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# Evaluation of Survey Methodologies for Monitoring Olympia Oyster (*Ostrea lurida* Carpenter, 1864) Populations in British Columbia

# Évaluation des méthodes de relevés pour la surveillance des populations d’huîtres plates du Pacifique (*Ostrea lurida* Carpenter, 1864) en Colombie-Britannique

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# Abstract

Olympia oysters were harvested commercially from the late 1880s to 1930 when stock declines and a shift in market preference ended the fishery. In 2000 they were listed as a species of special concern by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) and under the Species at Risk Act (SARA) in 2003. As a requirement of SARA a management plan for the Olympia oyster was completed in 2009. One of the required actions identified was the development of survey protocols to measure their relative abundance along the BC Coast. This paper reviewed seven different survey protocols and tested four of them in the field. The results from these reviews have lead to recommendations on survey protocols depending on the population structure and density. On beaches where Olympia oyster populations are discrete or scattered a Two-Stage design with some GPS mapping should be employed. The number of quadrats used at each beach should range from 50 to 100 depending on the population density. On beaches where Olympia oysters are in extremely low abundance (a few individuals under rocks) or in complex habitats such tidal pools or along rock walls, a visual assessment of these populations will be necessary.

# Résumé

La pêche commerciale des huîtres plates du Pacifique s'est pratiquée de la fin des années 1880 jusqu'en 1930, moment où la baisse des stocks et un changement de préférence du marché ont entraîné sa fin. En 2000, l'espèce a été inscrite à la liste des espèces préoccupantes par le Comité sur la situation des espèces en péril au Canada (COSEPAC) et, en 2003, elle a été inscrite en vertu de la *Loi sur les espèces en péril* (LEP). Selon une exigence de la LEP, un plan de gestion a été mis en place en 2009 pour l'huître plate du Pacifique. L'une des mesures requises qui y sont indiquées consiste en l'élaboration de protocoles de relevés afin d'évaluer son abondance relative sur la côte de la C.-B. Dans ce document, on a examiné sept protocoles de relevés différents et quatre ont été mis à l'essai sur le site. Les résultats de ces études ont mené à des recommandations sur les protocoles de relevés en fonction de la structure et de la densité de la population. Sur les plages avec des peuplements distincts d'huîtres plates du Pacifique ou avec des populations éparpillées, il faudrait utiliser un système en deux étapes de cartographie avec un GPS. Le nombre de quadrats utilisés à chaque plage devrait varier entre 50 et 100, selon la densité de la population. Sur les plages où l'abondance d'huîtres plates du Pacifique est extrêmement faible (quelques spécimens sous des pierres) ou dans les habitats complexes comme les cuvettes de marée ou le long des murs de pierre, il sera nécessaire de faire une évaluation visuelle de ces populations.

# Introduction

## *Background*

The Olympia oyster, *Ostrea lurida* Carpenter, 1864 (=*Ostrea conchaphila*[1]) is one of four species of oysters established in British Columbia (BC), Canada, and the only naturally occurring oyster (Bourne, 1997; Gillespie 1999, 2009). Pacific oysters, *Crassostrea gigas*, Eastern oysters, *Crassostrea virginica*, and European flat oysters, *Ostrea edulis*, have all established wild populations after introductions for aquaculture (Gillespie 1999, Gillespie 2007).

The Olympia oyster once supported subsistence and commercial fisheries, and was used to establish a mariculture industry on the west coast of the United States (Couch and Hassler 1989). Historical harvests of Olympia oysters by First Nations has been documented along the west coast in San Francisco Bay, CA (Shaw 1997), and in Puget Sound, WA (McKernan *et al*. 1949). In the late 1800's European settlers began to commercially harvest Olympia oysters and commercial landings continued to about 1930 (Bourne 1997; Qualye 1988) until stocks became depleted and the industry moved towards other larger oyster species that had already been introduced to the West coast. Since that time it is believed that Olympia oysters have maintained a stable population in BC, but have not recovered to the same abundance levels observed prior to the late 1800's (Gillespie 1999, 2009). Life history characteristics of *O. lurida* (low fecundity, limited dispersal, and sporadic spawning) may have contributed to its inability to recover from commercial over-harvest (Gillespie 2009). Some recent papers (*e.g.*, White *et al*. 2009), claim that *O. lurida* mature rapidly (<1yr) and produce abundant larvae (250,000/female) however colder temperatures in northern areas may result in one or two spawnings in mid summer, later age at maturity, and less productive populations (Couch and Hassler 1989, Gillespie 2009). Loss of preferred habitat to the Pacific oyster for post settlement recruitment, introduction of non-indigenous predators, parasites, and pollution have all been suggested as potential limiting factors preventing the recovery of oyster populations in some locations.

Since the closure of the fishery in 1930 Olympia oysters have received little attention in the scientific community. There are limited quantitative abundance estimates and most information regarding their distribution is sporadic and qualitative. The descriptions are subject to the opinion of the author and the use of vague terms such as 'present' or 'abundant' makes it difficult to extrapolate population densities and document long-term changes to their distribution.

Olympia oysters were listed as a species of special concern by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 2000 and under the Species at Risk Act (SARA) in 2003 (Canada Gazette 2003). A management plan for Olympia oysters was completed in 2009 (DFO 2009). One of the required actions identified in the

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[1] Harry (1985) proposed that only a single species of *Ostrea* was native to the Pacific coasts of North and Central America based on morphological characteristics; the name *Ostrea conchaphila* Carpenter, 1857, had chronological precedence. Recent work by Polson *et al*. (2009) presented chemical evidence that two species were involved. Therefore the name *O. lurida* was resurrected for the northern species.

management plan was the need to develop survey protocols to measure relative abundance of Olympia oyster populations. Once these survey protocols have been developed, index sites for long-term studies of Olympia oyster will be designated. Then surveys with these new protocols will be completed on these sites at least once every five years. This paper will describe and review a number of protocols that could be used to measure the abundance of Olympia oysters as well as review the data from field investigations testing the different survey protocols (Figure 1).

## *Objectives*

Specific objectives of this report include:

1. Evaluate different survey protocols used to estimate relative abundance of Olympia oyster populations and identify advantages and disadvantages of each type of survey design. Review field data for protocols that were tested.

2. Categorize different habitat and population types that support Olympia oysters and evaluate which protocol(s) are appropriate to each category. Recommend methods for long-term monitoring of cryptic populations in complex habitat, which may preclude standard quantitative assessment methods.

3. Recommend quantitative protocols (*e.g.*, survey design and layout, quadrat size, sampling rates) to obtain reliable estimates of small-scale distribution (bed size) and quantitative estimates of abundance.

# The Olympia Oyster

## *Description*

The Olympia oyster is a relatively small oyster with a deeply cupped lower (left) valve and a flat upper (right) valve that fits within the margins of the lower valve (Gillespie 1999). The reported maximum length is approximately 80-90 mm diameter (Harbo 1997). However, populations of Olympia oysters from BC, Puget Sound and the Pacific states are generally 60 mm or less (Baker 1995, Gillespie and Bourne 2005, Gillespie 2009). Although the maximum age of Olympia oysters is unknown they are thought to reach at least 10 years of age (Baker 1995). Further studies exploring the utility of shells or ligaments for ageing of oysters would be beneficial.

## *Distribution*

The Olympia oyster is found along the west coast of North America from BC to central Baja California (Mexico). The historic northern limit, based on a record by Dall (1914), is somewhat suspect as the specimen location was not recorded and despite investigation their presence has not been confirmed (Foster 1991, Polson and Zacherl 2009). Gale Passage in the Central Coast of BC is the northernmost documented location and this site likely represents the current northern range limit of Olympia oysters (Gillespie 2009, Polson and Zacherl 2009) (Figure 2).

Originally described as separate species by Carpenter in the mid 1800's, *Ostrea lurida* was synonymised with *O. conchaphila* based on morphological examinations conducted by Harry (1985), extending the southern limit of this species from Mexico to Panama. Recent molecular evidence presented by Polson et al. (2009) supports the distinction of separate species with populations north of central Baja California being once again referred to *O. lurida* and southern populations from south of Baja California to Panama as *O. conchaphila. O. lurida* populations are known to persist in parts of Washington, Oregon, and California however, limited information is available on the location of Olympia oyster populations and abundance in Mexico and further south.

In BC, Olympia oyster populations exist along the West Coast of Vancouver Island, the Strait of Georgia and some locations in the Central Coast. The most northerly locations are found in Gale Passage in the Central Coast (Gillespie 2009). Abundance data collected to date have been anecdotal and for the most part describe locations supporting populations of Olympia oysters that were large enough to attract commercial fisheries. There have been few quantitative estimates of population size in BC and even less information is available on population trends. However, the population in BC appears to be stable at low levels relative to historic accounts (Gillespie 2009).

## *Biology & Habitat*

Olympia oysters are larviparous, alternating hermaphrodites that do not self-fertilize. They mature first as males and then alternate between male and female each reproductive cycle throughout their life (Coe 1932). Gonadal development and spawning are both dependent on thresholds in water temperature. Oyster reproduction occurs between 12.5 and 16ºC. Spawning events appear to be sporadic and are not consistent from year to year. Spawning has been documented in the spring in southern locations (Baker 1995), but along the BC coast most likely spawning takes place later in the summer. Spawning events are initiated by release of sperm balls from male oysters. The sperm balls disintegrate in the water column and release spermatozoa, stimulating synchronous spawning. Female spawning occurs when eggs are extruded into the mantle cavity and fertilized by sperm which have been brought into the mantle cavity in the respiratory current (Gillespie 1999). The fertilized eggs develop into veliger larvae in the oyster's mantle chamber before they are discharged 10 to 12 days later (Couch and Hassler 1989). The release of larvae into the water column is called swarming. The planktotrophic larvae swim actively and feed on organic material in the water column. The larvae metamorphose into spat and after 2-3 weeks in the water column oyster spat settles on hard substrates, preferably shell (Couch and Hassler 1989, Gillespie 2009).

Olympia oysters have specific habitat requirements and temperature tolerances in comparison with other oysters found on the West coast. They are found primarily in lower intertidal and subtidal zones of estuaries and saltwater lagoons (Quayle 1969, 1988), but are also found on mud-gravel tidal flats, in splash pools, near freshwater seepage, in tidal channels, bays and sounds, or attached to pilings or the undersides of floats (Couch and Hassler 1989, Harbo 1997, Gillespie 1999). These locations provide oysters with standing water that will insulate them from extremes in air temperature and only expose them to the elements at very low tidal levels. Exposure to freezing temperatures which may occur during evening tides in the winter can cause significant mortalities. Documented occurrences of winter kill are suggested as the final depletion (to commercially insignificant levels) of oyster populations (after a history of overfishing) in Ladysmith Harbour in 1929 and Boundary Bay in approximately 1940 (Quayle 1969, Gillespie 1999). High summer temperatures can also affect young-of-the-year and cause mortalities. Olympia oysters are not found in exposed areas along the outer coast. They are often attached to hard substrate, but may occur free on the substrate, as singles or in clusters (Baker 1995). In some locations they have been found attached to the underside of hard substrates.

Olympia oyster populations can usually be classified into one of three habitat descriptions: discrete and/or scattered beds, complex beds, or low abundance sites. Discrete oyster beds consist of a well defined bed with scattered individuals of varying densities. The oysters can be abundant and considered a dominant species within these sites. Individuals are easily accessible, either loosely piled as singles or attached to one another in small clusters. The bed area can be mapped and quantitative assessments can be made with confidence at these locations. Complex oyster beds are characterized by oysters located underneath rocks, or attached to pilings and other structures. Sites that are complex are hard to survey and do not conform to a straight forward random surveys using fixed-size quadrats. It is usually difficult to access all individuals in these sites without causing damage to the oysters or their habitat. Low abundance sites contain very few oysters that are difficult to find even after a great deal of search effort. Low abundance, complex and discrete oyster beds are found at various locations in the Strait of Georgia including some areas in the mainland inlets of the Sunshine Coast and the East Coast of Vancouver Island. Discrete sites are found on the West Coast of Vancouver Island, in Ladysmith Harbor, in the Strait of Georgia, and some areas on the Sunshine Coast.

## *Ecology*

Olympia oysters are preyed upon by crabs, snails, seastars and birds (Gillespie 2009). Native predators include Dungeness (*Cancer magister*), red rock (*C. productus*) and slender (*C. gracilis*) crabs. Ochre star (*Pisaster ochraceus*), sun star (*Pycnopodia helianthoides*) and White-winged Scoters (*Melanitta fusca*) are also predators of Olympia oysters. Introduced predators include Eastern or Japanese oyster drills (*Urosalpinx cinerea* and *Ocinebrellus inornatus*) and European green crab (*Carcinus maenas*)(Palacios and Ferraro 2003, Buhle and Ruesink 2009).

Olympia oysters may be susceptible to many of the parasites that afflict shellfish aquaculture operations such as the protist *Mikrocytos mackini* (Denman Island Disease) which has been recorded in BC (Bower 2001). Denman Island Disease causes mortalities of larger Pacific oysters in the spring. In 2008, Olympia oysters from five geographic locations in BC from the West Coast of Vancouver Island and the Central Coast (Watt Bay, Port Eliza North, Port Eliza West, Queen Cove, Snowden Island) were tested for the presence of pathogens and disease using both histology and Polymerase Chain Reaction (PCR) assays (Meyer *et al*., in press). During this study, histology detected five parasitic/symbiotic organism and six unknown pathologens at such low prevalence and intensity they were assumed to have little or no major impact on the oyster's health. In addition, PCR assays were conducted for seven known bivalve pathogens, all of which produced negative results. Overall, this study did not find any pathogens or disease of concern in any of the *O. lurida* populations examined.

## *Fisheries*

Anecdotal information from Bourne (1997) and others suggests that commercial harvest in BC occurred from the late 1880s to 1930. By the 1930's there was evidence of stock decline (Bourne 1997). During the 1930's there was a shift in market preference towards introduced Eastern oysters and finally Pacific oysters. Quantitative data on historic populations in BC are almost non-existent. The fishery was small, and annual landings probably never exceeded 300 mt (Bourne 1997). This is likely an over-estimate as documented landings are combined for Olympia, Eastern and Pacific oysters (Figure 3). Within BC the majority of the commercial harvest took place within the Strait of Georgia (Boundary Bay and Ladysmith Harbour).

# Review of Survey Protocols

## *1. Haphazard Sampling*

### Survey Method and Analysis

Previous Olympia oyster assessments have been completed opportunistically by DFO staff as they encountered populations during broad-brush intertidal bivalve surveys along the coast. Gillespie and Bourne (2005) estimated densities by walking through oyster beds and tossing a small bucket (0.04 m² sampling area) to determine sampling locations (Gillespie 2009). The sampling bucket is "blindly" thrown to reduce bias in selecting sampling locations. Sample location selection is not truly random, but haphazard. This method was used when there was a need to quickly collect data but time or resources were not available to complete a more structured survey design[2]. This method can also be used to acquire a rough estimate of population densities with a limited amount of effort. In this survey the bucket was tossed, inverted and pressed into the substrate to mark the quadrat boundaries. Counts of live and dead shell within each quadrat were made. At each beach a sub-sample of 50 oysters was measured for overall shell length (Quayle 1988), and the size frequency distribution was recorded and graphed. Measured oysters were also examined for the presence of larvae. Summaries for each site included quantitative estimates of density for live oysters and dead shell, their range values, the number of samples collected, size frequency distribution and documentation of the presence and frequency of larvae detection. The number of samples collected at each beach was not consistent and varied due to the amount of time available. No estimates of bed area were made.

[2] This method is similar to that used by the BC Ministry of Agriculture, Fisheries and Food to estimate biomass of Pacific oysters for commercial harvest (S. Pilcher, BC MAFF, pers. comm.). In the provincial protocol, a quadrat is dropped over the shoulder by a surveyor as they walk through the oyster bed. Oysters in the quadrat are collected and a total weight is measured. The protocol does not include counts of oysters, nor are individual biological parameters (size, weight, etc.) collected. This protocol does not aid in meeting the objectives of Olympia oyster surveys, so it was not formally included.

Advantages:
1. Quick and simple, low cost sampling method.  Can be completed with only one investigator during one low tide.  The amount of time needed at each site is determined by the surveyor or by the time available/remaining during one tide.
2. It may be possible to survey more than one beach during one tide.
3. Survey design does not require time to measure the bed area or formally determine sampling locations.

Disadvantages:
1. Although the "blind" toss will produce sampling units that are spaced out across the bed area, there will be a tendency for the surveyor to place more quadrats near the middle of the bed and fewer quadrats along the edges of the oyster bed. This tendency will introduce a bias into the data and the sampling units will not accurately represent the entire oyster bed area.
2. This method is not statistically rigorous because the sampling is not truly random. The investigators will add bias to the situation by choosing the direction of the toss.  Each subplot along the beach does not have an equal chance of selection with each toss.
3. Bed area is not estimated therefore no estimate of total abundance can be calculated.  Analysis is restricted to the estimated density per square metre and the size frequency distribution.

## *2. Calculated Random Sample*

Allen and Davis (unpubl. manuscr.) developed a protocol to map Olympia oyster bed area, estimate density and quantify community dynamics. This method involves measuring the bed area to determine the number of sampling units required and evenly distributing sampling units throughout the oyster bed (Figure 4). When collected through a series of monitoring programs, good estimates of oyster density and size distributions will document young-of-the-year recruitment, oyster growth, survival patterns and cohort diversity.  Simple standardization may create problems associated with an unknown pattern in the population and result in an overestimated standard error.  However, this survey design attempts to eliminate this problem by randomizing the initial transect position and the first sample position on each transect, producing independent and random replicate samples of the population.

### Survey Method and Analysis

Surveys are conducted during a four hour window that is centered around the low tide. It is important to note that this protocol may involve more than one low tide depending on the oyster bed area, the surveyor's familiarity with the site, the calculated sample size, habitat complexity and available personnel.  The perimeter of the oyster bed is initially explored in order to become familiar with the size and shape of the oyster population. Once the area to survey has been identified, a start position for the survey is selected along the edge of the population.  This point will be on the long axis of the survey area and should be recorded with GPS and marked with a stake for future monitoring. Statistical notation required for Calculated Random sampling is provided in Appendix Table 1.

Measurements of the survey area can be completed with either survey tape or with a laser rangefinder. The oyster bed area is measured to determine the number and placement of transects through the bed. The longest distance across the bed is the baseline length ( $L_B$ ). Several measurements should be made of the width of the bed, especially potential transect lengths, in order to calculate the average width ( $\overline{L}_T$ ). The baseline length and the average width are used to estimate bed area ( $A_B$ ):

$$A_B = L_B \overline{L}_T \tag{1}$$

$$\text{where } \overline{L}_T = \frac{\sum_{n_T}^{i=1} L_{Ti}}{n_T} \tag{2}$$

and $L_{Ti}$ is the length of transect *i* and $n_T$ is the number of transects.

If the area has not been surveyed previously test samples are taken to estimate the minimum sample size. Quadrats are blindly thrown over the shoulder to determine sampling locations and oyster counts are made. The process is repeated fifteen times. Population density ( $d_h$ ) and sample variance ( $s_h^2$ ) from these haphazard samples are used to determine the minimum sample size ( $n_{\min}$ ), which is calculated for a desired precision ( $p$ ). The precision recommended by Allen and Davis for Olympia oyster populations was ± 30%; where the 95% confidence interval around the density estimate is restricted to ± 30% of the estimate. Although oyster populations can be highly variable or exist in very low densities the precision value of ± 30% has been used as an arbitrary reference when determining the sample size ( $n_q$ ) for the survey. The goal of the rapid assessment is to ensure that the oyster bed area is not over or under sampled during the survey and that a consistent amount of effort is allocated to each site. A surveyor can increase the precision of their estimates for diversity by increasing the number of samples collected or by decreasing the relative precision from ± 30% to ± 20% or 15%.

Minimum sample size given the estimated density and variance is calculated:

$$n_{\min} = \left( \frac{4 * s_h^2}{\overline{d}_h^2 * p^2} \right) \tag{3}$$

Once the bed area and the minimum sample size are determined the block size ( $r$ ), or the area represented by each sample can be calculated:

$$r = \left( \frac{A_B}{n_q} \right) \tag{4}$$

Calculations can also be completed to determine the standard distance between transects ( $TD$ ) and the standard distance between samples or quadrats ( $SD$ ):

$$TD = \left( \frac{L_B}{n_T \left( \frac{L_B}{L_B + \bar{L}_T} \right)^{0.85(Ln(n_T))}} \right) \quad (5)$$

$$SD = \left( \frac{r}{TD} \right) \quad (6)$$

The distance from the edge of the oyster bed to the first transect line is randomized. All other transects are placed perpendicular to the baseline length ( $L_B$ ) at set transect distances ( $L_{Ti}$ ). Placement of the first quadrat within each transect is randomized; all other quadrats are placed systematically at set distances ( $SD$ ) from the initial quadrat. This ensures the quadrats are evenly distributed across the bed area and that the beach is not over- or under-sampled.

This survey method provides estimates of oyster density, bed area and total population size and has also been used to provide important information for restoration efforts (Allan and Davis, unpubl. manuscr.). Habitat parameters such as predator and competitor abundance and substrate preference can also be analyzed. Quantified descriptions of the emergent oyster substrate can be used to assess the condition of the oyster habitat (availability, composition, quantity, and quality) using measures of substrate type, elevation, area, density, wet or dry conditions, and space available for recruiting oysters.

Advantages:

1. This method spreads the survey effort across the entire bed and only surveys the bed area.
2. A minimum sample size for a target precision is specified.
3. Precision of the estimates can be increased by adding additional samples to the survey or by decreasing the relative precision value when calculating the minimum number of samples required. However, this increase in precision will also increase the resources needed to complete the survey.
4. This survey method provides estimates of density, bed area and total abundance.

Disadvantages:

1. Costly and time consuming survey protocol.
2. Determining the boundary of the bed can be subjective as densities on the outside edges (especially in the high intertidal) can become very low.
3. This method used an unbalanced Two-Stage design that was analyzed as a Simple Random Sample. A more appropriate estimate of two-stage variance is provided by Kronlund and Gillespie (1998).

## *3. GPS Bed Area Simple Random Sample*

### Survey Method and Analysis

This method was conducted by Allen and Davis in Port Eliza, BC (?), in 2009 and involves mapping the boundary of the oyster bed with a differential Geographic Positioning System (GPS) unit using ArcPad software. Once the boundary of the bed was defined, ArcPad's Geographic Information System (GIS) capabilities were used to assign random quadrat locations (Figure 5). The GPS unit was then used to place random quadrats within the defined oyster bed. To date, no analysis has been completed on this data and therefore it will not be explored further in this report. The design of this survey is similar to a Simple Random Sample analysis.

Advantages:

1. The bed area can be measured and mapped. This mapping then can be used in other spatial analyses.
2. Quadrat locations can be easily determined in the field with ArcPad.
3. Analysis uses a Simple Random Sample method.
4. This method provides estimates of bed area, density and total abundance with confidence intervals.

Disadvantages:

1. Determining the boundary of the bed can be subjective as densities on the outside edges (especially in the high intertidal) can become very low.
2. The cost of the GPS unit and ArcPad software maybe to prohibitive.
3. Electronic malfunction can disrupt the survey.

## *4. Timed Random Search*

### Survey Method and Analysis

Polson and Zacherl (2009) developed a survey protocol to establish presence or absence of Olympia oysters as well as determine their densities and percent cover. Their study was the first quantitative assessment of Olympia oyster density along the oysters' entire suspected range; from Alaska to Mexico. Beaches were surveyed in two steps. The first step involved 2-hour timed surveys conducted during negative tides. For the purpose of their study, Polson and Zacherl used reference marks for known tidal height at survey locations. If there was no known reference mark, one was determined by taking the average of three low mark measurements[3]. During the 2-hour timed survey, search effort focused on portions of the beach that provided suitable or favourable habitat which consists of areas containing either natural or artificial hard substrata. The timed aspect of the survey allows the investigator to stop the survey at any point when unsuitable habitat is encountered and walk through the area until more

[3] Note that Canada and the US differ in how tidal height is estimated. The US uses tidal height calculated from mean lower low water (MLLW), while Canada uses tidal height calculated from chart datum (CD).

ideal habitat is found. At this point the survey can be continued. The ability to start and stop the survey is ideal for surveying patchy habitat.

Investigators started at one end of the beach and zigzagged through the area from the upper to the lower intertidal distribution (Figure 6). Approximately every 50 meters a 50 x 50 cm (0.25m$^2$ ) quadrat was laid down on the oyster bed and the density estimated and ranked. GPS waypoints were taken at each quadrat location. Oyster densities were ranked and the overall density calculated using a ranking system that ranged from 0-4; with 0 indicating no oysters present, a rank of 1 denotes oysters are rare with only 1-2 per quadrat observed. A rank of 2 or 3 was assigned for populations with quadrat counts ranging from 3-10 and 11-100, respectively. Finally quadrats with greater than 100 oysters were classified as abundant and received a rank of 4.

Average Maximum Density

Following the timed walk, oyster densities were ranked and calculations completed to determine the area of the beach with highest rank. A 50 x 4m transect belt was placed in the middle of the high rank area. The transect belt was designated X and Y coordinate values and random numbers were used to generate 10 replicate (n=10) quadrats measuring 50 x 50 cm (0.25 m$^2$) within the transect belt. Within each quadrat the number of oysters was counted and the percentage of oyster coverage estimated. Observations were also made on the presence of Pacific oysters and the presence of multiple size classes of Olympia oysters (either a yes or no). Statistical notation required for the Timed Random Search is provided in Appendix Table 1.

The average rank for each beach is calculated as the mean rank over the entire beach. This unit-less value can be used to compare different sites or monitor relative change in population abundance on one beach over time.

$$0 \leq rank \geq 4 \tag{7}$$

$$\mu_{rank} = \left( \frac{\sum (rank)}{n_q} \right) \tag{8}$$

Polson and Zacherl (2009) analyzed the data from their transect belt by determining the average value for the 10 replicate quadrats within the high density transect belt at each beach. The average maximum densities are used to calculate the coefficient of variation and measure the dispersion or spatial distribution of individuals in a population. The variation measures how uniform or patchy a population is relative to others. This value can be used to compare sites or to monitor index sites over time.

$$\bar{d} = \left( \frac{\sum_{i=1}^{N} d_i}{n_q} \right) \tag{9}$$

$$CV = \left( \frac{\sigma_{\bar{d}}}{\bar{d}} \right) \tag{10}$$

Advantages:

1. This method is low in cost and does not require a large team of surveyors.
2. The ability to stop and start the clock makes this method more versatile in sparse oyster beds or sites that contain areas that are not suitable for oysters such as freshwater streams. It allows the researchers to focus their efforts on the productive areas of the beach and will reduce confidence intervals of the estimate.
3. Placing the transect belt across the area with the highest ranks guarantees that survey time is actually spent in areas where there are oysters.
4. This survey will produce a statistical measure of variation for each beach surveyed. This measure can be used to compare numerous beaches.

Disadvantages:

1. Investigators must pay attention to their foot pace, ensuring they have good coverage and make consistent rankings throughout the site.
2. There is some misrepresentation of beach density by sampling only the high density location, producing an overestimate of density.
3. The transect belt sampling assumes that only one area of the beach has a high density of oysters. At some sites decisions would need to be made as to which high ranking area should be surveyed.
4. The time used to search a beach is not dependent on beach size (geographical characteristics) or oyster bed area. At large sites this method may lead to undersampling. At small or patchy sites this method may lead to oversampling.
5. The term of average maximum density is a misleading and confusing term to describe the estimate of oyster abundance at a particular beach.
6. This survey would difficult to repeat on the same beach in different years.
7. Density, bed area and total abundance are not estimated.

## *5. Stratified Random Sampling*

Other bivalve species, such as Manila and butter clams have been monitored over time in order to assess bivalve resources in BC (Gillespie *et al*. 1998a,b, 2001; Kingzett and Bourne 1998; Gillespie 2000). These surveys were completed using a Stratified Random sampling design as described in Gillespie and Kronlund (1999) and Kronlund *et al.* (1998). The level of stratification for sampling is based on the level of precision required for the results. The design of a survey is dependent on the physical characteristics of the beaches chosen to survey and prior knowledge of the site is helpful in determining the number of strata to designate. Stratification can be useful when there is a need to divide the beach into manageable survey units or when habitat or population characteristics define obvious boundaries. Quadrats are randomly placed throughout the survey area or stratum using a random number generator. Randomization provides a fair and repeatable means of avoiding bias in the selection of sampling sites (Kronlund *et al*. 1998). Oyster counts and measurement of biological samples can be used to estimate abundance and recruitment at the site.

## Survey Method and Analysis

A Stratified Random sample is a survey design where the sampling units are divided into non-overlapping groups and a simple random sample is drawn from each group (Figure 7).

Stratified sampling involves partitioning the population into strata and selecting a sample from each stratum. When the sampling method within each stratum is Simple Random Sampling, the design is called a stratified random sample (StRS). The key feature of StRS is that a sample is selected from each stratum independently of other strata.

The primary aim of stratification is to group sampling units such that the units within a stratum are as similar as possible. For example, prior knowledge may suggest that an area of high density should be partitioned into a stratum separate from areas of the beach with lower densities. Thus, one stratum may differ markedly from another, but the within stratum variability would be small. If no prior knowledge of the beach exists it is possible to stratify by substrate or tidal elevation. Statistical notation required for Stratified Random sampling is provided in Appendix Table 1.

## Estimating the Population Mean

One objective of a survey is to estimate the mean density of oysters in the survey area. The population mean is estimated as:

$$\bar{y}_{st} = \frac{1}{N}\sum_{h=1}^{H} N_h \bar{y}_h \qquad (11)$$

The variance of the mean is estimated as:

$$\hat{V}(\bar{y}_{st}) = \frac{1}{N^2}\sum_{h=1}^{H} N_h^2 \left(\frac{N_h - n_h}{N_h}\right)\frac{s_h^2}{n_h} \qquad (12)$$

where

$$s_h^2 = \frac{\sum_{i=1}^{n_h}(y_{hi} - \bar{y}_h)^2}{n_h - 1} = \frac{\sum_{i=1}^{n_h} y_{hi}^2 - \left(\sum_{i=1}^{n_h} y_{hi}\right)^2 \Big/ n_h}{n_h - 1} \qquad (13)$$

An estimate of the total number of oysters in the survey area can be obtained by expanding the mean estimate over the total surveyed area:

$$\hat{\tau}_{st} = N\bar{y}_{st} = \sum_{h=1}^{H} N_h \bar{y}_h \qquad (14)$$

The variance associated with this estimate can be calculated as:

$$\hat{V}\left(\bar{y}_{st}\right) = \frac{1}{N^2}\sum_{h=1}^{H} N_h^2 \left(\frac{N_h - n_h}{N_h}\right)\frac{s_h^2}{n_h} \tag{15}$$

where

$$s_h^2 = \frac{\sum_{i=1}^{n_h}\left(y_{hi} - \bar{y}_h\right)^2}{n_h - 1} = \frac{\sum_{i=1}^{n_h} y_{hi}^2 - \left(\sum_{i=1}^{n_h} y_{hi}\right)^2 \Big/ n_h}{n_h - 1} \tag{16}$$

## Confidence Intervals

Confidence intervals for population parameters can be computed in a variety of ways for Stratified Random sampling. The choice of the method may depend on the sample size within each stratum, or on whether normality is assumed.

When the sample size within each stratum is greater than 30 units, then the normal approximation may be used. For the population mean:

$$\bar{y}_{st} \pm t_{\alpha/2,d}\sqrt{\hat{V}\left(\bar{y}_{st}\right)} \tag{17}$$

For the population total:

$$\hat{\tau}_{st} \pm t_{\alpha/2,d}\sqrt{\hat{V}\left(\hat{\tau}_{st}\right)} \tag{18}$$

where t is the upper $\alpha$/2 point of Student's t distribution with d degrees of freedom computed using Satterthwaite's approximation. If sample sizes are large, then the t-value may be replaced with $z_{\alpha/2}$.

When sample sizes are small (as a rule of thumb, less than 30) an adjustment to the degrees of freedom for the t-statistic is appropriate. The adjustment is called Satterthwaite's approximation (Satterthwaite 1946):

$$d = \frac{\left(\sum_{h=1}^{H} a_h s_h^2\right)^2}{\left[\frac{\sum_{h=1}^{H} \left(a_h s_h^2\right)^2}{n_h - 1}\right]} \tag{19}$$

where

$$a_h = \frac{N_h \left(N_h - n_h\right)}{n_h} . \tag{20}$$

If all stratum sizes are equal and all sample sizes are equal, then the degrees of freedom are n-H, where $n = \sum_{h=1}^{H} n_h$ .

An alternative to assuming the normal distribution is to use resampling (bootstrap) techniques to compute a non-parametric estimate of the confidence interval. This method is described by Rao and Wu (1988), Sitter (1992), and Kronlund et al. (1998).

## Allocation of Survey Effort

There are a variety of ways to assign sample sizes to each stratum given n, the total number of quadrats that can be sampled. One simple approach is proportional allocation where a constant sampling fraction is used for the population (Gillespie and Kronlund 1999). The sample size within each stratum is given by the following equation:

$$n \ = \frac{nN_h}{N}, h = 1,...,H \ . \tag{21}$$

Sample sizes are normally assigned using proportional allocation of a fixed sample size. Other allocation methods that minimize variance for a fixed total sample size (optimal allocation) exist, but require prior survey data (Cochran 1977, Thompson 1992).

Advantages:

1. Produces statistically valid results to estimate abundance.
2. Since randomization occurs independently within each stratum, errors in the survey could be confined to one stratum.
3. Because the survey area is measured, estimates of total abundance for the surveyed section of the beach can be produced.

Disadvantages:

1. Requires pre-survey planning and knowledge of the survey location.
2. Can be labour intensive, may take more than one tide to complete.
3. This method may not distribute the survey effort across the entire bed but just randomly assign locations in a stratum so there is chance that this method may not sample the entire bed

4. With this method, only the area surveyed will be measured and in most cases the oyster bed will extend outside the survey boundaries. This method does not provide a complete measurement or mapping of the entire bed area.

## *6. Two Stage Sampling*

One strategy for distributing the sampling over a large area is to conduct randomization in two stages (Figure 8). A two-stage random sample is a survey design with first and second stage sampling units. The sample is obtained by first selecting a simple random sample of first stage units (FSUs) by partitioning the survey area into large plots. Then, a simple random sample of second stage units (SSUs) or quadrats are selected from each of the first stage units already selected, where each large plot is partitioned into smaller plots. This survey design is described in Gillespie and Kronlund (1999) and Kronlund *et. al.* (1998).

### Survey Method and Analysis

Stratified multi-stage sampling designs are commonly encountered in large area surveys. In a stratified two-stage design the sizes of first and second stage units can vary among strata. Within each stratum, however, first stage units are of equal size as are second stage units. The population of first stage units is partitioned into strata. Random selection of first stage units within each stratum is independent and a random sample of quadrats is chosen from each selected first stage unit.

In a stratified two-stage sample there are several features to note about the design:

1. the strata can be of unequal size;
2. the size of first stage units can vary among the strata but are FSUs are of equal size within a stratum;
3. the number of second stage units within each first stage unit varies among strata but is equal within a stratum.

The size and shape of strata and first stage units is very flexible, although rectangles are most convenient for randomization and physical layout. Thus, the restriction that FSUs are of equal size within a stratum is not difficult to accommodate in the field by carefully choosing the size and shape of each stratum. In fact, it may be easiest to use FSUs of equal size for all strata, but allow the stratum sizes to vary.

In Simple Random and Stratified Random designs, the variability of the estimate of the mean or total occurs because different samples of quadrats (the first stage units) give different values of the estimate. In contrast, two-stage designs have two sources of variability:

1. variability in estimates because of different samples of first stage units;
2. variability in the contribution of each first stage unit because of different samples of quadrats in each first stage unit.

Statistical notation required for Two-Stage sampling is provided in Appendix Table 1.

## Estimating the Population Mean

One objective of a survey is to estimate the mean density of oysters in the survey area. The sample information can be used to make inferences about the population mean. The equations required to compute the mean and the estimated variance of the mean are shown below.

$$\bar{y}_{ts} = \left(\frac{N}{M}\right)\frac{\sum_{i=1}^{n} M_i \bar{y}_i}{n} \tag{22}$$

where:

$$\bar{y}_i = \frac{1}{m_i}\sum_{j=1}^{m_i} y_{ij} \tag{23}$$

The estimator of the variance of the population mean:

$$\hat{V}(\bar{y}_{ts}) = \left(\frac{N-n}{N}\right)\left(\frac{1}{n\bar{M}^2}\right)s_1^2 + \frac{1}{nN\bar{M}^2}\sum_{i=1}^{n} M_i (M_i - m_i)\left(\frac{s_i^2}{m_i}\right) \tag{24}$$

where

$$s_1^2 = \frac{\sum_{i=1}^{n}(M_i \bar{y}_i - \bar{y}_i)^2}{n-1} \tag{25}$$

and

$$s_i^2 = \frac{\sum_{j=1}^{m_i}(y_{ij} - \bar{y}_i)^2}{m_i - 1}, i = 1,...,n \tag{26}$$

and

$$\bar{M} = \frac{M}{N} \tag{27}$$

Two-stage sampling may also be used in situations where stratification is desirable. Formulation for Stratified Two-Stage survey estimators can be found in Gillespie and Kronlund (1999).

The population mean can be estimated as:

$$\bar{y}_{sts} = \frac{\sum_{h=1}^{H} N_h M_h \bar{y}_h}{\sum_{h=1}^{H} N_h M_h} = \sum_{h=1}^{H} W_h \bar{y}_h \tag{28}$$

where

$$W_h = \frac{N_h M_h}{\sum_{h=1}^{H} N_h M_h} \quad \text{and} \quad \bar{y}_h = \frac{1}{n_h m_h} \sum_{i=1}^{n_h} \sum_{j=1}^{m_h} y_{hij} \tag{29}$$

The variance associated with the mean estimate is calculated as:

$$\hat{V}(\bar{y}_{sts}) = \sum_{h=1}^{H} W_h^2 \left[ \frac{1 - {n_h}/{N_h}}{n_h} s_{1h}^2 + \frac{\left({n_h}/{N_h}\right)\left(1 - {m_h}/{M_h}\right)}{n_h m_h} s_{2h}^2 \right] \tag{30}$$

where

$$s_{1h}^2 = \frac{\sum_{i=1}^{n_h} \left(\bar{y}_{hi\cdot} - \bar{y}_{h\cdot\cdot}\right)^2}{n_h - 1} \tag{31}$$

and

$$s_{2h}^2 = \frac{\sum_{i=1}^{n_h} \sum_{j=1}^{m_h} \left(y_{hij} - \bar{y}_{hi\cdot}\right)^2}{n_h (m_h - 1)} \tag{32}$$

## Estimating the Population Total

An estimate of the total number of oysters in the survey area can be obtained using:

$$\hat{\tau}_{sts} = \left( \sum_{h=1}^{H} N_h M_h \right) \bar{y}_{sts} \tag{33}$$

### Estimator of the variance of the total:

$$\hat{V}(\hat{\tau}_{sts}) = \left( \sum_{h=1}^{H} N_h M_h \right)^2 \hat{V}(\bar{y}_{sts}) \tag{34}$$

## Confidence Intervals

Confidence intervals for population parameters can be computed Stratified Two-Stage sampling. The confidence interval for the population mean can be calculated as:

$$\bar{y}_{sts} \pm z_{\alpha/2} \sqrt{\hat{V}(\bar{y}_{sts})} \tag{35}$$

The confidence interval for the population total can be calculated as:

$$\hat{\tau}_{sts} \pm z_{\alpha/2}\sqrt{\hat{V}(\hat{\tau}_{sts})} \qquad (36)$$

where $z$ is the upper $\alpha/2$ point of the standard Normal distribution.

Advantages:

1. Large areas can be surveyed using stratification.
2. By reducing variation within strata, the overall precision of estimates of population mean or total may be increased.
3. This survey is a repeatable method for one site over many years.

Disadvantages:

1. Prior knowledge or preliminary sampling is required in order to estimate minimum sampling rate (number of quadrats).
2. This method can be labour intensive and depending on the area may take more than one tide to complete.
3. This method does not give bed area measurements.

## *7. Visual Assessment*

### Survey Method and Analysis

At some North Coast locations, such as Watt Bay or Gale Passage, and in locations within the Strait of Georgia such as Goldstream, very sparse populations of Olympia oysters are found in complex habitats either attached to the underside of rocks or in specialized habitats such as tidal lagoons. These beaches are difficult to survey without crushing oysters or significantly disturbing habitat. In order to quantitatively enumerate these oysters, rocks would need to be turned over, or divers would be required to examine lagoons. At these locations a qualitative assessment is the only feasible method to monitor populations. A survey protocol can be developed to assess the change over time or rather record the continued presence of oysters at these sites. Documentation of site characteristics would depend on the information needs and priorities of a monitoring program. A qualitative assessment could include the following information: documenting the presence/absence of competitor or invasive species through time; determining the presence or absence of Olympia oysters over time and whether their distribution is patchy or abundant; description of the beach or habitat as simple or complex; and providing photographic documentation of individuals, populations, habitat and substrate. Although bed area could be estimated, it would only provide information about the spatial distribution, not abundance of oysters at the site over time. This type of assessment will allow for continued monitoring without sacrificing individuals.

A qualitative assessment should include a descriptive ranking similar to the qualitative rankings of Gillespie (1999, 2009). Definitions of the descriptive rankings should be formalized in order to ensure consistency in application over several years and different beaches. These rankings included four main categories including:

- Historic where presence was documented from literature prior to 1970;

- Present where documented presence occurred after 1970, including information from literature or personal communications, or where a small number of oysters are observed (often cryptic in complex habitat or lagoons); and
- Abundant where oysters are plentiful in number and are obvious to the casual observer.

Advantages:
1. This method is low in cost and does not require a large team of surveyors.
2. Allows complex sites to be included in a comprehensive monitoring program.
3. Survey method does not destroy individuals in order to enumerate them.

Disadvantages:
1. Inability to get an estimate of bed area, density or total abundance prevents quantitative measurement of change over time.

### *Review of Quadrat size, Sample Number and Biological Investigations*

In the above quantitative survey designs quadrat size varied from 0.25m x 0.25m = 0.0625m$^2$ and 0.5m x 0.5m = 0.25m$^2$ and methods for sample size selection also varied. Biological data collections such as length measurements, fecundity levels and tissue samples were also suggested in some survey methods. It was determined that a standard quadrate size, number of quadrats and biological sample size needed to be developed and these investigations are described in this methods and results section.

## Field Survey Methods and Results

During the daylight low tides of 2009, eight beaches on the South Coast of BC were surveyed using four of the survey methods described above to determine the best protocol for assessing Olympia oyster populations (Table 1, Table 2, Figure 1). Four of the seven survey methods reviewed were tested in the field. These methods were: Timed Random Search, Two-Stage, Calculated Random Sample and GPS Bed Area Simple Random Sample. Initially, the survey team field tested a version of the Allan and Davis Calculated Random Sample design where transects of varying lengths were spread across the oyster bed at Harris Point in Barkley Sound. This method was again employed and compared to the Timed Random Search survey method in Ladysmith Harbour. Once these and other field tests of the methods were completed, all the available data was analyzed using the analytical methods/steps described in the Two-Stage design, so that it could be included in the overall analysis of survey density, precision and sample number. In addition, the Timed Random Search was also analyzed using the original methods describe by Polson and Zacherl 2009. At the time of writing this paper, the data from the GPS Bed Area Simple Random Sample survey was not available for analysis and therefore, only a review of the survey protocol was included in this report. It was a decision of the authors not to analyze the Calculated Random Sample survey data using the Allan and Davis method because it was determined that the Two-Stage analysis was a more accurate and rigorous analysis than the one presented in the Calculated Random Sample method.

Trial and error throughout the survey season guided the decision process for the best survey method available. Once analyses for the various survey methods were completed and further literature review was conducted, the final survey methodologies for each population type was refined.

## *Timed Random Search Analysis & Results*

Data from the Timed Random Search and transect belt completed at the Limberis beach in Ladysmith Harbour were analyzed following the methods laid out by Polson and Zacherl (2009) and is detailed in the review of survey protocols section above.

The timed walk had four investigators with the first located closest to the water and fourth located highest on the beach (Table 3). The average rank across all four samples and across the Limberis beach was 1.82 with a standard error of 0.44. Polson and Zacherl (2009) also ranked one beach in Ladysmith Harbour with similar results of 1.9 (SE 0.25). A transect belt with 30 quadrats was completed in the area of the timed walk with the highest observed oyster density. In the Polson and Zacherl Time Random Walk transect belt survey method, only 10 quadrats were included in their survey therefore our analyses were conducted in three different ways: using all 30 quadrats, randomly selecting 10 of the 30 quadrats sampled, and resampling 10 quadrats 100 times, then carrying out the statistics on there 3 methods (Table 4).

Analysis of all three methods displayed very similar average maximum densities. The average maximum density calculated from the resampled data was 9.1 (SE 1.8), 9.6 (SE 2.2) for the 10 randomly selected quadrats and 9.2 (SE 1.1) for the all 30 quadrats. These average maximum densities were higher than the results of the Polson and Zacherl (2009) survey conducted in Ladysmith Harbour in 2005 (Avg Max. Den. 2.8 SE 1.5) but similar to densities found at sites in California. The coefficient of variation from the 2009 survey (Table 4) was lower than most of the coefficient of variations presented by Polson and Zacherl (2009).

The beach was also surveyed using a Two-Stage design to be directly comparable to other surveys. When comparing the Limberis Ladysmith survey and the two different analyses that were completed, the transect belt survey had higher quadrat means then the Two-Stage analysis which is to be expected since the transect belt survey was conducted in the most dense area of the beach (Table 5).

## *Two-Stage Sampling Design Analysis & Results*

Most of the remaining surveys and analyses followed the Two-Stage Sampling design as in the review of survey protocols section of this report. Each was standardized to 1 $m^2$ quadrats. Abundance, mean density, an estimate of population total, variance, 95% confidence intervals and a survey precision was calculated for all of these surveys (Table 5). Summaries of population totals are presented in Table 5 but the implications of the population totals are not discussed in detail in this report.

The Two-Stage sampling design was tested on a variety of discrete and/or scattered beds. By analyzing all the surveys and each stratum separately we can compare the methods and determine the best survey for each type of discrete Olympia oyster population.

The results of these analyses are found in Table 5 and Table 6. In general, high density areas produced better or more accurate analyses according to the precision calculated in the Two-Stage Sampling analysis.

## Calculation of Optimal Number of Samples

Two methods were used to determine optimal sample size for analysis of Olympia oyster populations.

### *Methods for Desired Precision Based on Previous Variation*

Kingzett and Bourne (1998) completed the analysis described below to obtain estimates of precision based on historic butter clam survey data from Seal Island.

The number of sampling units required to achieve a given precision in a study may be predicted with knowledge of the variation within a population (typically from an initial sample) for randomly distributed populations. For populations where the negative binomial distribution is a suitable model, the index of dispersion statistic common **(**k**)** may be used. To calculate the required number of samples for a given precision, the standard error to arithmetic mean ratio index of precision (D) was used. The value of (D) represents the standard error as a percentage of the mean. Percentage confidence limits of (D) about the mean were calculated by incorporating the Student's t-distribution statistic (t) in the equations (t=1.96 for 95% confidence interval). For a negative binomial distribution the number of required samples (n) was solved for various levels of desired accuracy (D) using the following formula (Elliot 1977):

$$N = \frac{t^2}{d\left(\frac{1}{\mu} + \frac{1}{k}\right)} \qquad (37)$$

The index of dispersion ( $k$ ) was approximated using the following formula

$$k = \frac{\mu^2}{\sigma^2 - \mu} \qquad (38)$$

### *Results for Desired Precision Based on Previous Variation*

The calculated optimal number of quadrats to derive a specified precision about the mean on derived values of common **(**k**)** (dispersion of the negative binomial distribution) are shown in Table 7. All estimates give the approximate number of samples that would be needed to obtain precision of the mean with 95% confidence.

Estimated sample numbers for each survey give a general indicator about the precision that should be obtainable in future surveys when the mean and standard deviations are unknown (Table 7, Figure 9, Figure 10, Figure 11). In order to obtain the precision of 10% of the mean, numerous samples are required. However, decreasing the precision to 30% allows for a more reasonable number of samples that could be accomplished in one survey (between 44 and 99 samples). Therefore, beaches that have more discrete beds with high densities, a minimum of 44 quadrats would give approximately 30% precision. Conversely, beaches with discrete and/or scattered individuals and a low mean density, such as Swy-a-lana lagoon, at least 99 quadrats would be required to obtain 30% precision.

## ***Simple Random Subsampling of Olympia Oyster Quadrat Counts***

Cochran (1977) suggested a simple method to determine the number of sampling units required: take five samples at random and calculate the mean, take five more samples and recalculate the mean, continue to add samples in units of five and plot the means versus the sample size. A suitable sample size has been reached when fluctuations in the value of the mean are reduced to acceptable levels (in the present study +/- one oyster/quadrat). A variation of this technique was used to perform an analysis on all beach surveys to determine the amount of randomly selected quadrats that would approximate the estimates of the population mean and variation. This analysis follows the methods described by Kingzett and Bourne (1998) conducted on Seal Island Butter clams but, in this case is completed on quadrat counts of live Olympia oysters from all beaches surveyed.

The analysis described above was completed using ExcelStatPro that randomly selected a series of sample quadrats without replacement from the original survey data for, Swy-a-lana Lagoon, Harris Point, Limberis, Ladysmith Harbour, Port Eliza Beach 2 and 3, Baker Bay and Gorge Inlet. In order to simulate the original population, the total number of sampled quadrats was assumed to represent the total number of quadrats available to be sampled. Random samples were taken starting at five and increased in increments of five until the total number (100%, or the next lowest denominator of five) were sampled. This procedure was repeated twenty times for each level of subsamples. The behaviour of the subsample means with increasing sample size were then compared against the original estimates.

*Sample Means:* For each series of subsamples taken from the original data set, estimates of the bias (+/- mean estimate oyster per quadrat) of the random subsample mean from the original total mean estimate were calculated. A mean and standard deviation of the derived bias from the original mean level were calculated and plotted.

## ***Results Simple Random Subsampling of Olympia Oyster Quadrat Counts***

Results of repeated random subsampling from the Olympia oyster quadrat count data are plotted for each beach in Figure 12 and Figure 13. The plots produced by the subsampling routine varied slightly each time it was repeated, as the program selects quadrats at random. Subsampling at each sampling interval was repeated 20 times to give a range of results showing the mean bias or mean calculated standard deviation plus or minus the standard deviation of the mean estimates indicated by the error bars.

The dotted lines on Figure 12 and Figure 13 indicate one sampling unit of bias from the mean.

## Preliminary Quadrat size investigations

Preliminary investigations into quadrat size were completed throughout the 2009 field season. Two different quadrat sizes were used throughout the season, 0.25m x 0.25m = 0.0625 $m^2$ and 0.5m x 0.5m = 0.25 $m^2$ (Table 1, Table 2). In general, smaller quadrats were used in areas displaying a high density of Olympia oysters and larger quadrats were used in lower density areas. It was observed that, in most cases, the number of oysters in the smaller quadrats was quite low even on high density beaches; therefore it would not be too onerous a task to count all the Olympia oysters within the larger quadrat (
Figure ***16***).

## Biological Sampling

There is a limited understanding of the age of Olympia oyster and a lack of ageing studies. Length-frequency distributions can be used to compensate for this lack of data and can provide information on the strength and frequency of recruitment events. It is essential that the oysters are randomly selected for this measurement. Actively selecting large oysters will infer there is a proportionally more oysters in the older age class and that there has been limited recruitment in recent years. Consistent measurement of the shell length is also vital. Each oyster should be measured from the anterior end (hinge) to the farthest distance on the posterior end (across the longest side from the hinge across the shell). The sample size measured should be appropriate to achieve a reasonable level of precision in measurement and accurately represent the oyster population of a specific beach. During haphazard surveys completed by DFO over the past 10 years, 50 oysters were measured from each site to create a length-frequency distribution. This number was selected for convenience by the investigators. Other studies have suggested that a minimum sample size of 10 times the number of length classes in the sample appears to be a reasonable comprise between effort and precision (Gerritsen and McGrath 2006). Using this guideline for Olympia oysters (max length of up to 60 mm in BC), 6 age classes of 10 mm bins would give a sample size of 60 oysters. But, if time permits more samples should be measured and more investigations should be completed to determine the appropriate samples size. Also, if abundance levels permit, during late summer and early fall oysters should also be opened to determine fecundity levels and if oysters are abundant tissues samples could be collected for subsequent historical or molecular analysis.

# Discussion

There are many potential survey protocols for Olympia oysters, in this document we reviewed seven different methods and tested four of these in the field: Timed Random Search, Two-Stage Sampling Design, Calculated Random Sample and GPS Bed Area Simple Random Sample.

Olympia oyster surveys were initially completed opportunistically by DFO staff in a haphazard approach when they came upon a beach where *O. lurida* populations were found. This method is not statically rigorous because the sampling is not truly random and is subject to bias due to sampler behaviour. Hence, this protocol would not be a good choice as a long-term monitoring protocol.

The Calculated Random Sample design was tested at various locations. This design adjusts transect length, so that all transects reach across the entire length of the oyster bed. This is different from other Two-Stage designs where transects are of the same length, with some reaching the edge of the bed and others extending beyond the bed boundaries. In theory, adjusted transect lengths make sense but in practice they are difficult to achieve because the edge of the bed is hard to define. In addition, the authors also found that in practice transect lengths did not vary considerably on many of the beaches. Therefore, placing a grid with constant transect lengths covering as much of the bed as possible may be a simpler and more reliable option. However, by using this method, the bed area is not documented; only the density within the survey area is documented. Changes in bed area are not directly documented, but could possibly be inferred by spatial analyses of the resulting data.

In 2009, a new survey method using GPS technology was tested by the Puget Sound Restoration Fund (PSRF) in Port Eliza. A differential GPS unit was used to map the entire bed area and random quadrats are placed within this area. This method offers considerable potential for Olympia oyster surveys as it allow for accurate measurement of bed area as well as random placement of quadrats, providing a measure of bed area and reliable estimates of mean density and total abundance.

The Timed Random Search method was tested as a potential survey design in low abundance sites. However, by employing a ranking system at regular intervals during a timed walk through suitable habitat, this method is reliant on the investigators selecting the correct ranking for a 50 meter section of the beach. In practice this proved to be difficult because the potential ranking could change over 50 meter interval depending one where the investigator stopped. The transect belt survey portion of this method was conducted in the in the area of highest density which lead to overestimation of density relative to a two-stage estimate on Limberis beach in Ladysmith Harbour. A review of this survey protocol determined that this approach is a more suitable method for comparing many beaches over a large geographic area and a more intensive survey would be required at low density sites and sites that would be repeated over a number of years.

The Stratified Random Sample and the Two-Stage designs have similar survey components, however the Two-Stage design was preferred because it gave the surveyors the ability to spread the sampling across the entire survey area and was easier to set up. Given that Olympia oyster populations are patchy, it is important to ensure the whole bed area is included in the survey. So for this reason and for the ease of setup up, the Two Stage Design was pursued and the Stratified Random Sample design not tested in the field during this study.

The Two-Stage design and analysis is a rigorous survey from which reliable and accurate population abundance estimates can be obtained. This method can fulfill the objective of this study and provides a survey methodology that can be repeated at a particular location over many years. One of the disadvantages of this survey design is its lack of ability to measure bed area. However, by combining this method with GPS bed mapping it could become an extremely robust method to monitor Olympia oyster populations.

In review of the survey protocols listed and reasons stated above the authors recommend that the population dynamics of the beach determines which survey protocol should be used. For example, at beaches with discrete and/or scattered Olympia oysters the Two-Stage protocol should be used. Beaches with complex beds or very low abundance, where a survey would harm the population or is logistically impossible to complete, a visual assessment can be completed.

Preliminary investigations into quadrat size (25x25 $cm^2$ vs. 50x50$cm^2$), suggests that the larger of the two quadrat sizes (50x50 $cm^2$) is more effective at measuring abundance without increasing the work load significantly. The extra accuracy obtained from the larger quadrat size negates the effort involved in the few instances where counting may become too onerous. Future surveys testing nested quadrats experiments will add to this analysis and are suggested as future work.

Calculations of optimal sample number for a beach were challenging as the beaches and the strata on each beach will vary from high density to low density, so it was determined that a range of samples numbers should be suggested (50 to 100 quadrats). As with all surveys the more of the beach surveyed with actual quadrats the more accurate your results will be. For example, Swy-a-lana lagoon is an area with a discrete bed of scattered individuals but is broken into two areas (outside and inside of the lagoon). The outside of the lagoon has a lower density then inside, and even though a higher percentage of the area was sampled (using 50 x 50 $cm^2$ quadrats) compared with sampling on inside of the lagoon we obtained greater survey precision from the inside strata. This same trend is observed in Gorge Inlet where a stratum 1 has a low density and a stratum 2 is a discrete bed with high density. At this beach the same percentage of area is surveyed in each strata but the survey precision was extremely good in strata 2 with the higher density population compared with strata one. These examples support the suggestion that lower density Olympia oyster populations require more quadrats to gain a better precision and a more accurate estimate of population abundance. As stated in the authors recommendations below, beaches with discrete beds and high densities a lower number of samples can be completed (approximately 50 quadrats) and beaches with scattered low densities a higher sample number (approximately 100 quadrats) is suggested to obtain a precision of approximately 30%.

In this paper we evaluated a number of different survey protocols, identified the advantages and disadvantages of each design and have provided suggestions based on the knowledge gained from these reviews and by analysing our own field survey results.

Both quantitative and qualitative protocols have been recommended and determining which survey is used is dependant on a number of simple factors that can be easily visualized in the field such as type of Olympia oyster habitat, population characteristics and abundance. Olympia oyster abundance will need to be surveyed at the same locations for a number of years before we can obtain information on abundance trends. As a requirement of the management plan, index sites will be determined by an established set of criteria and surveyed at least every five years with the protocols recommended in this report. Utilizing the survey methods recommended in this report will aid in the understanding of Olympia oysters abundance across BC, and will provide consistent population trend data and other valuable information for future reviews of the Management plan in 2013 and the next COSEWIC status report.

# Recommendations

Recommended survey designs are dependent on habitat and oyster population characteristics at sites requiring surveys. Therefore:

1. **Quantitative survey methods are recommended where Olympia oysters are abundant and habitat is relatively simple.** Either two-stage or Simple Random Sampling (with or without stratification) should be used. Investigate the utility of incorporating GPS technology, which has the advantage of providing a measure of bed area (not available in TS or StTS sampling).

2. **Qualitative survey methods are recommended where Olympia oysters are present at extremely low densities or are cryptic, particularly if surveys would require disturbance of oyster habitat.** The potential damage caused by turning rocks to detect oysters attached to the underside outweighs the benefits of quantitative information, particularly at sites on or near the limits of distribution of Olympia oysters. In these cases, verification that the populations still exist may be the most responsible means of monitoring these populations.

3. **Larger quadrat size is desirable, particularly in low density situations, but smaller quadrats may be adequate at high densities because of reduced processing time. In the absence of information to the contrary, a quadrat size of 0.25 $m^2$ is recommended in the interim.** Quadrat size and sampling rates require further examination before standards can be established. Surveys reviewed in this document suggest that a sampling rate of approximately 50 quadrats is sufficient to attain 30% precision when oyster density is high and the oysters are highly aggregated. In situations where oysters are present at low densities and not aggregated, sample sizes of up to 100 quadrats may be required to achieve this level of precision.

4. **Additional biological and ecological information should be collected in conjunction with abundance surveys.** Size frequency data should be collected as a matter of course. In the absence of age data, size frequency distributions provide the only information available on recruitment strength. This information will not provide complete age distribution information, but may allow assessment of relative recruitment rates between sites or years. If oysters are abundant and removals would not jeopardize the persistence of a population,

oysters that were sampled for length data could also be opened to examine reproductive maturity (presence of larvae) or to take tissues for subsequent histological or molecular analyses. Samples from late summer or fall may be more instructive for reproductive studies, as samples taken early in the summer likely contain numerous individuals that have not matured.

# Future work

1. Long-term monitoring will be necessary to assess recruitment and growth and to better understand population dynamics of Olympia oysters. Selection of index sites for long-term monitoring should consider habitat and population characteristics that would allow for quantitative, rather than qualitative, assessment of Olympia oyster populations.

2. Ageing studies examining oyster shells or ligaments could provide a better understanding of age composition, recruitment and mortality rates and support age-based assessments of Olympia oyster populations.

3. Acquire differential GPS technology and GIS software required to allow detailed surveys that provide GIS-mapped estimates of bed area in addition to estimates of mean density and total abundance.

4. Develop a protocol to define the edge of the bed with standards which are repeatable to reduce the potential error in area estimates.

5. Complete a nested quadrat experiment to determine optimal quadrat size for Olympia oyster surveys.

6. Develop a methodology for the selection of index sites for Olympia oysters.

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# Literature Cited


Allen, B.L. and J.P. Davis. Unpubl. manuscr. Intertidal *Ostrea conchaphila* population assessment procedures; applications for surveying natural populations and restoration areas.

Baker, P. 1995. Review of ecology and fishery of the Olympia oyster, *Ostrea lurida* with annotated bibliography. J. Shellfish Res. 14(2):501-518.

Bourne, N. 1997. Molluscan fisheries of British Columbia. NOAA Tech. Rep. NMFS 128: 115-130.

Bower, S.M. 2001. Synopsis of infectious diseases and parasites of commercially exploited shellfish: viral gametocytic hypertrophy of oysters. http://www.pac.dfo-mpo.gc.ca/sci/shelldis/title_e.htm

Buhle, E.R. and J.L. Ruesink. 2009. Impacts of invasive oyster drills on Olympia oyster (*Ostrea lurida* Carpenter 1864) recovery in Willapa Bay, Washington, United States. J. Shellfish Res. 28(1): 87-96.

Canada Gazette. 2003. Species at Risk Act. Ch. 29. Canada Gazette, Part III, Vol. 25, No. 3: v + 97 p. Available: http://www.sararegistry.gc.ca/approach/act/sara_e.pdf.

Cochran, W.G. 1977. Samplng techniques. 3rd Ed. John Wiley and Sons, New York. xvi + 428 p.

Coe, W.R. 1932. Development of the gonads and the sequence of sexual stages in the California oyster (*Ostrea lurida*). Bull. Scripps. Inst. Oceanogr., Univ. Calif. Tech. Ser. 3:119-144.

Couch, D. and T.J. Hassler. 1989. Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates (Pacific Northwest) -- Olympia oyster. U.S. Fish Wildl. Serv. Biol. Rep. 82(11.124). U.S. Army Corps of Engineers, TR EL-82-4. 8pp.

Dall, W.H. 1914. Notes on west American oysters. Nautilus 28(1):1-3.

DFO. 2009. Management plan for the Olympia oyster (*Ostrea conchaphila*) in Canada. Available at: http://www.sararegistry.gc.ca/document/default_e.cfm?documentID=1763.

Foster , N.R. 1991. Intertidal Bivalves. A guide to the common marine bivalves of Alaska. Univ. of Alaska Press. 152 pp.

Gerritsen and McGrath. 2006. Precision estimates and suggested sample sizes for length-frequency data. Fish. Bull. 106-120 (2007).

Gillespie, G.E. 1999. Status of Olympia oyster, *Ostrea conchaphila*, in Canada. DFO Can. Stock Assess. Sec. Res. Doc. 99/150. 36 p.

Gillespie, G.E. 2000. Preliminary review of experimental harvest rates in the depuration fishery for intertidal clams. DFO Can. Stock Assess. Sec. Res. Doc. 2000/122. 57 p.

Gillespie, G.E. 2007. Distribution of non-indigenous intertidal species on the Pacific Coast of Canada. Nipp. Suis. Gakk. 73(6): 1133-1137.

Gillespie, G.E. 2009. Status of Olympia oyster, *Ostrea lurida* Carpenter 1864, in British Columbia, Canada. J. Shellfish Res. 28(1): 59-68.

Gillespie, G.E. and N.F. Bourne. 2005. Exploratory intertidal bivalve surveys in British Columbia-2002. Can. Manuscr. Rep. Fish. Aquat. Sci. 2733. 199p.

Gillespie, G.E. and A.R. Kronlund. 1999. A manual for intertidal clam surveys. Can. Tech. Rep. Fish. Aquat. Sci. 2270. 144 p.

Gillespie, G.E., A.R. Kronlund and G.D. Heritage. 1998a. Intertidal clam stock estimates for selected depuration harvest beaches – 1994. p. 3-46. *In*: B.J. Waddell, G.E. Gillespie and L.C. Walthers [eds.]. Invertebrate working papers reviewed by the Pacific Stock Assessment review Committee (PSARC) in 1995. Pt. 1. Bivalves. Can. Tech. Rep. Fish. Aquat. Sci. 2214.

Gillespie, G.E. A.R. Kronlund and G.D. Heritage. 1998b. Assessment of Manila clam stocks at Savary Island, British Columbia – 1995. p. 245-317. *In*: B.J. Waddell, G.E. Gillespie and L.C. Walthers [eds.]. Invertebrate working papers reviewed by the Pacific Stock Assessment review Committee (PSARC) in 1995. Pt. 1. Bivalves. Can. Tech. Rep. Fish. Aquat. Sci. 2214.

Harbo, R.M. 1997. Shells and Shellfish of the Pacific Northwest. Harbour Publishing, Madiera Park, B.C. 270 p.

Harry, H.W. 1985. Synopsis of the supraspecific classification of living oysters (Bivalvia: Gryphaeidae and Ostreidae). Veliger 28(2): 121-158.

Kingzett, B.C. and N.F. Bourne. 1998. Assessment of Intertidal Clam Populations Surveys at Seal Island, British Columbia, 1940-1992. pp. 47-125. *In*: B.J.Waddell, G.E. Gillespie, and L.C. Walthers [eds.]. Invertebrate Working Papers reviewed by the Pacific Stock Assessment Review Committee (PSARC) in 1995. Part 1. Bivalves. Can. Tech. Rep. Fish. Aquat. Sci. 2214.

Kronlund, A.R., G.E. Gillespie, and G.D. Heritage. 1998. Survey methodology for intertidal bivalves. pp. 127-243. *In*: B.J.Waddell, G.E. Gillespie, and L.C. Walthers [eds.]. Invertebrate Working Papers reviewed by the Pacific Stock Assessment Review Committee (PSARC) in 1995. Part 1. Bivalves. Can. Tech. Rep. Fish. Aquat. Sci. 2214.

McKernan, D.L., V. Tarta and R. Tollefson. 1949. An investigation of the decline of the native oyster industry of the state of Washington, with special reference to the effects of sulfite pulp mill waste on the Olympia oyster (*Ostrea lurida*). State of Washington, Department of Fisheries. April, 1949. 49pp.

Meyer, G.R., G. Lowe, E. Kim, C. Abbott, S. Johnson and S. Gilmore. In press. Health of Olympia oysters (*Ostrea lurida*, Carpenter, 1864) in British Columbia, Canada. J. Shellfish Res.

Palacios, K.C. and S.P. Ferraro. 2003. Green crab (*Carcinus maenas* Linnaeus) consumption rates on and prey preferences among four bivalve prey species. J. Shellfish Res. 22(3): 865-871.

Polson, M.P., W.E. Hewson, D.J. Eernisse, P.K. Baker, and D.C. Zacherl. 2009. You say *conchaphila*, I say *lurida*: molecular evidence for restricting the Olympia oyster to temperate western North America. J. Shellfish Res. 28(1): 11-21.

Polson, M.P. and D.C. Zacherl. 2009. Geographical distribution and intertidal population status for the native West Coast oyster, *Ostrea lurida* Carpenter 1864, from Alaska to Baja. J. Shellfish Res. 28(1): 69-77.

Quayle, D.B. 1969. Pacific oyster culture in British Columbia. Fish. Res. Board Can. Bull. 169. 192p.

Quayle, D.B. 1988. Pacific oyster culture in British Columbia. Can. Bull. Fish Aquat. Sci. 218. 241 p.

Rao, J.N.K. and C.F.J. Wu. 1988. Resampling inference with complex survey data. J. AMer. Stat. Soc. 83(401): 231-241.

Satterthwaite, F.E. 1946. An approximate distribution of estimates of variance components. Biometrics Bull. 2: 110-114.

Shaw, W. 1997. The Shellfish Industry of California – Past, Present, and Future. NOAA Tech. Rep. NMFS 128: 57-74.

Sitter, R.R. 1992. A resampling procedure for complex survey data. J. Amer. Stat. Assoc. 87: 755-765.

Thompson, S.K. 1992. Sampling. John Wiley and Sons, New York. xv + 343 p.

White, J.L., E.R. Buhle, J.L. Ruesink and A.C. Trimble. 2009. Evaluation of Olympia oyster (*Ostrea lurida* Carpenter 1864) status and restoration techniques in Puget Sound, Washington, United States. J. Shellfish Res. 28(1): 107-112.

*Table 1. Comparison of design, analysis and population application of seven survey methods reviewed in this report.*

| | Design | | | | | | Analysis of Data | | | | Population Classification | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Survey Method** | **Systematic quadrats** | **Random quadrats** | **Can Be Stratified** | **Assign Rank/Class** | **Count individuals within quadrat** | **Repeatable survey** | **Estimate of Density / Square meter** | **Estimate of Abundance** | **Bed Area** | **Statistical Rigor** | **Discrete and/or Scattered** | **Low Abundance** | **Complex habitat** |
| **Haphazard Sampling** | ~ | ~ | ~ | ~ | √ | ~ | √ | ~ | ~ | ~ | √ | ~ | ~ |
| **Calculated Random Sample** | √ | ~ | ~ | ~ | √ | √ | √ | √ | √ | √ - could be improved | √ | ~ | ~ |
| **GPS Bed Area Simple Random Sample** | ~ | √ | ~ | ~ | √ | √ | √ | √ | √ | √ | √ | ~ | ~ |
| **Timed Random Search** | √ | ~ | √ | √ | √ | ~ | √ | ~ | ~ | √ | √ | √ | ~ |
| **Stratified Random Sampling** | ~ | √ | √ | ~ | √ | √ | √ | √ | √ | √ | √ | √ | ~ |
| **Two Stage Sampling** | √ | ~ | √ | ~ | √ | √ | √ | √ | √ | √ | √ | √ | ~ |
| **Visual Assessment** | ~ | ~ | ~ | group beaches into categories | ~ | √ | ~ | ~ | ~ | ~ | √ | √ | √ |

*Table 2. Summary of surveys completed and the methods for each survey.*

| Location | Survey Method | Date | Strata # | Total Area Surveyed (m2) | Baseline length (m) | # of Transect | Transect length (m) *average length | # of quadarts per transect | Actual Number of quadrats | Quadrat size ($cm^2$) | Actual area surveyed by quadrats $m^2$ (Quadrat area x Quadrate #) | % of the Area Surveyed |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Harris Point - Barkley Sound | Stratified Two Stage Design (with Allan and Davis Varying Transect Length) | April 27, 2009 | 1 | 960 | 60 | 10 | 16* | 3 | 30 | 25x25 | 1.88 | 0.20% |
| Limberis - Ladysmith Harbour | Stratified Two Stage Design (with Allan and Davis Varying Transect Length) | June 23, 2009 | 1 | 2250 | 50 | 10 | 45* | 5 | 55 | 50x50 | 13.75 | 0.61% |
| Limberis - Ladysmith Harbour | | June 23, 2009 | 2 | 4500 | 100 | 10 | 45* | 5 | 50 | 50x50 | 12.50 | 0.28% |
| Limberis Transect Belt - Ladysmith Harbour | Polson Timed Walk & Transect Belt | June 22, 2009 | 1 | 225 | 50 | 10 | 4.5 | 3 | 30 | 50x50 & 25x25 | 1.88 & 7.50 | 0.84% & 3.33% |
| Gorge Inlet - Victoria | Stratified Two Stage Design | July 7, 2009 | 1 | 125 | 25 | 5 | 5 | 3 | 15 | 50x50 | 3.75 | 3.00% |
| Gorge Inlet - Victoria | Stratified Two Stage Design | July 7, 2009 | 2 | 125 | 25 | 5 | 5 | 3 | 15 | 50x50 | 3.75 | 3.00% |
| Baker Bay - Sunshine Coast | Stratified Two Stage Design (with Allan and Davis Varying Transect Length) | July 23, 2009 | 1 | 550 | 100 | 10 | 5.5* | 3 | 24 | 25x25 | 1.50 | 0.27% |
| Swy-a-lana Lagoon Nanaimo | Stratified Two Stage Design | August 20, 2009 | 1 | 550 | 55 | 15 | 10 | 3 | 45 | 50x50 & 25x25 | 2.81 & 11.25 | 0.51% & 2.05% |
| Swy-a-lana Lagoon Nanaimo | | August 20, 2009 | 2 | 330 | 55 | 8 | 6 | 3 | 24 | 50x50 | 6.00 | 1.82% |
| Port Eliza Beach 2 | Stratified Two Stage Design (with Allan and Davis Varying Transect Length) | August 6, 2009 | 1 | 2525 | 100 | 15 | 25.25* | 5 | 60 | 25x25 | 3.75 | 0.15% |
| Port Eliza Beach 3 | Stratified Two Stage Design | August 5, 2009 | 1 | 4970 | 55 | 10 | 90 | 5 | 49 | 25x25 | 3.06 | 0.06% |
| Port Eliza Beach 3 | Stratified Two Stage Design | August 5, 2009 | 2 | 4950 | 71 | 10 | 70 | 5 | 49 | 25x25 | 3.06 | 0.06% |

*Table 3. Summary of Olympia oyster count and rank data from the Timed Random Search survey in Ladysmith Harbour (Limberis). In the field 25x25 cm2 quadrats were completed. To compare this data with the Polson and Zacherl method it had be extrapolated to 50x50 cm2 quadrats.*

| | Sampler 1 | | | Sampler 2 | | | Sampler 3 | | | Sampler 4 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Distance (m) | Walking Count | Live Olympia Oysters Count from 50x50 $cm^2$ Quadrat | Estimated rank | Walking Count | Live Olympia Oysters Count from 50x50 $cm^2$ Quadrat | Estimated rank | Walking Count | Live Olympia Oysters Count from 50x50 cm Quadrat | Estimated rank | Walking Count | Live Olympia Oysters Count from 50x50 cm Quadrat | Estimated rank 50x50 | Average Estimated rank |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00 |
| 50 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 15 | 8 | 2 | 0.50 |
| 100 | 26 | 0 | 0 | 36 | 16 | 3 | 56 | 48 | 3 | 104 | 16 | 3 | 2.25 |
| 150 | 166 | 44 | 3 | 379 | 76 | 3 | 185 | 96 | 3 | 151 | 4 | 2 | 2.75 |
| 200 | 223 | 20 | 3 | 332 | 40 | 3 | 154 | 4 | 2 | 49 | 8 | 2 | 2.50 |
| 250 | 180 | 12 | 3 | 135 | 8 | 2 | 42 | 4 | 2 | 22 | 0 | 0 | 1.75 |
| 300 | 527 | 68 | 3 | 611 | 44 | 3 | 111 | 28 | 3 | 51 | 32 | 3 | 3.00 |
| **Average Rank** | | | | | | | | | | | | | **1.82** |
| **Standard Error** | | | | | | | | | | | | | **0.435** |
| **n** | | | | | | | | | | | | | **7** |

*Table 4. Summary of data from transect belt survey at Limberis in Ladysmith Harbour.*

| Summary Statisitics | Analysis using all 30 quadrats completed | 10 random quadrats selected from the 30 completed quadrats | Resampled data of 10 quadrat randomly selected without replacement 100 times |
|---|---|---|---|
| Mean = Average Maximum Density | 9.3 | 9.6 | 9.1 |
| Standard error of the mean | 1.1 | 2.3 | 1.9 |
| n = Number of Quadrats | 30 | 10 | 10 (resampled 10 times) |
| Standard deviation (n) | 6.0 | 6.8 | 6.0 |
| Variation coefficient | 0.6 | 0.7 | 0.6 |
| Variance (n) | 35.6 | 46.1 | 33.0 |

*Table 5. Summary of results of Two-Stage survey analyses.*

| Location | Strata | Survey Quadrat Size ($m^2$) | Total Area Surveyed ($m^2$) | Population Mean (adjusted to $m^2$ quadrats): Quadrat Mean ($\#/m^2$) | Quadrat Variance | Upper 95% Confidence Interval | Lower 95% Confidence Interval | Upper 95% Confidence Interval | Population Total: Population Total | Population Total Variance | Upper 95% Confidence Interval | Lower 95% Confidence Interval | Survey precision |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Harris Point - Barkley Sound** | **1** | **25x25** | **960** | **85.3** | **448.5** | **126.8** | **43.8** | **126.8** | **81,920** | **413,368,149** | **121,770** | **42,070** | **48.6%** |
| Limberis - Ladysmith Harbour | 1 | 50x50 | 2250 | 23.6 | 4.4 | 27.7 | 19.5 | 27.7 | 53,018 | 22,246,653 | 62,263 | 43,774 | 17.4% |
| Limberis - Ladysmith Harbour | 2 | 50x50 | 4500 | 27.4 | 27.6 | 37.7 | 17.1 | 37.7 | 123,120 | 559,668,600 | 169,488 | 76,752 | 37.7% |
| **Limberis - Ladysmith Harbour** | **1&2** | **50x50** | **6750** | **26.1** | **12.8** | **33.1** | **19.1** | **33.1** | **176,138** | **581,915,253** | **223,419** | **128,857** | **26.8%** |
| Limberis Transect Belt - Ladysmith Harbour | 1 | 50x50 | 225 | 37.1 | 27.4 | 47.3 | 26.8 | 47.3 | 8,340 | 1,388,580 | 10,650 | 6,030 | 27.7% |
| Limberis Transect Belt - Ladysmith Harbour | 1 | 25x25 | 225 | 52.3 | 141.5 | 75.6 | 29.0 | 75.6 | 11,760 | 7,161,720 | 17,005 | 6,515 | 44.6% |
| Gorge Inlet - Victoria | 1 | 50x50 | 125 | 16.0 | 49.2 | 29.8 | 2.2 | 29.8 | 2,000 | 769,422 | 3,719 | 281 | 86.0% |
| Gorge Inlet - Victoria | 2 | 50x50 | 125 | 291.7 | 1186.2 | 359.2 | 224.2 | 359.2 | 36,467 | 18,533,911 | 44,905 | 28,029 | 23.1% |
| **Gorge Inlet - Victoria** | **1&2** | **50x50** | **250** | **153.9** | **308.9** | **188.3** | **119.4** | **188.3** | **38,467** | **19,303,333** | **47,078** | **29,855** | **22.4%** |
| **Baker Bay - Sunshine Coast** | **1** | **25x25** | **550** | **385.3** | **6371.1** | **541.8** | **228.9** | **541.8** | **211,933** | **1,927,251,464** | **297,978** | **125,888** | **40.6%** |
| Swy-a-lana Lagoon Nanaimo | 1 | 25x25 | 550 | 3.9 | 2.0 | 6.7 | 1.2 | 6.7 | 2,151 | 596,683 | 3,665 | 637 | 70.4% |
| Swy-a-lana Lagoon Nanaimo | 1 | 50x50 | 550 | 3.4 | 0.9 | 5.2 | 1.5 | 5.2 | 1,858 | 273,987 | 2,884 | 832 | 55.2% |
| Swy-a-lana Lagoon Nanaimo | 2 | 50x50 | 330 | 13.00 | 4.43 | 17.123 | 8.877 | 17.123 | 4,290 | 481,941 | 5,651 | 2,929 | 31.7% |
| **Swy-a-lana Lagoon Nanaimo** | **1&2** | **50x50** | **880** | **6.99** | **0.98** | **8.923** | **5.050** | **8.923** | **6,148** | **755,928** | **7852** | **4444** | **27.7%** |
| **Port Eliza Beach 2** | **1** | **25x25** | **2525** | **199.5** | **591.4** | **247.1** | **151.8** | **247.1** | **503,653** | **3,770,531,035** | **624,006** | **383,300** | **23.9%** |
| Port Eliza Beach 3 | 1 | 25x25 | 4970 | 255.0 | 4891.6 | 392.1 | 117.9 | 392.1 | 1,267,451 | 120,827,856,295 | 1,948,753 | 586,150 | 53.8% |
| Port Eliza Beach 3 | 2 | 25x25 | 4950 | 171.8 | 1351.4 | 243.8 | 99.7 | 243.8 | 850,188 | 33,111,487,847 | 1,206,840 | 493,535 | 41.9% |
| **Port Eliza Beach 3** | **1&2** | **25x25** | **9920** | **213.5** | **1564.3** | **291.0** | **136.0** | **291.0** | **2,117,639** | **153,939,344,142** | **2,886,647** | **1,348,631** | **36.3%** |

*Table 6. Summary of quadrat size, total area surveyed, density, actual area surveyed by quadrats, and precision of the mean.*

| Location | Strata | Survey Quadrat Size ($m^2$) | Total Area Surveyed ($m^2$) | Actual area surveyed by quadrats $m^2$ (Quadrat area x Quadrate #) | % of Area Surveyed | Quadrat Mean (# Olympia oysters/$m^2$) | Survey precision |
|---|---|---|---|---|---|---|---|
| **Harris Point - Barkley Sound** | **1** | **25x25** | **960** | **1.88** | 0.20% | **85.3** | **48.6%** |
| Limberis - Ladysmith Harbour | 1 | 50x50 | 2250 | 13.75 | 0.61% | 23.6 | 17.4% |
| Limberis - Ladysmith Harbour | 2 | 50x50 | 4500 | 12.5 | 0.28% | 27.4 | 37.7% |
| **Limberis - Ladysmith Harbour** | **1&2** | **50x50** | **6750** | | | **26.1** | **26.8%** |
| Limberis Transect Belt - Ladysmith Harbour | 1 | 50x50 | 225 | 7.5 | 3.33% | 37.1 | 27.7% |
| Limberis Transect Belt - Ladysmith Harbour | 1 | 25x25 | 225 | 1.88 | 0.84% | 52.3 | 44.6% |
| Gorge Inlet - Victoria | 1 | 50x50 | 125 | 3.75 | 3.00% | 16.0 | 86.0% |
| Gorge Inlet - Victoria | 2 | 50x50 | 125 | 3.75 | 3.00% | 291.7 | 23.1% |
| **Gorge Inlet - Victoria** | **1&2** | **50x50** | **250** | | | **153.9** | **22.4%** |
| **Baker Bay - Sunshine Coast** | **1** | **25x25** | **550** | **1.5** | 0.27% | **385.3** | **40.6%** |
| Swy-a-lana Lagoon Nanaimo | 1 | 25x25 | 550 | 2.81 | 0.51% | 3.9 | 70.4% |
| Swy-a-lana Lagoon Nanaimo | 1 | 50x50 | 550 | 11.25 | 2.05% | 3.4 | 55.2% |
| Swy-a-lana Lagoon Nanaimo | 2 | 50x50 | 330 | 6 | 1.82% | 13.00 | 31.7% |
| **Swy-a-lana Lagoon Nanaimo** | **1&2** | **50x50** | **880** | | | **6.99** | **27.7%** |
| **Port Eliza Beach 2** | **1** | **25x25** | **2525** | **3.75** | 0.15% | **199.5** | **23.9%** |
| Port Eliza Beach 3 | 1 | 25x25 | 4970 | 3.06 | 0.06% | 255.0 | 53.8% |
| Port Eliza Beach 3 | 2 | 25x25 | 4950 | 3.06 | 0.06% | 171.8 | 41.9% |
| **Port Eliza Beach 3** | **1&2** | **25x25** | **9920** | | | **213.5** | **36.3%** |

*Table 7. Summary of the estimated precision analysis in which k is a calculated variable used in the analysis.*

Mean and standard deviation are calculated from quadrat counts of Olympia oysters for each survey. The total quadrat number column is the actual number of quadrats in each survey and the estimated precision columns are the number of quadrats required to obtain each precision.

| | | | | | | | | Estimated Precision (# of quadrats) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | Survey Mean | Survey STD | k | Area surveyed | Quadrat Size | Total Quadrat # | Actual survey Precision | 10% | 20% | 30% | 40% | 50% | 60% | 70% | 80% | 90% | 100% |
| Swyalana 1&2 | 1.75 | 2.67 | 0.57 | 880 | 50x50 | 69 | 29% | 895 | 224 | 99 | 56 | 36 | 25 | 18 | 14 | 11 | 9 |
| Harris Point | 5.33 | 7.26 | 0.6 | 960 | 25x25 | 30 | 49% | 713 | 178 | 79 | 45 | 29 | 20 | 15 | 11 | 9 | 7 |
| Ladysmith Limberis 1&2 | 6.52 | 7.79 | 0.78 | 6750 | 50x50 | 105 | 27% | 549 | 137 | 61 | 34 | 22 | 15 | 11 | 9 | 7 | 5 |
| Port Eliza Beach 2 | 12.46 | 15.29 | 0.7 | 2525 | 25x25 | 49 | 24% | 579 | 145 | 64 | 36 | 23 | 16 | 12 | 9 | 7 | 6 |
| Port Eliza Beach 3 Strata 1&2 | 13.34 | 17.19 | 0.63 | 9920 | 25x25 | 109 | 36% | 638 | 160 | 71 | 40 | 26 | 18 | 13 | 10 | 8 | 6 |
| Baker Bay - Sunshine Coast | 24.08 | 25.66 | 0.91 | 550 | 25x25 | 24 | 41% | 436 | 109 | 48 | 27 | 17 | 12 | 9 | 7 | 5 | 4 |
| Gorge Inlet Strata 1&2 | 72.93 | 24.27 | 0.98 | 250 | 50x50 | 30 | 22% | 400 | 100 | 44 | 25 | 16 | 11 | 8 | 6 | 5 | 4 |

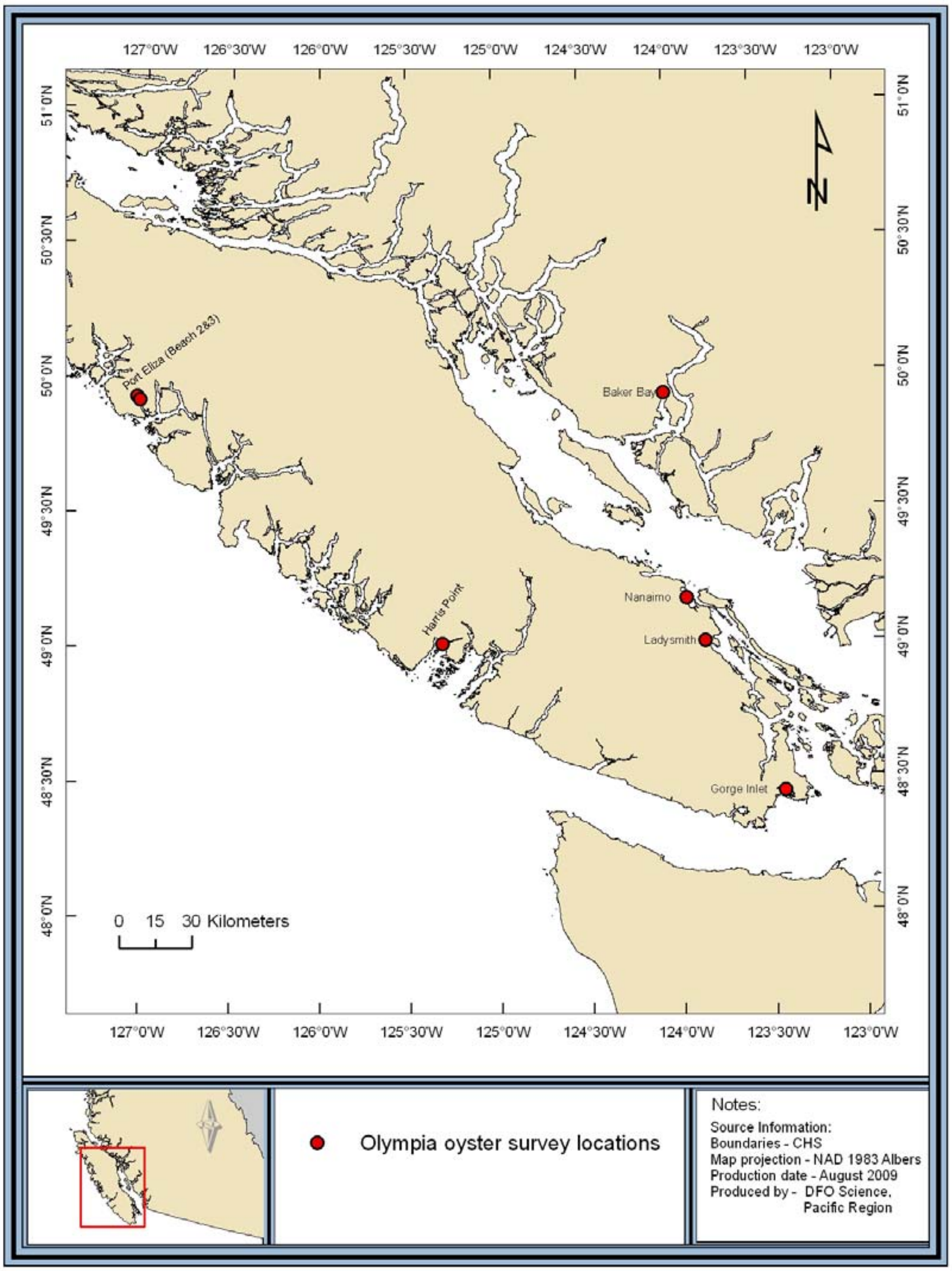


*Figure 1. Location of Olympia oyster survey s conducted in 2009.*

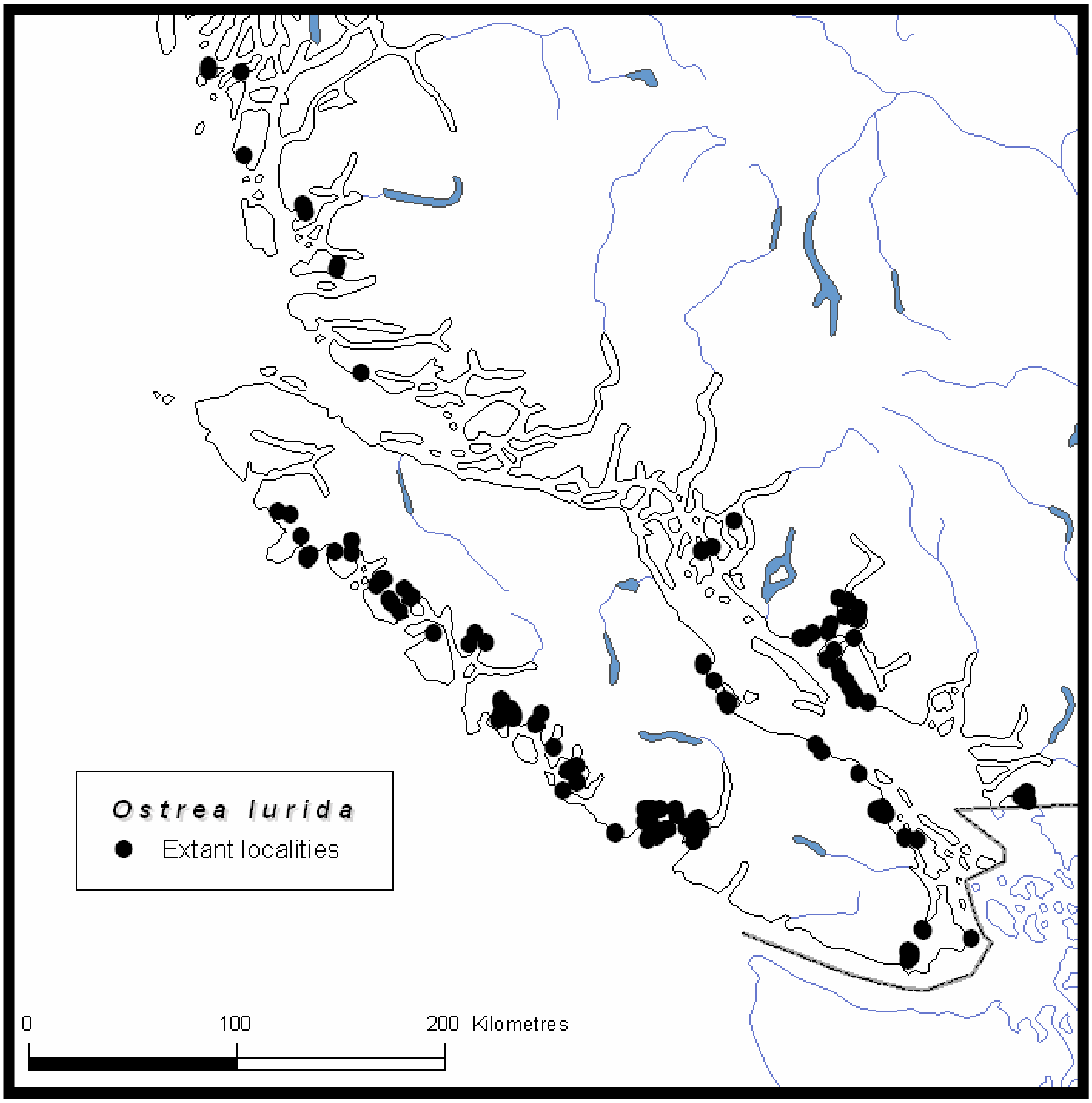


*Figure 2. Known Olympia oyster locations in British Columbia (as of December 2009).*

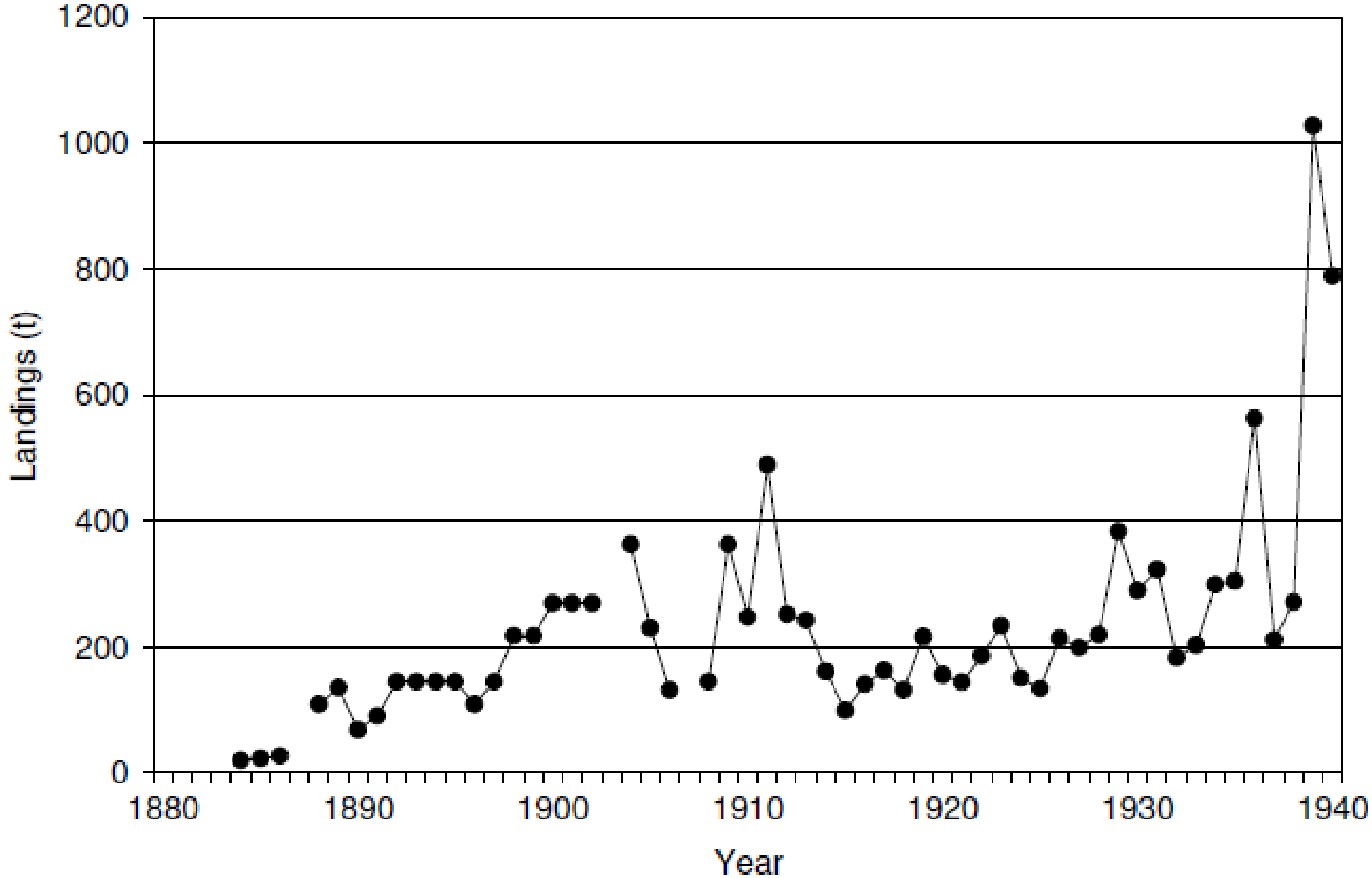


*Figure 3. Annual combined landings (t) of Olympia, Eastern and Pacific oysters from British Columbia, 1884-1940 (Gillespie 2009, data from Quayle 1988).*

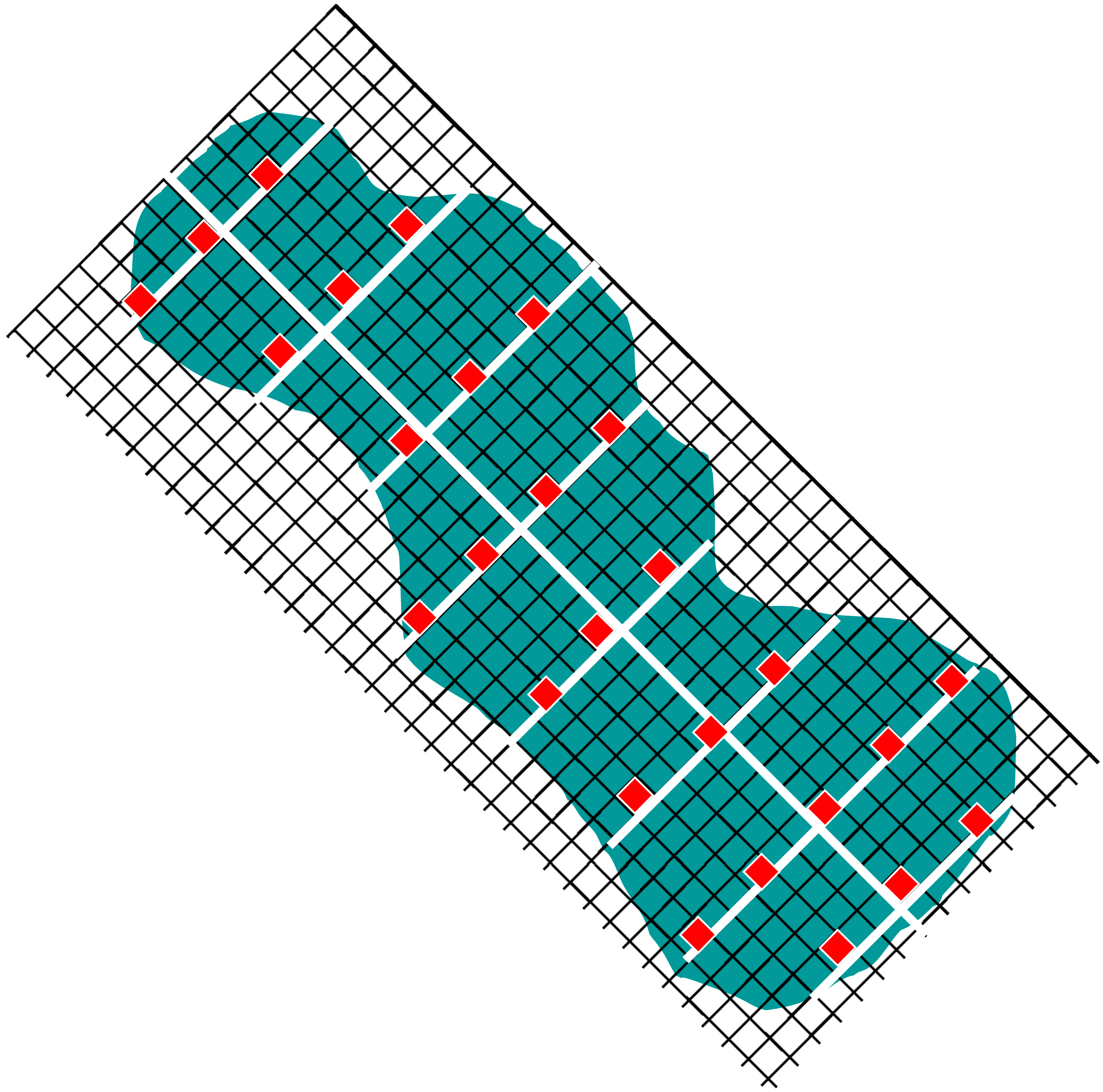

*Figure 4. Calculated Random Sample survey design with random transects that reach the edge of the bed.*

Baseline is set through the center of the bed and transects are perpendicular to baseline, quadrats are the evenly spaced red squares. The grid shows all the possible sampling locations.

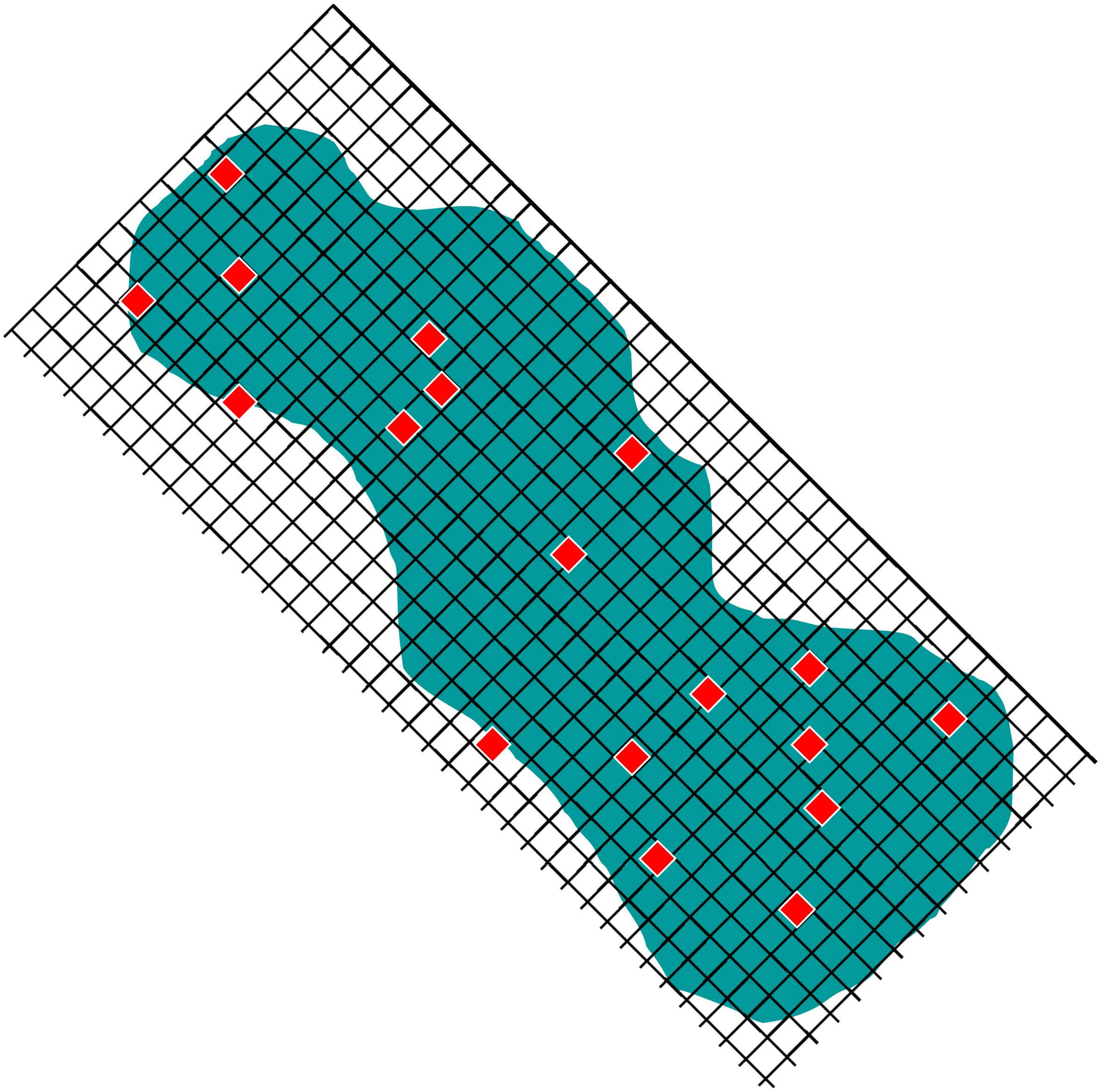

*Figure 5.  GPS Bed Area Simple Random Sample survey design.*

Quadrats are the squares randomly place throughout the sample area.  The grid shows all the possible sampling locations.

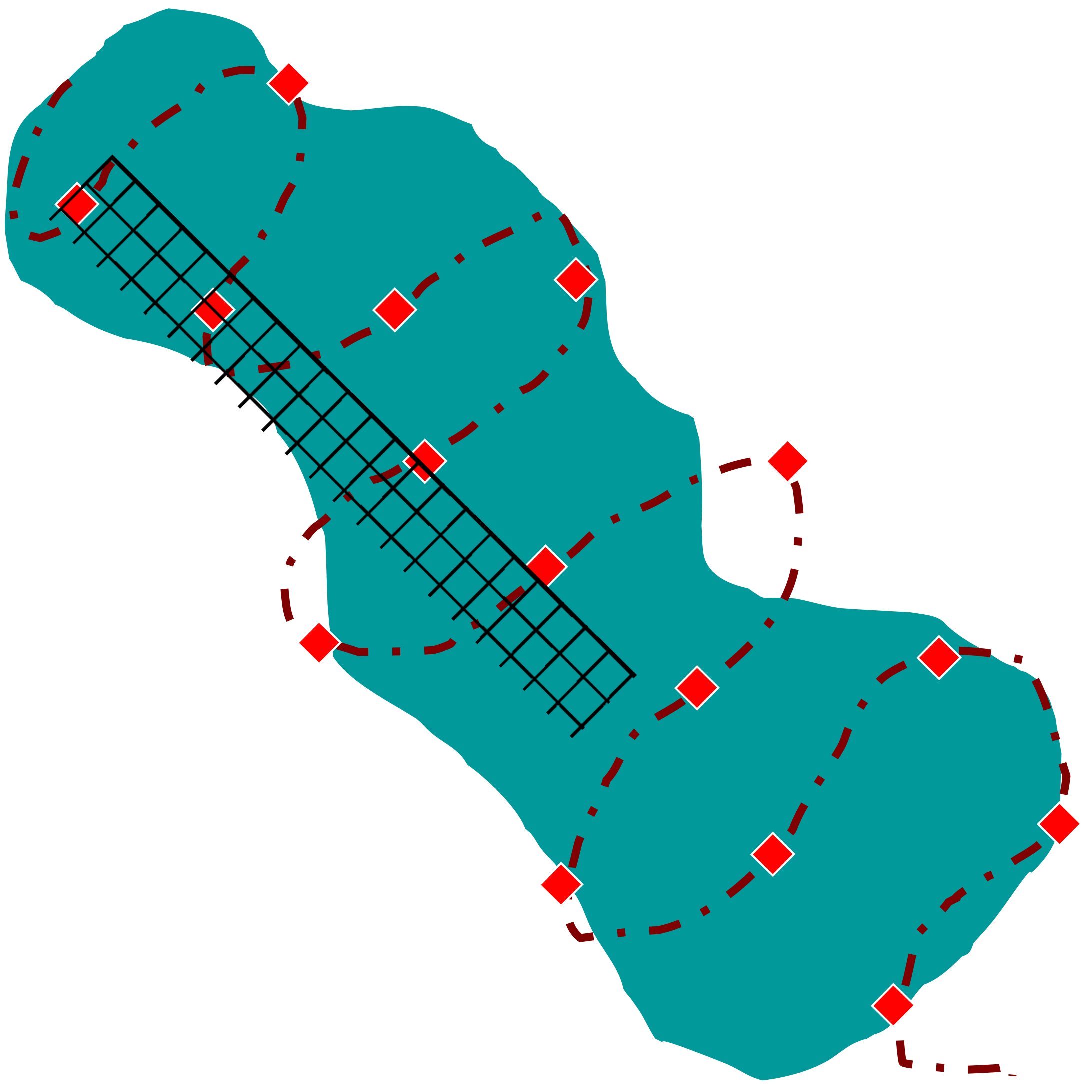

*Figure 6.  Timed Random Search survey design.*

The zigzag pattern is the walk with the squares quadrats at every 50 m.  The grid is the transect belt survey that is placed through the highest density area.

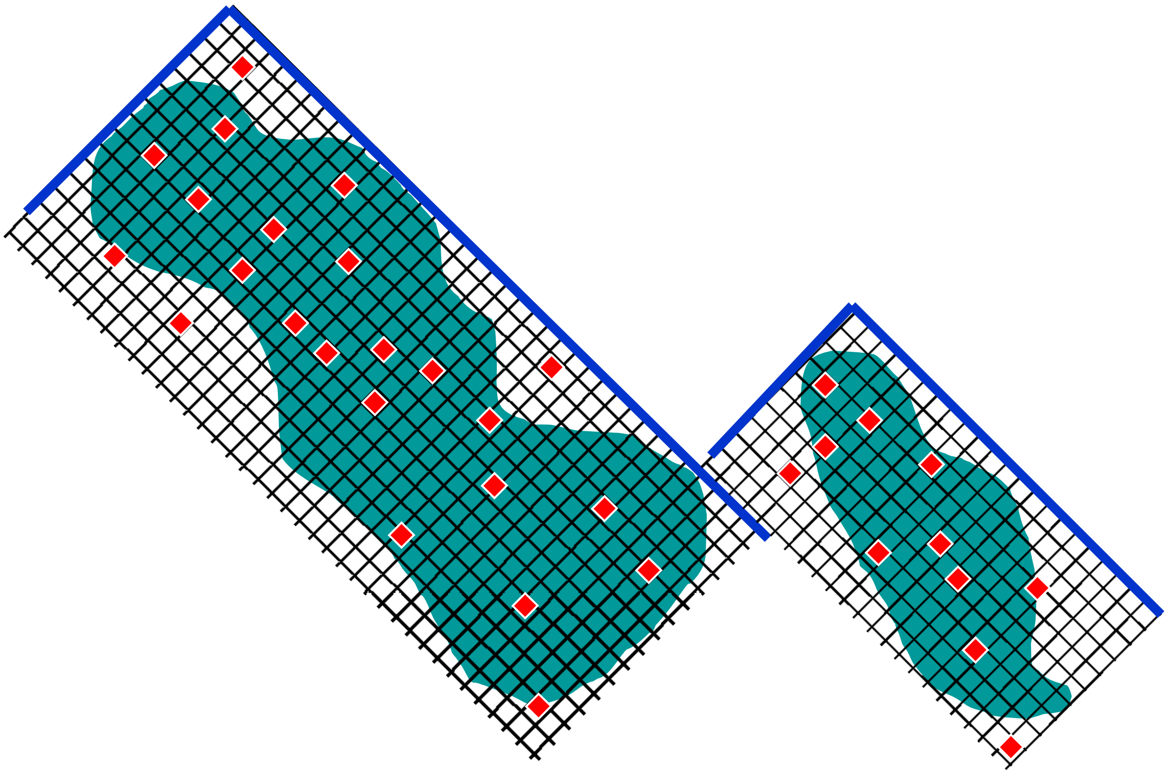

*Figure 7 Stratified Random Sample survey design.*

The baseline is established along one side of the bed, setting up an x and y axis, then random quadrats are selected throughout the survey area.  The grid show all the possible sampling locations and the two areas show that this design can be setup on multiple strata on the same beach.

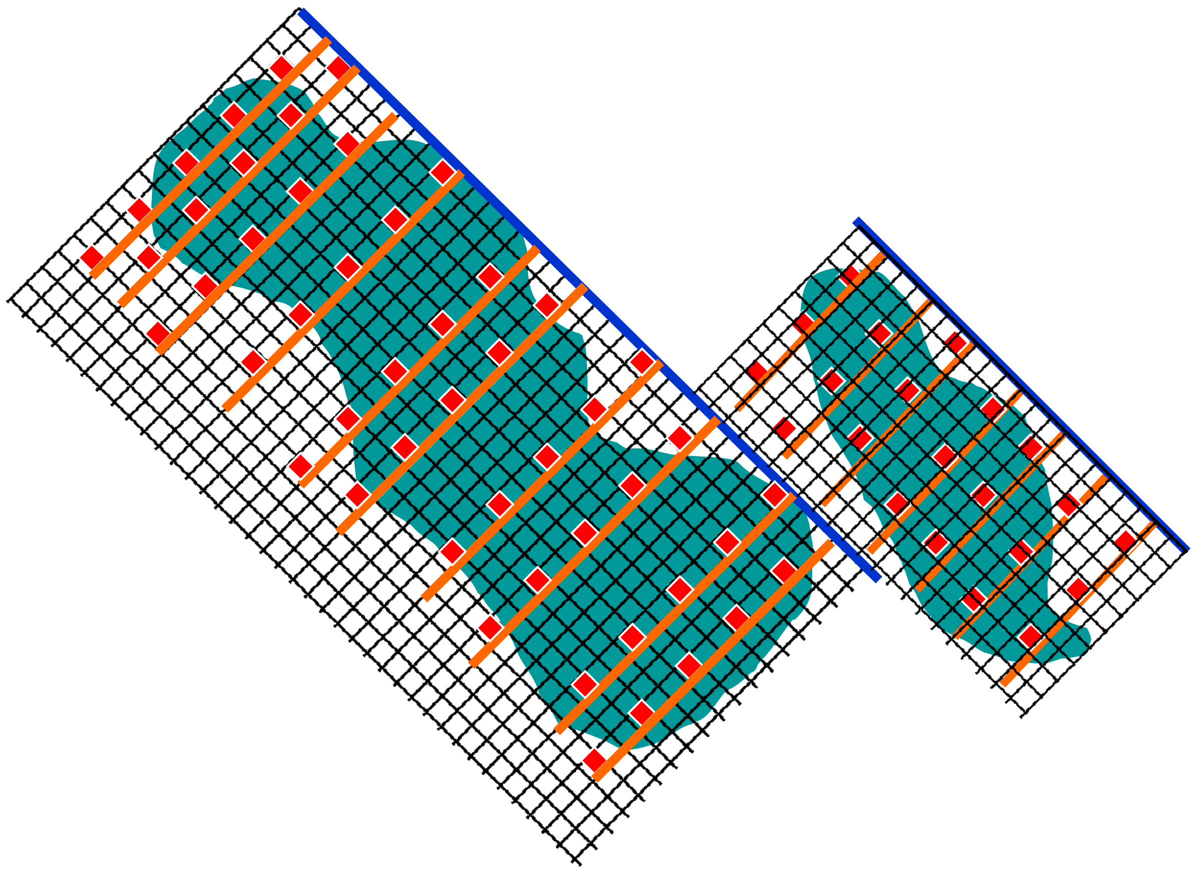

*Figure 8. Two-Stage sampling design.*

The baseline is established along one side of the bed and the transects are perpendicular to the baseline with quadrat (squares) systematically spaced along the transects. The grid show all the possible sampling locations and the two areas show that this design can be setup on multiple strata on the same beach.

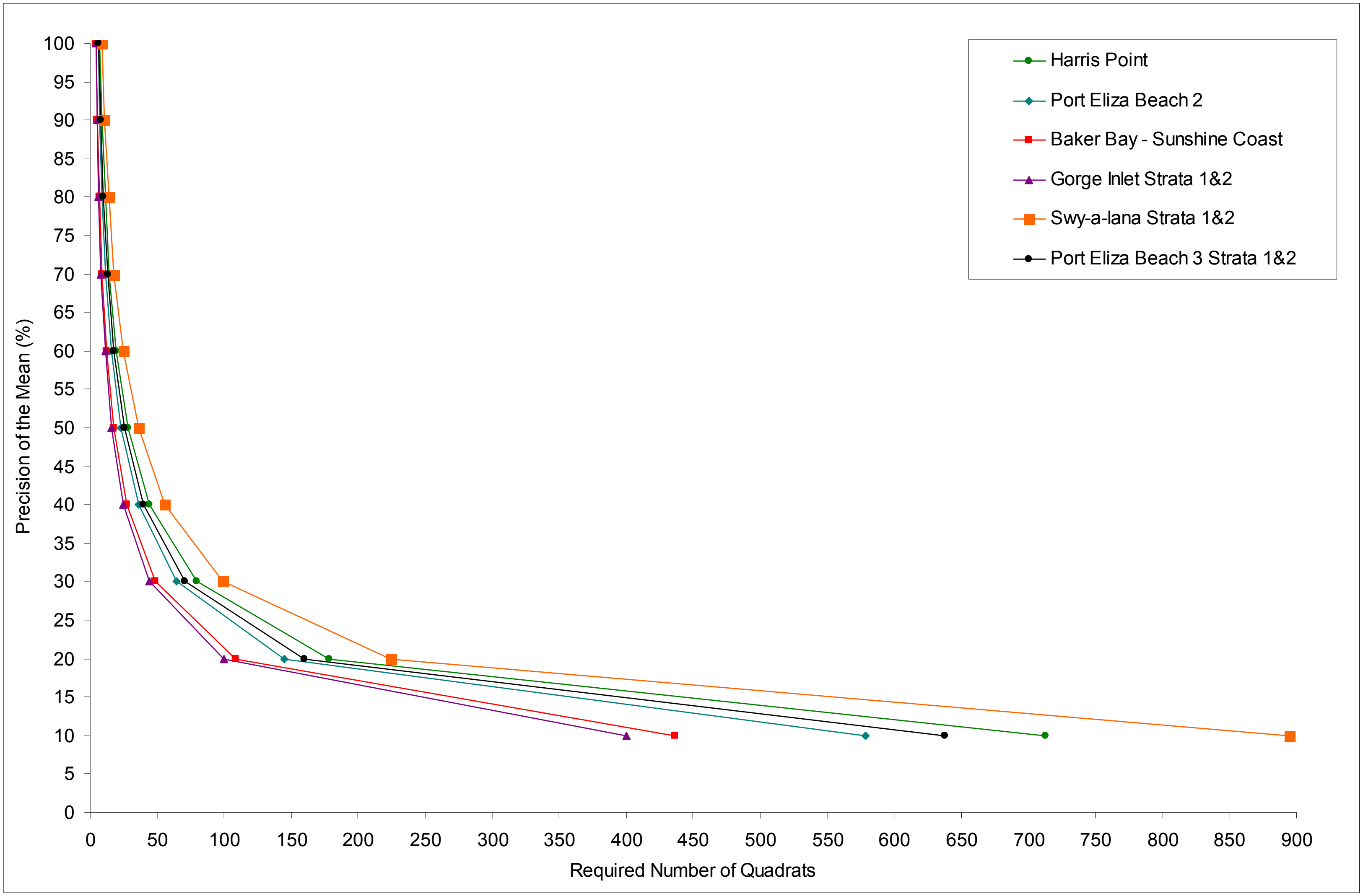


*Figure 9. Results of the precision analysis for each beach surveyed.*

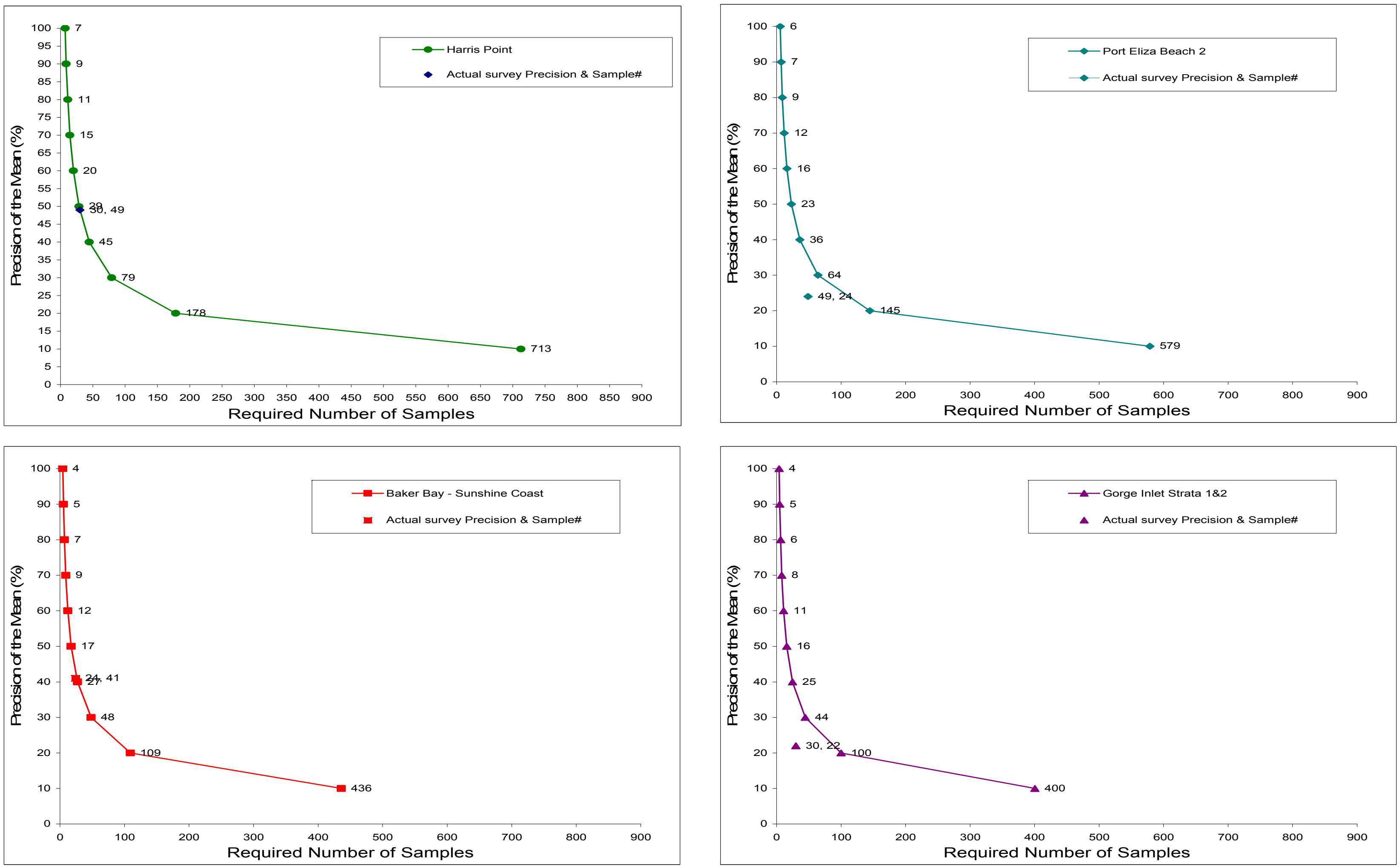


*Figure 10. Results of the precision analysis for Harris Point, Port Eliza Beach 2, Baker Bay and Gorge Inlet. Point labels along the line refer to the number of samples needed at each level of precision. The lone point on the graph is the actual precision from that survey.*

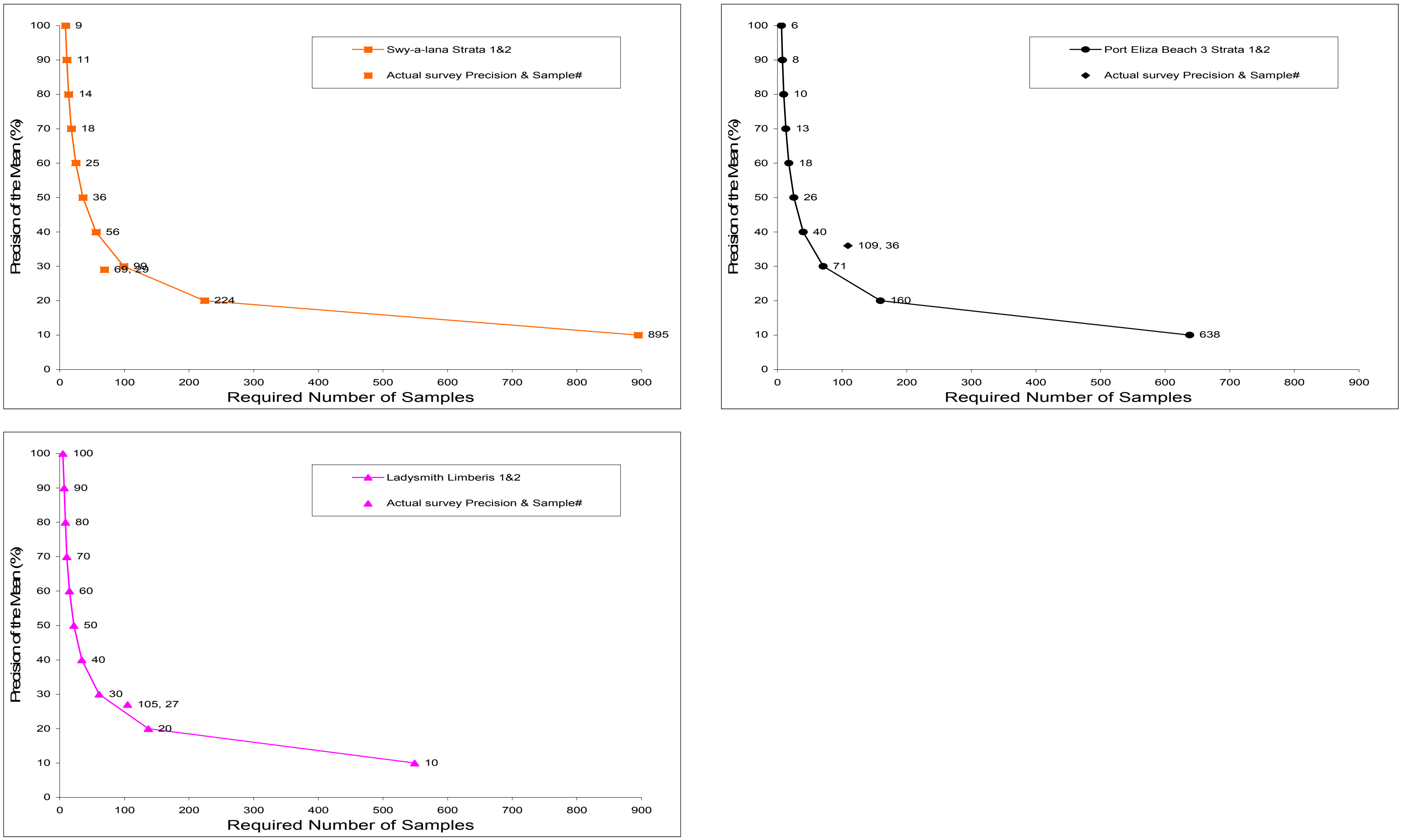


*Figure 11. Results of the precision analysis for Swy-a-lana, Port Eliza Beach 3 and Limberis in Ladysmith Harbour. Point labels along the line refer to the number of samples needed at each level of precision. The lone point on the graph is the actual precision from that survey.*

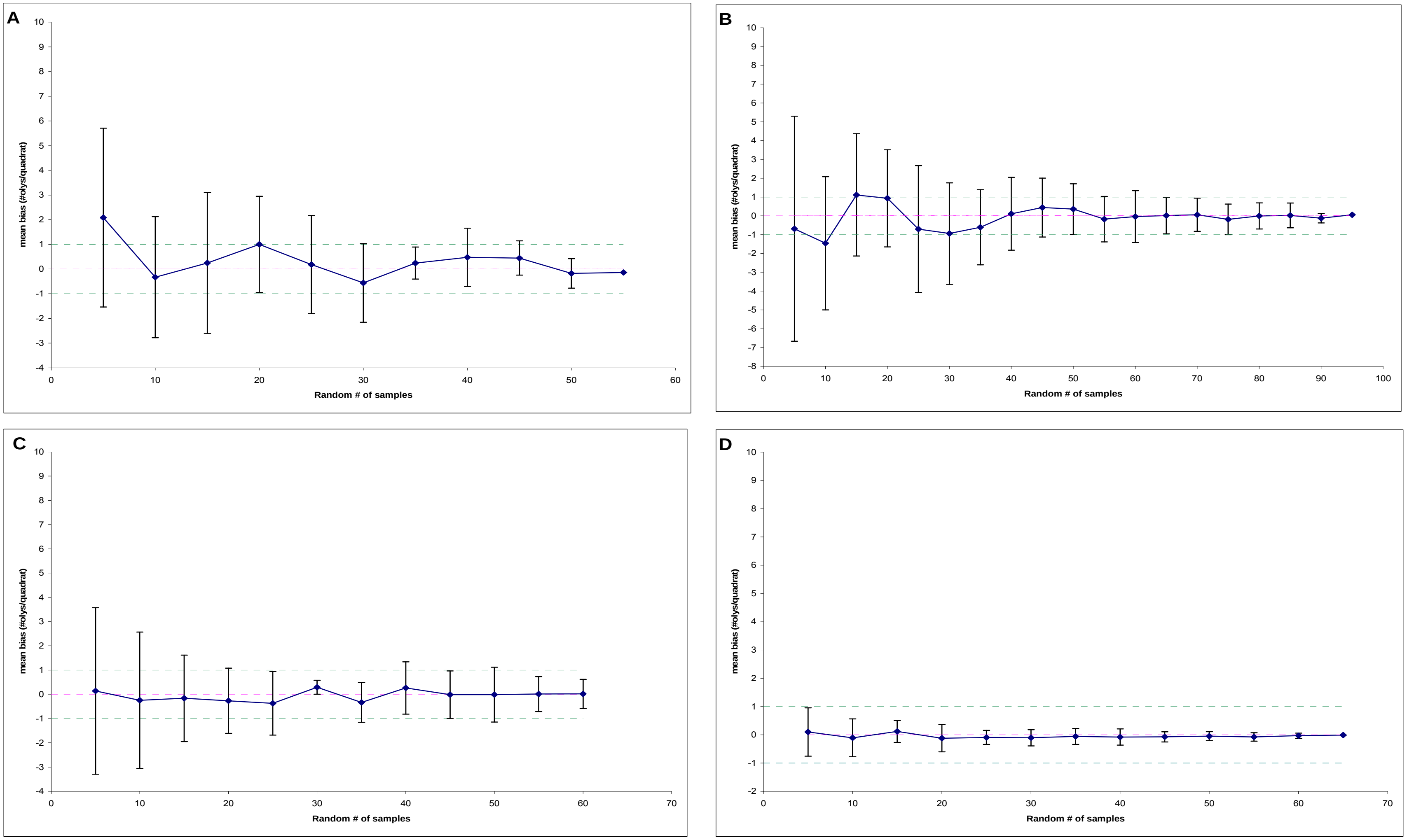


*Figure 12. Results of random subsampling for A) Port Eliza Beach 2, B) Port Eliza Beach 3, C) Limberis in Ladysmith Harbour and D) Swy-a-lana. Means and standard deviations (error bars) of the bias about the survey mean from 20 runs of random subsamples. Dotted line is + one Olympia oyster per quadrat*

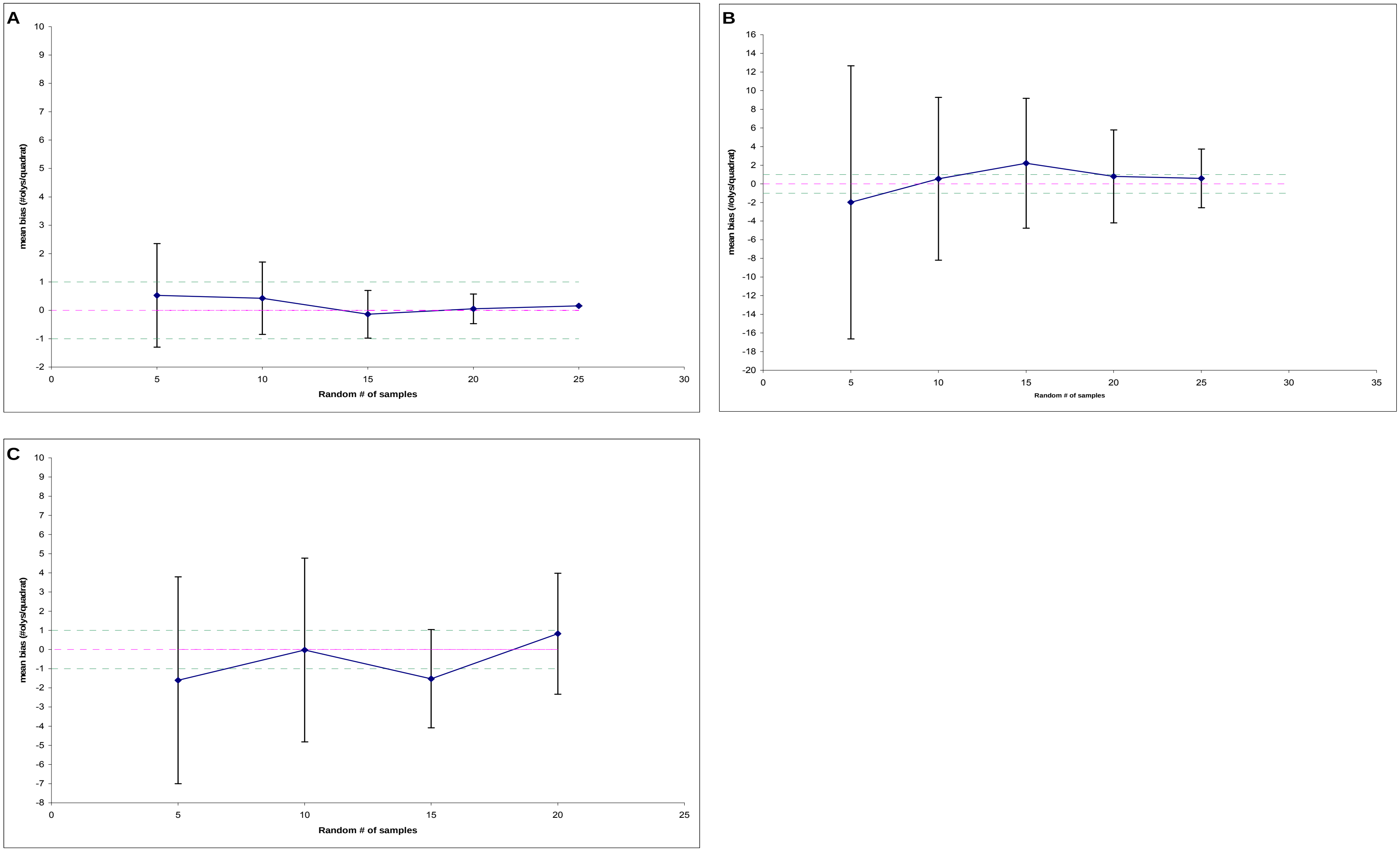


*Figure 13. Results of random subsampling for A) Harris Point- Barkley Sound, B) Gorge Inlet and C) Baker Bay-Sunshine Coast. Means and standard deviations (error bars) of the bias about the survey mean from 20 runs of random subsamples. Dotted line is + one Olympia oyster per quadrat.*

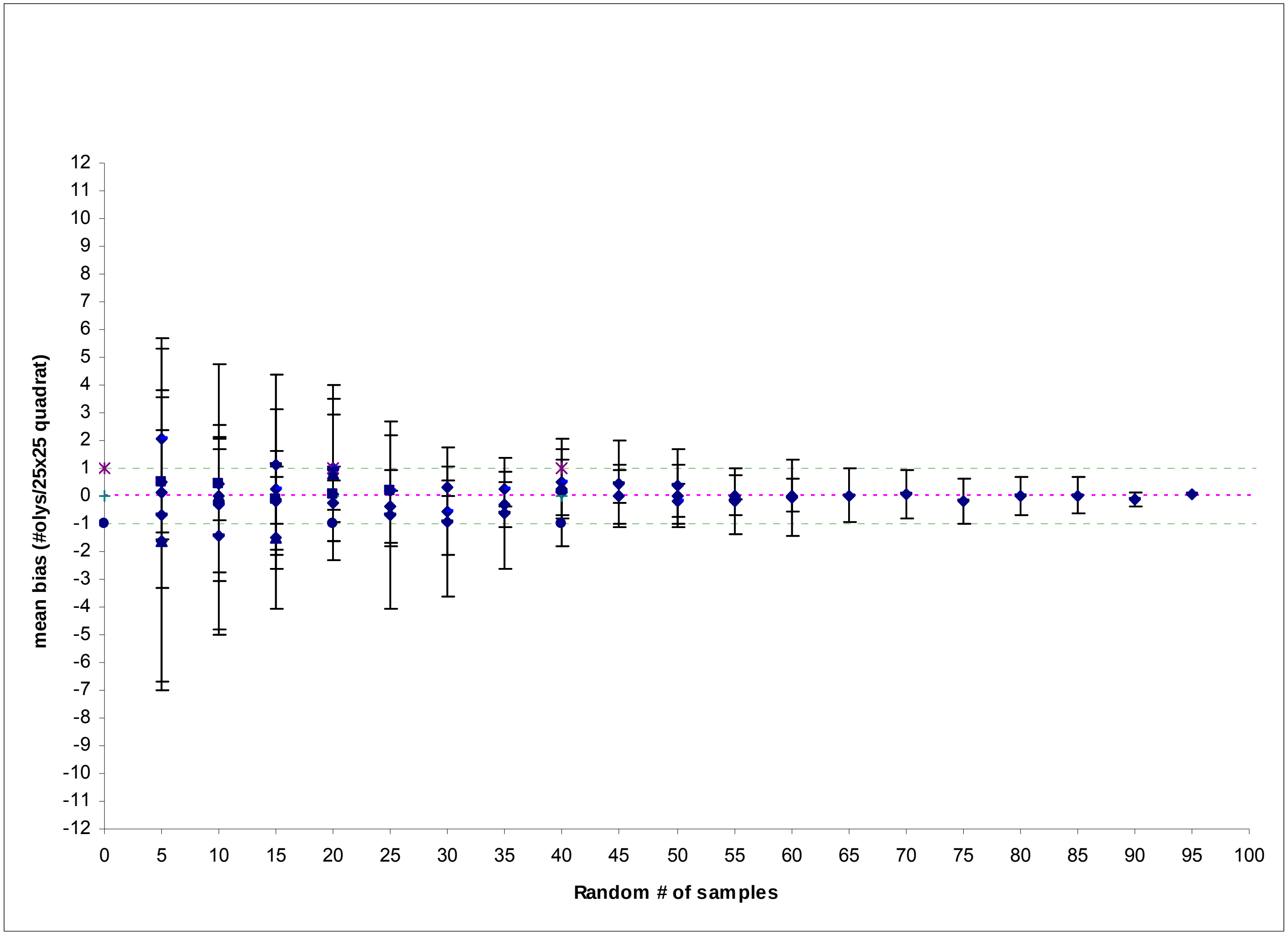


*Figure 14. Overlay of the results of the subsampling of all survey (Port Eliza 2, Port Eliza 3, Limberis Ladysmith, Harris Point and Baker Bay) where 25x25 cm2 quadrats were completed. Means and standard deviations (error bars) of the bias about the survey mean from 20 runs of random subsamples. Dotted line is + one Olympia oyster per quadrat.*

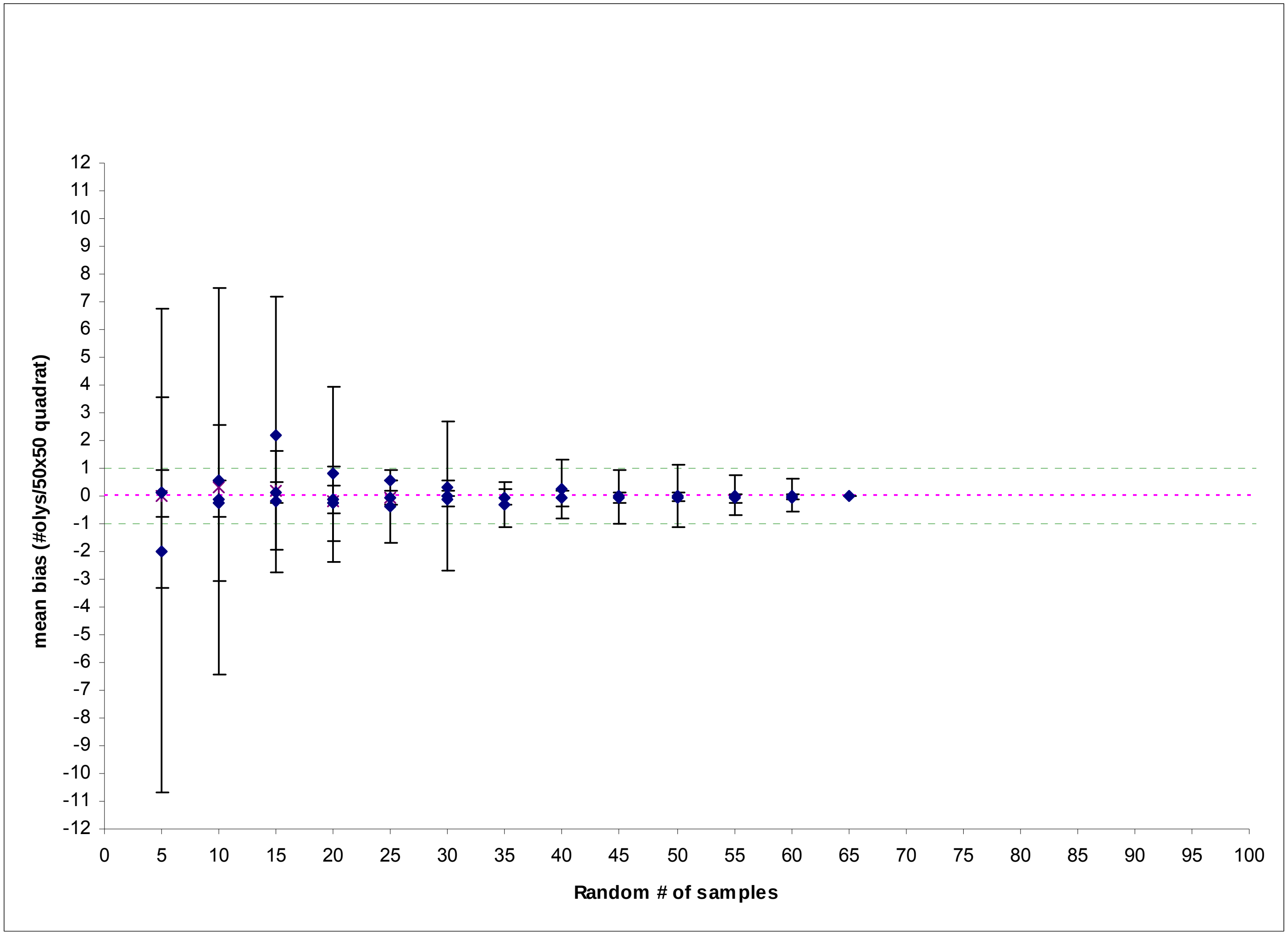


*Figure 15. Overlay of the results of the subsampling of all survey (Swy-a-lana Lagoon, Limberis Transect Belt, Gorge 1& 2) where 50x50 cm2 quadrats were completed. Means and standard deviations (error bars) of the bias about the survey mean from 20 runs of random subsamples. Dotted line is + one Olympia oyster per quadrat.*

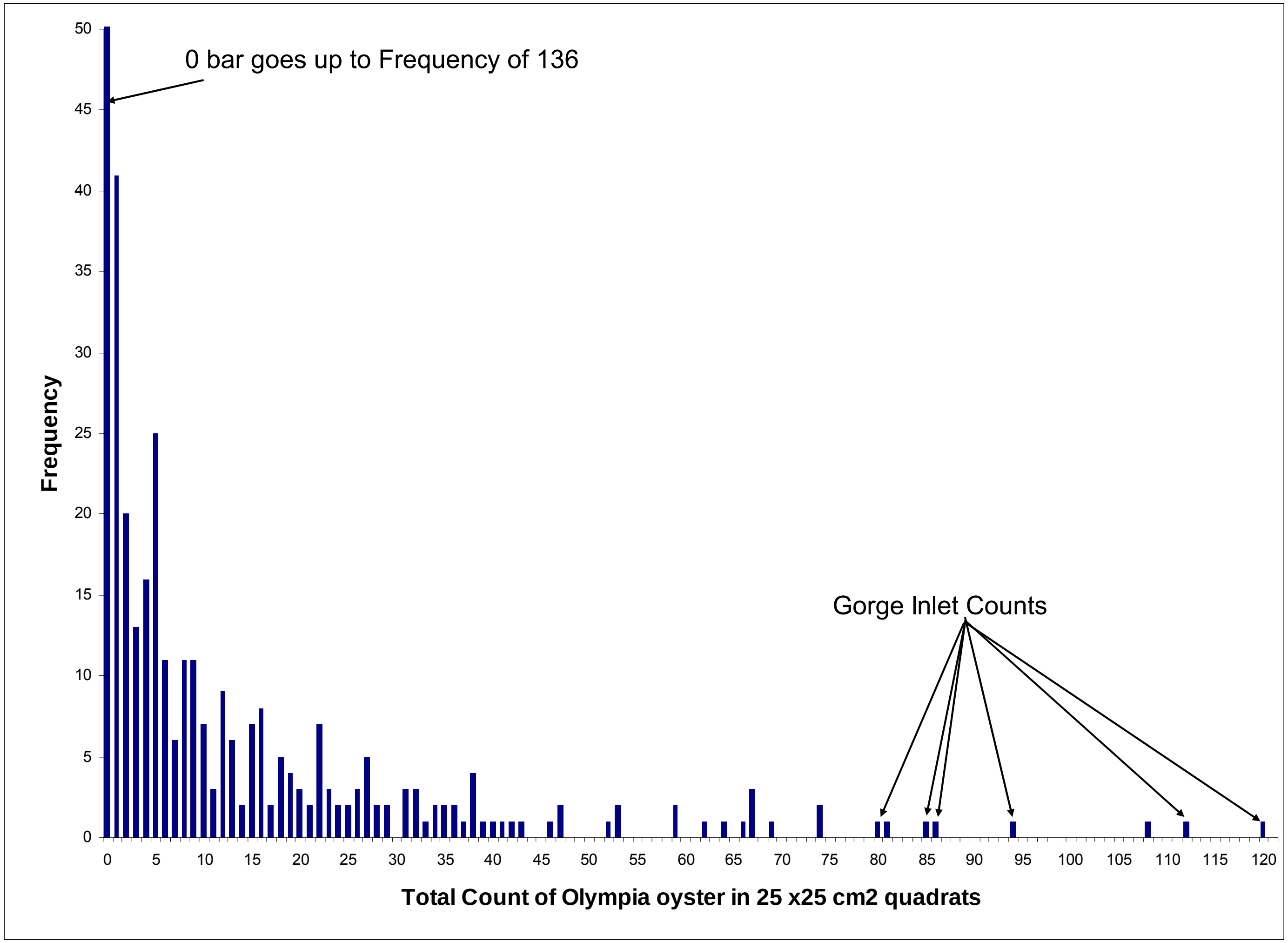


*Figure 16. Frequency of the counts from all the quadrats of the beach surveys (Baker Bay, Gorge Inlet, Harris Point, Limberis Ladysmith, Port Eliza Beach 2 & 3, Swy-a-lana Lagoon Nanaimo) that used 25 x 25 cm2 quadrats. The zero frequency count bar is cut off and goes up to 136.*

# Appendices

*Appendix Table 1. Statistical notation for reviewed survey analyses.*

| Symbol | Description |
|---|---|
| | **Timed Random Search** |
| $\mu_{rank}$ | average rank |
| $\sum (rank)$ | sum of all ranks |
| $n_q$ | number of quadrats |
| $\overline{d}$ | average maximum density |
| $CV$ | coefficient of variation |
| $\sigma_{\overline{d}}$ | standard deviation of the mean density |
| | **Stratified Random Sampling** |
| $h$ | stratum index |
| $i$ | y-value index |
| $N$ | total number of sampling units (quadrats) in the population |
| $N_h$ | total number of sampling units in stratum *h* |
| $n$ | number of units (quadrats) in the sample, or sample size |
| $n_h$ | number of units in the sample from stratum *h* |
| $y_{hi}$ | y-value *i* in stratum *h* (number of oysters) |
| $\mu$ | population mean |
| $\tau$ | population total |
| $\overline{y}_{st}$ | estimated population mean |
| $\hat{V}(\overline{y}_{st})$ | estimated variance of the population mean |
| $\hat{\tau}_{st}$ | estimated population total |
| $\hat{V}(\hat{\tau}_{st})$ | estimated variance of the population total |
| $s_h^2$ | sample variance in stratum *h* |
| $z_{\alpha/2}$ | *t*-value may be replaced with this estimator for large sample sizes |

## Two-Stage Sampling

| | |
|---|---|
| $h$ | stratum index |
| $i$ | first stage unit index |
| $j$ | second stage unit (quadrat) index |
| $N_h$ | number of first stage units in the $h^{th}$ stratum |
| $M_h$ | number of second stage units (quadrats) in each FSU for the $h^{th}$ stratum |
| $W_h$ | relative weight of stratum $h$ in terms of second stage units (quadrats) |
| $n_h$ | number of first stage units selected from stratum $h$ |
| $m_h$ | number of quadrats in each first stage unit in stratum $h$ |
| $y_{hij}$ | y-value $j$ in first stage unit $i$ from stratum $h$ |
| $\mu$ | population mean |
| $\tau$ | population total |
| $\bar{y}_{sts}$ | estimated population mean |
| $\hat{V}(\bar{y}_{sts})$ | estimated variance of the population mean |
| $\hat{\tau}_{sts}$ | estimated population total |
| $\hat{V}(\hat{\tau}_{sts}) =$ | estimated variance of the population total |
| $s_{1h}^2$ | sample variance **among** first stage units in the stratum $h$ |
| $s_{2h}^2$ | sample variance **within** the first stage units in stratum $h$ |

*Appendix Table 1. (continued).*

**Calculated Random Sample**

| Allen and Davis (unpub. manuscr.) notation | Our notation | Description |
|---|---|---|
| $L_B$ | $L_B$ | Length of baseline for survey area |
| $L_{Ti}$ | $L_{Ti}$ | Length of transect *i* |
| $\overline{L}_T$ | $\overline{L}_T$ | Mean transect length |
| SUM | | Sum of baseline length and mean transect length |
| $n_q$ | $n_q$ | Number of quadrats |
| $n_T$ | $N_h$ | Number of transects |
| $d_h$ | | Population density |
| $r$ | $r$ | Ratio of area sampled to bed area |
| $A_B$ | $A_B$ | Bed area |
| $TD$ | $TD_i$ | Standard distance between transect *i* |
| $SD$ | $SD_i$ | Standard distance between sample/quadrat *i* |
| $n_{min}$ | $n_{\min}$ | Minimum sample size |
| d | $\overline{d}_h$ | Mean density from haphazard sample to determine $n_{\min}$ |
| $s^2$ | $s_h^2$ | variance from haphazard sample to determine $n_{\min}$ |
| $p$ | $p$ | Desired precision expressed as a proportion (*e.g.*, ±30% = 0.3) |