or no mobility, such as geoducks embedded in the substrate, are particularly vulnerable to hypoxic (low dissolved oxygen) or anoxic (no dissolved oxygen) water conditions.

There is no unified consensus on critical threshold of DO needed for marine animal health, but hypoxia is often defined at levels below 2 mg of DO per liter of water. Many species experience reduced growth and altered behavior below this level and may experience mortality during prolonged exposure. The Environmental Protection Agency (EPA, 2000) and the Virginia Providence database for Chesapeake Bay calculated a minimum average concentration of DO for a 24 hour period at 2.24 and 2.27 mg/L, respectively, to protect juvenile and adult fish, crustacean, and molluscan species from dying. The critical threshold for larval and juvenile growth for Chesapeake Bay is higher at about 5.0 mg/L. Below this level behavior and growth of larvae and juvenile marine animals may be affected, but may not result in immediate death.

In southern Hood Canal, water quality data is available from an ECOLOGY station (HCB004) near Sisters Point from 1990 to the present (Figure 1.2).

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Figure 1.2 Water quality stations and geoduck index stations in Hood Canal.

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At a water depth of 10 meters (-32.8 feet, MLLW) a total of 189 water samples were taken and values of DO were recorded (Figure 1.3). Two important considerations are the concentrations of DO and the duration of low DO concentrations. Fifteen out of 189 DO values, or 7.9% of all samples, recorded during this period were below the hypoxia threshold of 2 mg/L cited in the literature for Chesapeake Bay. Since continuous values are not available from this data, the precise duration of the hypoxia is not known. Values of DO at this location were below the critical criteria of 5.0 mg/L for normal larvae and juvenile marine animal growth on 95 out of 189 (or 50.3%) sampling events (Figure 1.3). Some persistent periods of low DO appear to have occurred at this water depth between late August 1997 to April 1998, late August 1999 to November 1999, October to December 2000, June 2004 to March 2005, September 2005 to October 2005, and mid August 2006 to October 2006. While the seasonal periodicity of low DO is similar for the entire sample period, the duration of consecutive low DO values is longer beginning in 1997 and the average DO concentration is lower. The average DO for sample events at this water depth between December 1990 and June 1997 is 6.4 mg/L. The average DO for sample events at 10 meters water depth between July 1997 and March 2007 is 4.6 mg/L.



Figure 1.3 Dissolved oxygen values (mg/L) recorded in South Hood Canal at ECOLOGY Station (HCB004).

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In north-central Hood Canal, water quality data is available from an ECOLOGY station (HCB010) near the Quatsap Point geoduck index station from 2005 to the present. At this station 13 DO values have been taken at the 10 meter water depth. A minimum value of 3.98 mg/L was recorded in October of 2006. The average value for all data collected at this depth is 7.62 mg/L. At this writing there does not appear to be persistent low DO events at this site.

In northern Hood Canal, water quality data is available from two ECOLOGY stations (HCB006, HCB008), near the Lofall/Vinland geoduck index station from 1989 to the present (Figure 1.4). At these stations 132 DO values have been taken at the 10 meter water depth. A minimum value of 3.77 mg/L was recorded in October of 2000. The average value for all data collected at these stations from water depth is 7.58 mg/L. At the time of this writing, there does not appear to be persistent low DO events at this site.



Figure 1.4 Dissolved oxygen values (mg/L) recorded in North Hood Canal at ECOLOGY Stations (HCB006, HCB008).

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1.6 UPDATE ON INVASIVE TUNICATE FOUND IN SOUTHERN HOOD CANAL

An observation of interest in the last report of geoduck studies in Hood Canal (Sizemore, 2006) was the presence of an invasive tunicate, *Ciona savignyi*, on the Tahuya geoduck tract in southern Hood Canal. In October 2005, the tunicate was observed on 54 standard geoduck survey transects (Figure 1.5). A follow-up survey was done in October 2007 by replicating 34 transects in the western portion of this tract. No invasive tunicates were observed replicate transects during the 2007 survey. The potential causes for the sudden invasive colonization in 2005 and the apparent collapse of this same population is not well understood.



Figure 1.5 Distribution of invasive tunicate, Ciona savignyi, observed during a 2005 survey of the Tahuya geoduck tract.

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1.7 CONCLUSIONS AND RECOMMENDATIONS

This initial round of establishing geoduck index stations provides additional information about the relative abundance of geoducks in Hood Canal and supports other survey data indicating a strong latitudinal cline (or gradient) of increasing abundance in Hood Canal from south to north. During the period of this study, it was possible to establish a geoduck index station in northern Hood Canal at Lofall/Vinland, and re-survey this site. There has not been hypoxic conditions recorded in northern Hood Canal at a 10 meter water depth during this sampling period, and this geoduck index station indicates that unfished geoduck abundance has been stable in northern Hood Canal. Additional data points in future years are needed to establish on-going geoduck abundance trends in central and southern Hood Canal and to monitor trends in all three subregions. It is recommended that additional surveys of the established index sites should be done. The limitation of these discrete index sites is that they may only represent a small geographic area, and caution should be taken if attempting to extrapolate these results over broader regions. From geoduck tract survey information, it is known that small geographic distances can result in large differences in geoduck abundance. To account for small-scale differences, or to average these differences for a sub-region, additional representative index stations are needed. Surveys of existing fished and unfished geoduck tracts should also be done, to continue the time series of abundance on the tracts and to complement abundance estimates from index sites.

Only one parameter of water quality (dissolved oxygen) from one water depth (10 meters) was used to compare conditions between southern, central and northern Hood Canal. This water depth was chosen to approximate conditions that geoducks at an index site might experience. This informal comparison shows that critical thresholds of DO are rarely observed in northern Hood Canal and geoduck abundance at an index site (and also the subregion surveyed tract's biomass) is relatively high. In north-central Hood Canal the water quality data is limited to recent observations, and the recorded DO at this water depth is similar to northern Hood Canal, such as similar minimum DO and mean DO. However, geoduck density at the index site, and also geoduck density observed on tract surveys, indicates that successful geoduck recruitment or adult survivorship (or both) is affected in central Hood Canal. In southern Hood Canal the DO levels recorded are strikingly different than central and northern Hood Canal, with levels often falling below 5 mg DO/liter of water which may affect growth of certain species and sometimes reaching hypoxic conditions, below 2 mg DO/liter of water. The relative geoduck abundance is dramatically different between southern and northern Hood Canal index sites, with the southern index site having only 1.3% of the density found at the northern index site. For each geoduck index site, optimal density and substrate conditions were selected, which resulted in slight differences in water depths between sites. Geoduck density has been observed to vary with depth, and this may be a factor in density differences between these three index sites. To control for this variable, more exploration is needed in southern Hood Canal to determine if a deeper (10 meter water depth) index station can be established. Also, since differences in age structure with depth were observed, additional index stations should be established at various depths. As data is gathered in future years at these index sites and at geoduck tracts, a formal analysis of geoduck population trends and associated environmental conditions may be possible.

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During a standard geoduck survey, at the Tahuya River delta in 2005, an invasive tunicate (Ciona savignyi) was observed on most transects between -18 to -70 ft. (MLLW). None of these tunicates were observed during the same time of year on replicate transects done in 2007. It is common for invasive species to temporarily colonize and re-colonize an area before successfully establishing. Many invasive species do not establish at all. Williamson and Fitter (1996) identify three levels leading to establishment of a non-native species; 1) "imported" meaning a species is brought into a country, 2) "introduced" meaning a species is found in the wild, and 3) "established" meaning a population is self-sustaining. General probabilities are given that 10% of imported species will become introduced and 10% of introduced species will become established. Using this system, the invasive tunicate (Ciona savignyi) has been introduced and may be on the path to becoming established. To determine if future colonization events occur, this same area should be surveyed using the same methods at the same time of year. Using this type of data, it may be possible to determine if a "boom and bust" cycle of this invasive tunicate occurs and when (or if) the species permanently establishes. Given the observation of introduction of this tunicate, efforts should be undertaken to determine vectors for introduction and how it may be affected by environmental conditions found in southern Hood Canal.

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2.0 SECTION II. Geoduck Age Structure (University of Washington) by Juan L. Valero, B. Lee, D. Armstrong, L. Orensanz, A. Parma, R. Hilborn, B. Sizemore, T. Palzer.

A report on RCW 79.135.070 - A Study of age of Hood Canal geoducks.

2.1 INTRODUCTION

There have been increased concerns on historically low dissolved oxygen concentrations in Hood Canal, a fjord-like side basin of Puget Sound in Washington State, USA. During the last decade marine fauna showed signs of stress, including mass mortalities (Roberts et al. 2005, Devol et al. 2007), changes in behavior and distribution of mobile fauna (Palsson et al. 2006). Geoduck clams (Panopea abrupta) are distributed in Hood Canal from the intertidal to the subtidal. The first surveys of geoduck abundance in Hood Canal started in the early 1970s and have been continued by the Washington Department of Fish and Wildlife. Surveys are done by scuba divers and focus on areas between the -18 to -70 feet (MLLW, corrected to mean low water) water depth contours. Preliminary survey results showed a declining trend in geoduck abundance in three unfished geoduck tracts, selected to be representative of Hood Canal sub-regions (Sizemore and Blewett, 2006). Since this declining trend in abundance has been found in areas that have not been fished previously, other potential sources of mortality should be identified to explain the population trends. In other resources, individual size provides additional information about population structure that can be used to understand population dynamics. Geoducks grow relatively rapidly in their first years of life and attain maximum size at an early age, therefore individual size is not a very informative source of information regarding population structure. On the other hand, geoducks can be aged by reading growth layers in their shells after being sectioned, similar to growth rings found in trees. Aging methods are more expensive and time consuming than methods used to determine individual size structure. However, they provide excellent information regarding population structure, in particular when combined with large sample sizes at appropriate spatial scales. Resultant age frequency distributions when combined with other sources of information (such as individual size, population estimates, environmental variables) can be used to estimate individual growth, mortality, recruitment and population trends. In this context, estimating age frequency distributions across Hood Canal was identified as a research priority to understand changes in geoduck population structure and provide additional information that can be used to understand geoduck population dynamics and changes in geoduck population trends.

This report summarizes results on estimation of geoduck age structure in Hood Canal as part of project required by RCW 79.135.070. Geoduck samples were obtained at three unfished locations selected to be representative of Northern, Central and Southern Hood Canal subregions. The selection criteria for this index stations is described in Sizemore and Blewett (2006). Results of this project are currently being used in ongoing lines of research focusing on changes in geoduck population trends, identifying sources of mortality and estimating times to recovery of affected areas.

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2.2 MATERIALS AND METHODS

Samples for determination of age structure were obtained at three subtidal locations near Vinland in Northern Hood Canal, Hamma Hamma in Central Hood Canal and the Tahuya River delta in Southern Hood Canal (Figure 2.1). Geoducks were collected by scuba divers using standard commercial harvest gear to remove them from the substrate. Availability of geoducks to the sampling gear depends on visual detection by the divers and geoducks have been reported to be fully selected between ages 6 (Campbell et al., 2004) and 8 (Bradbury et al., 2000). That is geoducks below age 8 have a chance of being underrepresented in the samples. The collections were made in the 0 feet to 60 feet (MLLW) water depth range following 10 feet contour strata. Information about shells available for each site is summarized in Table 2.1. Geoducks shells were cleaned and the left valve was prepared for aging. Geoduck age estimation was done by counting internal shell annual rings to a resolution of 1 year (Figure 2.2) following protocols previously established (Honeycutt and Valero, 2003). Randomly selected subsamples comprising around 20% of the shells aged for each site were re-aged blind, that is without providing prior estimates, to assess variability in age estimation. We used ANOVA to compare average ages across sites. We report age frequency distributions in ten year bins for clearer visualization of data. We also calculated the expected age frequency distribution by simulating a hypothetical geoduck population under equilibrium assumptions and the natural mortality estimate used in Washington State for assessment and management purposes, M = 0.02/year (Bradbury et al., 2000, Bradbury and Tagart, 2000) to compare potential departures of the actual age distribution data to equilibrium assumptions. In addition, we compared the age frequency distributions obtained for Hood Canal with a historical Puget Sound age frequency distribution obtained from Goodwin and Shaul (1982).

2.3 RESULTS

A total of 1095 geoduck shells were able to be assigned final ages from the 1470 processed for ageing, representing 74% of assignment of final ages. The percentage of assignment of final ages varied slightly between sites (Table 2.1), Hamma Hamma geoducks being the most difficult to age. The percentage of successful assignment of age is similar to what it was found for geoduck samples from other regions in Puget Sound. The remainder shells were processed for ageing but we were unable to provide reliable final age estimates in a 1-year resolution. Unsuccessful assignment of ages is a result of a number of factors including damaged shells and distorted patterns of growth lines. We found no relationship between unsuccessful assignment of ages and preliminary age estimates. Re-ages of random subsamples for each of the three sites reveals high consistency between assignment of age performed and considered final (Age axis in Figure 2.3) and following assignments of age without previous knowledge of previous age estimates (Re-Age axis in Figure 2.3). The consistency between regions can be seen in the low maximum and average errors (Table 2.2) and the low departure from the 1:1 line representing no differences between subsequent ages between (Figure 2.3).

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Table 2.1 Summary information for the age collection sites. N sample: number of geoducks available for ageing, N aged: number of geoducks successfully aged. % aged: percentage successfully aged.

Site	N sample	N aged	% aged
Vinland	508	413	81%
Hamma-Hamma	554	381	69%
Tahuya	408	301	74%
All sites	1470	1095	74%

Table 2.2 Summary of re-age analysis. N aged: number of geoducks successfully aged, N re-aged: number of geoducks randomly re-aged, % re-aged: percentage of geoducks randomly re-aged, Er_max (yr): maximum absolute error in years, Er_avg (yr): average error in years.

Site	N aged	N re-aged	% re-aged	Er_max (yr)	Er_avg (yr)
Vinland	413	80	19%	2	0.08
Hamma-Hamma	381	63	17%	9	-0.27
Tahuya	301	60	20%	10	0.30
All sites	1095	203	19%		

Average age differs significantly among sites (ANOVA, p < 0.001, Table 2.3). Pair-wise comparisons between sites show that all sites differed among themselves (Tukey Test, P < 0.05, Table 2.4) with Tahuya geoducks being the youngest in average age, around 13 to 21 years younger than the other sites (Table 2.5). Hamma Hamma was characterized mostly by individuals around 38 years old, with relatively fewer older individuals than Vinland (Table 2.5, Figure 2.4) and relatively fewer younger individuals than Tahuya (Table 2.5, Figure 2.4). Other age summary statistics by site are reported in Table 2.5.

Table 2.3 ANOVA summary. df: degrees of freedom, SS: sums of square error, MS: meansquare error.

	df	SS	MS	F value	Р
Site	2	69975	34988	135.72	2.20E-16
Residuals	1088	280485	258		

Table 2.4 Tukey Test summary for differences in average age among sites. TY: Tahuya,HH: Hamma-Hamma, VIN: Vinland.

	diff	lwr	Upr	Р
ТҮ-НН	-20.37	-23.28	-17.47	< 0.05
VIN-HH	-8.01	-10.69	-5.32	< 0.05
VIN-TY	12.37	9.51	15.23	< 0.05

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Table 2.5 Summary statistics of geoduck age estimates at the three index sites. Avg age (yr): average geoduck age in years, SD: standard deviation, Med age (yr): median age in years, Max age (yr): maximum age in years.

Site	Avg age (yr) SD		Avg age (yr) SD Med		Med ag	d age (yr)Max age (yr)	
Vinland	30	18.5	27	107			
Hamma-Hamma	38	11.4	38	80			
Tahuya	17	17.4	11	77			

All Hood Canal sites showed departures from the expected age structure under equilibrium assumptions and from a historical Puget Sound age frequency distribution (Figure 2.4), although Vinland was closest to the expected age distribution under equilibrium assumptions (Figure 2.4). It is noteworthy that the ability to collect young geoducks decreases at younger ages as it was mentioned in the previous section. This may account for some level of under representation of individuals at or below the 10 year bin (Figure 2.4). The geoduck age distribution of Hamma-Hamma was characterized by a larger fraction of individuals 40 years and older and smaller fractions of younger than expected in equilibrium and in the historical age distribution. Conversely, the age structure of Tahuya geoducks is characterized by a smaller than expected fraction of 30 year old and older geoducks, and larger than expected fractions of younger geoducks (Figure 2.4).

2.4 DISCUSSION

We found differences in age structure between Hood Canal subregions, particularly regarding differences in average age by subregion and in the relationship between average age and depth. A final interpretation of these patterns exceeds the scope and goals of this report and is highly dependent on ongoing research that is being conducted as continuation of this project. However, we discuss some emerging patterns of the aging results and its relation to other concurrent and ongoing research. The age structure of a particular location is a result of a combination between recruitment to the site (arrival of new settlers) and subsequent mortality of the age classes already present in the site. Both recruitment and mortalities (both natural and fishing mortalities) can vary from year to year and therefore result in departures on the age structure from what would be expected under equilibrium. However, since the three sampled sites have not been previously fished, only natural mortality will be affecting survival of different year classes. Although it is expected that age structure of a particular location will depart from what is expected in equilibrium or under historical integrated age distributions, the marked observed departures observed in the three sites reported here are remarkable. First, it is interesting to note that some of the differences in age structure are consistent with preliminary trends in geoduck abundance, particularly in Southern Hood Canal (Sizemore and Blewett, 2006). That is, the steep decline in geoduck abundance in Tahuya and the lower than expected proportion of older geoducks in the age composition may be indicative of episodic mortalities or increased average mortalities. To assess this hypothesis, additional samples of dead geoducks were collected earlier this year in Southern Hood Canal in areas characterized with high densities in the early part of the time series of abundance but low current densities. The rationale being that if geoducks were abundant in areas where they are currently absent, then we should be able to find evidence of accumulation of dead geoducks on those areas. These new samples were not planned in the

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original work associated with HB 1896, but were identified as an important component under the Hood Canal Molluscan Study, and we are moving forward with their analysis given their relevance to understand processes responsible for the observed patterns in geoduck abundance and age structure. The source of the additional geoduck mortalities to the expected baseline natural mortality still remains to be identified. Among the hypothetical sources of mortality are episodic low dissolved oxygen events such as the ones observed at least after 1990 (see Figure 2.3, Section I). During the last decade marine fauna showed signs of stress, including mass mortalities (Roberts et al. 2005, Devol et al. 2007), changes in behavior and distribution of mobile fauna (Palsson et al. 2006). Animals embedded in the substrate, such as geoducks, could be particularly vulnerable to low or no dissolved oxygen water conditions since they lack the ability to escape to areas with better water conditions.

If, as evidenced by the survey of geoduck abundance and age structure composition, geoduck populations have been subjected to additional sources of mortality, a further question that remains to be answered is the recovery of the affected areas. Recovery will result from a combination of recruitment to a particular site and survival of existing geoducks on that site. Recruitment to a particular region may be dependent on geoduck larvae originating from the same region or from different regions. Currently there is a lack of information regarding geoduck larval connectivity. However, areas with longer water retention times are more likely to depend on local recruitment . Whereas, areas with high flushing are more likely to depend on recruitment from other regions. Southern Hood Canal has the longest retention time in Puget Sound (Babson et al., 2006), on the order of the geoduck larval duration. A combination of longer water retention times, decreased population sizes and high mortalities, as suggested for southern Hood Canal, could potentially result in longer recovery periods of affected areas.

Furthermore, results obtained from work funded by HB 1896 are being incorporated in a more extensive ongoing project focusing on evaluating the population abundances and ecological roles of geoducks and sea cucumbers in Hood Canal (Principal Investigator: Dr. Tim Essington, School of Aquatic and Fishery Sciences, University of Washington). This project is collaboration between the University of Washington, the Hood Canal Salmon Enhancement Group, Washington Department of Natural Resources and Taylor Shellfish. One of the goals of our current work is implementing an age structured model incorporating available age structure, time series of geoduck abundance, environmental forcing and catch history data (for areas where catch has occurred, which is not the case of the three sites reported here) to estimate trends in abundance, population structure, recruitment strength and different sources of mortality: baseline natural mortality, episodic events (e.g. low oxygen) and fishing mortality. We are in the early stages of this ongoing work but preliminary results show the potential of this approach to understand the dynamics of geoduck clams in Hood Canal, the potential impacts of disturbance (human and natural) and the recovery of affected areas (Valero et al. 2006).

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Figure 2.1 Index stations where samples for geoduck age determination were taken. VIN: Vinland, HH: Hamma-Hamma, TY: Tahuya

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Figure 2.2 Cross section of geoduck shell showing annual growth rings.

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Figure 2.3 Results of re-ages of randomly selected geoduck shells for each index station and for the combination of all sites. The line represents the 1:1 relationship.

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Figure 2.4 Estimated geoduck age structure for sampled sites in Hood Canal and a historical Puget Sound age distribution (PS) grouped by 10 year bins (columns). The line represents the expected age structure under equilibrium assumptions.

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3.0 SECTION III. Geoduck Geochemical Analysis by Dr. Yongwen Gao, Makah Tribe

A report on RCW 79.135.070 - A Study of shell oxidation rate of Hood Canal geoducks

3.1 INTRODUCTION

The Interagency Agreement (IAA 06-258) between the Makah Tribal Council (MTC) and the Washington Department of Natural Resources (DNR) required MTC to use stable isotope ratios (${}^{18}O/{}^{16}O$ or $\delta^{18}O$, and ${}^{13}C/{}^{12}C$ or $\delta^{13}C$), trace elemental concentrations (e.g., Ca, Sr, Mg, Fe, Mn, and Cd), and organic carbon and sulfur from shells to examine the life history of geoduck and the possible connection with low dissolved oxygen (DO) in Hood Canal. The principle of using geoduck is that the carbonate shells are deposited in, or very close to, oxygen isotopic equilibrium with the ambient seawater (e.g., Urey 1947; Epstein et al. 1953; Grossman and Ku 1986), so the ${}^{18}O/{}^{16}O$ ratios of geoduck shells record the environmental conditions that an individual clam encountered. The carbon isotope ratios of geoduck shells do not reach equilibrium conditions, but they are still useful because they reflect metabolic status of the animal and may show trophic level changes as the animal grows (DeNiro and Epstein 1978; Fry 1988; Schwarcz et al. 1998). Similar principles can be applied to trace elemental concentrations. In particular, the Fe/Mn ratios may suggest the sedimentary environment (oxidation or reduction) in geological history. Concentrations of organic carbon and sulfur may indicate the nutrient loading and bacteria activities as documented in the literature (e.g., Brand 1983; Berner 1989).

Like other bivalves and fish otoliths, there are clear growth rings or layers that precipitated in geoduck shells as annual growth records, and these annuli can be used as a proxy for long-term marine ecosystem studies (e.g., Romanek et al. 1987; Weidman et al. 1994; Gao 2002). Noakes and Campbell (1992) identified the potential to use geoduck clams as indicators of climate change. Strom et al. (2004) developed precise methods for ageing geoduck and demonstrated that the growth patterns in geoduck shells may relate to sea surface temperature changes in North Puget Sound. These previous studies, however, are based primarily on physical markers in geoduck shells. The geochemical methods that combine stable isotopes and trace elements in shell carbonate should provide a powerful new tool for geoduck research and provide independent chemical records on marine environmental changes in Hood Canal. Since geoducks are sedentary animals and have a long life-span over 160 years, the geochemical approach on geoduck shells should be a more effective way than that of otoliths in Puget Sound (cf. Gao et al. 2001).

3.2 METHODOLOGY

Selected shell samples were cut along the hinge, exactly matching the cutting lines from the University of Washington ageing. The hinge portion was placed in a self-made mold consisting of an aluminum base and strips with reference lines, similar to molds used in otolith studies (cf. Gao and Beamish 1999). After positioning shell hinges in the mold, black-pigmented fiberglass resin was added and allowed to harden overnight. The hardened blocks were removed from the

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mold and cut into thin sections to a thickness of 0.5-1 mm. The saw we used is a MV-600C Metcut system, with variable speed and sliding weight control. Each shell section was polished using an ECOMET 3 variable speed grinder-polisher with 240/400/600/2400 self-adhesive paper. The polished shell section was attached onto a Premium frosted slide by Krazy glue and cleaned using 91% rubbing ethanol. Microsampling was conducted using the Dremel method (Gao 1999) at the Otolith Laboratory of Makah Fisheries Management in Neah Bay, Washington. At least 50 µg of powder material from the shell surface were extracted for stable isotope analysis. Once a sample was taken, the powder was carefully tapped onto aluminum foil and placed into a metal cup. The shell section and the sampling bit were subsequently cleaned using an Aero-Duster gas.

Twenty seawater samples were collected each from Vinland, H. Hamma, and Tahuya by the Department of Fish and Wildlife (WDFW). Of which 10 samples were collected from the water surface (0-5 m), while the other 10 samples collected from bottom (about 20-30 m). Immediately after the completely-full bottle coming out of water, the sample was tightly capped and sealed with a 3 M ScotchTM vinyl electrical tape, and then labeled and kept in dark boxes. At the same time and site, temperature, salinity, and dissolved oxygen were measured by an YSI Salimeter. Other relevant information, such as sampling date and time, location, personnel, and water current, were also recorded. After field sampling, all water bottles were kept refrigerated until the oxygen isotope analysis was conducted.

Analysis of powder and seawater samples was performed in the Stable Isotope Laboratory of the University of Michigan Ann Arbor, using a Finnigan MAT Kiel preparation device that is coupled directly to the inlet of a Finnigan MAT 251 triple-collector gas-ratio mass spectrometer. All the measurements were reported in the standard δ notation (‰): $\delta^{18}O =$ {[(¹⁸O/¹⁶O)_A / (¹⁸O/¹⁶O)_S] - 1} x 1000, for instance, where *A* is shell sample and *S* is standard (VPDB, Vienna Peedee belemnite). Calibration of isotopic enrichments to VPDB standard is based on daily analysis of NBS-19 powdered carbonate and the analytical precision is better than 0.08‰ for both $\delta^{18}O$ and $\delta^{13}C$.

All trace elemental analyses for shell samples were performed in the Laboratory of Environmental Geochemistry of Brock University in Ontario, Canada, using a newly-updated Varian Atomic Absorption Spectrometer. Based on weight, shell powder samples were digested in 5 mL or 9 mL of 5% (v/v) HCL for 90 minutes. After rinsing the digestion fluid through a funnel and into a volumetric flask, the solutions were brought to the working volume of 14 mL with deionized water. All discussions were based on elemental concentrations recalculated on a 100% (insoluble residue-free) carbonate basis (Brand 1983). All prepared samples were measured for 6 elements (Ca, Sr, Mg, Fe, Mn and Cd). Precisions of the technique compared with NIST SRM 633 (N=4) and average precision based on duplicate analyses are: Ca (3.95%), Mg (2.15%), Sr (5.07%), Fe (5.61%), and Mn (2.98), respectively. There is no certified value for Cd, and the Cd concentrations were not used in this report.

Analyses of organic carbon and sulphur in shell carbonate were not complete because the contents of organic carbon in geoduck shells are too small. We did test 10 powder samples successfully for total carbon and sulphur analyses, although the results are not presented in this report.

3.3 RESULTS

The δ^{18} O values of geoduck shells ranged from -1.34 to +0.95% VPDB, while the δ^{13} C ranged from -2.19 to +0.35% VPDB. The differences between isotope values from the three different

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sampling sites (i.e., northern, central, and southern Hood Canal; Figure 3.1) were presented in Table 3.1 and Table 3.2. The overall δ^{18} O values of seawater ranged from -2.03 to -1.55% VSMOW, and no significant differences (ANOVA, *p*=0.971) between Vinland, H. Hamma and Tahuya samples.



Figure 3.1 Three geoduck sampling sites and the ECOLOGY stations in Hood Canal

Table 3.1 Analysis of variance on $\delta^{13}C$

SOURCE	DF	SS	MS	F	Р
FACTOR	2	16.068	8.034	38.34	0.000
ERROR	244	51.130	0.210		
TOTAL	246	67.198			

Table 3.2 Analysis of variance on δ^{18} O

SOURCE	DF	SS	MS	F	Р
FACTOR	2	33.244	16.622	131.50	0.000

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ERROR	244	30.843	0.126
TOTAL	246	64.086	

The mean values and the range of element Ca, Sr. Mg. Fe, and Mn were presented in Table 3.3. Due to the nature that trace elements substitute for Ca^{2+} in the crystal structure, we also analyzed the Sr/Ca and Mg/Ca and Fe/Mn ratios in shell carbonate. The differences between the three sampling sites were presented in Table 3.4.

Element	Minimum (ppm)	Maximum (ppm)	Mean (ppm)	
Ca	341,419	399,928	386,609	
Sr	796	2583	1464	
Mg	23	396	89	
Fe	0.7	384.1	38.5	
Mn	11.5	210.9	27.3	

Table 3.3 Summary of element analyses in shell carbonate

 Table 3.4 Analysis of variance on elemental ratios in shell carbonate

Element	SOURCE	DF	SS	MS	F	Р
Sr/Ca	FACTOR ERROR TOTAL	2 135 137	8.398 100.325 108.723	4.199 0.743	5.65	0.004
Mg/Ca	FACTOR ERROR TOTAL	2 135 137	0.0075 3.4891 3.4966	0.0038 0.0258	0.15	0.865
Fe/Mn	FACTOR ERROR TOTAL	2 135 137	13.14 214.50 227.63	6.57 1.59	4.13	0.018

3.4 ENVIRONMENTAL CHANGES FROM GEOCHEMICAL DATA

The stable isotope values of shell carbonate showed some difference between the three sampling sites, but the patterns in δ^{18} O and δ^{13} C were distinctly different. The annual δ^{18} O variations of Tahuya (TY) samples were consistent with those of Vinland (VL), but different from those of H. Hamma (HH) (Figure 3.2).

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In contrast, the δ^{13} C variation trends of Tahuya samples were consistent with those of H. Hamma, but different from those of Vinland (Figure 3.3). This is understandable because H. Hamma is geographically close to Tahuya, so the food sources of geoducks might be similar. The more negative δ^{18} O values in the H. Hamma geoducks indicated that significant freshwater mixed into the central Hood Canal, making different δ^{18} O variations between H. Hamma and Tahuya (and Vinland).

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Figure 3.3 Carbon isotope comparisons between the three sampling sites

Analyses of ¹⁸O/¹⁶O ratios in seawater indicated there were no significant differences between the three sampling locations. However, correlation between δ^{18} O in seawater and salinity showed two different variation patterns (Figure 3.4). The δ^{18} O_W-salinity relationship of Tahuya samples was similar to that of Vinland, but different from that of H. Hamma. The difference appeared in agreement with the δ^{18} O variations in shell carbonate, indicating that the H. Hamma River might be responsible for the isotopic difference.

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Figure 3.4 Seawater δ^{18} O-salinity relationships for the three sampling sites

Trace elements, particularly Sr/Ca, Mg/Ca, and Fe/Mn ratios, are useful indicators for water conditions and marine environmental changes over time. The Sr/Ca ratios from Tahuya shells showed distinct changes in 1994 (refer to the next section of this report), which corresponds to increases in both Fe/Mn and Mg/Ca ratios. The changes also occurred in Vinland samples. We do not know what happened in 1994 in Hood Canal, however, the distinct Fe/Mn ratios in Tahuya would be an indicator that the sedimentary conditions of the southern Hood Canal had been changing since 1994, and reaching the minimum in 1999.

In summary, geoduck shell samples from the northern, central, and southern Hood Canal showed distinct differences in δ^{18} O and δ^{13} C, and trace elemental concentrations.

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Both seawater and shell δ^{18} O values indicated that H. Hamma samples are different from Tahuya and Vinland due to the freshwater input. However, the δ^{13} C values and trace elemental ratios showed that Tahuya samples are distinctly different from those of Vinland, indicating that the food sources and water conditions of the southern Hood Canal were different from those of the northern sub-region.

3.5 DISSOLVED OXYGEN AND ECOLOGY RECORDS

The Washington Department of Ecology (ECOLOGY) has two kinds of monitoring stations, core and rotating. Core stations are permanent stations that are sampled every year whereas rotating stations are sampled in a couple of years. Based on the requirements of HB 1896, three geoduck collection sites near the ECOLOGY stations were chosen throughout the northern, central, and southern sub-regions of Hood Canal (cf. Figure 3.1):

(1) Northern Hood Canal: A core station is located near King Spit/Bangor (#HCB006), corresponding to the Vinland collection site in this report.

(2) Central Hood Canal: There are no core stations in the sub-region, so a rotating station near the H. Hamma River (#HCB003) was chosen, corresponding to the H. Hamma collection site.

(3) Southern Hood Canal: A core station is located near Great Bend/Sisters Point (#HCB004), corresponding to the Tahuya collection site.

Courtesy of Tina Blewett from WDFW and Skip Albertson from ECOLOGY, we received 68,762 CTD observations valid from 1990-2005. The data sets include detailed information such as observation date, time, station, depth, and records of temperature (°C), salinity (psu), density (sigma-t), pH, DO (mg/L), Xmiss (%), and Chlorophyll-a (μ g/L). Thus all the ECOLOGY profile data were analyzed after depth corrections in this report. Because the DO records for both Vinland and Tahuya core stations showed consistent variations from 1990-2005, we took Tahuya as an example. There were no core stations in the central Hood Canal, so we did not compile the ECOLOGY data from H. Hamma.

Over the last 16 years, the DO records for the Tahuya station was decreasing from 1993-94, with the lowest levels in 1998 (Figure 3.5). As compared with DO records δ^{18} O values from shell carbonate showed very similar patterns, with the lowest values in 1997. The similarity indicated some consistent variations between DO concentrations and δ^{18} O variations in Hood Canal.

The δ^{13} C values from geoduck shells also showed a distinct decrease from year 1998, corresponding to the lowest DO records (Figure 3.6). In particular, the δ^{13} C variations displayed a pattern over two periods: a stable period from 1991-98, and an increase from 2000-04, which was also consistent with the DO records. Thus we may conclude that both δ^{18} O and δ^{13} C variations were consistent with DO records in Hood Canal.

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Trace elemental concentrations of geoduck shells, particularly Sr/Ca and Fe/Mn ratios, were consistent with the DO records in Tahuya (Figure 3.7). The Sr/Ca ratios decreased from 1994, and reached a minimum value in 1999, which was very similar to the DO changes observed. The Fe/Mn ratios (Figure 3.8) reached the lowest values in 1994, and then increasing until the low valley occurred in 1999. Both Sr/Ca and Fe/Mn ratios indicated that the low DO concentrations might be related to water and sedimentary conditions in Hood Canal.

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Figure 3.7 Comparison between DO and Sr/Ca ratios in Tahuya

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Comparison between temperature of corrected water profile and DO records did not show consistency or similarity (Figure 3.9), although the seawater temperature decreased in 2001 for the southern Hood Canal.

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Salinity values showed a two-stage variation between 1995 and 1997, some similarity to the DO records (Figure 3.10), but the consistency was very weak, if any.

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In conclusion, the large database and ECOLOGY records provided a valuable means for comparison between DO concentrations and geochemical data and physical oceanographic parameters. The overall comparisons showed that the DO concentrations in the southern Hood Canal were consistent with δ^{18} O, δ^{13} C, and trace elemental ratios, but not with temperature and salinity. This consistency appeared to suggest that the low DO concentrations in Hood Canal were more related to habitat and water conditions, at least for the southern sub-region.

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3.6 RECOMMENDATIONS

This is the first time geochemical research has been conducted on geoduck shells in Hood Canal. We have overcome many technical and analytical difficulties during the project, and have learned a lot from the subject and practice. We have two recommendations for future research:

(1) Total carbon and sulphur analyses:

Although it is difficult to analyze organic carbon from shell annuli, total carbon and sulphur analyses still provide useful information about the nutrient loading and bacteria activities of the environment, particularly for the southern Hood Canal. Therefore, future research should complete the task and examine the nutrient loading factor over time.

(2) Back-calculation of oxygen isotopic temperature:

One of the most important applications' of stable oxygen isotope analyses is that we can backcalculate the isotopic temperature over time. The task needs precise measurements on seawater ${}^{18}\text{O}/{}^{16}\text{O}$ ratios, and temperature and salinity records in situ. Analysis of the second batch of seawater samples is underway, so isotopic temperature calculation back to the last 50 years in Hood Canal is strongly recommended.

3.7 REFERENCES

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