A Manual for Intertidal Clam Surveys

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ABSTRACT

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

This manual describes a protocol for intertidal clam surveys. Standards are established for survey design, field and laboratory methods, data formats, and reporting requirements. Other topics include biology and life history of commercial hardshell clam species in British Columbia. The statistical basis of the survey methodology is based on probability sampling. Design and analytical techniques for simple random sampling, stratified random sampling, two-stage sampling, and stratified two-stage sampling are reviewed, and worked examples and case studies provided.

RESUME

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

Le présent manuel décrit un protocole visant les relevés des bivalves fouisseurs intertidaux. Il établit des normes pour la conception des relevés, les méthodes de travail sur le terrain et en laboratoire, la présentation des données et les exigences concernant les rapports. Les autres sujets traités sont la biologie et le cycle vital des bivalves à coquille dure exploités commercialement en Colombie-Britannique. La base statistique de la méthodologie de relevé est l'échantillonnage probabiliste. Les auteurs examinent la conception et les techniques d'analyse de l'échantillonnage aléatoire simple, de l'échantillonnage aléatoire stratifié, de l'échantillonnage à deux degrés et de l'échantillonnage stratifié à deux degrés, et fournissent des exemples de travaux et des études de cas.

Chapter 1 - Introduction

This manual describes a survey protocol for intertidal clams that establishes minimum acceptable standards of work and a methodology based on statistical sampling theory. The manual was produced by Fisheries and Oceans Canada (DFO) to provide to stakeholder groups the requirements for design, analysis, and reporting of surveys for stock assessment.

Commercial clam fisheries date to the turn of the century, and subsistence fisheries for intertidal bivalves pre-date commercial fisheries in British Columbia (B.C.). Despite this long history of exploitation, little is known of the population dynamics of clams in B.C. or the resilience of these clam stocks to harvest. Specifically, there are few data on natural mortality, life history, larval dispersal, stock identity, or recruitment patterns for any commercial clam species harvested in B.C. Surveys of clam populations can help to establish short term harvest rates and will contribute towards the data required to develop sustainable harvest strategies.

Our intent is that this manual serve as a basis for clam surveys conducted by DFO, First Nations, and third party consultants. The manual describes the DFO survey protocol, approved by the Pacific Stock Assessment Review Committee (Gillespie *et al.* 1998b; Kronlund *et al.* 1998), including a discussion of survey design, field and laboratory methods, data management, and reporting requirements. By necessity, some of the statistical material is technical. In some cases guidance from DFO staff or qualified professionals may be required. However, we have tried to demonstrate statistical computations using simulated and real data whenever possible. Discussion of each survey design includes explanatory figures and a case study based on real data to illustrate the various field and analytical techniques.

Our current preference is to use survey (probability) sampling as a basis for computing estimates of density and biomass for intertidal clam populations. However, it is not our intent to restrict surveys to the four designs discussed in this manual. We understand that surveys may have to be tailored to address the specific constraints of a beach, or the objectives of proposed work. For example, the design of broad-brush ecological surveys may differ from stock assessment surveys. Suitably modified, the survey protocol could be applied to other intertidal species of interest. The best choices of sampling rate and quadrat size may differ among species, and must be determined on a case-specific basis. Our intent is to describe generally applicable methods and to set the standards for conducting and reporting stock assessment surveys.

Other survey strategies have been used in British Columbia (Bourne and Farlinger 1982; Bourne and Adkins 1985; Adkins and Harbo 1991; Adkins 1992; Heizer 1992; Adkins and Joe 1993; Heizer 1996; Kingzett and Bourne 1998) and elsewhere (Franklin and Pickett 1979; Gustafson 1995, 1996; Campbell 1996). However, the lack of a randomization basis for these sampling schemes requires the use of model-based estimates which may be biased and are difficult to compute. Furthermore, some of the methods have been subjective in design, and as such do not provide a repeatable basis for surveys.

We have tried to minimize the use of formulas in this manual. Whenever possible, we have used figures and examples to illustrate key points. Nevertheless, surveys are a technical undertaking that require specialized terminology and statistical methods for proper planning, conduct, and analysis. Chapters that describe specific sampling designs begin with a table of symbols required to calculate population estimates for that design.

We expect that the survey protocol will change with field testing and input from stakeholder groups. In addition, changing management requirements may require revision of the survey protocol. We welcome your feedback on how to improve the presentation of material in this manual, and how to make the it easier to understand. Information on how to contact DFO is listed at the end of this chapter.

What's in this manual?

This manual covers the following topics:

- why clam surveys are necessary;
- who to contact for further assistance;
- a summary of the survey protocol and DFO requirements (Ch. 2);
- a summary of available biological information on intertidal hardshell clams in British Columbia (Ch. 3);
- an introduction to survey sampling, selection of a random sample, and the factors that affect survey estimates (Ch. 4) ;
- basic sampling designs: simple random sampling (Ch. 5) and stratified random sampling (Ch. 7) with case studies (Ch. 6 and 8);
- field and laboratory methods including worked examples of DFO data forms (Ch. 9 and 10);
- data requirements including examples of spreadsheet formats, and a list of DFO species codes (Ch. 11);
- analysis of biological data including frequency distributions, length-weight relationships and growth curves illustrated with a case study (Ch. 12 and 13);
- complex sampling designs: two-stage sampling (Ch. 14) and stratified two-stage sampling (Ch. 16) with case studies (Ch. 15 and 17).

Why do clam surveys?

Determination of the size of clam populations and their biological characteristics are central to the assessment and management of these resources. Surveys represent one means of obtaining at least some of this information. Typically, an intertidal clam survey is conducted by digging small plots of the beach at specified locations and recording data such as the number, weight, and species of clams removed from each plot. The plots are called **quadrats** and are usually rectangular in shape (Elliot 1977). Information collected during clam stock assessment surveys is of three basic types:

1. **Quadrat counts and weights**. The number of clams per quadrat is used to estimate total number of animals (**abundance**) in the survey area. Weight per quadrat is used to estimate total weight of animals (**biomass**) in the survey area. The precision of the estimates of total number or weight can also be calculated. For species with a size limit, these estimates are produced separately for **legal** and **sublegal** portions of the population. Abundance and biomass estimates provide guidance on the amount of clams that can be harvested sustainably. The average number, or average weight, of clams in each quadrat is a useful statistic for comparing populations on different beaches.
2. **Clam length, weight and age**. The length, weight, and age of individual clams can be used to develop an impression of the status of a clam population. For example, if few clams are above the legal size limit, then harvest opportunities will be limited in the short term. Suppose the legal size clams are all about the same age and close to the legal size limit on a beach. This may indicate that the particular beach is fished frequently and that the fishery is effectively removing clams as soon as they reach the legal size limit. If few sublegal clams are present, then the currently legal size clams may have to be partitioned over a number of years of harvest until new recruitment and growth re-supplies the legal stock.
3. **Clam growth**. The rate at which an individual clam grows can be determined by measuring the distance between the "growth rings" (**annuli**) on the shell. These **length-at-annulus** data can be used to estimate the growth rate of individual clams over time. In turn, data from many clams can be used to estimate the growth rate of a population of clams. A slow growth rate might indicate overcrowding of clams on the beach or poor feeding conditions. Beaches where clams rapidly reach legal size might be, on average, more productive and thus produce greater yields with less risk to the stock if harvested.

The results of a single survey can be used to assess a clam population in the year the survey was conducted. However, as data are gathered from geographically separated beaches over a period of years, the accumulating knowledge may help to improve management of clam populations through increased understanding of recruitment patterns, productivity, mortality rates and harvest impacts.

Conventions used in this manual

This manual is organized by numbered chapters containing sections (no numbers). Chapter or section headings marked with an asterix (*) contain technical material and may be omitted on first reading. Key words defined in the glossary are printed in **boldface** type. Numbering of tables, figures, and examples includes the chapter number, *e.g.* Figure 1.2 is the second figure in chapter 1. Examples appear in *italicized* type.

Each chapter that contains a description of a survey design includes a table of symbols and equations required to calculate estimates for the design. Worked examples based on simulated and real data are presented. The chapter following each survey design contains a case study based on real survey data. The following abbreviations are used in the manual:

Unit	Abbreviation	Unit	Abbreviation	Unit	Abbreviation
centimeter	cm	gram	g	weight	wt
meter	m	kilogram	kg	hectare	ha
year	y				

How to contact DFO

Contact DFO staff regarding this manual under the following circumstances:

- if you are requesting approval of survey designs;
- if you are submitting survey results;
- if you need clarification on topics in the manual;
- if you wish to report errors in the manual or provide feedback on the manual.

For information on clam stock assessment and surveys, please contact:

Intertidal Clam Assessment Program
Pacific Biological Station
3190 Hammond Bay Road
Nanaimo, BC V9R 5K6

Phone: (250) 756-7000
Fax: (250) 756-7138
e-mail: gillespieg@pac.dfo-mpo.gc.ca

If you have questions or comments regarding fishery openings for clams or the management of clam fisheries, please contact:

DFO Parksville Office
457 E. Stanford Avenue
Parksville, BC V9P 1V7
Phone: (250) 954-2675

North Coast Division Office
417 2nd Avenue West
Prince Rupert, BC V8J 1G8
Phone: (250) 627-3415
Fax: (250) 627-3056

Chapter 2 - Synopsis of Survey Protocol

The survey protocol for intertidal clams in British Columbia is based on statistical survey sampling. The objectives of the protocol are:

- to provide a fair and repeatable methodology for surveying intertidal clams;
- to establish standards for the design, conduct, analysis and reporting of clam surveys;
- to describe field procedures that have been tested through experience.

This chapter should be viewed as a road map to the requirements of the survey protocol. It is intended to direct you to specific details of the protocol described in subsequent chapters. This manual describes acceptable standards for the conduct, analysis, and reporting of clam assessment surveys required by DFO. For some survey problems, rigid application of the protocol will be impractical; in these situations contact DFO for assistance. We anticipate revisions to the protocol as data and experience accumulate, and as management or assessment requirements change.

Statement of protocol

The following headings identify the basic elements of the survey protocol:

How to survey

Recommendations for survey design, sampling rate, analysis and the DFO role are listed in Table 2.1. Each recommended survey design is based on the size of the survey area. All survey designs must be reviewed by Stock Assessment Branch, DFO, prior to a survey permit being issued. A general introduction to survey sampling is presented in Chapter 4. Subsequent chapters contain worked examples and case studies designed to illustrate analysis and reporting of results.

When to survey

DFO requires that surveys for stock assessment of Manila and littleneck clams be completed during daylight low tides no greater than 1 m above chart datum. Because butter clams live in the lower intertidal zone and subtidally, assessment surveys for butters should be planned for the lowest available tide series, in order to assess the largest possible portion of the intertidal population. These tides are generally available only between April and September each year.

Quadrat size

Standard quadrat size and depth of samples for each species are:

Species	Quadrat Dimensions (cm)	Quadrat Area (m^2)	Sample Depth (cm)
Manila	50 x 50	0.25	20
Littleneck	50 x 50	0.25	20
Butter	100 x 100	1.00	30

Note that samples should be dug deeper if clams are still being removed from the diggings at the minimum depth.

Sampling rate

Sampling rate depends on the size of the survey area and the specific survey design (Table 2.1). Minimum sampling requirements are 30 quadrats/ha for Manila and littleneck clam surveys, with no less than 10 quadrats per stratum; and 10 quadrats/ha for butter clam surveys. Sampling rates are based on available data and the experience of DFO staff. As data accumulates, the recommended sampling rates may be revised.

Field methods

Field methods are described in detail in Chapter 9. In particular, ensure that:

1. the survey area is carefully mapped including stratum boundaries and reference lines, so the survey could be repeated, if necessary;
2. quadrat sizes are carefully measured and all quadrats are dug;
3. samples collected should include all sizes of all species of clam from each quadrat;
4. samples are carefully labeled for later identification; and
5. samples are stored correctly until they can be processed.

Sample processing

Laboratory and data management procedures are outlined in Chapters 10 and 11. In particular, ensure that:

1. samples are handled separately;
2. all required information for each sample is correctly recorded on each data form.

Biological samples

Clams for length, weight, age, and length-at-annulus measurements submitted for processing must be:

1. cleaned with shells numbered for age determination and length-at-annulus measurements (note: do not boil shells to clean them; this causes the shells to break down, and can make ageing impossible);
2. marked on their inside surface with serial numbers that are accurate and clear for later reference to length and weight data;

If biological samples are processed by non-DFO staff, then shells must be submitted to DFO for verification and quality control. The survey report must accompany all biological samples submitted to DFO.

Data requirements

Submit the following raw data with the survey report:

1. clean, legible copies of completed number and weight, biological and length-at-annulus data forms (Ch. 10);
2. electronic data as an Excel spreadsheet file (Ch. 11); and
3. ensure that all data fields in Table 11.1 through Table 11.4 are recorded in electronic copies.

Report

The survey report provides concise documentation of survey design, methods, analyses, results and interpretation. The report should contain sufficient detail so that a person not involved with the survey could assess the results, and be able to repeat the survey based on the report. A thorough report is particularly important for beaches where surveys are conducted annually to ensure that surveys are comparable over time. The report should summarize the following survey details:

1. Personnel and contacts
    - names and contact information of the people who planned and supervised the survey, as well as the authors of the report.
2. Site description
    - a description of the location (e.g. Goldstream Estuary, Finlayson Arm) with approximate latitude and longitude;
    - a general description of the beach including substrate type, slope, stream channels, rocky areas, muddy areas, distribution of clams or other animals (*e.g.* mussel beds);
    - dates and times of field activities;
    - the time and height of low tide on the survey dates and the reference table used to determine the tides.
3. Survey design and randomization
    - a description of survey design and randomization procedures;

- detailed documentation of stratum boundaries and quadrat locations, including maps, diagrams, and bearings from stratum nodes to permanent landmarks;

4. Field results
   - details of quadrats missed due to errors or flooding;
   - detailed information or comments not summarized on the data sheets;
   - descriptions and reasons for departures from the survey design.
5. Biomass and abundance estimates
   - a table that lists the area of each stratum, where applicable, the number of quadrats, the mean density (number of clams/quadrat);
   - a table containing mean biomass (kg/quadrat) by species and size category with associated variances and standard errors;
   - a table showing estimates of total abundance (number of clams) and total biomass (weight of clams) for each species and each size category, with associated 95% confidence intervals.
6. Biological data
   - a figure showing length plotted against weight;
   - tables summarizing length and age frequencies (if applicable);
   - figures summarizing length and age frequencies (if applicable).
7. Appendices containing original data
   - clean, legible copies of the original data sheets;
   - electronic copies of the data as EXCEL spreadsheets.

Submitting data to DFO

Ensure the following steps are complete before submitting survey data to DFO:

1. Check that the data has been recorded completely and correctly.
2. Ask a co-worker to verify that the data is complete and correct.
3. After the data are keypunched into an electronic format (spreadsheet), print the files to check against the original data sheets for data-entry errors.
4. Copy the electronic data files to diskette.
5. Make a duplicate copy of all data sheets, all diskettes, and the survey report.

Submit the original data sheets and diskettes with the survey report to DFO.

A comment on sampling rates

Inspection of the examples in Table 2.1 reveals that a total of 90 quadrats is required for a 3 ha survey area, or 30 quadrats/ha. In contrast, 270 quadrats are specified for a 15 ha site or 18 quadrats/ha. Small survey areas appear to be unfairly penalized in terms of a higher sampling rate. Our rationale for the difference is based on the following considerations:

- Smaller sites may be less resilient to accidental over-harvesting than larger sites if survey estimates are too high. Thus, it is important to have good estimates of population size.
- Smaller sites can be more rapidly depleted, given equal fishing effort, than larger sites. Thus, improved estimates of population size due to a higher sampling rate can help to select the best management measures for preventing excessive harvest.
- Estimates of abundance or biomass obtained from a small site will, in general, be known more precisely than estimates from a larger site obtained with a lower sampling rate. Thus, managers may set allowable harvest at a small site with more confidence.

The possibly high uncertainty associated with estimates from a large site means that managers may take a precautionary approach to setting harvest levels. For example, a harvest rate of 25% of the estimated legal biomass might be applied to a small site based on survey results. However, managers may set a lower rate of, for example, 15% of the estimated legal biomass for a large site because of the imprecision of survey estimates.

Table 2.1 Summary of DFO survey protocol.

	Survey area less than 5 hectares
Design (recommended)	Stratified random sampling
Sampling rate	30 quadrats per hectare, minimum 10 quadrats per stratum (10 per ha for butter clams).
Analysis	Chapter 7 - Stratified Random Sampling
DFO Role	Sampling plan submitted to DFO **prior** to conduct of survey. Instruction of novice technicians when requested, on-site cooperative participation at the discretion of DFO, quality control (audit of data and results)
Example	A 3 ha site might be partitioned into 3 strata: one of 1.5 ha (45 quadrats), one of 1.0 ha (30 quadrats), and one of 0.5 ha (15 quadrats).
Notes	For a single stratum, use the analysis in Chapter 5 - Simple Random Sampling.
	Survey area 5 to 15 hectares
Design (recommended)	Stratified two-stage sampling using simple random sampling at both stages and equal size first stage units (FSUs) within each stratum.
Sampling rate	Approximately 30 quadrats per hectare within each FSU, minimum 10 quadrats per FSU (10 quadrats per ha for butter clams).
Analysis	Chapter 16 - Stratified Two-Stage Sampling
DFO Role	Sampling plan submitted to DFO **prior** to conduct of survey. On-site cooperative participation at the discretion of DFO, quality control (audit of data and results).
Example	A 15 ha beach might be stratified into three areas of 5 ha. Partition each stratum into 10 FSUs of 0.5 ha. Select six FSUs from 10. From each selected FSU select 15 quadrats. Total quadrats=3*6*15=270.
	Survey area greater than 15 hectares
Design (recommended)	To be determined prior to conduct of survey by DFO staff or qualified consultant. Probable (stratified) multi-stage design.
Sampling rate	To be determined on a case-specific basis.
Analysis	Dependent on design.
DFO Role	Sampling plan submitted to DFO **prior** to conduct of survey. On-site cooperative participation at the discretion of DFO, quality control (audit of data and results).

Chapter 3 - Hardshell Clams

This manual is primarily concerned with surveys of the commercially important intertidal hardshell clam species of B.C. which include Manila clams, native littleneck clams and butter clams. This chapter contains information on identifying these species, with a brief summary of their distribution, habitat preference and life histories.

The various species of clams are identified by characteristics of their internal and external anatomy. We concentrate on those traits that differentiate the three main species of intertidal hardshell clams harvested commercially in B.C. (Table 3.1, Figure 3.1). Identification will not always be clear, since shell shape varies considerably with different growth patterns. Stunted, poorly growing clams are rounder than clams that grow normally. Clams that have survived injury are often mis-shapen, with wavy shell margins or unusual ridges in the surface of the shell. The traits discussed here are based on normal growth patterns. Taxonomy follows Coan and Scott (1997).

The three species have similar life cycles (Table 3.2). The sexes are separate, and spawning involves release of gametes into the water column, where fertilization occurs. After a planktonic period of 3-4 weeks, depending on species, temperature and available food, the larvae settle and take up an infaunal existence. Exchange of adults among beaches does not occur, *i.e.*, adult populations are considered closed. There may be mixing of clams within suitable habitat, but no exchange of adults between isolated patches of habitat (*i.e.*, between beaches) occurs. Recruitment, or animals coming into a population, is variable. In some cases, a number of years can pass without significant influx of new animals to a harvestable population (Quayle and Bourne 1972; Gillespie *et al.* 1998b). This may be due to poor settlement of larvae at a beach, or due to a high rate of juvenile mortality that reduces the number of clams growing large enough to enter the fishery.

Manila clam (*Venerupis philippinarum* [Adams and Reeve, 1850])

Manila clams, also called Japanese littlenecks, have been reported under other scientific names, including *Tapes philippinarum*, *Venerupis japonica* [Deshayes, *in* Gray, 1853], *Venerupis semidecussata* [Reeve, 1864] and *Paphia bifurcata* Quayle, 1938. Both the common name Manila clam and the trivial name *philippinarum* are holdovers from the type material, which was incorrectly attributed to the Philippine Islands. For a complete synonymy, see Bernard (1983).

Identification

Manila clams (Figure 3.2) are generally longer than they are high, resulting in an oblong profile (as compared to the more circular profile of the littleneck clam). The valves are thick, and marked with both concentric and radial sculpture. The radial striae are more pronounced on the posterior part of the valve. The lunule is present as a flat area or depressed pit anterior to the umbo, and lacks the ridge present in littleneck clams. The internal surface of the valve is smooth, and lacks the crenulations (fine, comb-like teeth) on the ventral margin which are present in the littleneck clam. The interior of the valve is white or yellow in color, with deep purple at the posterior end. The siphonal tips are split. Maximum size is approximately 75 mm (Quayle 1960; Quayle and Bourne 1972; Abbott 1974; Kozloff 1974; Harbo 1997).

Distribution and habitat

The Manila clam was inadvertently introduced to British Columbia prior to 1936, at Ladysmith Harbour during the importation of Pacific oysters (*Crassostrea gigas* [Thunberg 1793]) from Japan (Quayle 1941). They quickly spread throughout the Strait of Georgia, and were accidentally introduced on the west coast of Vancouver Island, and intentionally to other sites on the central coast of British Columbia and the Queen Charlotte Islands (Quayle and Bourne 1972). Intentional introductions in the central coast and Queen Charlotte Islands failed to produced sustainable populations (Gillespie and Bourne 1998). Recent recruitment of Manila clams into the Bella Bella area is believed to have been carried into the area as pelagic larvae from Quatsino Sound (Bourne 1982).

Although the speed of their dispersal throughout the Strait of Georgia may indicate considerable dispersal of larvae in a given year, Quayle's comment (1944) that "*only one or two season's spawnings were required to make this previously unknown species the dominant clam of the area between the tidal limits*" may indicate that a significant proportion of the larvae spawned in a given location are retained and form the basis of recruitment for that beach. Thus, it may be a dangerous strategy to remove a large proportion of available spawning stock from a beach, under the assumption that recruits come from "somewhere else".

In western North America, Manila clams are found from the central coast of British Columbia (at approximately 52°59'N; Gillespie and Bourne, unpublished data) to California (Quayle 1960; Bourne 1982; Bourne *et al.* 1994). They are found in the upper half of the intertidal zone in British Columbia (Quayle 1960) in mixed substrates of mud, sand and gravel. They live in shallow, transitory burrows in the substrate, and are susceptible to extremes of temperature, including catastrophic mortalities ("winter kills"). These can occur when night-time low tides coincide with low air temperatures and prevailing winds (Bower *et al.* 1986; Bower 1992). Surveys of subtidal bivalves (Bernard 1983) have not found significant subtidal populations of Manila clams in British Columbia.

Life history

Manila clams spawn in the summer, generally mid- to late June to September in the Strait of Georgia (Bourne 1982). Size at first maturity is 20-25 mm (Holland and Chew 1974). Fecundity increases exponentially with length, with estimates ranging from 432,000 eggs at 20 mm to 2,350,000 eggs at 40 mm (Yap 1977) for a population in Hawaii. The maximum size of 75 mm is attained after approximately 8-10 years. Maximum age in British Columbia is 14 years (Bourne 1987). Age at recruitment to legal size varies from beach to beach and between areas on a single beach, depending on tidal height and substrate conditions. Under optimal conditions, Manila clams can reach legal size in approximately 3-4 years in the Strait of Georgia (Quayle and Bourne 1972; Bourne 1982), 4 years on the west coast of Vancouver Island (Bourne and Farlinger 1982) and 4-5 years in the Central Coast (Bourne and Cawdell 1992; Bourne *et al.* 1994; Bourne and Heritage 1997; Heritage *et al.* 1998).

Native littleneck clam (*Protothaca staminea* [Conrad, 1837])

Native littleneck clams have also been given the common names carpet shell and rock cockle. Although they appeared under numerous scientific names in the late 1800s and early 1900s, their taxonomy has been stable since the 1920s (Bernard 1983).

Identification

Native littleneck clams (Figure 3.3) are round or slightly oval in profile. The outer surface of the valves is marked with both radial and concentric sculpture. The valves may be white, brown, or white marked with angular (chevron) patterns of brown. The lunule is present, but the dorsal margins of the valves are raised into a sharp ridge, not depressed to form a pocket as in Manila clams. The interior surface of the valve is white, and lacks the purple marking found in Manila clams. The interior ventral margin of the valve is crenulated (has numerous small fine teeth), not smooth as in Manila clams (Quayle 1960; Quayle and Bourne 1972; Abbott 1974; Kozloff 1974; Harbo 1997).

Distribution and habitat

Littleneck clams are distributed from the Aleutian Islands to Cabo San Lucas, Baja California Sur (Chew and Ma 1987), but are generally abundant only north of Oregon. They are found in mixed gravel, sand and mud substrates, generally in the lower intertidal zone (Quayle and Bourne 1972). Although recorded from the intertidal to depths of 10 m (Bernard 1983) there are no significant subtidal populations of *P. staminea* in British Columbia (Quayle and Bourne 1972).

Life history

Littleneck clams spawn from April until October in British Columbia. Larvae are pelagic for approximately 3 weeks, and settle at a size of 260-280 μ (0.26-0.28 mm). Size at maturity is 22-35 mm (Quayle 1943), and maximum size (approximately 75 mm) is attained after approximately 10 years (Quayle and Bourne 1972). Maximum age in British Columbia is 14 years (Bourne 1987). Like Manila clams, the growth rate for littleneck clams varies among beaches, and among different areas on the same beach. Under optimal conditions, littleneck clams can reach legal size (38 mm) in 3-4 years in the Strait of Georgia (Quayle and Bourne 1972).

Butter clam (*Saxidomus gigantea* [Deshayes, 1839])

Butter clams were the target species of the commercial clam fishery in British Columbia until a market shift to steamer clams (Manilas and littlenecks) in the late 1970s (Bourne 1986; Gillespie *et al.* 1998b). The common name Washington butterclam was used by Coan and Scott (1997) to distinguish the northern *S. gigantea* from it's southern congenor *S. nuttalli* Conrad, 1837, the California butterclam. Butter clams have also been referred to under the scientific names *Venerupis gigantea* Deshayes, 1839, *Venus maximus* Philippi, 1846, and *Saxidomus giganteus brevis* Dall, 1916.

Identification

Butter clams (Figure 3.4) are large; maximum size is approximately 130 mm. The valves are heavy and solid, oval in profile in young animals becoming square in profile with age. The valves are marked with strong concentric sculpture and deep winter checks. The valves are yellow in very young animals, becoming white with age, although they are often stained grey or black by the substrate. The internal surface of the valve is white and smooth, but not glossy. The external hinge ligament is large and prominent, as are the umbones. In older clams the posterior edges of the valves do not close completely, exposing the siphon tips in the gape (Quayle 1960; Quayle and Bourne 1972; Abbott 1974; Kozloff 1974; Harbo 1997).

Distribution and habitat

Butter clams are found from the Aleutian Islands to California (Quayle 1960). They generally inhabit the lower intertidal zone, but have been found subtidally to approximately 40 m (Bernard 1983). They generally live in more stable substrates than Manila and littleneck clams, usually sand, broken shell and small gravel. As adults, butter clams inhabit more or less permanent burrows up to 30 cm in depth.

Life history

Butter clams spawn from April to October in British Columbia (Quayle and Bourne 1972). The sexes are separate. Size at maturity in British Columbia is 33-43 mm (Quayle and Bourne 1972). Age of recruitment to legal size (63 mm) is 4.5-5 years in Barkley Sound, 5-6.5 years in the Strait of Georgia, 6.5-8.5 years in Alert Bay and 9 years in Prince Rupert (Quayle and Bourne 1972).

Summary

The commercially important species of intertidal clams are Manila, littleneck and butter clams. Manila clams, which are not native to British Columbia, are found primarily in the upper third of the intertidal zone, while littleneck clams are found below the half-tide level. Butter clams are found primarily in the lower third of the intertidal zone. Manila and littleneck clams are relatively short-lived and fast-growing, while butter clams live longer, grow more slowly, and grow larger (Table 3.2). Manila clams begin spawning later in the summer than the other species, and all spawning is complete by September. All have larvae which are pelagic for at least three weeks before settling in suitable habitat, where they remain for the rest of their lives.

Table 3.1 Features used to identify Manila, littleneck and butter clams.

Species	Sculpture	Lunule	Ventral Margin	Internal Shell Color	Siphons
Manila	radial and concentric	present, flat or depressed	smooth	glossy white or yellow with purple stain	split at tip
Littleneck	radial and concentric	present, with raised ridge	rough, crenulated	glossy white	fused
Butter	concentric only	absent	smooth	white, not glossy	fused

Table 3.2 Life histories, habitat and demography of British Columbia intertidal hardshell clams.

	Manila clam	Littleneck clam	Butter clam
Scientific name	*Venerupis philippinarum*	*Protothaca staminea*	*Saxidomus gigantea*
Max. size (mm)	75	75	130
Max. age (y)	14	14	20
Legal size (mm)	38	38	63
Age of recruitment (y)	3.0-3.5	3.5-4.0	5.0-9.0
Growth	rapid for first 4 y, slow after	rapid for first 4 y, slow after	rapid for first 5 y, slow after
Size at maturity (mm)	20-25	22-35	33-43
Age at maturity (y)	1.0-2.0	1.5-2.5	3.0-5.0
Fecundity	~2,500,000	NA	NA
Spawning season	June-September	April-September	April-September
Larvae	pelagic	pelagic	pelagic
Larval period	3 wk	3 wk	3-4 wk
Size at settlement (mm)	NA	0.26-0.28	NA
Habitat	gravel/mud/sand	gravel/mud/sand	sand/shell/mud
Depth	upper to middle third of intertidal	middle intertidal to 10 m	lower intertidal to 20 m
Geographic distribution	central BC to California	Alaska to California	Alaska to California
Natural mortality rate[1]	0.328	0.328	0.283
Recruitment	high - variable	variable	low - variable

[1] estimated using Hoenig's (1983) equation (see Gillespie *et al.* 1998b).

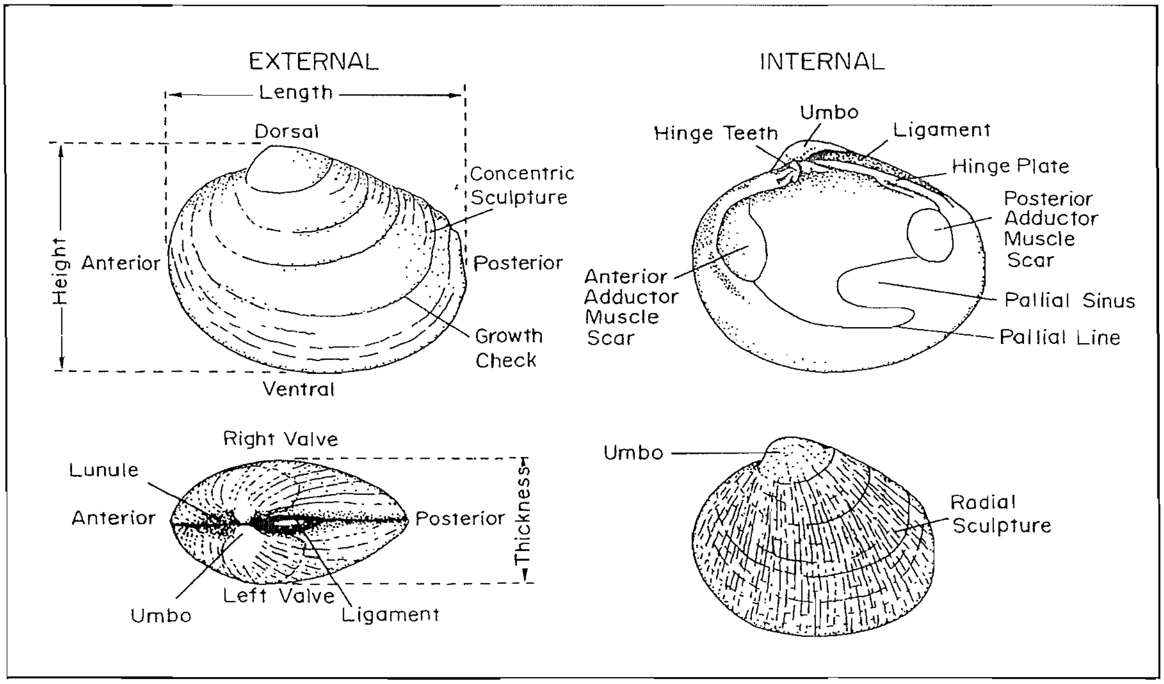

Figure 3.1 Major anatomical features of intertidal clams and the linear measurements of length, height, and thickness. Redrawn from Quayle and Bourne (1972).

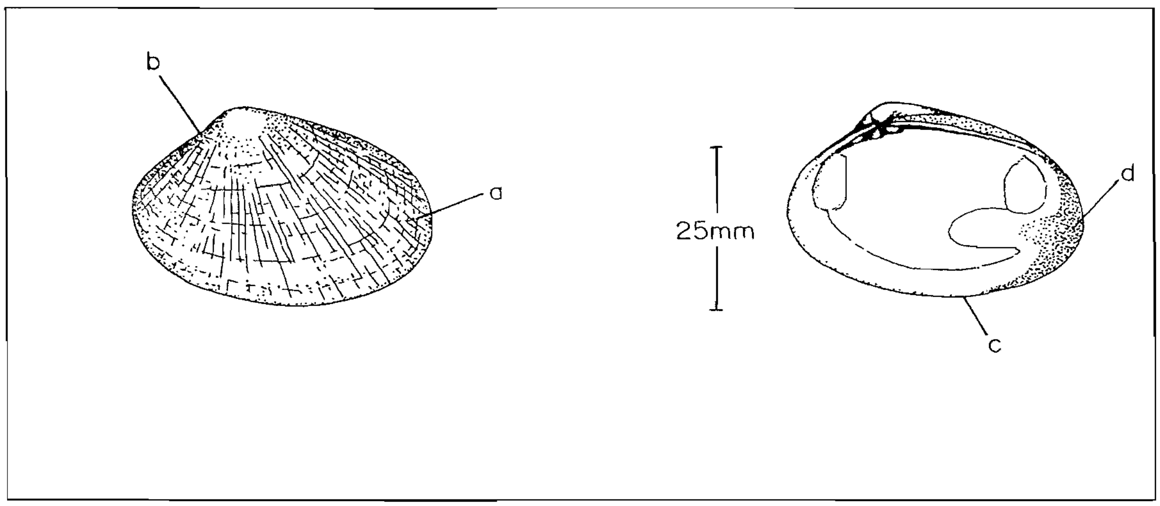

Figure 3.2 Identification features of Manila clams. Manila clams possess both radial and concentric sculpture (a), the shell margin in the area of the lunule is flat or depressed to form a pit (b), the ventral margin of the shell is smooth (c) and there is a purple stain at the posterior end of the inside of the shell (d). Redrawn from Keen (1963).

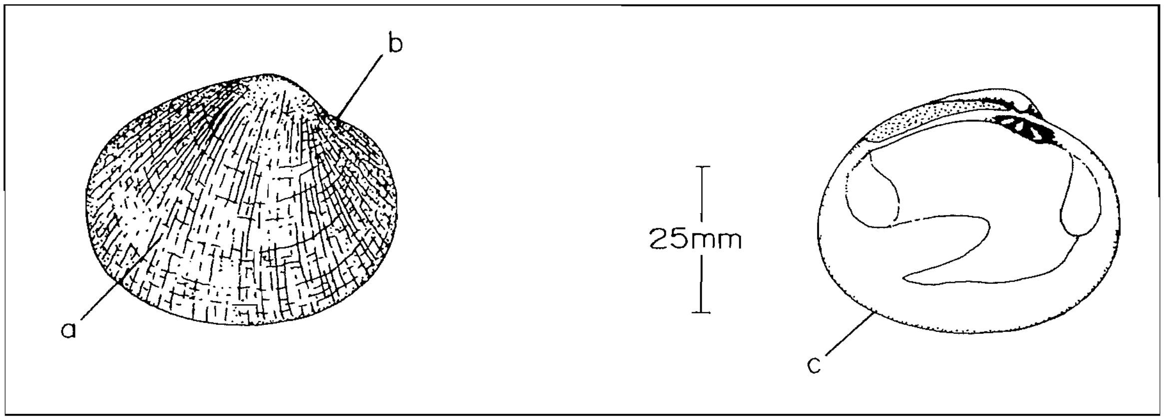

Figure 3.3 Identification features of littleneck clams. Littleneck clams possess both radial and concentric sculpture (a), the shell margin in the area of the lunule is raised to form a ridge (b) and the ventral margin of the shell is crenulated (c). Redrawn from Keen (1963).

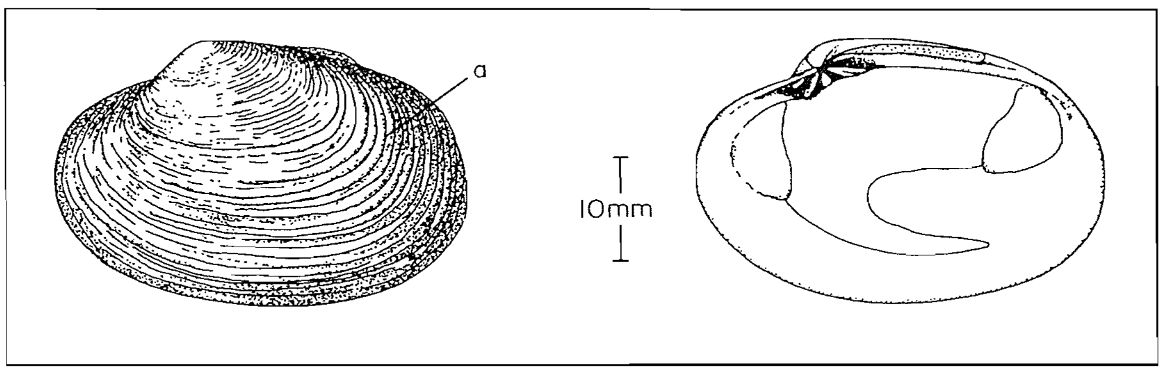

Figure 3.4 Identification features of butter clams. Butter clams possess only concentric sculpture (a). Redrawn from Keen (1963).

Chapter 4 - Probability Sampling

This chapter reviews concepts in probability sampling theory. These concepts are the basis of all survey designs described in this manual.

What you will learn

- what is meant by "probability sampling";
- what is randomization, and why it is important;
- how to randomize;
- assumptions of probability sampling;
- how the distribution of clams affects sampling estimates;
- how sample size affects the sampling estimates.

In this chapter, we use computer simulations to show how a population of clams might be spread over a beach. A computer is used to "sample" the simulated population of clams to mimic the process of actually conducting a survey. This allows key concepts to be demonstrated and provides data for example calculations.

For more information

Readers interested in more information on sampling theory are referred to excellent books by Thompson (1992) and Shaeffer *et al.* (1979). A standard, but statistically intense, treatment is provided by Cochran (1977).

What is probability sampling?

Surveys of intertidal clams usually involve digging at specified locations on a beach. Clams removed from each location are counted and weighed by species and size category. Since clams are generally hidden beneath the substrate to some degree, the digging is costly both in terms of time and money. Furthermore, the beach area is usually extremely large compared to the number of locations that can be sampled. The problems facing the surveyor are threefold:

1. What is the best strategy for deciding where to dig?
2. How many locations on the beach should be dug?
3. How can the total number or total weight of animals in the entire survey area be estimated based only on data from the few sampled locations?

One solution to these problems is called **survey sampling**, or **probability sampling.** The basic idea of sampling is to observe some part of a population in order to estimate "something" about the entire population. For example, we might be interested in determining the average number of clams per square meter on a beach. Since the entire beach is too large to excavate every clam, one approach might be to divide the beach into square plots and select some of the plots for digging. The "population" to be sampled in this case is the entire collection of square plots. If we select some of the plots for digging in such a way that the chance of choosing each plot is known, then the resulting set of plots is called a **probability sample**. The plots are often called **sampling units** or **quadrats** (Elliot 1977). An extremely important concept to grasp is that the sampling unit is a quadrat, since quadrats are randomly selected, not the individual clams. The resulting sample consists of all the clams excavated from the selected quadrat. One or more **variables** are measured for each unit in the sample, *e.g.* the number of clams or the weight of clams in a quadrat. Additional variables such as substrate type or depth below the high tide line might also be recorded for each quadrat. These data are attributes of the quadrats, rather than attributes of clams. A variable is sometimes called a **y-value**. The basic sampling method is illustrated graphically in Example 4.1 using simulated data.

Example 4.1 Simple random sampling.

> *The survey region shown in* Figure 4.1 *is divided into 100 distinct squares. Each square is called a* ***quadrat****. The locations of 500 "clams" in the survey region are indicated by the "+" symbols, although the locations of all clams on a beach is never known in practice. The objective of the survey is to estimate the average density of clams in the entire region by counting the clams in a sample of quadrats. Since there are 100 possible quadrats, the average number of clams per quadrat is 500/100=5. The total of 500 and average density of 5 clams per quadrat are the true, although unknown, values for the population.*
>
> *A sample of ten quadrats (hatched squares) was selected at random using a computer program. The sample data consist of a count of the number of clams from each quadrat in the sample (*Table 4.1*).*

The sample of ten counts can to be used to estimate the average density in the population and, remarkably, a measure of the certainty of the estimate. The data resulting from the 10 quadrats "sampled" in Figure 4.1 are shown in Table 4.1. The average number of clams per quadrat in the sample is 5.1, which compares well with the true value of 5 clams per quadrat. The average can also be used to estimate the total number of clams in the population by multiplying by the number of quadrats in the survey area. Thus, the number of clams in the population is estimated to be 100(5.1)=510, a number fairly close to the actual population size of 500.

As stated above, the objective of sampling is to deduce something about the entire population by measuring only a subset of the population. The "something" is called a population **parameter** such as the **mean** number of clams per quadrat or the total weight of clams on the beach. The true values of these parameters are unknown, since we cannot dig out every clam on the beach. By taking a sample, the data can be used to calculate an **estimate** of the population mean or total.

Table 4.1 also contains a column called "Quadrat Label". These numbers are used uniquely identify each quadrat. The labels were assigned by numbering the quadrats in Figure 4.1 from lower left beginning with "1" and proceeding by rows to the upper right quadrat labeled "100". The table also contains the *x* and *y* coordinates of the lower left corner of each quadrat in the sample.

Notice that the sample estimates of the population average and total do not match the true values exactly. The difference is called **sampling error** and occurs because only part of the population of 100 quadrats is included in the sample. If we were to select a different set of ten quadrats for a sample, a different estimate of the average and total would result.

To illustrate this point, Figure 4.2 shows the results of estimating the average density of clams from 50 different samples of 10 quadrats. The vertical solid line represents the true mean density of 5 clams per quadrat. The dots indicate the mean calculated for each of the 50 samples. The estimates tend to agree with the population value of 5 clams/quadrat, although a few are badly wrong. The horizontal lines represent a measure of the uncertainty of the averages called a **confidence interval**. The interpretation of the confidence interval can be confusing, but basically the wider the confidence interval, the more uncertainty associated with an estimate. For the confidence intervals shown in Figure 4.2, the interval should overlap the true value of the mean 95% of the time on repeated sampling. Note that the *x*-axis of the figure has been deliberately set wider than necessary to allow comparison with similar figures shown later.

What is randomization?

The sample shown in Figure 4.1 is said to be selected at random **without replacement**. Furthermore, the probability of selecting each quadrat is known. The term "without replacement" simply means that a quadrat can be included in the sample one time only. In this section, several equivalent methods for selecting a random sample without replacement are described.

Consider the simulated "clam beach" shown in Figure 4.1 . A sample could be selected by labeling 100 pieces of paper with the numbers 1 through 100, mixing the papers in a container, and picking 10 pieces of paper from the container without looking. The pieces of paper are not returned to the container after each selection. Each piece of paper has the same chance of being selected and all possible samples of size 10 have the same chance of selection. In practice, we would not actually use pieces of paper since a clam beach may involve thousands of possible quadrats. The basic randomization procedure for selecting a sample of size 10 from 100 possible quadrats can be summarized as follows.

What to do

1. Assign labels to all quadrats, *e.g.* the numbers from 1 to 100;
2. Generate a number between 1 and 100 at random and include the corresponding quadrat in the sample;

3. Repeat step (2) a total of 10 times. If a number previously drawn is selected ignore it and draw again.

More generally, the total number of sampling units in the population is denoted by N, and the sample size by n.

How to select a random sample

A random sample can be selected from a population using a table of random numbers or by using a computer program called a random number generator. A portion of a random number table is shown in Table 4.2. Random number tables can usually be found in statistical textbooks (*e.g.* Snedecor and Cochran 1980). The use of a random numbers table is illustrated in Example 4.2.

Example 4.2 Random selection without replacement:

> *Suppose there are N=100 units in the population and a random sample of size of n=10 units is required. In this situation, the 100 sampling units can be labeled with the numbers 1,2,3,...,99, 00, with 00 representing 100. A set of random numbers can be obtained by picking a starting point and reading two columns of numbers in any direction. For example, the second row of the third column in* Table 4.2 *was used as the starting point for the 10 random numbers {61, 24, 86, 98, 15, 10, 6, 93, 76, 31} obtained by reading down the table. The number 24 occurs twice in the sequence of numbers between the starting point and the final number 31. The second occurrence of 24 was ignored.*

Generally, if n units are to be selected at random from a population of N units, then label the units from 0 to N-1, with 0 representing the N-th unit. Since multiple occurrences of numbers in the random numbers table are ignored, the same unit cannot be selected more than once. This procedure is called random sampling without replacement. Many statistical packages and spreadsheets have the ability to generate series of random numbers between 1 and N either without replacement or **with replacement**.

When selecting a random sample of quadrats, it is not necessary to actually list the label and location of each quadrat. An equivalent way to choose quadrats at random is to generate the coordinates of quadrats at random. The lower left corner of each of the quadrats in Figure 4.1 can be represented by an x and y distance. For example, the coordinates of each of the sampled quadrats are listed in Table 4.1. The method used to select these coordinates is described in Example 4.3. The practical utility of this approach will become more apparent when selecting quadrat locations in two-stage designs.

What to do

1. Determine the number of quadrats X along the x-axis of the survey area;
2. Choose n numbers at random between 0 and X-1;

3. Determine the number of quadrats Y along the y-axis of the survey area;
4. Choose n numbers at random between 0 and Y-1;
5. Pair the two sets of numbers to make n pairs of coordinates for quadrats.

Example 4.3 Selecting pairs of coordinates at random.

> *There are 10 quadrats along each axis of the survey area shown in* Figure 4.1 . *For the x-coordinates, select 10 numbers* ***with replacement*** *from 0,1,2,...,9 using a random number table. For example, the set of numbers {3,4,4,8,2,0,9,2,5,6} was chosen for the sample shown in the figure. Repeat the procedure for the y-coordinates. The set of y-coordinates {9,4,1,4,0,6,8,9,3,8} was chosen for the sample. Pair the two sets of 10 numbers without changing their order. Discard any duplicate pairs and choose additional x and y coordinates from the random number table, if required.*

Living with aggregations

Clams are not evenly distributed over the intertidal zone; their distribution tends to be clustered or patchy depending the substrate and tidal exposure, among other factors. Aggregations of clams can be readily observed on sandy beaches by looking for clusters of siphon holes. The clam beach shown in Figure 4.1 was simulated so that clams are uniformly distributed over the survey area. For this situation, random sampling worked well. However, as the degree of clustering among the clams increases, the variability among quadrat counts and weights also increases. This results in wider confidence intervals for the mean and total and less certainty for management decisions.

Fortunately, probability sampling can still be used when the clams are aggregated. For example, Figure 4.3 shows a simulated clam beach where the clams are mildly clustered. We can compare how well random sampling works for this simulated population of 500 clams with the results obtained from sampling the beach in Figure 4.1 .

Example 4.4 shows the results of the random sample shown in Figure 4.3. While the estimated mean and total for this particular sample do not differ markedly from the results in Example 4.1, the best comparison is made by taking many samples using a computer. The average clam density and 95% confidence intervals computed for 50 different samples of 10 quadrats are plotted in Figure 4.4. The confidence intervals tend to be wider than those in Figure 4.2; there is more uncertainty about the estimates.

Example 4.4 Sampling a moderately aggregated population.

> *The simulated clam population shown in* Figure 4.3 *exhibits a mild amount of clumping. The shaded squares indicate a simple random sample of n=10 quadrats from the population. In this case the estimated average is 4.8 clams per quadrat and the estimated total is therefore (100)(4.8)=480 clams.*

The problem of wider confidence intervals gets worse as the clams become more aggregated. A moderate degree of clumping is shown in Figure 4.5, again for a population of 500 clams. Here the random sample indicates an average density of 4.0 clams per quadrat (the estimate of the total is 400 clams). However, the confidence intervals shown for 50 different samples from the simulated data in Figure 4.5 are extremely wide (Figure 4.6), often plus or minus the estimate of the average.

The problem of increasing variability can reduced by increasing the number of quadrats in the sample. For example, Figure 4.7 shows the average clam density and confidence intervals for 50 different samples of size n=30 from the simulated clam beach shown in Figure 4.5. The width of the confidence intervals is much narrower than those shown in Figure 4.6 for 50 samples of size n=10.

Why use randomization?*

Our current preference is to use probability sampling to obtain estimates of abundance and biomass for intertidal clam populations. Randomization provides a fair and repeatable means of avoiding bias in the selection of sampling locations, whether accidental or intentional. This advantage is particularly desirable where the data are likely to be used by parties with conflicting interests; fishery managers, commercial fishers, and First Nations biologists may use the data or estimates for disparate purposes. Furthermore, the collection of data following a probability sampling scheme does not preclude the use of alternative methods of data analysis. However, data collected using an *ad hoc* or haphazard scheme cannot be analyzed using probability sampling methods. Thus, desirable statistical properties of probability samples are lost.

Surveys conducted in the past by DFO and various third party surveys have used a systematic grid or a purposive sample in the sense of Kish (1965). Surveys of this type can be analysed using model-based approaches, but are subject to bias problems related to the selection of the sample.

A systematic sample, or even a systematic random sample (Shaeffer *et al.* 1979) has been a frequent choice for past surveys. In most cases, these samples were analysed as though a simple random sample had been selected. The Washington State Department of Fisheries survey protocol (Campbell 1996) employs a systematic random sample in two dimensions analysed as a simple random sample. We have avoided this approach for several reasons:

- no unbiased variance estimator is defined for systematic random sampling;
- statisticians have not developed variance estimators for general application to two-dimensional systematic sampling.
- the variance estimator for systematic random sampling is known to be biased downwards, producing an overly optimistic estimate, when assumptions of complete spatial randomness are violated (Kish 1965);
- a uniform grid may not be practical for beaches with elongated shape, uneven topography, or erratic shorelines.

We hasten to add that systematic sampling can be applied providing it is not used at the first stage of randomization in a multi-stage design. Where systematic random sampling is used in surveys, it should be applied at the last stage of a multi-stage sampling design. For example, the two-stage designs presented in Chapter 14 could by extended to a third stage by including a systematic random sample at the final stage to space quadrats out. However, this scheme is beyond the scope of material described in this manual.

Various investigators have used a so-called purposive sample, *i.e.* using "expert opinion" to judge where samples should be placed. The resulting data has usually been analysed by computing a simple mean and 95% confidence interval for the mean based on assuming a normal distribution (Snedecor and Cochran 1980, p. 66). The flaws in this approach include:

- there is no guarantee of unbiased selection of the sample;
- the estimators of the mean and variance are derived from the properties of an underlying normal error distribution, which may not apply;
- the construction of a confidence interval assumes that the quadrat counts or weights follow a normal distribution;
- experts may disagree on what is the "best" sample.

Bias in sample selection may occur in either direction, depending on the objectives of the surveyor. The protection provided by randomization does not apply in the case of systematic or purposive sampling. It is unlikely that counts of clams per quadrat, or weights, will be normally distributed due to the frequent occurrence of zeros and occasional occurrence of high values. By comparison, estimates of the mean or total, and their variances, derived from probability samples do not require the assumption of normality.

The behavior of the confidence interval under violations of the normality assumption is similar for both probability sampling and for alternative approaches that assume normality. If the data are not normally distributed, then a 95% percent confidence interval will contain the true population value far less often than expected. In other words, the confidence interval will be too narrow, suggesting an overly optimistic level of precision in the survey estimates. However, there are alternative methods of constructing confidence intervals from probability samples that do not require the normality assumption (*e.g.* Efron and Tibshirani 1993; Rao and Wu 1988; Sitter 1992).

Assumptions in sampling*

The assumptions of probability sampling, as discussed in this manual, are that:

- the entire population can be divided into a known finite number of units such that each unit can be labeled with numbers $\{1,2,\ldots,N\}$;
- the units included in the sample are selected with known probability corresponding to a specified sampling design;
- the y-value for each sampling unit is a fixed quantity, not a random variable;

- the y-values in the sample are measured without error, that is, they are known exactly.

Estimates of population parameters and corresponding standard errors can be computed without assuming any underlying statistical distribution. Thus, probability sampling is basically a non-parametric estimation method. For example, it is not true that a normal distribution must be assumed to estimate a population mean and standard error.

However, inferences about a population parameter made using a confidence interval *may require* assuming a statistical distribution. A common method of computing confidence intervals from sampling data assumes that the estimator of a statistic is approximately normal. Alternative methods, such as bootstrap techniques (Efron and Tibshirani 1993), do not require a statistical distribution to be assumed, but are beyond the scope of this manual.

Probability sampling is complemented by model-based approaches where data are collected by some strategy and a fit to the data is sought via an iterative modeling process. For example, a regression model may describe the relationship between clam density and distance from the high tide line. In spatial sampling situations, such as those for intertidal clams, correlation among values of clam density at different sites may influence the choice of sampling design, estimation method, and shape of the sampling unit. Although the uncertainty of population estimates can often be reduced by using a model-based approach, these methods usually require rather sophisticated analytical procedures and a detailed examination of model adequacy for each case. Furthermore, the model may reduce the robustness of the analysis by introducing assumptions which are not exactly true. An ideal approach uses models to suggest efficient procedures in conjunction with a sampling design to protect against bias (Thompson 1992).

Statistical notation

Let a set of data be represented by the observations $y_1, y_2, \ldots, y_n$. The "..." is a convenient way to indicate that measurements exist between the second and the last number in the data set. Another way to represent the numbers in a data set is to use an index for the subscript label. For example, the expression $y_i, i = 1,2,\ldots,n$ means $y_1, y_2, \ldots, y_n$.

Summation of the numbers in a data set is indicated by the capital Greek letter sigma (Σ) as follows:

$$\sum_{i=1}^{n} y_i = y_1 + y_2 + \ldots + y_n \quad .$$

The sum of the squares of the numbers in a data set appears as follows:

$$\sum_{i=1}^{n} y_i^2 = y_1^2 + y_2^2 + \ldots + y_n^2 \quad .$$

This sum of squares is distinct from the square of the sum which appears as follows:

$$\left(\sum_{i=1}^{n} y_i\right)^2 = (y_1 + y_2 + \ldots + y_n)^2 \ .$$

These three forms of summation are used in every sampling estimator presented in this manual. For example, the computing formula for the sample variance of a set of data takes the form:

$$s^2 = \frac{\sum_{i=1}^{n} y_i^2 - \left(\sum_{i=1}^{n} y_i\right)^2 \Big/ n}{n-1} \ .$$

Example 4.5 Summation operator.

Suppose the data set $y_1 = 23, y_2 = 45, y_3 = 7, y_4 = 12, y_5 = 3$ *represents the number of clams found in n=5 quadrats. Each number has a unique subscript label, e.g. the second number in the data set is* $y_2 = 45$. *The sum of the numbers in the data set is*

$$\sum_{i=1}^{5} y_i = 23 + 45 + 7 + 12 + 3 = 90 \ .$$

The sum of squared numbers is

$$\sum_{i=1}^{5} y_i^2 = (23)(23)+(45)(45)+(7)(7)+(12)(12)+(3)(3)=2756.$$

The square of the sum is

$$\left(\sum_{i=1}^{5} y_i\right)^2 = (23+45+7+12+3)^2=(90)(90)=8100.$$

Sometimes there may be multiple subscripts for a y-value, for example, $y_{ij}, i = 1,2,\ldots,I$ and $j = 1,2,\ldots,J$. Dot notation is used to indicate summation over a particular index, for example

$$y_{i\bullet} = \sum_{j=1}^{J} y_{ij} \ .$$

indicates summation of the j index.

The Greek letters mu, sigma, and tau (μ, σ, τ) are used in this manual. They represent means, standard deviations, and totals, respectively.

Samples are used to compute **estimates** of population **parameters**. For example, the mean number of clams per quadrat in a sample is an estimate of the true mean density. The formula used to compute the mean is called an **estimator**. A "bar" over a letter indicates an average as in $\bar{y}$. A "hat" indicates that the symbol is an estimator of the parameter. For example, the symbol $\hat{\tau}$ represents an estimator of τ. The calculated numerical value is the estimate.

Table 4.1 Data from the random sample of the clam beach in Figure 4.1 .

Observation	Quadrat Label	(x,y) Coordinate	Number of clams
1	94	(3, 9)	6
2	45	(4, 4)	3
3	15	(4, 1)	4
4	49	(8, 4)	7
5	3	(2, 0)	8
6	61	(0, 6)	6
7	90	(9, 8)	7
8	93	(2, 9)	3
9	36	(5, 3)	3
10	87	(6, 8)	4

Table 4.2 Selecting 10 numbers from 100 using a random numbers table.

289760	014940	908084	911403	692220
993124	257196	2692**61**	582197	617162
006015	524715	0966**24**	589309	876352
109345	596016	6094**86**	479906	964260
726365	009794	733624	047233	997891
057261	709775	5985**98**	979615	509723
158140	574013	5070**15**	730059	439321
097109	125540	8690**10**	931134	607419
362531	806417	5061**06**	934780	824292
652844	913558	0009**93**	356459	313881
841367	629582	0892**76**	689227	341128
075043	349477	0648**31**	222091	759599
940573	627080	349061	962092	404677
140428	000636	685010	091937	830381
871194	987659	534297	070377	537713
471189	692810	537668	650742	628164
855535	098537	613600	674795	123399
995481	421642	089282	381219	803815
441963	335025	583463	044463	145468
902253	753027	177409	177691	734113

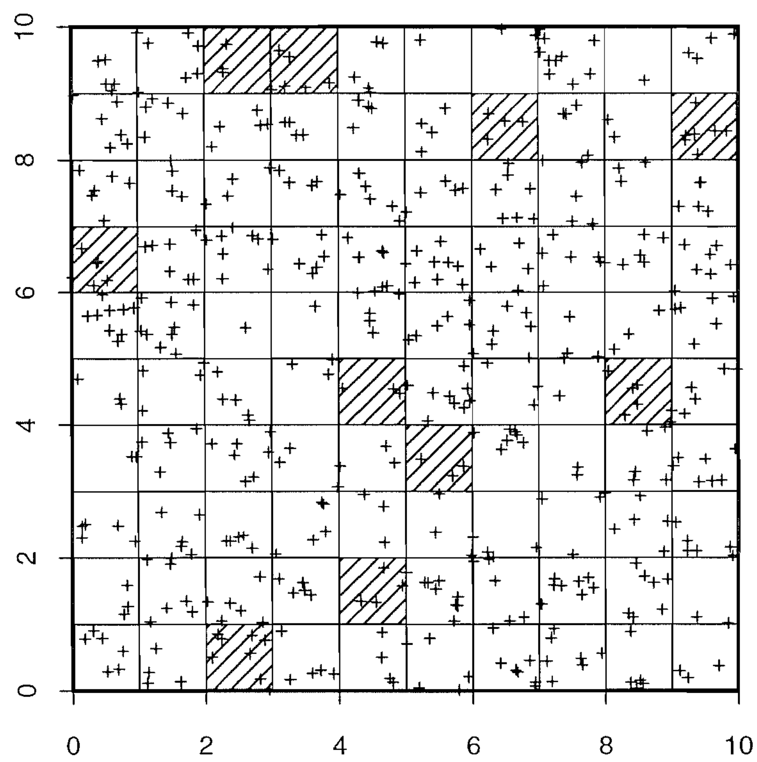

Figure 4.1 A simple random sample of a simulated clam beach. The "+" symbols indicate the positions of individual clams. Quadrats selected at random for inclusion in the sample are hatched.

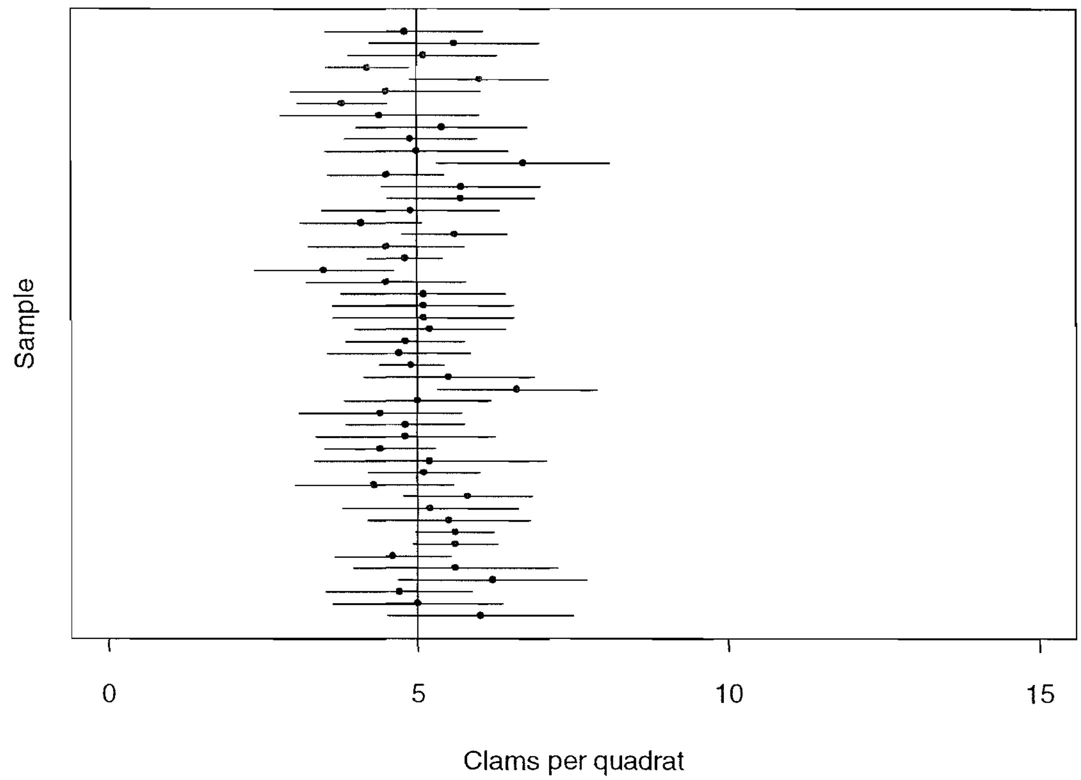

Figure 4.2 Estimates of the average density (dots) of clams and 95% confidence intervals (horizontal lines) from 50 repeated samples of the clam distribution shown in Figure 4.1. The true average is 5 clams per quadrat (vertical line).

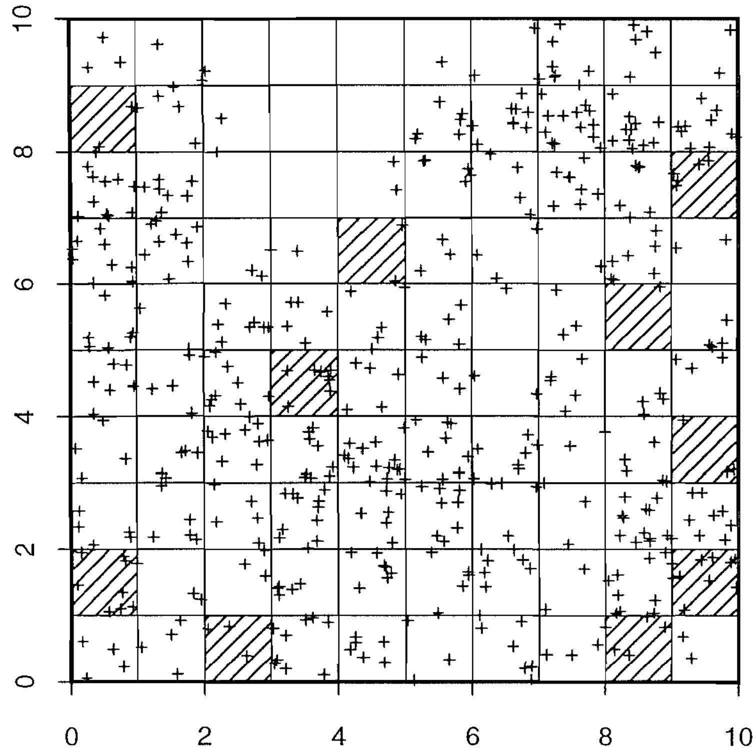

Figure 4.3 Simple random sampling from a clam beach showing mild clumping. The "+" symbols indicate the positions of individual clams. Quadrats selected for the sample are hatched.

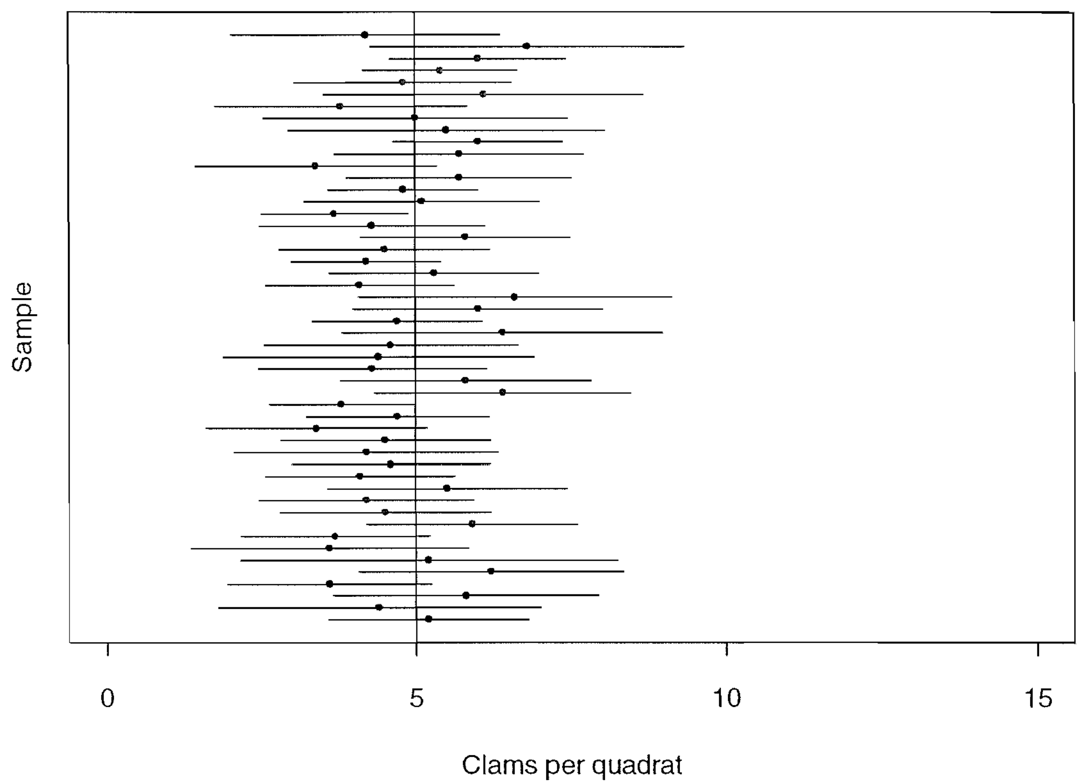

Figure 4.4 Estimated average density of clams (dots) and 95% confidence intervals (horizontal lines) resulting from 50 repeated samples of size n=10 from the clam beach shown in Figure 4.3. The true value is 5 clams per quadrat (vertical line).

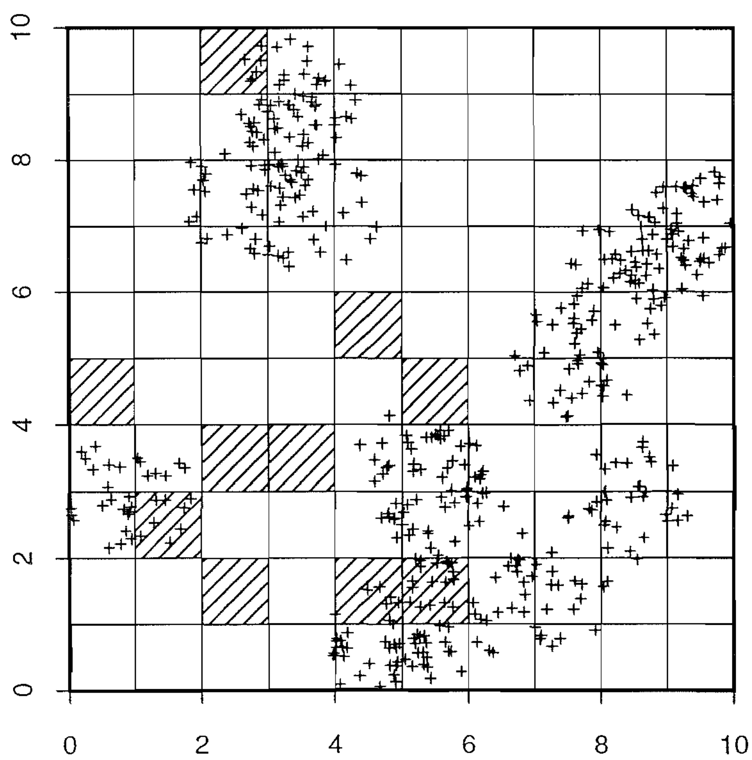

Figure 4.5 Simple random sampling from a clam beach showing a moderate degree of clumping. The "+" symbols indicate the positions of individual clams. Quadrats selected for the sample are hatched.

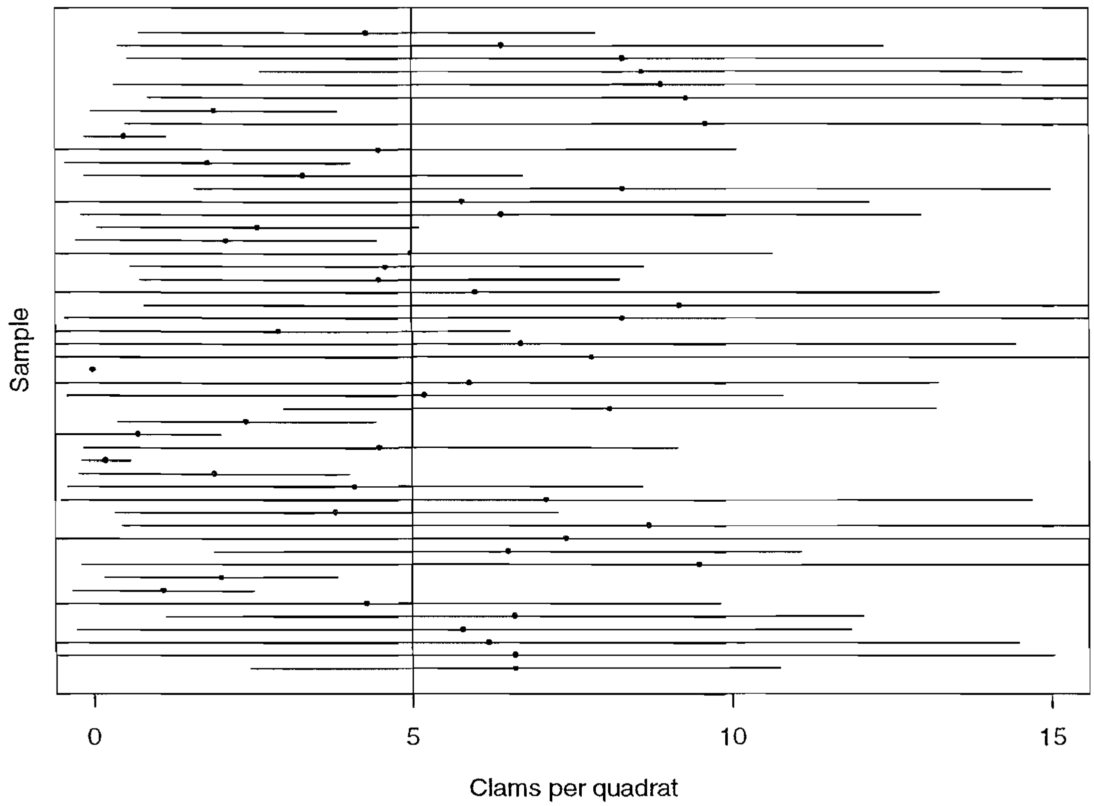

Figure 4.6 Estimates of the average density (dots) and 95% confidence limits (horizontal lines) resulting from 50 repeated samples of size n=10 from the clam beach shown in Figure 4.5. The true average is 5 clams per quadrat (vertical line).

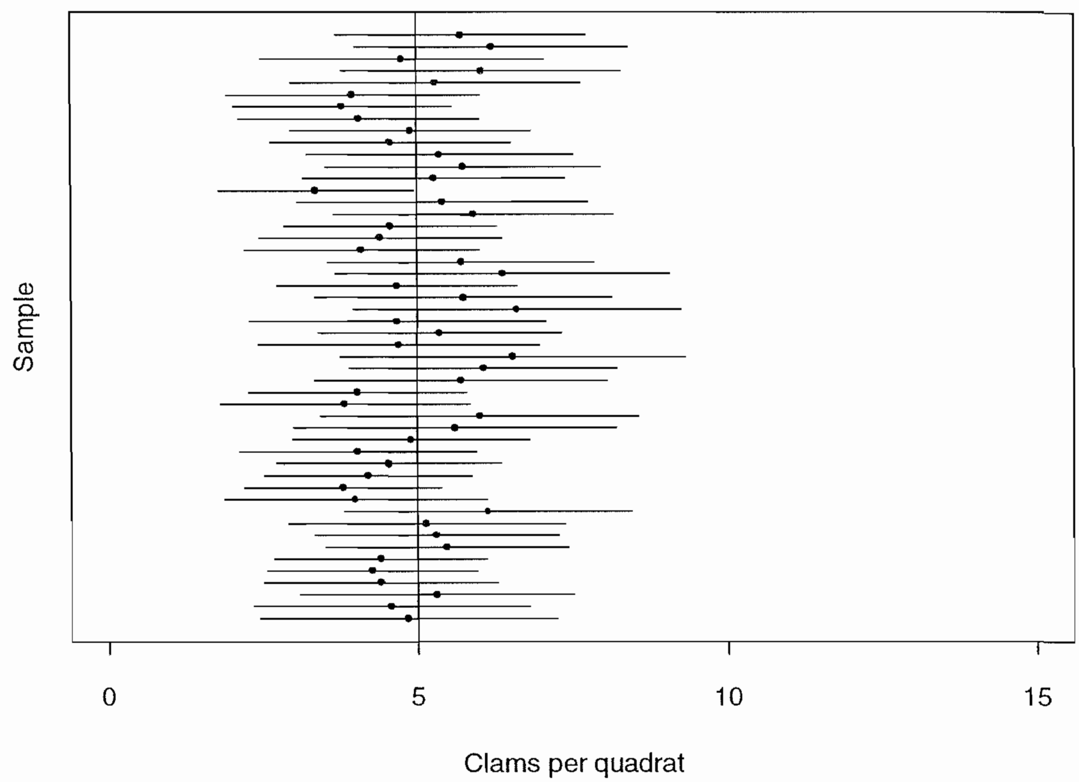

Figure 4.7 Estimates of the average density (dots) and 95% confidence limits (horizontal lines) resulting from 50 repeated samples of size n=30 from the clam beach shown in Figure 4.5. The true average is 5 clams per quadrat (vertical line).

Chapter 5 - Simple Random Sampling

Simple random sampling is the basic building block for a wide variety of sampling designs. More complicated designs usually involve simple random sampling (SRS) at some stage of their construction. We suggest that this chapter be read carefully prior to the chapters on the stratified random design and two-stage designs.

What you will learn

- how to select a simple random sample;
- estimating the population mean and variance;
- estimating the population total and variance;
- construction of confidence intervals.

It is the randomization procedure that determines the survey design and analysis; the sample data alone cannot be used to identify the sampling design. For each sampling design in this manual, a formal definition is supplied that describes how the design is randomized.

> ***Definition 1:*** *A simple random sample is a survey design where **n** distinct units are selected from the N units in the population such that every possible sample of **n** units has the same probability of selection.*

A diagram of a simple random sample is shown in Figure 5.1. For this example, a population of 400 squares, or sampling units, was simulated using a computer. A simple random sample of n=20 units (shaded squares) was selected from the total population of N=400 possible units.

The notation for simple random sampling is summarized in Table 5.1. This chapter assumes that you have read Chapter 4 and are familiar with the terminology introduced for probability sampling.

Selecting a simple random sample

Simple random sampling is the only design where every possible sample of n units from N has the same probability of being selected. In practice, we use a random number table or a computer program to generate a set of n units to be included in the sample. Also, this type of simple random sample is said to be selected **without replacement** since a particular sampling unit cannot be selected more than once.

What to do

1. Determine the number of distinct sampling units, N, in the population;
2. Assign each sampling unit a number in the sequence $1,2,...,N$;
3. Choose the required number of sampling units, n, such that each unit has the same chance of selection.

Alternately, the procedure described previously for selecting random pairs of coordinates could be used.

The distribution of clams ('+' symbols) shown in Figure 5.2 was simulated using a computer. A simple random sample of size n=40 was selected without replacement from N=400 possible quadrats. The sample data are listed in Table 5.2.

Estimating the population mean

One objective of a survey is to estimate the average, or mean density of clams in the survey area using only the data from the sample. The equations required to compute the mean and the estimated variance of the mean are shown in Table 5.3. The variance of an estimate measures the degree of closeness of the observations. If the observations are similar, the variance is small. Regardless of survey design, the **standard error (SE)** of an estimate is the square root of the variance of that estimate. The standard error of the estimate is used in the calculation of a **confidence interval (CI)**. The confidence interval is a commonly used measure of uncertainty associated with an estimate.

Example 5.1 Simple random sampling estimates of the population mean.

For the data listed in Table 5.2, *the mean and variance can be estimated using the equations in* Table 5.3. *The sample mean* $\bar{y}$ *is the average of the y-values in the sample:*

$$\begin{aligned}\bar{y} &= \frac{1}{n}\sum_{i=1}^{n} y_i = \frac{1}{n}(y_1 + y_2 \ldots + y_n) \\ &= \frac{1}{40}(2 + 5 + \ldots + 2) \\ &= 2.85\end{aligned}$$

Thus, there is an average of 2.85 clams per quadrat.

The estimated variance of the mean is more complicated. First compute the sample variance as follows:

$$s^2 = \frac{\sum_{i=1}^{n} y_i^2 - \left(\sum_{i=1}^{n} y_i\right)^2 \Big/ n}{n-1}$$

$$= \frac{1}{39}\left[658 - \frac{114^2}{40}\right] = \frac{1}{39}[658 - 324.9]$$

$$= 8.541$$

Then, the estimated variance of the mean is given by:

$$\hat{V}(\bar{y}) = \left(\frac{N-n}{N}\right)\frac{s^2}{n}$$

$$= \left(\frac{400-40}{400}\right)\frac{8.541}{40}$$

$$= 0.192$$

Note that the units of the variance estimate are clams per quadrat squared since the units of the sample variance s^2 is in terms of clams per quadrat squared.

Finally, the standard error is given by:

$$SE(\bar{y}) = \sqrt{\hat{V}(\bar{y})} = \sqrt{0.192} = 0.438$$

Estimating the population total

An estimate of the total number or total weight of clams in the survey area can be obtained by estimating the population total, τ. The equations are similar to the estimators for the population mean; the estimator of the total is N times the estimated mean, while the estimator of the variance of the total is N^2 times the estimator for the variance of the mean (Table 5.4).

Example 5.2 Simple random sampling estimates of the population total.

For the data listed in Table 5.2, *the total and variance of the total can be estimated using the equations in* Table 5.4. *The estimate of the total is simply the sample mean $\bar{y}$ times the number of sampling units N:*

$$\hat{\tau} = N\bar{y}$$

$$= (400)(2.85)$$

$$= 1140$$

The estimated variance of the total is more complicated, but can be computed using the variance of the population mean:

$$\hat{V}(\hat{\tau}) = N^2\left(\frac{N-n}{N}\right)\frac{s^2}{n}$$
$$= (400)^2(0.192)$$
$$= 76.869$$

Note that the units of the sample variance are in clams squared.

Confidence intervals*

Estimates of the population mean or total are statistics whose numeric values vary from sample to sample. For example, if the same beach were sampled on 10 different occasions, then 10 different estimates of the mean density of clams would be obtained. Hopefully the 10 sample means would be similar since they are all estimates of the same fixed, though unknown, population parameter. One way to assess the uncertainty in an estimate from a single sample is to construct a **confidence interval**.

A confidence interval uses the sample data to define the endpoints of an interval that has a high probability of containing the value of the population parameter. The level of probability is determined by the **confidence coefficient** symbolized by 1-α. When α=0.05, for example, the confidence coefficient is 0.95. The confidence interval is called a 100(1-α)% or 95% confidence interval in this case.

One method of computing a confidence interval is to assume that the estimate (the mean or total) is normally distributed. For small samples less than n=50, approximate confidence intervals can be computed using the t-distribution and the equations in Table 5.5. When the sample size is large, the t-value can be replaced by the upper $\alpha/2$ point of the standard normal distribution, z. Readers unfamiliar with the t-distribution and the standard normal distribution are referred to introductory statistics books *e.g.* Snedecor and Cochran (1980).

Example 5.3 Confidence intervals for the population mean and total.

The results from Example 5.1 *can be used to compute a 95% confidence interval for the population mean. Recall that α=0.05 for a 100(1-0.05)=95% confidence interval. The interval for the population mean can be computed as follows:*

$$\bar{y} \pm t_{0.025,9}\sqrt{\left(\frac{N-n}{n}\right)\frac{s^2}{n}}$$
$$2.85 \pm (2.023)\sqrt{0.192}$$
$$2.85 \pm 0.887$$

Thus, the bounds of a 95% confidence interval for the population mean are given by (1.96, 3.74) clams per quadrat.

The 95% confidence interval for the population total is similarly computed as follows:

$$\hat{\tau} = \pm t_{0.025,9}\sqrt{N(N-n)\frac{s^2}{n}}$$

$$1140 \pm (2.023)\sqrt{400(400-40)\frac{8.541}{40}}$$

$$1140 \pm 354.679$$

Thus, the bounds of a 95% confidence interval for the population total are (785,1495) clams.

Table 5.1 Notation for simple random sampling.

Symbol	Description
i	the y-value index
N	total number of sampling units (quadrats) in the population
n	number of units (quadrats) in the sample, or sample size
y_i	the variable or y-value (*e.g.* number of clams, weight of clams)
μ	the true population mean
τ	the true population total
$\bar{y}$	the estimated population mean
$\hat{V}(\bar{y})$	the estimated variance of the population mean
$\hat{\tau}$	the estimated population total
$\hat{V}(\hat{\tau})$	the estimated variance of the population total
s^2	the sample variance

Table 5.2 Data from the simple random sample shown in Figure 5.2

Observation	Quadrat Label	Coordinates	Clam Count	Observation	Quadrat Label	Coordinates	Clam Count
1	237	[16,11]	2	21	325	[4,16]	0
2	320	[19,15]	5	22	308	[7,15]	0
3	205	[4,10]	4	23	387	[6,19]	1
4	224	[3,11]	3	24	215	[14,10]	5
5	316	[15,15]	8	25	220	[19,10]	3
6	40	[19,1]	0	26	37	[16,1]	2
7	243	[2,12]	2	27	17	[16,0]	2
8	14	[13,0]	4	28	327	[6,16]	0
9	90	[9,4]	6	29	45	[4,2]	0
10	271	[10,13]	0	30	285	[4,14]	0
11	213	[12,10]	3	31	240	[19,11]	2
12	12	[11,0]	2	32	158	[17,7]	9
13	357	[16,17]	4	33	115	[14,5]	5
14	385	[4,19]	0	34	196	[15,9]	1
15	377	[16,18]	2	35	108	[7,5]	4
16	123	[2,6]	9	36	276	[15,13]	1
17	95	[14,4]	3	37	284	[3,14]	0
18	10	[7,0]	0	38	174	[13,8]	8
19	143	[6,7]	0	39	347	[6,17]	1
20	155	[14,7]	11	40	113	[12,5]	2

Table 5.3 Simple random sampling estimators for the population mean.

Estimator of the population mean:

$$\bar{y} = \frac{1}{n}\sum_{i=1}^{n} y_i$$

Estimator of the variance of the mean:

$$\hat{V}(\bar{y}) = \left(\frac{N-n}{N}\right)\frac{s^2}{n}$$

where

$$s^2 = \frac{\sum_{i=1}^{n}(y_i - \bar{y})^2}{n-1} = \frac{\sum_{i=1}^{n} y_i^2 - \left(\sum_{i=1}^{n} y_i\right)^2 \Big/ n}{n-1}$$

Table 5.4 Simple random sampling estimators for the population total.

Estimator of the population total:

$$\hat{\tau} = N\bar{y} = \frac{N}{n}\sum_{i=1}^{n} y_i$$

Estimator of the variance of the total:

$$\hat{V}(\hat{\tau}) = \hat{V}(N\bar{y}) = N^2\left(\frac{N-n}{N}\right)\frac{s^2}{n}$$

where

$$s^2 = \frac{\sum_{i=1}^{n}(y_i - \bar{y})^2}{n-1} = \frac{\sum_{i=1}^{n} y_i^2 - \left(\sum_{i=1}^{n} y_i\right)^2 \Big/ n}{n-1}$$

Table 5.5 Confidence intervals for simple random sampling.

Approximate 100(1-α)% confidence interval for the population mean:

$$\bar{y} \pm t_{\alpha/2,n-1}\sqrt{\left(\frac{N-n}{N}\right)\frac{s^2}{n}}$$

Approximate 100(1-α)% confidence interval for the population total:

$$\hat{\tau} \pm t_{\alpha/2,n-1}\sqrt{N(N-n)\frac{s^2}{n}}$$

where t is the upper $\alpha/2$ point of Student's t distribution with n-1 degrees of freedom.

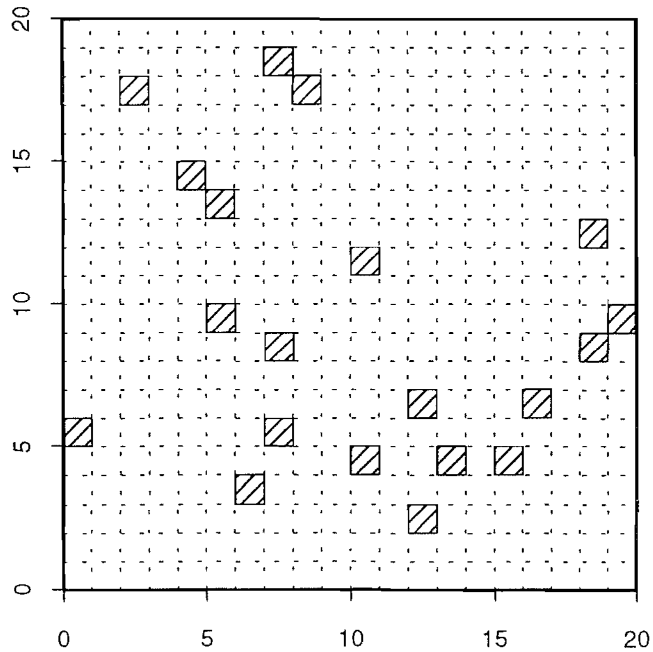

Figure 5.1 A simple random sample of 20 units from a population of 400 units.

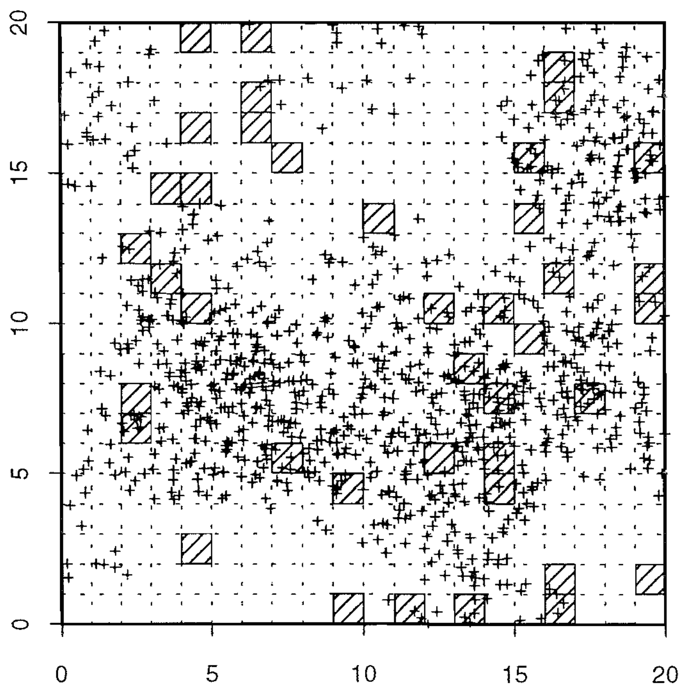

Figure 5.2 A simple random sample of n=40 quadrats from N=400 possible quadrats. The positions of 1000 clams are indicated by the '+' symbols.

Chapter 6 - Case Study 1

This case study reports the results of a clam survey on a small beach at Dunsmuir Islands as a prerequisite of proposed depuration harvests. For brevity, this case study presents the results of the survey and population estimates for Manila clams only. Only a subset of the Manila clam data is presented. Reports for actual surveys would also include population estimates of littleneck clams and presentation of biological information. Complete documentation of this survey is in Gillespie *et al.* (1998a).

Personnel and Contacts

The survey was designed and supervized by G. Gillespie (DFO PBS) and samples collected by G. Gillespie, G. Parker (DFO PBS) and A. Coronica (ICM Unique Seafoods).

Site Description

The Dunsmuir Islands are 2 small islands near the mouth of Ladysmith Harbour, southwest of Coffin Point (48°59.6'N, 123°47.3'W). The survey area (Figure 6.1) was on the northeastern shore of the islands, opposite Sibell Bay. The survey area was a triangular bank with a low ridge that ran perpendicular to shore. The substrate was mixed sand/gravel with the highest proportion of sand on the ridge and progressively more gravel towards the sides. The beach sloped gradually from the high tide mark, which was delineated by a rock wall. Manila and littleneck clams inhabited the top and slopes of the bank and butter clams became more plentiful near the low tide line. Butter clams were also present on a low bank to the south of the survey area.

Survey Design and Randomization

The survey consisted of a single triangular stratum, with a reference line of 150 m and a height of 75 m, for a total area of 5,625 m^2. The DFO protocol requires 17 samples be taken for a beach of this area, but a survey done in April 1994 (consisting of 8 samples) indicated high variability, so the beach was purposely sampled at a higher rate.

Quadrats were selected using simple random sampling. Quadrat locations were determined by first selecting from the available number of sampling units on the reference line (x-coordinate), then selecting from the available number of sampling units perpendicular to the reference line (y-coordinates). Random numbers were selected between 0 (for the lower left-hand corner of the first unit) and 299 (for the lower left-hand corner of the last unit) for the x-coordinates. Y-coordinates that fell outside the survey area were discarded during randomization, and selection continued until 25 pairs of coordinates were chosen (Table 6.1).

Field Results

The survey was completed on July 7, 1994. Low tide was 0.6 m at 1045 PDT. Twenty-five quadrats were dug and returned to the Pacific Biological Station for processing. All samples were processed the same day, while the clams were still alive (not frozen).

Biomass and Abundance Estimates

Summary statistics and stock estimates for Manila clams are in Table 6.2 and Table 6.3. Mean weights of Manila clams were 27.28 g for legal-size and 7.49 g for sublegal size. Mean weights for littlenecks were 36.71 g for legal-size and 10.06 g for sublegal-size.

The survey provided a good estimate of legal Manila stock size, reasonable estimates of littleneck stocks, and a poor estimate of sublegal Manila stock size. Confidence intervals were approximately 30% of the estimated totals for legal abundance and biomass, but consideraly wider for sublegal Manila abundance (77%) and biomass (104%). This variance was due to one quadrat (1-5) which had 135 sublegal Manila clams weighing 1,408 g. The next highest value was for quadrat 1-10, which had 33 sublegal Manilas weighing 197 g.

Biological Data

Due to the small number of samples taken in this survey, all clams collected were processed for length, weight and age. [Note: The raw data and resulting tables and figures are not included in this case study for brevity. A completed report would have figures of length and age frequency distributions.]

Original Data

Examples of the original data sheets for number and weight data and biological data are in Chapter 10. Examples of electronic data in spreadsheet formats are in Chapter 11. [Note: Original data sheets and EXCEL files would accompany the submitted report.]

Table 6.1 Quadrat location and number and weight data for Manila clams from the 1994 Dunsmuir Islands clam survey.

Quadrat	x (m)	y (m)	Quad. Size (m^2)	Species	# Legals	# Sublegals	Wt Legals (g)	Wt Sublegals (g)
1	2.0	1.0	0.25	82B	22	0	753	0
2	8.5	1.5	0.25	82B	29	3	717	35
3	12.0	5.0	0.25	82B	40	7	1255	51
....	....	....	....	....	....	....	....	....
24	132.0	1.5	0.25	82B	25	0	676	0
25	139.0	2.5	0.25	82B	14	0	486	0

Table 6.2 Density and total abundance estimates of Manila clams from the 1994 Dunsmuir Islands clam survey.

Legal Density (#/m^2)	SE	Sublegal Density (#/m^2)	SE	Total Legals ('000's)	95% CL ('000's)	Total Sublegals ('000's)	95% CL ('000's)
93.76	14.36	57.28	22.00	527.4	(365.8; 689.0)	322.2	(74.7; 569.7)

Table 6.3 Density and total biomass estimates of Manila clams from the 1994 Dunsmuir Islands clam survey.

Legal Density (kg/m^2)	SE	Sublegal Density (kg/m^2)	SE	Total Legals (kg)	95% CL (kg)	Total Sublegals (kg)	95% CL (kg)
2.558	0.380	0.429	0.222	14,388	(10,111; 18,665)	2,413	(-88; 4,914)

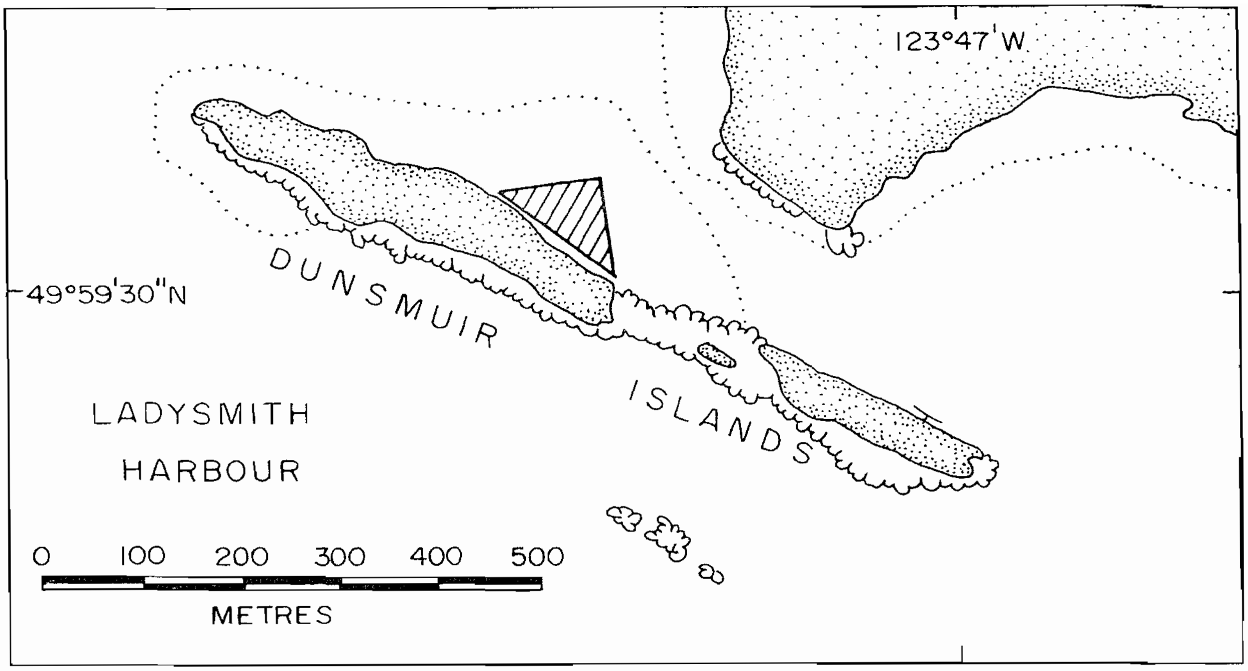

Figure 6.1 Layout of the 1994 Dunsmuir Islands clam survey.

Chapter 7 - Stratified Random Sampling

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

Definition 2: *A stratified random sample is a survey design where the sampling units are divided into* ***H*** *non-overlapping groups, called strata, and a simple random sample is drawn from each group.*

For example, a population of 400 units with 4 strata is shown in Figure 7.1. The sizes of the strata are $N_1 = 200, N_2 = 100, N_3 = 50,$ and $N_4 = 50$. Within each stratum, the shaded squares represent a simple random sample (see Chapter 5) selected without replacement so that $n_1 = 20, n_2 = 10, n_3 = 5,$ and $n_4 = 5$. Thus, the total sample size is n=20+10+5+5=40.

Advantages of stratification

- variance estimates of the mean or total may be more precise when compared with the variances obtained from simple random sampling;
- the cost of conducting the survey may be less than with simple random sampling because quadrats within a stratum are in close proximity;
- since randomization occurs independently within each stratum, corruption of the survey design due to problems experienced in the field may be confined to one stratum.

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 clam density should be partitioned into a stratum to separate it from areas of the beach with low densities of clams. Thus, one stratum may differ markedly from another, but the within stratum variability would be small.

What you will learn

- how to select a stratified random sample;
- estimating the population mean and total;
- computing confidence intervals for a stratified random sample;
- determining whether stratification was worthwhile.

Learning the material covered in this chapter requires that you have read and understood the material presented in Chapter 4 and Chapter 5. Statistical notation required for this chapter is contained in Table 7.1.

Selecting a stratified random sample

What to do

1. Determine the number of distinct sampling units $N_h = N_1, N_2, \ldots, N_H$ in each stratum;
2. Select a simple random sample of size n_h **independently** from each stratum, $h=1,2...,H$.

Example 7.1 A stratified random sample.

The simulated population of clams in Figure 5.2 *has been reproduced in* Figure 7.2. *Rather than choosing a simple random sample from the population, strata have been constructed to group the sampling units into three areas of similar clam densities. A stratified random sample of size* $n_1 = 20, n_2 = 15, n_3 = 5$ *was selected to yield the data in* Table 7.2.

Estimating the population mean

One objective of a survey is to estimate the mean density of clams in the survey area. The equations required to compute the mean and the estimated variance of the mean are shown in Table 7.3.

Example 7.2 How to compute the stratified mean and variance.

For the data simulated in Figure 7.2, *the mean and variance can be estimated using the equations in* Table 7.3. *The sample mean* $\bar{y}_{st}$ *is the weighted sum of the averages within each stratum:*

$$\begin{aligned}\bar{y}_{st} &= \frac{1}{N}\left[N_1\bar{y}_1 + N_2\bar{y}_2 + N_3\bar{y}_3\right] \\ &= \frac{1}{400}\left[(200)(3.3) + (150)(14.733) + (50)(15.0)\right] \\ &= 2.7\end{aligned}$$

Then, the estimated variance of the stratified mean is given by:

$$\hat{V}(\bar{y}_{st}) = \frac{1}{N^2}\sum_{h=1}^{3} N_h^2 \left(\frac{N_h - n_h}{N_h}\right)\frac{s_h^{\ 2}}{n_h}$$

$$= \frac{1}{400^2}\left[200^2\left(\frac{200-20}{200}\right)\frac{5.91}{20} + \ldots + 50^2\left(\frac{50-5}{5}\right)\frac{9.0}{5}\right]$$

$$= 0.125$$

Finally, the standard error of the stratified mean is given by:

$$SE(\bar{y}_{st}) = \sqrt{\hat{V}(\bar{y}_{st})} = \sqrt{0.125} = 0.354$$

Estimating the population total

An estimate of the total number or weight of clams in the survey area can be obtained by estimating the population total, τ. The equations are similar to the estimators for the population mean; the estimator of the total is N times the estimated mean, while the estimator of the variance of the total is N^2 times the estimator for the variance of the mean (Table 7.4).

Example 7.3 How to compute the stratified total and variance.

For the data listed in Table 7.2, *the total and variance of the total can be estimated using the equations in* Table 7.4. *The estimate of the total is simply the sample mean* $\bar{y}_{st}$ *times the number of sampling units N:*

$$\hat{\tau}_{st} = N\bar{y}_{st}$$

$$= (400)(2.7)$$

$$= 1080$$

The estimated variance of the total is more complicated, but can be computed using the variance of the population mean:

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

$$= (400)^2 (0.125)$$

$$= 19979.66$$

Note that the units of the variance are clams squared.

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 as noted in Table 7.5. When sample sizes are small (as a rule of thumb, less than 30 in each stratum) an adjustment to the degrees of freedom for the t-statistic is appropriate. The adjustment is called Satterthwaite's approximation (Satterthwaite 1946):

$$d = \left(\sum_{h=1}^{H} a_h s_h^2 \right)^2 \Bigg/ \left[\sum_{h=1}^{H} \left(a_h s_h^2\right)^2 \Big/ (n_h - 1) \right]$$

where

$$a_h = N_h (N_h - n_h) / n_h \quad .$$

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

Example 7.4 How to calculate Satterthwaite's approximation.

For the data shown in Table 7.2, the sample variances within each stratum are $s_1^2 = 5.905, s_2^2 = 9.352$ and $s_3^2 = 9.0$. *Satterthwaite's approximation is given by:*

$$d = \frac{\left[1800(5.905) + 1350(9.352) + 450(9.0)\right]^2}{\left[1800(5.905)\right]^2/19 + \left[1350(9.352)\right]^2/14 + \left[450(9.0)\right]^2/4}$$

$$= \frac{27305.19^2}{21433574}$$

$$= 34.785$$

The t-value for the confidence interval corresponding to a significance level of 0.95 with 35 degrees of freedom is 2.031.

Example 7.5 Computing confidence intervals for the stratified mean and total.

The results from Example 7.2 *and* Example 7.4 *can be used to compute a 95% confidence interval for the population mean. Recall that* α*=0.05 for a 100(1-0.05)=95% confidence interval. The interval for the population mean can be computed as follows:*

$$\bar{y}_{st} \pm t_{0.025,35}\sqrt{\hat{V}(\bar{y}_{st})}$$
$$2.7 \pm (2.031)\sqrt{0.125}$$
$$2.7 \pm 0.718$$

Thus, the bounds of a 95% confidence interval for the population mean are given by (1.98, 3.42) clams per quadrat.

The 95% confidence interval for the population total is similarly computed as follows:

$$\hat{\tau}_{st} = \pm t_{0.025,35}\sqrt{\hat{V}(\hat{\tau}_{st})}$$
$$1080 \pm (2.031)\sqrt{19979.66}$$
$$1080 \pm 287.018$$

Thus, the bounds of a 95% confidence interval for the population total are (793, 1367) clams.

For more information

An alternative to assuming the normal distribution is to use resampling techniques to compute a non-parametric estimate of the confidence interval. This method is described by Rao and Wu (1988), Sitter (1992), and Gillespie *et al.* (1998a) 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. The sample size within each stratum is given by the following equation:

$$n_h = \frac{nN_h}{N}, \qquad h = 1, \ldots, H.$$

The sample sizes for Example 7.1 were assigned using proportional allocation of a fixed sample size of n=40 quadrats. Other allocation methods that minimize variance for a fixed total sample size (optimal allocation) exist, but require prior survey data (Thompson 1992). Proportional allocation should be used unless DFO staff or a consulting statistician recommend alternatives.

Gain in precision from stratification*

One result of interest is to determine whether the stratification was successful in increasing precision when compared to a simple random sample. Based on the sample data, the problem is to compare the variance from the stratified estimator of the mean to the variance that would have resulted *had a simple random sample been selected*:

$$V_{srs} = \frac{(N-n)}{n(N-1)}\left[\frac{1}{N}\sum_{h=1}^{H}\frac{N_h}{n_h}\sum_{i=1}^{n_h} y_{hi}^2 - \bar{y}_{st} + \hat{V}(\bar{y}_{st})\right].$$

When this rather complex formula is computed, the ratio of the variance of the estimate of the mean from the stratified random sample can be compared to the estimate from a simple random sample of the total sample size to determine the **design effect**:

$$\text{design effect} = \frac{\hat{V}(\bar{y}_{st})}{V_{srs}}.$$

If the ratio is less than 1, then the stratification was successful in reducing the uncertainty of estimates, *i.e.* the survey precision increased due to stratification. For the example data listed in Table 7.2, the design effect is 0.125/0.236=0.529. Thus, stratification reduced the estimated variance by about 50% relative to a simple random sample.

Table 7.1 Notation required for stratified random sampling.

Symbol	Description
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 clams, weight of clams)
μ	the population mean
τ	the population total
$\bar{y}_{st}$	the estimated population mean
$\hat{V}(\bar{y}_{st})$	the estimated variance of the population mean
$\hat{\tau}_{st}$	the estimated population total
$\hat{V}(\hat{\tau}_{st})$	the estimated variance of the population total
s_h^2	the sample variance in stratum h

Table 7.2 Sample data from the stratified random sample in **Figure 7.2**. Observations are labeled with the stratum number and a quadrat number.

Observation	Quadrat Label	Clam Count	Observation	Quadrat Label	Clam Count
1-1	22	2	2-1	241	2
1-2	72	3	2-2	361	0
1-3	20	0	2-3	394	3
1-4	92	1	2-4	246	2
1-5	67	4	2-5	333	5
1-6	3	1	2-6	290	1
1-7	78	1	2-7	269	8
1-8	52	5	2-8	212	2
1-9	60	0	2-9	354	3
1-10	158	1	2-10	348	9
1-11	6	2	2-11	261	4
1-12	32	0	2-12	208	6
1-13	132	1	2-13	346	1
1-14	81	0	2-14	387	5
1-15	101	0	2-15	285	3
1-16	106	8	3-1	340	1
1-17	70	3	3-2	377	6
1-18	53	2	3-3	337	6
1-19	146	4	3-4	317	2
1-20	161	0	3-5	218	1

Table 7.3 Estimators for the population mean of a stratified random sample.

Estimator of the population mean:

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

Estimator of the variance of the mean:

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

where

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

Table 7.4 Estimators for the population total of a stratified random sample.

Estimator of the population total:

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

Estimator of the variance of the total:

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

where

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

Table 7.5 Confidence intervals for stratified random sampling.

Approximate 100(1-α)% confidence interval for the population mean:

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

Approximate 100(1-α)% confidence interval for the population total:

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

where t is the upper α/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 $z_{\alpha/2}$.

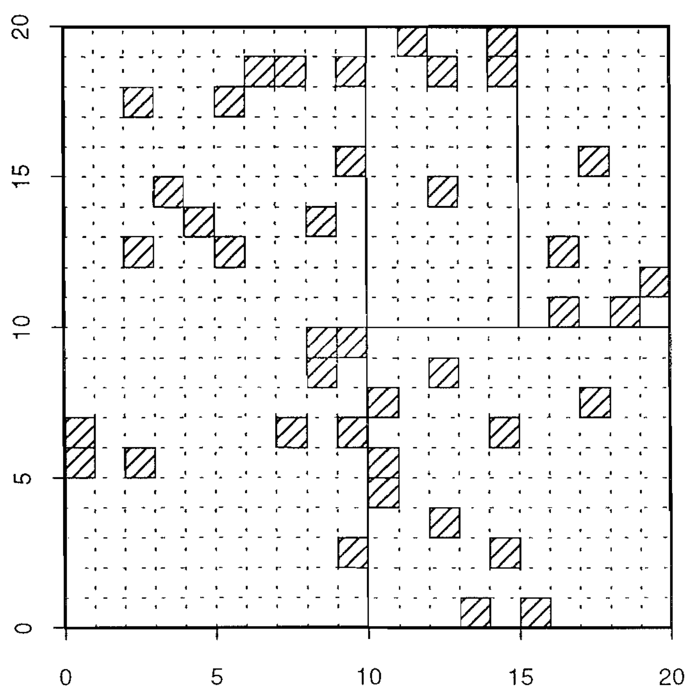

Figure 7.1 A stratified random sample from a population with four strata.

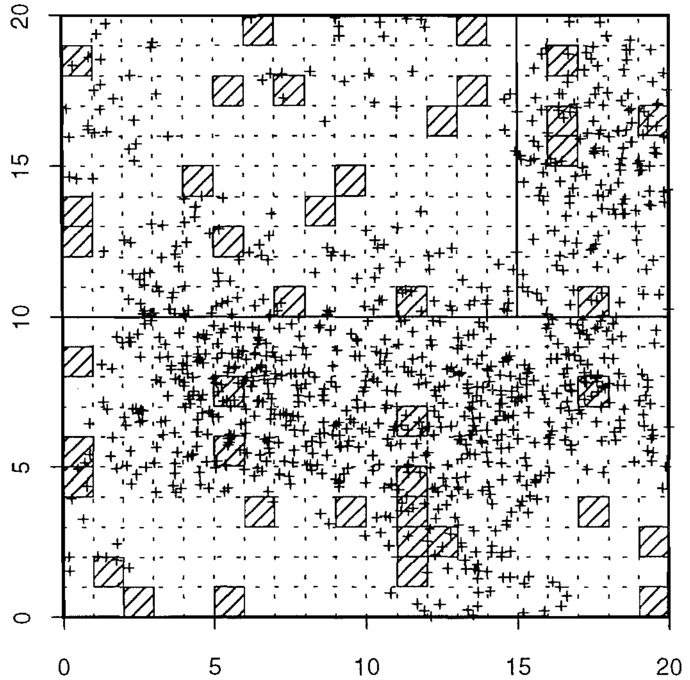

Figure 7.2 A stratified random sample with three strata. The positions of 1000 clams are indicated by the "+" symbols.

Chapter 8 - Case Study 2

This case study reports the results of a clam survey on the Goldstream Estuary as a prerequisite for proposed depuration harvests. For brevity, this case study presents the results of the survey and population estimates for Manila clams only. Reports for actual surveys would also include population estimates of littleneck clams, presentation of biological information and electronic and hard copies of original number and weight and biological data sheets. A full report of this survey is in Gillespie *et al.* (1998a).

Personnel and Contacts

The survey was designed and supervised by G. Gillespie (DFO PBS), and samples were collected by G. Parker, D. Heritage (DFO PBS) and E. Helgeson (Coopers Cove Oyster Farm).

Site Description

Goldstream Estuary lies at the head of Finlayson Arm, Saanich Inlet (48°29.7'N, 123°33.0'W). The harvest area is a large, gently sloping estuary cut by several active stream channels. The substrate is a thick layer of silt, except in and near the stream channels, where it is primarily gravel/sand. The harvest permit area extends from the overhead power lines adjacent to the mouth of Arbutus Creek to the southern tip of Sawluctus Island.

Survey Design and Randomization

The survey was a stratified random design of 5 strata (Figure 8.1) mapped over areas of harvestable abundance (clam beds). The stream channels were not surveyed, as these would not be accessible to diggers during high winter run-off. The total area surveyed was 3.84 ha (Table 8.1).

Quadrat locations were selected at random independently within each of the 5 survey strata. The southern boundary of each stratum was designated the reference line (see Chapter 9) for that stratum. Quadrat positions were established by randomly selecting pairs of coordinates, which correspond to distances along the reference line (x-coordinates) and distances from the reference line (y-coordinates).

Field Results

The survey was completed on August 4th and 5th, 1994. Low tides for these dates were 0.80 m at 0945 PDT and 0.72 m at 1015 PDT, respectively. Several quadrats were missed on the survey as they were flooded out before they could be dug, thus the sampling intensity was less than DFO protocols require (53 qudrats dug, 61 required under the protocol of 16/ha. used in

1994). The samples were returned to PBS, and processed for number and weight and biological data (Table 8.2).

Biomass and Abundance Estimates

Summary statistics and stock estimates for Manila clams are in Table 8.3 and Table 8.4. Mean weights of Manila clams were 25.96 g for legal-size and 5.41 g for sublegal size.

The survey provided good estimates of abundance and biomass of legal-size clams and biomass of sublegal-size clams, and reasonable estimates of sublegal abundance. Confidence interval ranged from 18-22% of the mean estimate for all categories except sublegal abundance of Manilas (33%).

Design effect values for estimates of Manila clam abundance and biomass are all less than 1.0 (Table 8.5). This indicates that precision of these estimates was increased by using a stratified design. [Note: Survey reports do not require calculation of design effect - it is used here to illustrate the benefits of stratification.]

Biological Data

Biological samples were chosen by randomly selecting quadrats from all available quadrats and processing all of the clams in each quadrat for length, weight and age. Quadrats were selected until a total of at least 500 Manila and littleneck clams were measured, and the final quadrat completed. [Note: The resulting frequency distributions are not included in this case study for brevity. Figures summarizing length and age frequency distributions would be included with actual survey report submissions.]

Original Data

[Note: Summaries of the original data are not included in this case study for brevity. Spreadsheet files and original data sheets would accompany survey report submissions.]

Table 8.1 Stratum areas (m^2), number of potential samples (N) and number of samples taken (n) for the 1994 Goldstream Estuary clam survey.

Stratum	Area (m^2)	N	n
1	10,000	40,000	16
2	4,500	18,000	6
3	8,900	35,600	13
4	5,000	20,000	7
5	10,000	40,000	11
Total	38,400	153,600	53

Table 8.2 Quadrat location and number and weight data for Manila clams from the 1994 Goldstream Estuary clam survey.

Quadrat	x (m)	y (m)	Quad. Size (m^2)	Species	# Legals	# Sublegals	Wt Legals (g)	Wt Sublegals (g)
1-1	2.0	83.5	0.25	82B	17	5	644	35
1-2	13.0	88.5	0.25	82B	11	0	311	0
1-3	15.0	7.5	0.25	82B	35	3	998	22
....	....	....	....	....	....	....	....	....
5-15	85.0	94.0	0.25	82B	5	1	195	5
5-16	99.0	62.5	0.25	82B	8	1	175	12

Table 8.3 Density and total abundance estimates of Manila clams from the 1994 Goldstream Estuary clam survey.

Stratum	Legal Density (#/m^2)	SE	Sublegal Density (#/m^2)	SE	Total Legals ('000's)	95% CL ('000's)	Total Sublegals ('000's)	95% CL ('000's)
1	109.00	19.83	28.50	5.49	1,090.0	(693.3; 1,486.7)	285.0	(175.3; 394.7)
2	21.33	5.33	19.33	8.48	96.0	(48.0; 144.0)	87.0	(10.7; 163.3)
3	119.08	19.88	64.31	13.16	1,059.8	(706.0; 1,413.6)	572.3	(338.1; 806.5)
4	86.86	23.28	32.57	9.77	434.2	(201.4; 667.0)	162.9	(65.2; 260.6)
5	85.46	11.03	22.18	15.75	854.5	(633.9; 1,075.1)	221.8	(-93.3; 536.9)
Total					3,534.6	(2,904.9; 4,164.3)	1,329.0	(891.1; 1,766.9)

Table 8.4 Density and total biomass estimates of Manila clams from the 1994 Goldstream Estuary clam survey.

Stratum	Legal Density (kg/m^2)	SE	Sublegal Density (kg/m^2)	SE	Total Legals (kg)	95% CL	Total Sublegals (kg)	95% CL
1	2.834	0.542	0.164	0.031	28,343	(17,494; 39,192)	1,638	(1,027; 2,249)
2	0.653	0.162	0.138	0.054	2,937	(1,479; 4,395)	621	(135; 1,107)
3	2.623	0.401	0.335	0.047	23,345	(16,209; 30,481)	2,982	(2,142; 3,822)
4	2.193	0.632	0.205	0.060	10,963	(4,642; 17,284)	1,026	(428; 1,624)
5	2.749	0.387	0.085	0.041	27,495	(19,763; 35,227)	855	(32; 1,678)
Total					93,082	(76,435; 109,729)	7,121	(5,568; 8,674)

Table 8.5 Design effect values for estimates of Manila clam abundance and biomass from the August 1994 Goldstream Estuary clam survey.

Estimated Value	Mean (per quadrat)	$\hat{V}(\bar{y}_{st})$ (per quadrat)	V_{srs} (per quadrat)	Design Effect
Legal Abundance	23.01	4.11	14.63	0.28
Legal Biomass (g)	606.00	2866.28	10234.02	0.28
Sublegal Abundance	8.65	1.92	3.22	0.60
Sublegal Biomass (g)	46.36	24.91	71.95	0.35

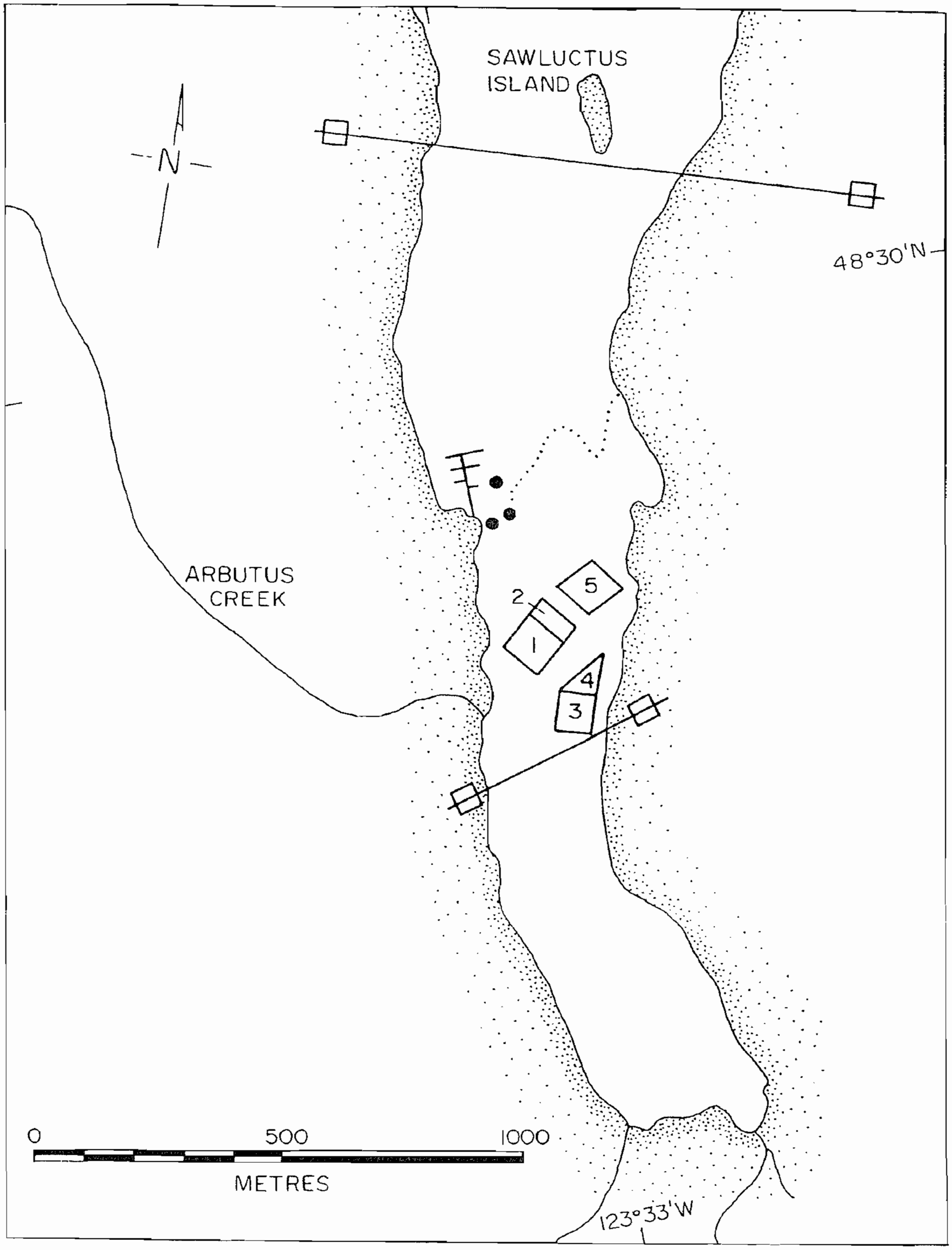

Figure 8.1 Layout for the 1994 Goldstream Estuary clam survey.

Chapter 9 - Field Methods

Field surveys have two components: (1) a pre-survey to obtain preliminary data to guide survey design, and (2) the main survey, in which samples are collected following the selected design.

What you will learn

- when to survey;
- how to do an exploratory pre-survey to delineate and measure the survey area;
- how to document important features of the survey area, such as stratum boundaries and reference line(s);
- how to determine quadrat locations on the beach;
- how to dig quadrats and gather samples;
- how to label and store samples for processing.

When to survey

Assessment surveys are to be conducted during daylight low tides of no more than 1.0 m above chart datum (generally April to early September in British Columbia). Low tide levels of 1.0 m above chart datum are sufficient to completely survey Manila clam populations, and allow access to most of the littleneck clam population on the beach. Surveys for butter clams should be planned for the lowest tide series available, since they live much lower on the beach than Manila or littleneck clams. Surveys during night tides are not supported because darkness and inclement weather:

- make it extremely difficult to accurately locate stratum boundaries and quadrats;
- increase the likelihood of missing clams in quadrats which biases survey estimates downwards; and
- can create dangerous working conditions travelling to and working on the beach.

Pre-survey

The first tasks in survey design are to understand the configuration of a beach and to get a sense of the general distribution of clams within the survey area. If a beach has been previously surveyed or recently harvested, there may be prior knowledge to assist survey design. If the beach is unfamiliar to the surveyors, a pre-survey should be conducted to:

- locate the limits of the clam population;
- identify general areas of high or low clam density;

- select **reference lines** from which to locate quadrats;
- map the survey area in detail.

Portions of the beach that harbour clams can be located by digging small test holes at intervals when walking up and down the intertidal zone and across the beach. Once clams have been located, markers can be placed at the limits of their distribution. It is important that the survey area encompass as much of the clam population as possible, excluding large areas of mud or rock which do not support clams. Surveyors should remember that clam populations are not static on a beach, and that areas of low density this year may contain higher densities in coming years due to settlement or shifting of substrate during storms. Ideally, the survey area should include all potential clam-bearing areas, regardless of the presence of commercial densities of clams.

On small beaches there may be little point to portioning the area into strata. On larger beaches, stratification is nearly always worthwhile, so try to identify portions of the beach which seem to contain very dense or very sparse concentrations of clams. Gross changes in substrate type or elevation may also suggest a likely stratification of the survey area. Keep in mind that it is most convenient to work with rectangular strata.

Reference lines are used to provide an easy method for locating the positions of quadrats. Essentially, a reference line provides an anchor point for locating the strata, clusters of first stage units or quadrats. They also help to prevent over and under-sampling the survey area when the shoreline includes bays and points. For example, a survey design may require that transects be laid out perpendicular to the shoreline at evenly spaced intervals (Figure 9.1). When the shoreline is concave, as in a bay, the result is a large number of quadrats in a small area in the center of the bay. When the shoreline is convex, as at a point, the result is a dearth of quadrats for the same area.

Campbell (1996) used the concept of a reference line, or line of best fit, to prevent over- and under-sampling problems on crooked shorelines. The reference line is laid out parallel to the long axis of the beach, and the transects aligned perpendicular to the reference line (Figure 9.2). We use a reference line to align rectangular strata on the survey beach (Figure 9.3). Although some small areas will be excluded from the survey, often the strata can be devised to take in most of the clam-bearing area of the beach with a minimum of poor habitat included.

Locate the endpoints of reference lines using permanent landmarks such as points of land, navigational markers, or easily identifiable large rocks. Also record the compass bearing of reference lines. Landmarks must be far enough apart to permit easy triangulation when relocating important boundaries or locations on the beach. Describe the landmarks and reference line in sufficient detail on diagrams of the beach to allow someone who has not participated in the survey to easily locate them at a later date. If conditions permit, the bounds of strata can be mapped for later quadrat location.

Survey layout

Equipment required to conduct field procedures for a survey relate to (1) the location and marking of quadrats, and (2) digging the clams from each quadrat. A list of suggested equipment for clam surveys is includes:

	Surveying Gear	
Surveying chains	Measuring tapes	Sighting compass
Stratum markers	Range markers	Bamboo or PVC stakes
Surveyors tape	Permanent marking pens	Field notebook
Pencils	Random number tables	Field vest
	Sampling Gear	
Quadrat frame	Scrapers	Potato forks
Shovel	Screens	Sample bags
Butcher's twine	Coolers	Buckets

We recommend that surveyors arrive at the beach at least 3 hours prior to low tide. As the tide ebbs, surveyors can determine reference lines or stratum boundaries and begin to place quadrats high on the beach. The surveyors move down the beach as the tide ebbs, and diggers begin to excavate quadrats as soon as they are placed. The diggers should follow the surveyors down the beach as low tide approaches, digging the lowest available quadrats. Quadrats that are passed up to keep up with the surveyor can be dug as the tide floods over the beach. The surveyors must judge when to stop placing quadrats and assist the diggers.

Small beaches can be completed in one tide or less. Distance between survey sites usually prevents completion of more than one survey per tide by the same crew. On large beaches, more than one day may be required to complete the survey. We have found that sampling one stratum low on the beach each day, and another high on the beach, to be an efficient way of dividing the survey over several days.

How to locate quadrats:

1. Designate one end of the reference line to be 0. We usually face seaward and use the left end as the origin.
2. Lay a surveyors chain or measuring tape along a reference line.
3. Locate the x-coordinate of the first quadrat to be sampled along the reference line.
4. Lay a second surveyors chain or measuring tape at right angles to the reference line.
5. Locate the y-coordinate of the first quadrat to be sampled.
6. Mark the lower left corner of the quadrat with a labeled stake, so that digging is seaward and to the right.
7. Repeat steps 2-6 for each quadrat in the sample, moving the chains or tapes as required.

When measuring reference lines longer than 200 m, we use range markers to maintain the correct bearing. Range markers consist of two poles each 2 m long, one marked with a triangle pointing up, and one marked with a triangle pointing down. Our triangles are fluorescent orange and red reflective markers used to mark long trailers or truck loads. The pole with a downwards-pointing triangle is driven into the beach at the starting point of the reference line. A measuring tape or surveyors chain is stretched across the beach, and a compass bearing is taken to confirm the orientation. The second pole is driven into the beach at least 30 m along the reference line. Then, to maintain alignment of the reference line while stretching subsequent lengths of tape, surveyors need only to look back at the range markers and line up the points of the two triangles.

Quadrat labels

The location of each quadrat in the sample is marked with a labeled stake. We use bamboo or PVC stakes about 0.5 m in length with labels made of plastic surveyor's flagging tape. We do not use paper labels, as abrasion caused by clams and grit scraping together in bags causes paper labels to break down very quickly. The labels are removed from the stake and bagged with the clams when the quadrats are dug. Mark each label with the beach name, survey date, and quadrat number using a permanent marking pen. Use the following numbering system for the quadrats:

- **Simple random sampling** (Ch. 5): simply number the quadrats from 1 to *n*;
- **Stratified random sampling** (Ch. 7): use a stratum number and a quadrat number separated by a dash. For example, 1-1 is the first quadrat and stratum 1, and 4-12 is the twelfth quadrat in stratum 4.
- **Two-stage random sampling** (Ch. 14): identify the first stage unit (FSU) and the quadrat number separated by a dash. For example, 2-4 is the fourth quadrat in the second FSU.
- **Stratified two-stage random sampling** (Ch. 16): identify the stratum, first stage unit and quadrat separated by dashes. For example, 2-5-6 is the sixth quadrat in the fifth FSU of stratum 2.

Quadrat coordinates (x, y) are recorded on the number and weight data sheet (see Ch. 10), so that quadrats can be mapped for spatial analyses, if desired.

Sample collection

Digging

Digging methods vary with the target species. Manila and littleneck quadrats are dug with a clam scraper, while butter clam quadrats are dug with a potato fork. Razor clam quadrats can be dug by driving a circular quadrat into the sand, emulsifying the substrate with pressurized water jet and dip netting the clams (Jones *et al.* 1998).

Each quadrat is marked with a stake, usually placed at the lower left corner of the quadrat so that digging occurs in a seaward direction and to the right of the quadrat marker. Use a quadrat frame to mark out the area to be sampled. Frames can be made of wood, metal or PVC pipe, with inside dimensions which match the appropriate quadrat size (50 x 50 cm for Manila and littleneck clams, and 1 x 1m for butter clams). With some experience, diggers can use dimensions marked from the end of the tines of their scraper to a point 50 cm up the handle to measure out quadrats. This frees diggers from carrying a quadrat frame during the survey. However, diggers must be extremely careful not to over- or under-estimate the size of the quadrat. Digging too large a quadrat will include clams from outside the bounds of the quadrat, making estimates of density, abundance and biomass too large. Likewise, digging too small a quadrat will exclude clams that are inside the quadrat bounds, artificially decreasing the survey estimates.

Even when quadrat boundaries are clearly marked, some clams will fall on the boundaries. The digger must decide if these clams are included in the sample. If more than half a clam is within the quadrat boundaries, it should be included in the sample. Too many clams incorrectly included or rejected from the quadrat will bias survey estimates in the same way as digging too small or large a quadrat.

When digging Manila and littleneck clams, a scraper is used to remove substrate to a depth of at least 20 cm. This is best done in a systematic fashion, working from the edge of the quadrat closest to the digger, and digging progressively further away. Clams are removed and placed in a bucket as the quadrat is dug. The diggings are then sifted through the fingers back into the quadrat, to recover any clams which may have been missed in digging.

A similar procedure is used for butter clams, but the quadrat is dug using a potato fork. This method can result in more breakage, as clams are pierced by the fork tines rather than turned out of the substrate in a scraping motion. Because the quadrat size is larger for butter clams, sifting the diggings for smaller clams can take considerably longer than for 0.25 m^2 quadrats. Once the quadat is dug, the diggings can be sorted with a scraper, and finally sifted back into the quadrat by hand. Butter clam quadrats should be dug to at least 30 cm depth.

Screening*

Although screening of samples is not a requirement of the current DFO protocol, we describe it here for completeness. Passing the substrate removed from a quadrat through a fine-mesh screen allows surveyors to find small clams (≤ 20 mm) or assess the effectiveness of hand-sorting of samples. When conducting stock assessment surveys, DFO staff are primarily interested in collecting clams which are > 20 mm in length. Although we retain all sizes of clams obtained by hand-picking the quadrats, we often do not expend extra effort to collect all of the clams smaller than 20 mm from the sampled quadrats, since we do not know how many 1-year-old clams will survive to recruit to the fishery two (or more) years later, and the effort required to collect and process them is considerable. The mortality rate of small clams is not known, but is assumed to be much higher than for large clams, as small clams are more vulnerable to predation by fish and crabs.

When assessing the effectiveness of hand-sorting, DFO staff use a 4x4 mm vexar mesh stretched across a 1x1 m wooden frame. After hand sorting, the diggings are placed in the screen, and washed with buckets of water until no mud or sand remains. The screened sample is then hand picked, and bagged. In the rare event that a night survey is unavoidable, or if diggers are unsure of their effectiveness in finding all of the clams in the sampled quadrat, they can use a screen of 10 x 10 mm mesh, which will retain clams >20 mm in length.

Bagging Samples

All species and sizes of clams are to be collected. Wash the clams to remove sand and mud, and bag them ***with the quadrat label from the stake***. Tie the neck of the bag to seal it, or twist the neck of the bag and tie it off with butcher's twine. Bags which are sealed by tying the neck of the bag are more difficult to untie. We carry two sizes of bags: small bags (32 x 20 cm) which can usually hold all of the clams removed a quadrats, and large bags (75 x 40 cm) for the exceptions. If two or more bags must be used, make more labels including the quadrat number and indicating "Bag 1 of 2", or "Bag 2 of 3", as appropriate. Diggers must carry a permanent marker and roll of flagging tape to replace, modify or duplicate quadrat labels as needed.

Small clams, particularly from screened samples, should be stored separately from the larger clams since they are easily crushed. We use plastic medicine vials to store small clams. To avoid losing the small clams, place the vials in the bag along with the larger clams and the label.

If no clams are found in the quadrat, place the sample label in a bag with a rock or piece of debris to ensure that the quadrat is correctly included in the estimates as a zero (0). Zeros are valid data and should not be excluded from the analysis either intentionally or through oversight.

Count and identify all of the bags from each quadrat before leaving the beach. Any missing quadrats should be located and dug, or recorded as flooded, so they can be accounted for in the survey report. For stratified designs, quadrat counts should be completed for each stratum as it is finished.

Sample storage

Samples must be kept under cover during a beach survey since direct sunlight can cause heat to build up in sealed bags and kill clams. Large coolers can be taken to the beach to store the clams until they can be transported from the beach. The coolers also provide a convenient way to pack the clams.

Samples should be processed live (unfrozen) whenever possible. Clam samples are best stored dry in a cool area, and processed as soon as possible. Clams which have not been maintained in cool temperatures will gape, which may cause drying of the clam's soft tissues, weight loss, and death of the clams. This results in biased estimates of growth patterns and stock biomass. Freezing and thawing of clams also causes water loss. If samples cannot be processed within two days, they should be frozen for later processing. Care must be taken to seal the samples, to protect the clams from freezer burn.

Summary

The following steps are required to complete the field component of a clam survey:

1. Conduct a pre-survey to locate concentrations of clams on the beach, delimit the bounds of the clam population, and map the survey area;
2. Develop a survey design and randomly select a sample of quadrats in the manner prescribed by the design;
3. Locate quadrats in the sample by measuring from the reference lines using a surveyors chains or a tapes;
4. Mark each quadrat with a labeled stake;
5. Dig each quadrat and bag the clams with the quadrat label, one bag per quadrat;
6. Store the clams out of direct sunlight while at the beach, and in a cool place once removed from the beach prior to processing;
7. Count the bagged clams at the end of the day, and make note of missing or flooded quadrats for the survey report.

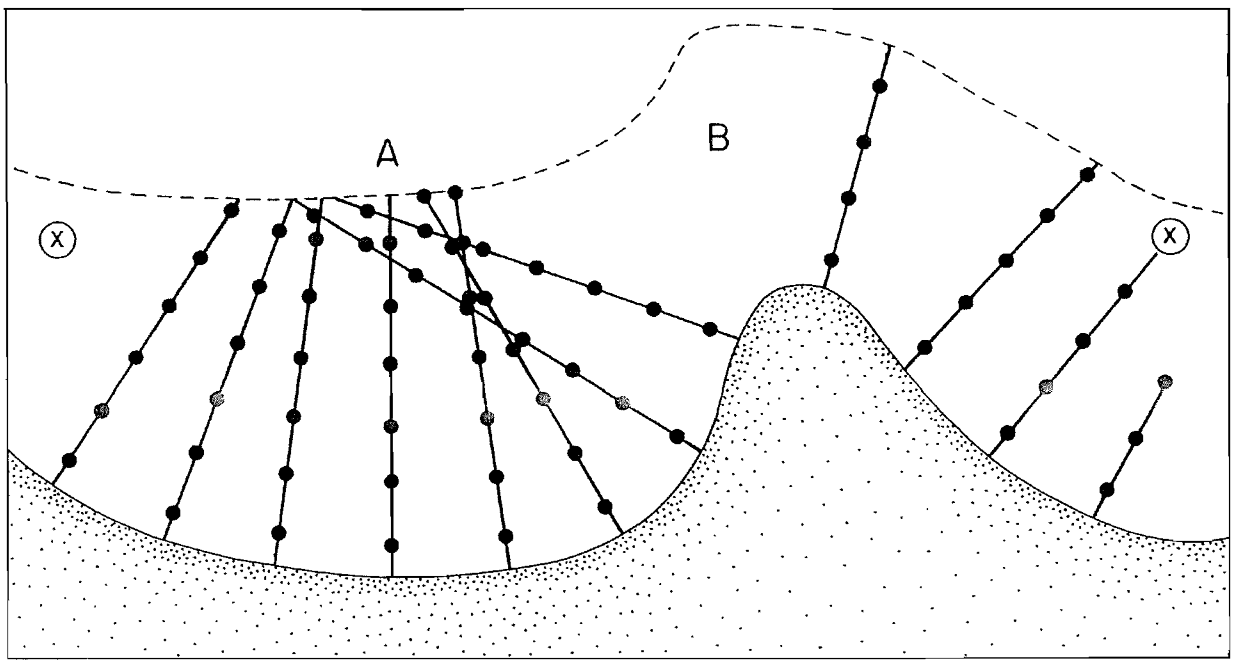

Figure 9.1 A survey design in which evenly-spaced transects are laid out perpendicular to the shoreline. The survey area boundaries are denoted by an ⊗. This design results in over-sampling in bays (A) and undersampling at points (B).

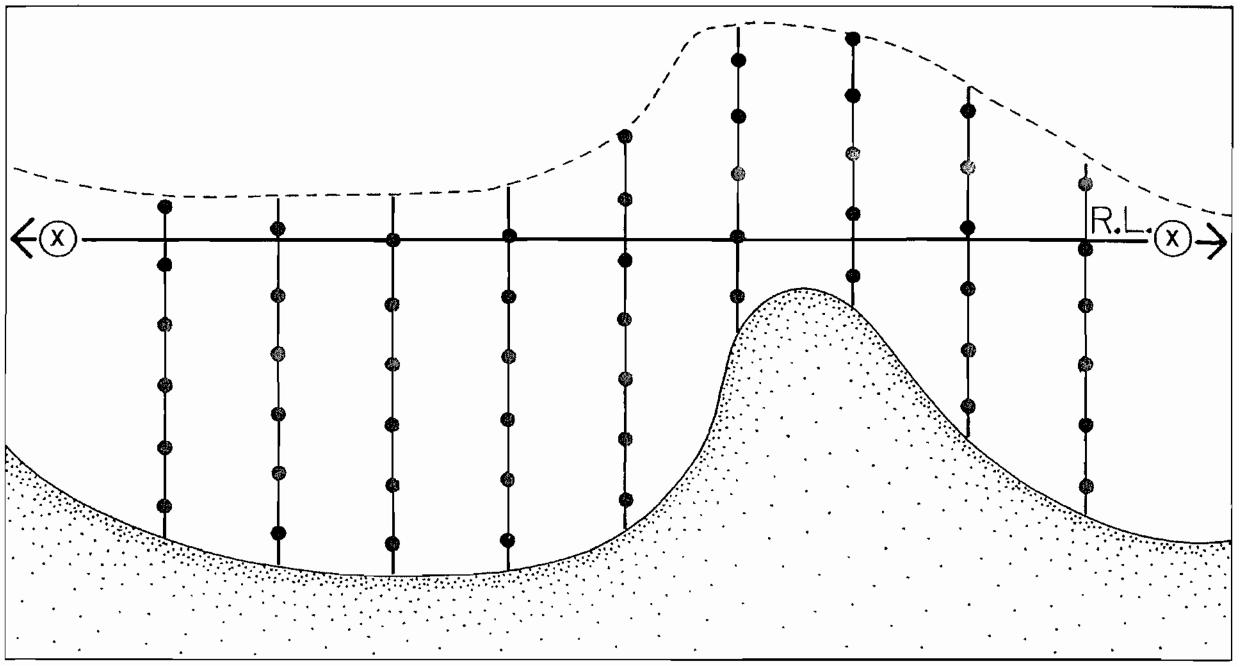

Figure 9.2 A survey design that aligns sampled transects perpendicular to a reference line (RL). The boundaries of the survey area are denoted by an ⊗.

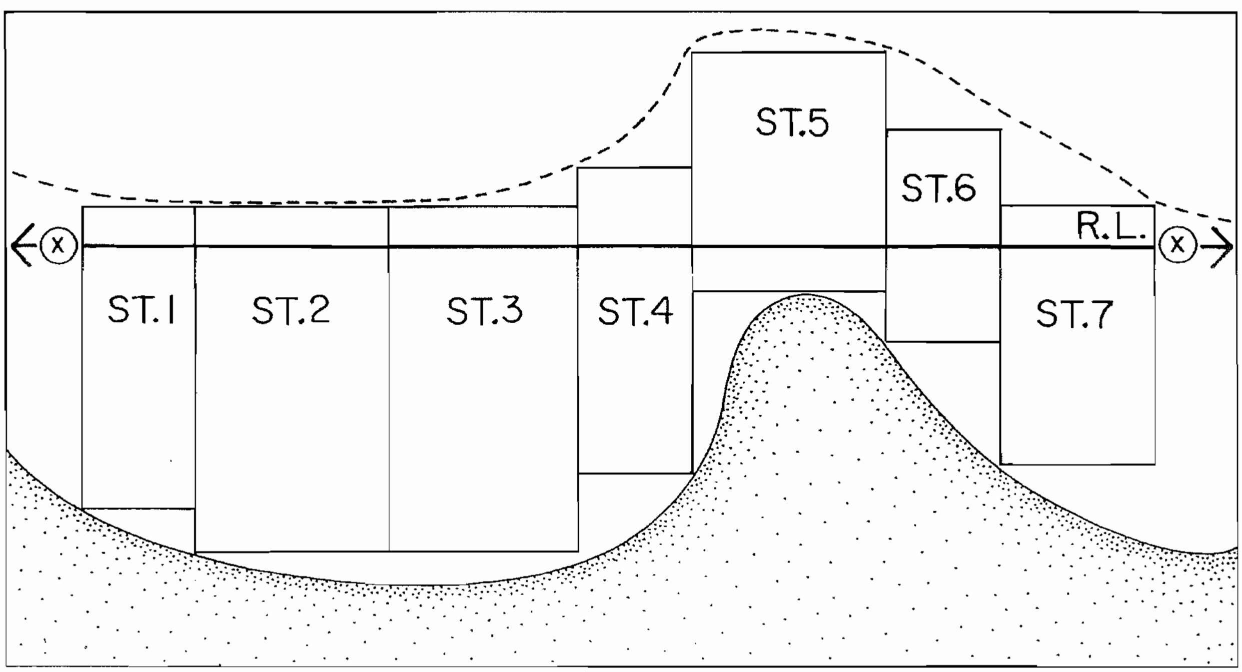

Figure 9.3 A survey design that aligns strata (ST) along a reference line (RL). The boundaries of the survey area are denoted by an ⊗.

Chapter 10 - Laboratory Methods

Laboratory processing involves identifying clams to species, sorting each species into legal and sublegal size categories, and recording data for analysis. Data collected during clam stock assessment surveys is of three basic types:

- quadrat counts and weights by species and size category;
- biological information including the length, weight and age of individual clams;
- length-at-annulus measurements of individual clams.

These data are associated with information that documents when, where and how the samples were collected. It is essential that all information for each quadrat is recorded correctly, since quadrats that are not identified correctly cannot be included in the analysis.

Equipment

The basic equipment for processing clam samples includes precision calipers, a precision balance, data sheets, a clipboard and pencils. Other useful equipment includes a legal size clam gauge, a colander or kitchen strainer for washing samples, and a countertop with a sink for a work surface.

The calipers should be of sufficient resolution to measure to a tenth of a mm. The measurement is then rounded to the nearest mm. For example, all clams measuring between 37.5 and 38.4 mm in length are considered to be 38 mm clams. If the calipers measure to a hundredth of a mm, ignore the second decimal place and round from only the first decimal place to the nearest whole number.

The balance should be of sufficient resolution to measure to a hundredth of a gram. The resolution required for aggregate and individual weights is different. Aggregate weights, the weight of all clams of a specified species and size category, are measured to the nearest whole gram. For example, weights between 49.5 and 50.4 g are recorded as 50 g. Weights of individual clams are measured to the nearest tenth of a gram. For example, weights between 9.95 and 10.04 g are recorded as 10.0 g.

Quadrat number and weight data

Aggregate counts and weights for each quadrat are measured after sorting the clams by species and size category (legal or sublegal). Legal size limits for B.C. intertidal clams are:

Species	Minimum Legal Size
Manila clam	≥ 38 mm
Littleneck clam	≥ 38 mm
Butter clam	≥ 63 mm
Razor clam	≥ 90 mm

How to collect quadrat number and weight data:

1. Work with only one quadrat at a time;
2. Record header information for the quadrat being processed on the Intertidal Clam Number and Weight Data sheet (Figure 10.1);
3. Wash the clams in a colander to remove any debris which may have been collected along with the clams;
4. Spread the clams on a table, and separate them by species;
5. Further separate those species which are managed by a size limit into legal and sublegal size groups;
6. Count the number of clams in each species and size category, and record the count in the appropriate columns;
7. Weigh the clams in each species and size category and record the weight in the appropriate columns;
8. Re-bag the clams and the label, and store for later biological sampling.

When working in the laboratory, we work in pairs, with one person processing samples and the other recording data. The recorder also watches carefully as the quadrats are processed to detect errors or problems before the data are recorded and the samples frozen or discarded. An example of a completed Intertidal Clam Number and Weight Data sheet is shown in Figure 10.1.

Enter the counts for each species in the "Number of Legals" and "Number of Sublegals" columns on the data sheet (Figure 10.1). Measure the total weight of clams (to the nearest whole gram) in each group. Record the weight in the "Total Weight of Legals" and "Total Weight of Sublegals" columns on the data sheet. The data for one species is recorded on one line of the data sheet. Thus, if a quadrat contains 4 species of clams (Manila, littleneck, macoma and softshell clams), there will be 4 lines of data recorded from that quadrat. All of the information for Manila clams (numbers and weights of legal and sublegal clams) goes on one line, all the information for littleneck clams on the next line, and so forth. Since the macoma and softshell clams do not have size limits, their numbers and weights are recorded in columns for legal numbers and weights.

Biological samples

The survey protocol requires measurement of length, total weight, and age for individual clams. For those species which are managed by a size limit, total length is the basis of legal size. Shell **length** is defined as the greatest linear distance between the anterior and posterior margins of the valve. The height of the valve is an appropriate measurement for some species, such as cockles (*Clinocardium nuttallii* [Conrad, 1837]) and scallops. **Height** is defined as the greatest linear distance from the umbo to the ventral margin of the valve. **Thickness** is defined as the greatest linear distance measured across a closed pair of valves of a clam. These measurements are shown in Figure 3.1 and are based on Quayle and Bourne (1972).

The total **weight** of the clam should be measured to the nearest 0.1 g. Clams which have been frozen and then thawed lose weight. Likewise, clams which have died, gaped and dried out will lose weight through dehydration. If samples have been frozen, they should be processed frozen, with the clams lightly washed in warm water to remove external ice from the shells.

The **age** of a clam is determined by examining the cocentric rings (**annuli**) on the external surface of the shell. Annuli are formed each winter as the growth rate of the clam slows due to the combined effects of low temperatures and food availability. However, they are also formed by other physiological stresses such as spawning (Quayle and Bourne 1972). Ageing is a procedure that involves interpretation of concentric rings on clam shells to determine which represent annuli (marks formed once per year) and which are checks from other physiological stresses. In warmer climates annuli cannot consistently be differentiated from spawning or temperature checks, and other methods must be used to estimate age. Age determination may be done by staff at the Pacific Biological Station in Nanaimo on a cost recovery basis. Contractors qualified to age clams can also be used provided shells are submitted to DFO for quality control. Contact DFO for more information.

Length and weight data, when combined with age data, allow the growth rates of clams to be calculated. Growth rates are used to estimate how long it will take for clams which have settled successfully on a beach to grow to legal size.

Although not required by the survey protocol, other important measurements from individual clams include height and thickness, and shell weight (meat weight=total weight-shell weight). In some bivalves (*e.g.* milky venus clams, *Compsomyax subdiaphana* [Carpenter, 1864], scallops) sex can be determined macroscopically by gonad color, and should be recorded.

How To Select A Biological Sample

Biological samples are selected at random from the quadrats processed for numbers and weights. Quadrats are selected until the total number of clams of the species of interest is at least 200 for beaches of less than 5 ha., or at least 500 for larger beaches. Do not stop measuring at the target sample size, instead finish the quadrat. This prevents the introduction of bias to the sample by measuring all of the large (or small) clams first, and then not measuring the remaining clams when the target sample size is reached.

The selection of clams for a biological sample should follow the survey design. For example, if the survey was a stratified design then clams from each stratum should be represented in the biological sample. For each stratum, select a quadrat at random from all quadrats dug from that stratum.

Ideally, each stratum should be included in the biological sample. However, on small beaches with few strata, some strata may have more than one quadrat in the biological sample to make up the minimum sample size. On large beaches with many strata and relatively large numbers of clams per quadrat, not all strata can be selected because of the cost of processing large biological samples. Randomization again offers a fair selection method which will minimize bias. First select a stratum at random without replacement so that as many strata are represented in the sample as possible. Then, select a quadrat from all possible quadrats sampled and repeat until the target sample size is reached (200 clams, 500 clams, *etc.*).

Example 10.1 How to select a biological sample from a stratified survey design.

> *A survey consists of three strata, each with thirty quadrats. A biological sample of 200 clams is required. Strata are selected without replacement, and quadrats within each stratum are selected. The number of clams in each quadrat is tallied from the number and weight data, and selection ceases when at least 200 clams are selected. The results of this sample selection are shown in* Table 10.1. *Note that all strata are selected at least once before any stratum is selected for the second time. Note also that more than 200 clams make up the sample, since all of the clams in quadrat 1-29 must be sampled to avoid selection bias.*

How to Collect Individual Biological Data

1. Work with only one quadrat at a time.
2. Record header information and serial numbers for clams being processed on the Intertidal Clam Biological Data sheet.
3. Spread the clams on a table, and sort them by species.
4. For each clam in the quadrat, measure and record the following data:
   - length to the nearest whole mm (*e.g.* 35);
   - weight to the nearest tenth of a gram (e.g. 27.3);
   - age, if you are qualified to age clams.
5. Place each clam on a countertop or in numbered egg carton to preserve the order of processing.
6. After all clams in the biological sample have been measured and weighed, shuck each out, clean out any flesh adhering to the shells, rinse in fresh water. **Be careful to retain the order of processing.**
7. After all shells have dried (usually overnight), use a permanent marker to write a serial number on the inside of both shells of each clam.
8. Place all shells in labeled zip-lock bags (each species in its own bag), and store in a sturdy cardboard box until they can be delivered to the Pacific Biological Station.

Serial Numbers

Because biological measurements might not be collected at the same time, serial numbers are required to ensure that length, weight, and age are correct for each clam. Because length and age must be compared for each clam to assess growth rates, it is not useful to have measurements of length, weight, or age that cannot be assigned to individual clams.

Serial numbers are entered on the biological data sheet (Figure 10.2), and must also be written on the inside of the shells, after they have been cleaned and dried. Since the hinge ligament will eventually break down, and pairs of shells will separate, the serial numbers must be written on each shell of the pair. Underline the serial numbers so that they cannot be accidentally mis-interpreted (*e.g.* 16, 89, 188, *etc.*). This avoids problems reading serial numbers that could be different numbers when viewed upside down (*e.g.* 16 upside down could be 91, 89 upside down could be 68, 188 upside down could be 881, *etc.*).

How to Collect Length-at-Annulus Data

1. Work with only one quadrat at a time.
2. Spread the clams on a table, and sort them by species.
3. Record header information and serial numbers for clams being processed on the Intertidal Clam Length-at-Annulus Data sheet.
4. For each clam in the biological sample, repeat the following steps:
   - Mark each annulus on the shell with a pencil.
   - Measure and record the length of each annulus from anterior to posterior ends. Record the length of the first annulus in the "Length at 1" column, the length of the second annulus in the "Length at 2" column, *etc.* Length is recorded to the nearest mm.
   - Measure the total length of the clam. Record it in the "Total Length" column.
5. Place all shells in labeled zip-lock bags and store in a sturdy cardboard box until they can be delivered to the Pacific Biological Station, Nanaimo.

Table 10.1 Biological sample selection from Example 10.1.

Stratum	Quadrat Label	Number of Clams
2	2-18	16
3	3-2	48
1	1-22	54
3	3-25	28
1	1-29	64
Total		210

Figure 10.1 Example of a completed number and weight data sheet.

INTERTIDAL CLAM NUMBER AND WEIGHT DATA

Location: DUNSMUIR IS. Sampler: GILLESPIE, PARKER Page 1 of 5

Beach No.	Date Collected Year	Month	Day	Low Tide Height +/-	ht. (0.1 m)	Statistical Area	Stat. Subarea	Data File Name
946	1994	07	07	+	0.6	17	7	

Sample Type: [✓] Grid [ ] Strip [ ] Other: ____

[✓] Live [ ] Frozen [ ] Thawed

Sampling Technique: [✓] Scraper [ ] Fork [ ] Other: ____

[ ] Screened [✓] Not screened

Species Codes:

Littleneck	81H	Horse clam	76D	*Macoma* sp.	77C
Manila	82B	*T. capax*	76E	*M. balthica*	77D
Butter	82E	*T. nuttallii*	76F	*M. inquinata*	77E
Razor	76I	Cockle	75C	*M. nasuta*	77F
Softshell	83G	Pac. oyster	69F	*M. secta*	77G
Varnish	79I	Native oyster	69H	Dpwtr. Venus	82C

Stratum	FSU	Quadrat No.	Sample time (PST)	Quadrat Position (0.0 m) Long Shore	Vertical	Sample Area (0.00 sq. m.)	Method	Species Code	Number of Legals	Number of Sublegals	Total Wt. (g) Legals	Total Wt. (g) Sublegals	Comments
1		17		90.0	13.0	0.25		81H	6	1	262	145	
								82B	22	5	578	32	
								77C	6	0	49	0	
		22		123.0	19.0			81H	9	1	427	5	
								82B	9	0	371	0	
								77C	15	0	87	0	
								75C	2	0	96	0	
								82E	2	0	250	0	
		12		64.0	40.0			82B	15	2	508	14	
								81H	27	38	828	353	
								75C	1	0	48	0	
								83G	2	0	148	0	
								77C	3	0	15	0	
		20		108.0	21.0			81H	22	0	933	0	
								82B	19	1	700	3	
								83G	1	0	58	0	
								77C	12	0	85	0	
								82E	1	0	102	0	
		11		64.0	31.0			76D	0	1	0	2	
		11		64.0	31.0			82B	44	24	1296	77	

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INTERTIDAL CLAM BIOLOGICAL DATA

Location: DUNSMUIR IS. Sampler: GILLESPIE, PARKER Page 1 of 29

Beach No.	Date Collected Year	Month	Day	Low Tide Height +/-	ht. (0.1 m)	Statistical Area	Stat. Subarea	Sample ID Number	Data File Name
946	1994	07	07	+	0.6	17	7		

Species Codes:

Littleneck	81H	Horse clam	76D	*Macoma* sp.	77C
Manila	82B	*T. capax*	76E	*M. balthica*	77D
Butter	82E	*T. nuttallii*	76F	*M. inquinata*	77E
Razor	76I	Cockle	75C	*M. nasuta*	77F
Softshell	83G	Pac. oyster	69F	*M. secta*	77G
Varnish	79I	Native oyster	69H		

Sample Type: ☑ Grid ☐ Strip ☐ Other: ______

☑ Live ☐ Frozen ☐ Thawed

Sampling Technique: ☑ Scraper ☐ Fork ☐ Other: ______

☐ Screened ☑ Not screened

Stratum	FSU	Quadrat No.	Sample time (PST)	Quadrat Position (0.0 m) Long Shore	Vertical	Sample Area (0.00 sq. m.)	Method	Species Code	Serial No.	Length (mm)	Height (mm)	Thickness (mm)	Weight (0.0 g)	Annuli	Condition
1		17		90.0	13.0	0.25		82B	1	56			40.0	9	
									2	56			36.2	10	
									3	47			24.4	6	
									4	40			14.6	4	
									5	34			9.7	3	
									6	40			16.2	4	
									7	36			12.1	3	
									8	42			17.3	5	
									9	51			37.0	7	
									10	48			24.1	6	
									11	47			24.6	6	
									12	38			12.8	4	
									13	34			8.9	3	
									14	37			12.8	4	
									15	30			6.2	3	
									16	39			31.2	5	
									17	59			55.9	10	
									18	33			18.3	3	
									19	37			14.7	4	
									20	62			59.8	11	

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Figure 10.2 Example of a completed clam biological data sheet.

INTERTIDAL CLAM LENGTH AT ANNULUS DATA

Location: GANGES HBR   Sampler: BOURNE   Page 1 of 4

Beach No.	Date Collected: Year	Month	Day	Low Tide Height: +/-	ht. (0.1 m)	Statistical Area	Stat. Subarea	Stratum	FSU	Quadrat	Species Code	Data File Name
944	1994	07	26	+	1.1	18	3	1		1	82B	

Species Codes.

Littleneck	81H	Softshell	83G	*T. nuttalli*	76F	*Macoma sp.*	77C	*M. secta*	77G
Manila	82B	Varnish	79I	Cockle	75C	*M. balthica*	77D		
Butter	82E	Horse clam	76D	Pac. oyster	69F	*M. inquinata*	77E		
Razor	76I	*T. capax*	76E	Native oyster	69H	*M. nasuta*	77F		

Serial Number	Length at 1	Length at 2	Length at 3	Length at 4	Length at 5	Length at 6	Length at 7	Length at 8	Length at 9	Length at 10	Length at 11	Length at 12	Length at 13	Length at 14	Length at 15	Length at 16	Length at 17	Length at 18	Length at 19	Length at 20	Total Length
1	6																				23
2	6	13																			23
3	4	15																			27
4	7	18	28																		31
5	4	23																			31
6	7	21	29																		35
7	7	20	31																		38
8	4	16	32																		36
9	7	22	30																		39
10	5	25	33																		38
11	5	18	27	38																	40
12	6	21	32	36	39	42	43														45
13	5	20	28	37	44	46															48
14	9	16	34	42	49	52															55
15	6	21	33	38	43																47
16	7	23	37	41	49	51															51
17	5	21	30	34	38	50	52	54													55
18	5	19	30	46																	50
19	6	22	33	46																	49
20	6	25	34																		45
21	10	23	47	51																	51
22	7	23	40	52	53																55
23	8	24	36	42	46	48															49
24	5	19	32																		40
25	6	25	41																		49
26	5	29	39																		44
27	7	25	32	40	44																48

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Figure 10.3 Example of a completed clam length-at-annulus data sheet.

Chapter 11 - Data Management

This chapter explains the procedure for submitting survey data to the Intertidal Clam Assessment Unit at the Pacific Biological Station in Nanaimo.

What you will learn

- how to complete the header information on each data sheet;
- instructions on entering data on each of the three Intertidal Clam data sheets: (1) numbers and weights data, (2) biological data, (3) length-at-annulus data;
- file formats for spreadsheet files;
- the procedure for submitting survey data to DFO.

The original survey data sheets should be submitted along with electronic versions as an EXCEL spreadsheet.

Header Information

Each data sheet has header information that provides vital information regarding when and how the samples were collected (Table 11.1 and Table 11.2). This section describes how to complete each of the fields in the header. Field names are listed in double quotes.

1. Beach numbers ("Beach No.") are codes applied to clam beaches cataloged in the South Coast Clam Atlases (Harbo *et al.* 1997 a,b,c). These codes will be added when data are submitted to the Pacific Biological Station (PBS).
2. Date of survey ("Date Collected") is the date (or dates) that samples were collected. It is not the date on which samples were processed and data sheets completed, unless survey and sample processing occurred on the same day. The year is entered as four digits (*e.g.*, 1999, 2000. *etc.*), and the month and day are each entered in their own columns.
3. Low ebb tide level ("Low Tide Height") on each day quadrats were dug is to be recorded to the nearest 0.1 m. Tides less than chart datum are marked as "-" and those above chart datum are marked "+" in in the +/- column.
4. The coastal waters of British Columbia have been divided into Pacific Fishery Management (Statistical) Areas and Subareas. If you don't know which Area and Subarea are correct, these codes can be added when data are submitted to PBS.
5. Stratum numbers ("Stratum No.") are entered in the appropriate column.
6. For two-stage designs, the appropriate first stage unit number is recorded in the "FSU" column. FSUs are numbered sequentially and independently in each stratum.
7. Quadrat numbers ("Quadrat No.") within each stratum or first stage unit are numbered sequentially.

8. The species found in each quadrat are recorded in the "Species" column. Codes for commercially important species are included on each biological data sheet, and a more complete set of codes is included in Table 11.5.
9. The size of the quadrat ("Quadrat Area") is recorded to the nearest 0.01 square meter. Standard quadrat size for Manila and littleneck clams is 50 cm on a side, or 0.25 m^2.
10. The survey method field ("Method") is a combination of survey layout, collection and storage methods. These codes will be added by DFO staff.
11. The "Long Shore (X) Coordinate" of the quadrat location the first number in the selected pair of quadrat coordinates. Record to the nearest 0.1 m.
12. The "Vertical (Y) Coordinate" of the quadrat location is the second number in the selected pair of quadrat coordinates. Record this distance to the nearest 0.1 m

Three different data sheets are used for recording number and weight data, biological data, and length-at-annulus data. Examples of completed data sheets are included in Ch. 10.

We cannot stress enough the requirement that data be recorded neatly, accurately and clearly. Use single vertical lines for the number "1", with no horizontal cap, and the European convention of a horizontal dash across the transverse body of the number "7", so that the two numbers can be easily discerned. Exaggerate the square corner and long horizontal cap on the number "5" to distinguish it from the number "6". Use a horizontal slash through the number "Ø" or be very careful to complete the circle, to distinguish zero from a poorly written number "8". Always record information in pencil, to allow corrections during sample processing.

Number and Weight Data

Number and weight data are arranged such that each line of data contains information for one species from a single quadrat, along with associated header information. Numbers and weights of legal and sublegal size clams of each species in each quadrat are to be recorded in the "Number of Legals", "Number of Sublegals", "Total Weight of Legals", or "Total Weight of Sublegals" fields. Weights are rounded to the nearest whole g.

Biological Data

Biological data is arranged such that data for each individual clam, and associated header information, are coded on one line. Total length and total weight of each clam are recorded in the "Length" and "Weight" fields. Total length is rounded to the nearest whole mm, while weight is rounded to the nearest 0.1 g. If ages were determined, the number of annuli counted for each clam is recorded in the "Annuli" field.

File formats

The information recorded on the data sheet must also be entered into a spreadsheet file, where each of the variables is recorded in a column. The variable names should appear in the first row of the column. Ensure that decimal points and the appropriate number of significant figures are included when coding data into spreadsheet files. Examples of the information from the data sheets in Figure 10.1, Figure 10.2 and Figure 10.3 are coded into spreadsheet file format in Figure 11.1, Figure 11.2 and Figure 11.3, respectively.

Beach	Year	Month	Day	Tide	Area	Subarea	Stratum	FSU	Quadrat	Time	LS Pos.	V. Pos.	Quad. size	Method	Species	# Legal	# Sublegal	Wt Legal	Wt Sublegal
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		81H	6	11	262	145
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	22	5	578	32
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		77C	6	0	49	0
946	94	7	7	0.6	17	7	1		22		123.0	19.0	0.25		81H	9	1	427	5
946	94	7	7	0.6	17	7	1		22		123.0	19.0	0.25		82B	9	0	371	0
946	94	7	7	0.6	17	7	1		22		123.0	19.0	0.25		77C	15	0	87	0
946	94	7	7	0.6	17	7	1		22		123.0	19.0	0.25		75C	2	0	96	0
946	94	7	7	0.6	17	7	1		22		123.0	19.0	0.25		82E	2	0	250	0
946	94	7	7	0.6	17	7	1		12		64.0	40.0	0.25		82B	15	2	508	14
946	94	7	7	0.6	17	7	1		12		64.0	40.0	0.25		81H	27	38	828	353
946	94	7	7	0.6	17	7	1		12		64.0	40.0	0.25		75C	1	0	18	0
946	94	7	7	0.6	17	7	1		12		64.0	40.0	0.25		83G	2	0	148	0
946	94	7	7	0.6	17	7	1		12		64.0	40.0	0.25		77C	3	0	15	0
946	94	7	7	0.6	17	7	1		20		108.0	21.0	0.25		81H	22	0	933	0
946	94	7	7	0.6	17	7	1		20		108.0	21.0	0.25		82B	18	1	700	3
946	94	7	7	0.6	17	7	1		20		108.0	21.0	0.25		83G	1	0	58	0
946	94	7	7	0.6	17	7	1		20		108.0	21.0	0.25		77C	12	0	85	0
946	94	7	7	0.6	17	7	1		20		108.0	21.0	0.25		82E	1	0	102	0
946	94	7	7	0.6	17	7	1		11		64.0	31.0	0.25		76D	0	1	0	2
946	94	7	7	0.6	17	7	1		11		64.0	31.0	0.25		82B	44	24	1296	77

Figure 11.1 Count and weight data from Figure 10.1 in spreadsheet file format.

Beach	Year	Month	Day	Tide	Area	Subarea	Stratum	FSU	Quadrat	Time	LS Pos.	V. Pos.	Quad. size	Method	Species	Serial #	Length	Height	Thickness	Weight	Annuli	Condition
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	1	56			40.0	9	
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	2	56			36.2	10	
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	3	47			24.4	6	
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	4	40			14.6	4	
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	5	34			9.7	3	
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	6	40			16.2	4	
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	7	36			12.1	3	
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	8	42			17.3	5	
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	9	51			37.0	7	
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	10	48			24.1	6	
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	11	47			24.6	6	
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	12	38			12.8	4	
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	13	34			8.9	3	
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	14	37			12.8	4	
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	15	30			6.2	3	
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	16	39			31.2	5	
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	17	59			55.9	10	
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	18	33			18.3	3	
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	19	37			14.7	4	
946	94	7	7	0.6	17	7	1		17		90.0	13.0	0.25		82B	20	62			59.8	11	

Figure 11.2 Biological data from Figure 10.2 in spreadsheet file format.

Figure 11.3 Length-at-annulus data from Figure 10.3 in spreadsheet file format.

Beach	Year	Month	Day	Tide	Area	SubA	Stratum	FSU	Quad	Spp	L at 1	L at 2	L at 3	L at 4	L at 5	L at 6	L at 7	L at 8	L at 9	L at 10	L at 11	L at 12	L at 13	L at 14	L at 15	L at 16	L at 17	L at 18	L at 19	L at 20	Total L	Serial #
944	94	7	26	1.1	18	3	1		1	82B	6																				23	1
944	94	7	26	1.1	18	3	1		1	82B	6	13																			23	2
944	94	7	26	1.1	18	3	1		1	82B	4	15																			27	3
944	94	7	26	1.1	18	3	1		1	82B	7	18	28																		31	4
944	94	7	26	1.1	18	3	1		1	82B	4	23																			31	5
944	94	7	26	1.1	18	3	1		1	82B	7	21	29																		35	6
944	94	7	26	1.1	18	3	1		1	82B	7	20	31																		38	7
944	94	7	26	1.1	18	3	1		1	82B	4	16	32																		36	8
944	94	7	26	1.1	18	3	1		1	82B	7	22	30																		39	9
944	94	7	26	1.1	18	3	1		1	82B	5	25	33																		38	10
944	94	7	26	1.1	18	3	1		1	82B	5	18	27	38																	40	11
944	94	7	26	1.1	18	3	1		1	82B	6	21	32	36	39	42	43														45	12
944	94	7	26	1.1	18	3	1		1	82B	5	20	28	37	44	46															48	13
944	94	7	26	1.1	18	3	1		1	82B	9	16	34	42	49	52															55	14
944	94	7	26	1.1	18	3	1		1	82B	6	21	33	38	43																47	15
944	94	7	26	1.1	18	3	1		1	82B	7	23	37	41	49	51															51	16
944	94	7	26	1.1	18	3	1		1	82B	5	21	30	34	38	50	52	54													55	17
944	94	7	26	1.1	18	3	1		1	82B	5	18	30	46																	50	18
944	94	7	26	1.1	18	3	1		1	82B	6	22	33	46																	49	19
944	94	7	26	1.1	18	3	1		1	82B	6	25	34																		45	20
944	94	7	26	1.1	18	3	1		1	82B	10	23	47	51																	51	21
944	94	7	26	1.1	18	3	1		1	82B	7	23	40	52	53																55	22
944	94	7	26	1.1	18	3	1		1	82B	8	24	36	42	46	48															49	23
944	94	7	26	1.1	18	3	1		1	82B	5	19	32																		40	24
944	94	7	26	1.1	18	3	1		1	82B	6	25	41																		49	25
944	94	7	26	1.1	18	3	1		1	82B	5	29	39																		44	26
944	94	7	26	1.1	18	3	1		1	82B	7	25	32	40	44																48	27

Table 11.1 Formats for intertidal clam survey header data.

Survey Header Data		
Variable	Format	Comments
Beach Number	Alphanumeric	Assigned by DFO.
Date Collected - Year	Integer	Four digits (*e.g.*, 1999).
Date Collected - Month	Integer	
Date Collected - Day	Integer	
Low Tide Height	Numeric	+/- nearest 0.1 m.
Statistical Area	Integer	
Statistical Subarea	Integer	
Method	Alphanumeric	Assigned by DFO.

Table 11.2 Formats for intertidal clam sample header information.

Sample Header Data		
Variable	Format	Comments
Stratum Number	Alphanumeric	
FSU Number	Alphanumeric	
Quadrat Number	Alphanumeric	
Time Sampled	Integer	
Sample Area	Numeric	nearest 0.01 m^2.
Long Shore (X) Coordinate	Numeric	nearest 0.1 m.
Vertical (Y) Coordinate	Numeric	nearest 0.1 m.

Table 11.3 Formats for intertidal clam count and weight data.

Count and Weight Data		
Variable	Format	Comments
Survey/sample header data	See Table 11.1 and Table 11.2	
Species Code	Alphanumeric	See Table 11.5
Number of Legals	Integer	
Number of Sublegals	Integer	
Total Weight of Legals	Integer	nearest whole g.
Total Weight of Sublegals	Integer	nearest whole g.
Survey/sample header data	See Table 11.1 and Table 11.2	

Table 11.4 Formats for intertidal clam biological data.

Variable	Biological Data Format	Comments
Survey/sample header data	See Table 11.1 and Table 11.2	
Species Code	Alphanumeric	See Table 11.5
Length	Integer	nearest whole mm.
Height	Integer	nearest whole mm.
Thickness	Integer	nearest whole mm.
Weight	Numeric	nearest 0.1 g.
Survey/sample header data	See Table 11.1 and Table 11.2	
Annuli	Integer	

Table 11.5 DFO standard species codes for intertidal bivalves.

Common Name	Scientific Name	Code
Northern horsemussel	*Modiolus modiolus* (Linnaeus, 1758)	66D
Japanese mussel	*Musculista senhousia* (Benson, *in* Cantor, 1842)	66F
California mussel (sea mussel)	*Mytilus californianus* Conrad, 1837	65I
Blue mussel	*Mytilus edulis* Linnaeus, 1758	66A
Mediterranean mussel	*Mytilus galloprovincialis* Lamark, 1819	66B
Foolish mussel	*Mytilus trossulus* Gould, 1850	66C
Pacific oyster	*Crassostrea gigas* (Thunberg, 1793)	69F
Olympia oyster (native oyster)	*Ostrea conchaphila* Carpenter, 1857	69H
Nuttall's cockle (heart cockle)	*Clinocardium nuttallii* (Conrad, 1837)	75C
Macoma clams	*Macoma* sp.	77C
Baltic macoma	*Macoma balthica* (Linnaeus, 1758)	77D
Pointed macoma	*Macoma inquinata* (Deshayes, 1855)	77E
Bentnose macoma	*Macoma nasuta* (Conrad, 1837)	77F
White sand macoma	*Macoma secta* (Conrad, 1837)	77G
Eastern softshell clam	*Mya arenaria* Linnaeus, 1758	83G
Varnish clam (dark mahogany-clam)	*Nuttallia obscurata* (Reeve, 1857)	79I
Geoduc	*Panopea abrupta* (Conrad, 1849)	84C
Native littleneck clam	*Protothaca staminea* (Conrad, 1837)	81H
Butter clam	*Saxidomus giganteus* (Deshayes, 1839)	82E
Pacific razor clam	*Siliqua patula* (Dixon, 1789)	76I
Manila clam (Japanese littleneck)	*Venerupis philippinarum* (Adams and Reeve, 1850)	82B
Horse clams (unspecified)	*Tresus* sp.	76D
Fat horse clam (fat gaper)	*Tresus capax* (Gould, 1850)	76E
Pacific horse clam (Pacific gaper)	*Tresus nuttallii* (Conrad, 1837)	76F

Chapter 12 - Analysis of Biological Data

This chapter describes procedures for analyzing length, weight, and age data collected from clam surveys and provides examples.

What you will learn

- how to summarize frequency distributions of length or age;
- how to plot length-weight relationships;
- methods for estimating growth rates using length-age relationships;
- estimating time to recruitment to the fishery.

Mathematical notation for the methods described in this chapter is listed in Table 12.1.

Frequency distributions

Frequency distributions are counts of the number of times an a particular observation occurs in a set of data. Although frequency distributions for small data sets can be compiled by hand, most frequency distributions should be prepared using a spreadsheet or statistical software. Consider the simulated biological data for 20 clams shown in Table 12.2. A frequency distribution of lengths would entail counting the number of times a clam in each 1 mm size class occurred in the data set. An example of compiling an age frequency distribution is shown as Example 12.1.

Example 12.1 Compiling an age frequency distribution.

> *Suppose that the simulated data in* Table 12.2 *are lengths, weights and ages of 20 clams collected from a clam survey. To compile an age frequency, count how many times each age appears in the sample (*Table 12.3*). A frequency distribution is easier to interpret when presented graphically, as a histogram (*Figure 12.1*). The age frequency results indicate that most clams in this sample are 3 or 4 years of age, and older clams do not appear often. A lack of clams younger than 3 years old may indicate poor settlement in the previous two years, or may indicate* ***size selectivity*** *(i.e., that small clams present in the population were not fully collected by surveyors).*

Length-weight relationships

A plot of weight against length for each clam in a biological sample is a useful starting point for proofing data. Length-weight data from Table 12.2 are plotted in Figure 12.2. Notice that one point is a considerable distance from the remaining points, which closely follow a curved relationship. The outlying data point in this example corresponds to clam number 15 in the table. This clam weighs much less than it should for its length. In this example, length was incorrectly recorded as 64 mm for a clam that was actually 46 mm in length.

Although not required by the survey protocol, a length-weight model (Table 12.4) can be fit to length-weight data using a "trend line" function in a spreadsheet program (Figure 12.3). In most cases, the estimate of b will be very close to 3, as weight is a cubic function of length (Thompson 1917).

The data should be clustered relatively close to the line, and outlying observations should be examined for recording or keypunching errors. With reference to the original data sheets, correct any outliers that arise due to keypunching errors. If the data were recorded on the data sheets incorrectly, then a decision must be made whether or not to include the suspect observations in further analyses.

Growth relationships*

A plot of length or weight against age can be constructed using individual lengths (weights) and ages, or mean length (weight) at age. The von Bertalanffy growth model is a reasonable choice for molluscan growth (Caddy 1989) and has been used for Manila clams (Bourne 1982; Gillespie *et al.* 1998). The model should be fit to the data using nonlinear fitting routines available either as standalone software (*e.g.* Mittertreiner and Schnute 1985) or included in most statistical software packages (*e.g.* MathSoft 1997).

The parameter estimates from the model (Table 12.4) can be used to compute the amount of time required for Manila or littleneck clams to reach legal size by substituting the estimates into the age of recruitment equation (Table 12.4) and setting L_t to 38 mm (Gillespie *et al.* 1998). Set L_t to the appropriate legal size limit for other clam species (*e.g.* 63 mm for butter clams, 90 mm for razor clams).

Growth rates can be estimated using two forms of the data: (1) total length at age, and (2) length at each annulus. Length-at-annulus data is preferred over total length-at-age data for growth analyses. The two data sets measure different things at different resolutions. Length-at-age describes total lengths of clams at given ages, while length-at-annulus incorporates historic information detailing size of each individual at each age.

Clam growth is characterized by rapid growth in the summer months when temperatures are higher and food is more plentiful, and greatly decreased growth in winter months. Therefore, a 4-year-old clam is considerably smaller in April than it is in September of the same year. However, since new annuli form in mid-winter, this clam sampled in April and September would be assigned the same age. Length-at-age data are inflated by the growth which has occurred since formation of the last annulus. Measuring length at each annulus eliminates this imprecision.

Intertidal clams spawn in the spring and summer, and settle in the summer and fall. The first annulus forms in the middle of the first winter of life, when a clam is not yet one year old.

For growth analyses, length-at-annulus data are corrected by reducing the assigned age by 0.5 years, to reflect the true age of a clam at the time of annulus formation.

For more information

For further reading on growth models in general, and the von Bertalanffy model in particular, see Ricker (1975).

Table 12.1 Symbols for the analysis of biological data.

Symbol	Description
a, b	parameters of length-weight relationship
L	length
L_t	length of a clam in year t
L_∞	expected length of a clam in the oldest age class (asymptotic length)
ln	natural logarithm
e	root of natural logarithm
K	rate of increase in size, Brody coefficient
t_0	theoretical time at which the clam's length is 0
W	weight

Table 12.2 Simulated biological data for 20 clams.

Clam	Length (mm)	Weight (g)	Age	Clam	Length (mm)	Weight (g)	Age
1	47	35.5	6	11	41	23.1	4
2	39	19.8	4	12	56	61.6	9
3	51	45.9	7	13	20	2.4	2
4	44	28.9	5	14	33	11.7	3
5	21	2.8	3	15	64	33.2	5
6	22	3.3	3	16	50	43.2	6
7	11	0.4	1	17	31	9.6	3
8	38	18.2	4	18	38	18.2	4
9	40	21.4	4	19	42	25.0	5
10	23	3.8	3	20	38	18.2	4

Table 12.3 Age frequency distribution for 20 clams.

Age	Frequency
1	1
2	1
3	5
4	6
5	3
6	2
7	1
8	0
9	1
10	0
Total	20

Table 12.4 Equations required for the analysis of biological data.

von Bertalanffy growth equation:

$$L_t = L_\infty\left(1 - e^{-K(t-t_0)}\right) \ .$$

Estimated age of recruitment to legal size:

$$t = t_0 - \left(\frac{1}{K}\right)\ln\left(1 - \frac{L_t}{L_\infty}\right) \ ,$$

where L_t is set to the legal size limit for the species.

Length-weight equation:

$$W = aL^b \ .$$

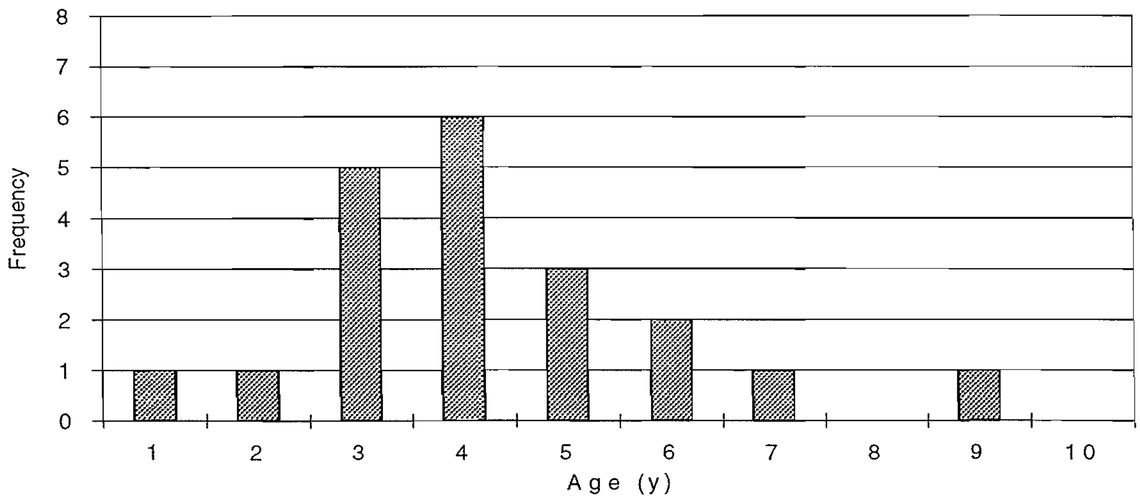


Figure 12.1 Age frequency distribution from Table 12.2.

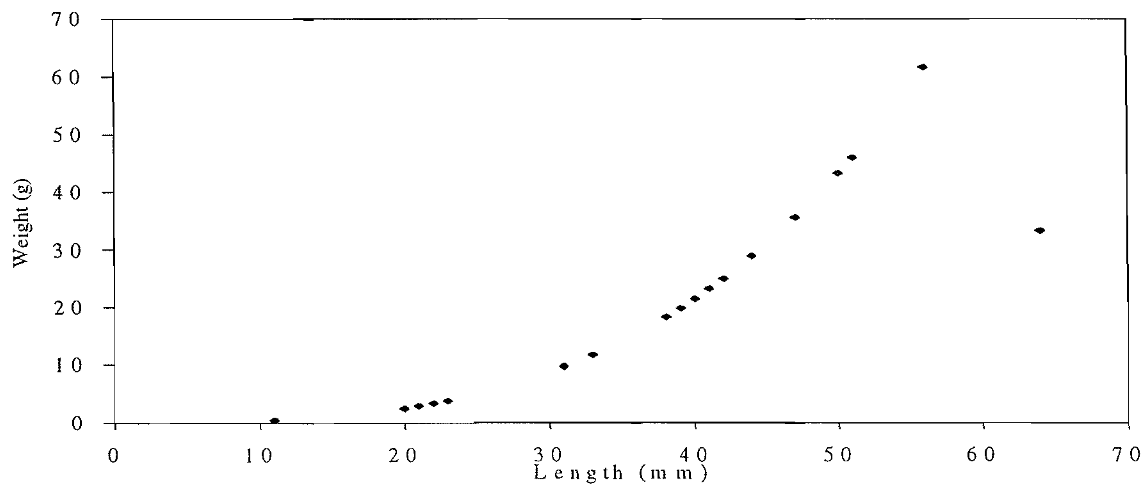


Figure 12.2 Plot of weight against length for the data in Table 12.2.

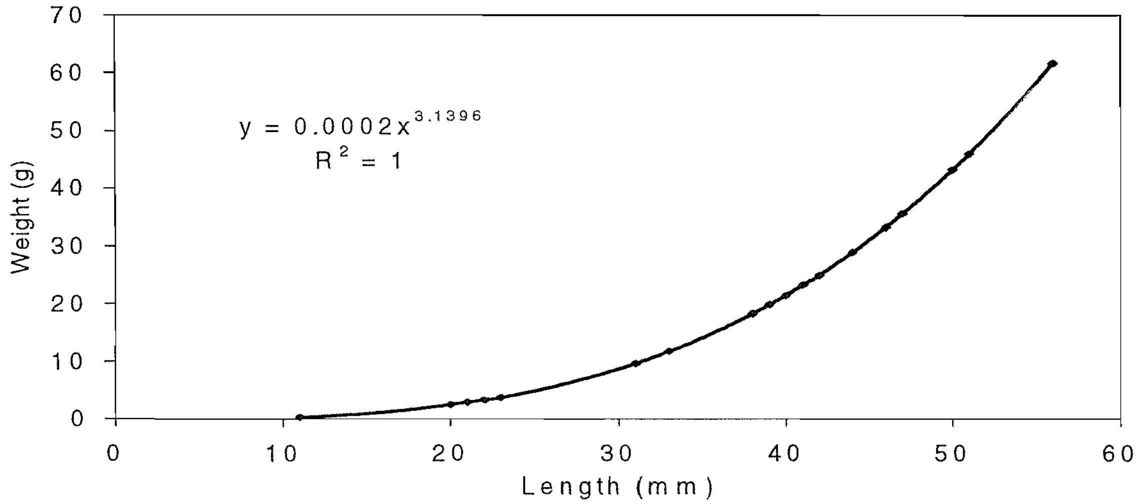


Figure 12.3 Results of fitting a length-weight model to the corrected data from Table 12.2.

Chapter 13 - Case Study 3

A major assessment of Manila clam stocks at Savary Island, B.C., was carried out in 1995 (Gillespie *et al.* 1998b; Kronlund *et al.* 1998). Biological data gathered for two of the beaches surveyed will be used to illustrate analyses of biological data.

Survey Area

Savary Island is located in northeastern Strait of Georgia, south of Lund (45°57'N, 124°47'W). The data analysed here were collected at two different beaches on the island: (1) beach 102 between First and Second Points, and (2) beach 103 between Second Point and Indian Point (Gillespie *et al.* 1998b).

Biological Sample Selection

The 1995 Savary Island survey was based on a stratified 2-stage design (see Chapter 16 for description of this design), with strip clusters of quadrats selected at the first stage, and a multiple systematic sample of quadrats between the high tide mark and the water selected at the second stage (Kronlund *et al.* 1998). A biological sample was selected by processing all clams from all quadrats on randomly selected first stage units within each stratum. Clams in the biological sample were measured for length, weight, age and length-at annulus data.

Results

Biological samples processed for length, weight and age included 735 Manila clams from beach 102 and 1,013 from beach 103. A subset of these animals yielded 1,160 and 3,358 lengths-at-annulus, for beaches 102 and 103, respectively.

Length-weight plots for both beaches do not reveal any obvious outliers that might indicate innaccurate data (Figure 13.1). The fitted power relationships are both nearly exactly cubic, but the majority of the points fall above the fitted line in the largest clams from beach 103, indicating that they are somewhat heavier than would be expected for their length. [Note: This can be an indication of poor growth, or **stunting.**]

Length frequency distribution at site 102 showed a large mode at 43 mm (Figure 13.2). Length frequency for site 103 showed a single broad mode at 24-32 mm (Figure 13.3). Age frequencies from both sites showed strong modes at 5 years of age (Figure 13.2 and Figure 13.3).

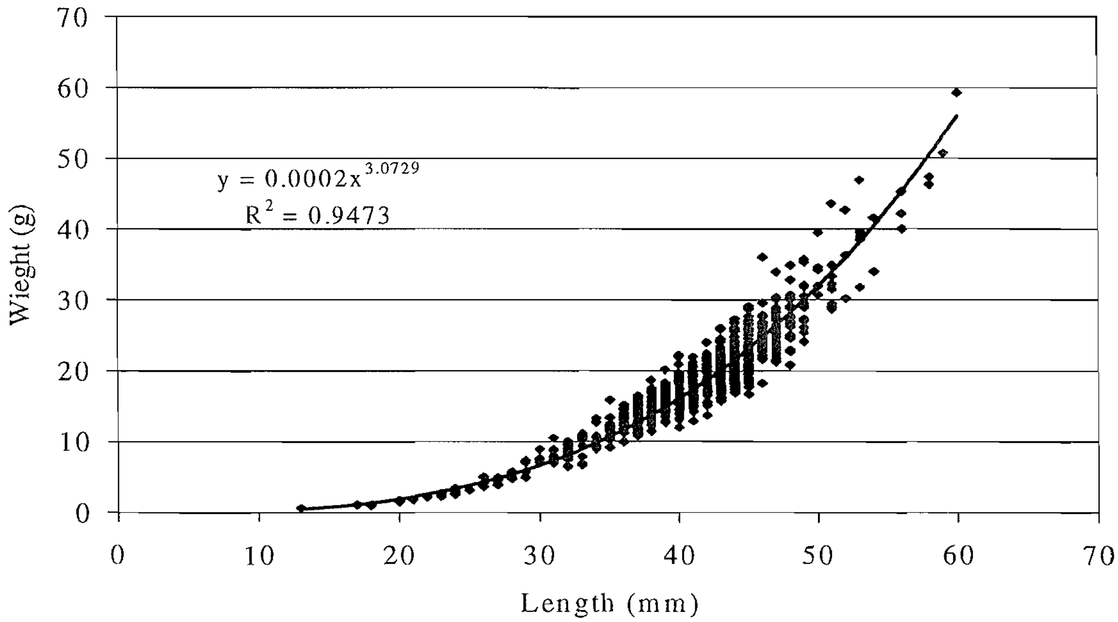
[Note: If lengths had been the only biological data used to assess the populations, one possible interpretation might be that beach 102 has a large number of legal-sized clams available for harvest, and that beach 103 has a large number of clams just less than legal size which will grow to harvest size in the next few years. However, length-based analyses alone can be misleading. Obviously, growth characteristics of the populations on the two beaches are different.]

Parameter estimates from von Bertalanffy models fit to length-at-annulus data from each beach were quite different, as was the estimated age at legal size (Table 13.1). The differences in growth are easier to see when the growth curves are overlaid (Figure 13.4). The growth curve for beach 102 crosses the horizontal line at 38 mm (representing legal size) at 4 years of age. The growth curve for beach 103 crosses the horizontal line between 6 and 7 years.

The low frequency of 2 and 3-year-old clams at both sites indicate low levels of settlement in recent years and serve warning that few clams are available to recruit to legal size over the next two years. The growth information indicates that clams grow poorly on beach 103, and will take at least 2.5 years longer to reach legal size. Most of them will not grow to legal size, either due to mortality, or possibly due to the effects of crowding or poor habitat.

Table 13.1 Parameter estimates from the von Bertalanffy growth model and age of recruitment to legal size for Manila clams from beaches 102 and 103, Savary Island, 1995 survey.

Beach	n	L_∞	K	t_0	Years to Legal Size
102	1,160	48.784	0.394	0.201	4.03
103	3,358	43.575	0.320	0.110	6.54



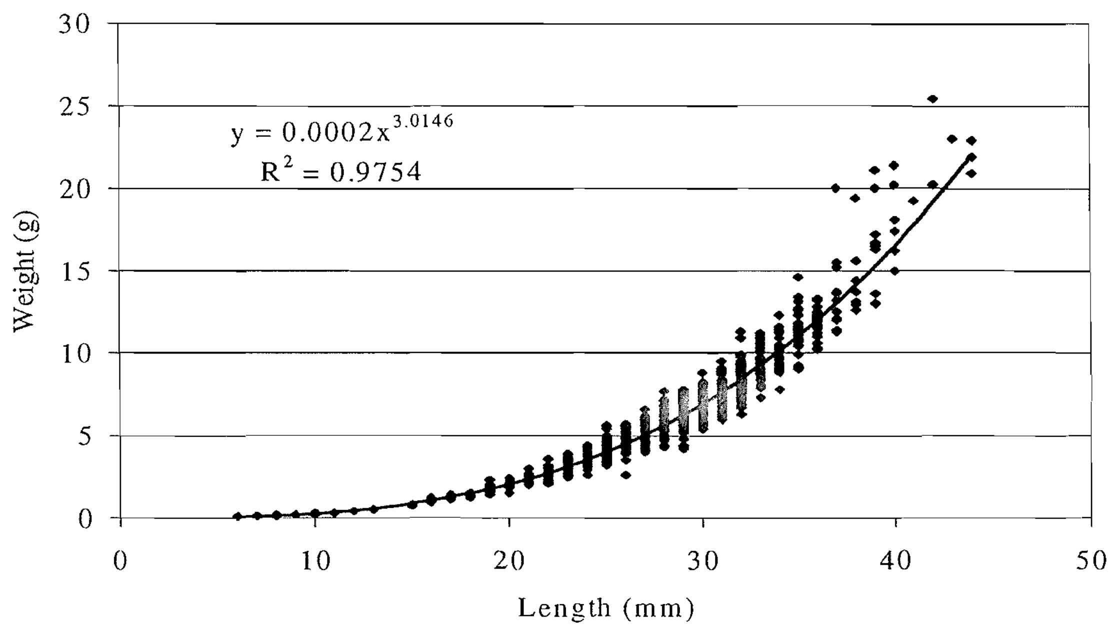


Figure 13.1 Length-weight plots of Manila clams from the 1995 survey of beaches 102 (upper plot) and 103 (lower plot) at Savary Island.

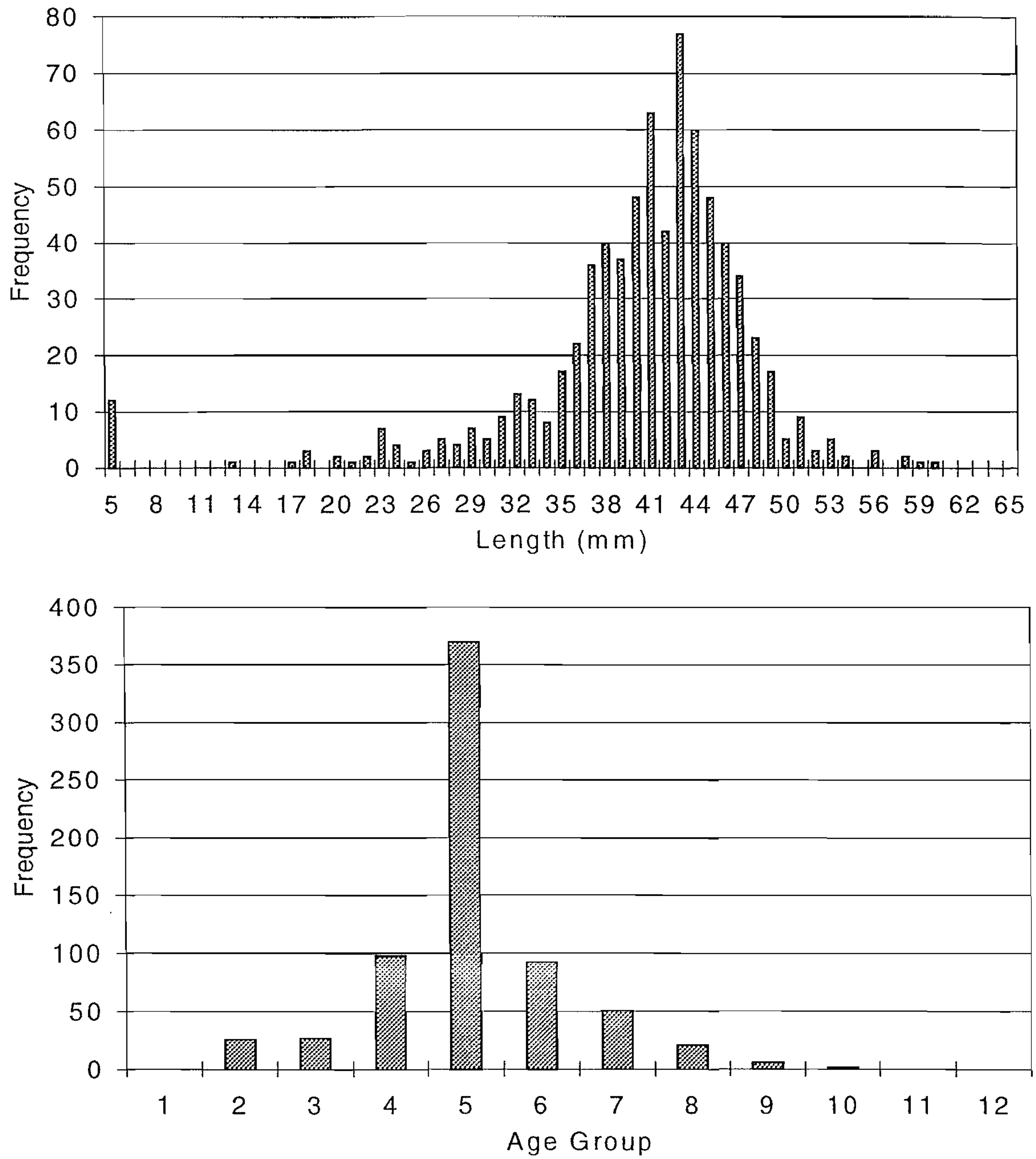


Figure 13.2 Length (upper panel) and age (lower panel) frequency distributions of Manila clams from the 1995 survey of beach 102 at Savary Island.

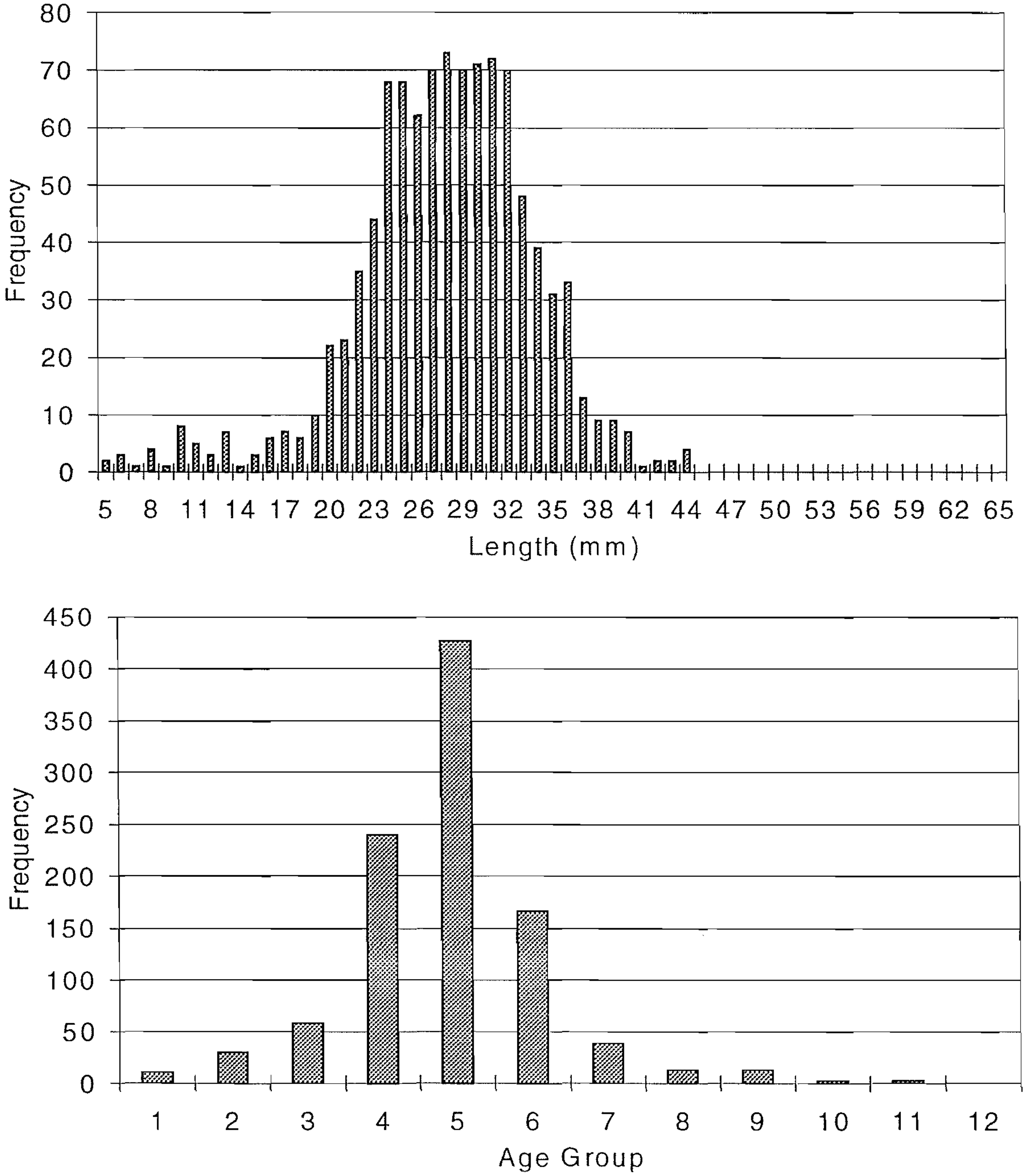


Figure 13.3 Length (upper panel) and age (lower panel) frequency distributions of Manila clams from the 1995 survey of beach 103 at Savary Island.

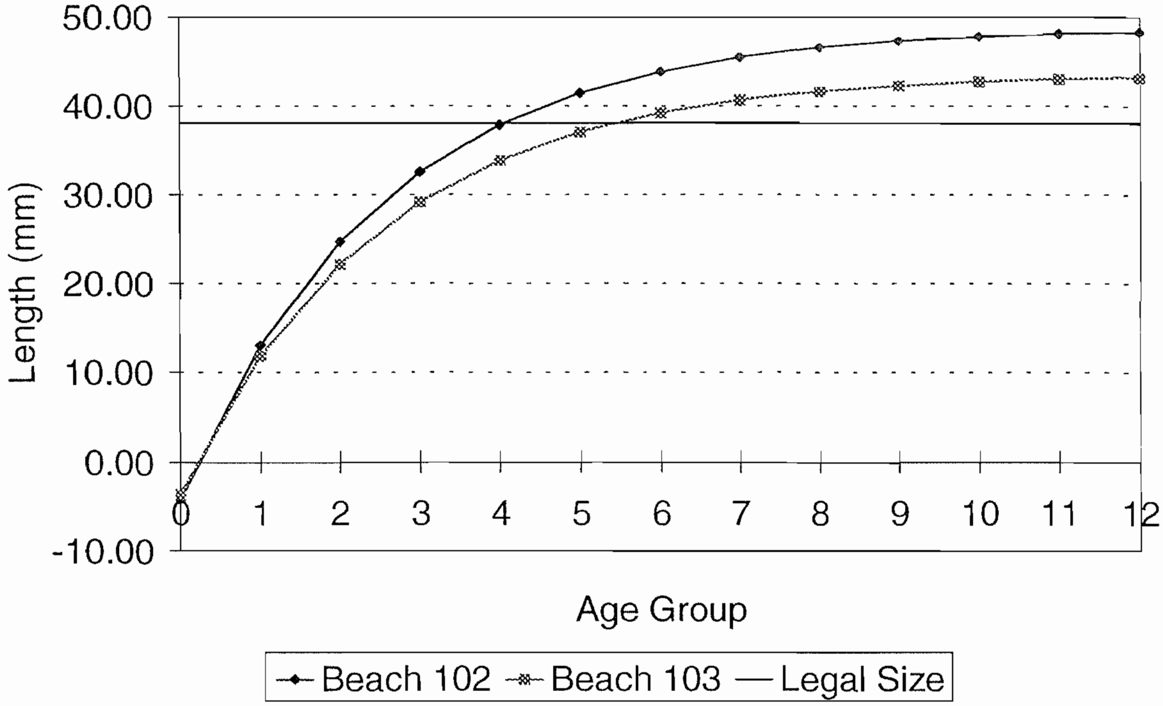


Figure 13.4 von Bertalanffy growth curves of Manila clams from the 1995 survey at Savary Island.

Chapter 14 - Two Stage Sampling

In essence, the various sampling designs are strategies for allocating survey effort over the population in an unbiased fashion. Simple random sampling, where a subset of n units is selected from a population of N units, is the basic method for many more complex designs. In SRS, the randomization occurs once when selecting the sample.

The sample size for most intertidal surveys is small relative to the beach area because of the costs of conducting a survey. A real hazard of a small sample size is that chance alone could result in a quadrats being selected from one end of a large beach. If costs could be ignored, the sample size could be increased to overcome this problem. However, available tides, manpower, and money limit all surveys.

One strategy for distributing the sampling over a large area is to conduct the randomization in two stages. First, the survey area is partitioned into large plots called **first stage units** (FSUs). Each large plot is in turn partitioned into smaller plots called **second stage units** (SSUs) or quadrats. When the sample is selected, a sample of first stage units is chosen at random. Then, a sample of quadrats is randomly chosen from each of the first stage units selected in the previous step. For a bivalve survey, the first stage units can be any shape, although rectangles are most convenient. For example, Figure 14.1 shows two possible schemes for two-stage sampling. In both diagrams a sample of n=5 first stage units has been chosen from a total of N=20 possible FSUs. Within each selected first stage unit, a sample of m=4 quadrats has been selected. In the upper diagram, the first stage units are rectangles with dimensions 5 by 5 quadrats. The lower diagram of Figure 14.1 shows FSUs that are long strips containing 20 quadrats in line.

Definition 3: *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. Then, a simple random sample of second stage units (quadrats) is selected from each of the first stage units already selected.*

In simple random sampling and stratified random sampling, 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.

Advantages of two-stage sampling

- survey effort can be spread over a large area;
- it is faster to locate quadrats within first stage units relative to locating quadrats at random over a larger area;
- mistakes may be confined to a single first stage unit;
- two variance components can be estimated to indicate whether more first stage units or more quadrats need to be sampled on subsequent surveys.

Although two-stage sampling may appear more complicated to randomize, the actual survey is no more difficult to lay out on a beach than simple random or stratified random sampling. Layout and analysis are easier if the first stage units are the same size. First stage units should be constructed so that each FSU contains measurements as different as possible; the ideal situation occurs when the among quadrat variance is high but the FSU means are nearly equal. This is the opposite condition to stratified random sampling where the quadrat measurements are intended to be as similar as possible and stratum means can differ.

What you will learn

- how to select a two-stage random sample;
- estimating the population mean and total;
- computing confidence intervals for a two-stage random sample;
- determining whether to sample more FSUs or more quadrats next survey.

Learning the material covered in this chapter requires that you have read and understood the information presented in Chapters 4 and 5. Statistical notation required for this chapter is contained in Table 14.1.

Selecting a two-stage random sample

What to do

1. Determine the number of distinct first stage sampling units, N;
2. Select a simple random sample of size n;
3. Determine the number of distinct quadrats $M_i, i = 1,2,\ldots,n$ in each of the first stage units selected in step (2);
4. Select a simple random sample of size $m_i, i = 1,2,\ldots,n$ from each of the first stage units selected in step (2).

Example 14.1 A two-stage random sample.

The simulated population of clams in Figure 5.2 *has been reproduced in* Figure 14.2. *Rather than choosing a simple random sample from the population,* *a two-stage sample was selected containing n=10 first stage units and m=4 quadrats per first stage unit. The total number of first stage units is N=16. Each FSU contains 25 quadrats. The sampling data are shown in* Table 14.2.

Choosing the first and second stage sample sizes

Two-stage sampling requires making a decision on the number of quadrats to sample within each FSU, and the total number of FSUs to sample. Sampling is performed in two stages to allow estimation of the two components of variance: (1) the variance among FSUs, and (2) the variance within FSUs (among quadrats). Estimates of these variances can help determine whether more FSUs should be sampled on subsequent surveys. Standard references that describe the required calculations include Cochran (1963, p.283, 313), Schaeffer *et al.* (1996, p. 285) and Thompson (1992, p. 132).

When there is no prior information from a survey site, sample at least three quadrats from each FSU and select as many FSUs as possible. The number of FSUs sampled is important since the variance of the estimated population mean depends on the variance among FSU means if *N* is large, as is the case for bivalve surveys. Furthermore, try to arrange the first stage units so that they contain an equal number of quadrats. This is usually possible if stratification is employed (see Chapter 16).

Estimating the population mean

One objective of a survey is to estimate the mean density of clams 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 in Table 14.3.

Example 14.2 How to compute the mean and variance for a two-stage design.

For the data simulated in Figure 14.2, *the mean and variance can be estimated using the equations in* Table 14.3. *The sample mean can be computed as follows:*

$$\begin{aligned}\bar{y}_{ts} &= \frac{N}{M}\left(\frac{M_1\bar{y}_1 + M_2\bar{y}_2 + \ldots + M_{10}\bar{y}_{10}}{n}\right)\\ &= \frac{16}{400}\frac{[25(2.75) + 25(3.00) + \ldots + 25(3.25)]}{10}\\ &= 2.65\end{aligned}$$

Then, the estimated variance of the two-stage mean is given by:

$$\hat{V}(\bar{y}_{ts}) = \left(\frac{16-10}{16}\right)\left(\frac{1}{10(25^2)}\right)1963.542 + \frac{1}{10(400)(25^2)}\sum_{i=1}^{n} 25(25-4)\left(\frac{s_i^2}{4}\right)$$

$$= 0.181$$

where

$$s_i^2 = \{6.25,\ 8.67, \ldots,\ 11.58\}.$$

Finally, the standard error of the two-stage mean is given by:

$$SE(\bar{y}_{ts}) = \sqrt{\hat{V}(\bar{y}_{ts})} = \sqrt{0.181} = 0.425$$

Estimating the population total

An estimate of the overall number or weight of clams in the survey area can be obtained by estimating the population total. The appropriate estimators are given in Table 14.4.

Example 14.3 How to compute the total and variance for a two-stage design.

For the data simulated in Figure 14.2, *the mean and variance can be estimated using the equations in* Table 14.4. *The sample total can be computed as follows:*

$$\hat{\tau}_{ts} = \frac{N}{n}(M_1\bar{y}_1 + M_2\bar{y}_2 + \ldots + M_{10}\bar{y}_{10})$$

$$= \frac{16}{10}[25(2.75) + 25(3.00) + \ldots + 25(3.25)]$$

$$= 1060$$

Then, the estimated variance of the two-stage total is given by:

$$\hat{V}(\hat{\tau}_{ts}) = 16(16-10)\frac{1963.542}{10} + \frac{16}{10}\sum_{i=1}^{10} 25(25-4)\left(\frac{s_i^2}{4}\right)$$

$$= 28930$$

where

$$s_i^2 = \{6.25,\ 8.67, \ldots,\ 11.58\}.$$

Confidence intervals*

Confidence intervals for population parameters can be computed in a variety of ways for two-stage random sampling. The assumption of normality yields the results given in Table 14.5.

Example 14.4 How to compute confidence intervals for the population mean and total for a two-stage design.

Recall that α=0.05 for a 100(1-0.05)=95% confidence interval. The interval for the population mean can be computed as follows:

$$\bar{y}_{ts} \pm z_{0.05/2}\sqrt{\hat{V}(\bar{y}_{ts})}$$
$$2.65 \pm (1.96)\sqrt{0.181}$$
$$2.65 \pm 0.833$$

Thus, the bounds of a 95% confidence interval for the population mean are given by (1.82, 3.48) clams per quadrat.

The 95% confidence interval for the population total is similarly computed as follows:

$$\hat{\tau}_{ts} = \pm z_{0.05/2}\sqrt{\hat{V}(\bar{y}_{ts})}$$
$$1060 \pm (1.96)\sqrt{28930}$$
$$1060 \pm 333.4$$

Thus, the bounds of a 95% confidence interval for the population total are (727, 1393) clams.

Further Reading

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

Table 14.1 Notation required for two-stage random sampling

Symbol	Description
i	index for the first stage units
j	index for the second stage units (quadrats)
N	total number of first stage units in the population
M	total number of second stage units (quadrats)
M_i	total number of second stage units in the i^{th} first stage unit
n	number of first stage units in the sample
m_i	number of quadrats the sample from the i^{th} first stage unit
y_{ij}	y-value j in first stage unit i (number of clams, weight of clams)
μ	the population mean
τ	the population total
$\bar{y}_{ts}$	the estimated population mean
$\hat{V}(\bar{y}_{ts})$	the estimated variance of the population mean
$\hat{\tau}_{ts}$	the estimated population total
$\hat{V}(\hat{\tau}_{ts})$	the estimated variance of the population total
s_i^2	the sample variance in the i^{th} first stage unit

Table 14.2 Data from a two-stage random sample.

Observation	First Stage Unit	Clam Count
1	2	3
2	2	2
3	2	0
4	2	6
5	7	3
6	7	0
7	7	1
8	7	0
9	13	0
10	13	0
11	13	0
12	13	0
13	3	2
14	3	3
15	3	0
16	3	7
17	11	2
18	11	4
19	11	0
20	11	3
21	10	5
22	10	4
23	10	7
24	10	4
25	14	2
26	14	10
27	14	6
28	14	5
29	16	8
30	16	3
31	16	2
32	16	0
33	15	4
34	15	3
35	15	1
36	15	2
37	12	0
38	12	3
39	12	0
40	12	1

Table 14.3 Estimators of the population mean and variance for a two-stage design.

Estimator of the population mean:

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

where

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

Estimator of the variance of the population mean:

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

where

$$s_1^2 = \frac{\sum_{i=1}^{n}(M_i\bar{y}_i - \bar{y}_1)^2}{n-1}$$

and

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

and

$$\overline{M} = \frac{M}{N}$$

and

$$\bar{y}_1 = \frac{1}{n}\sum(M_i y_i) = \frac{\hat{\tau}_{ts}}{N}$$

Table 14.4 Estimators of the population total and variance for a two-stage design.

Estimator of the population total:

$$\hat{\tau}_{ts} = M\bar{y}_{ts} = \frac{N}{n}\sum_{i=1}^{n} M_i \bar{y}_i$$

where

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

Estimator of the variance of the total:

$$\hat{V}(\hat{\tau}_{ts}) = N(N-n)\frac{s_1^2}{n} + \frac{N}{n}\sum_{i=1}^{n} M_i(M_i - m_i)\frac{s_i^2}{m_i}$$

where

$$s_1^2 = \frac{\sum_{i=1}^{n}(M_i\bar{y}_i - \bar{y}_1)^2}{n-1}$$

and

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

Table 14.5 Confidence intervals for two-stage random sampling.

Approximate 100(1-α)% confidence interval for the population mean:

$$\bar{y}_{ts} = \pm z_{\alpha/2}\sqrt{\hat{V}(\bar{y}_{ts})}$$

Approximate 100(1-α)% confidence interval for the population total:

$$\hat{\tau}_{ts} = \pm z_{\alpha/2}\sqrt{\hat{V}(\hat{\tau}_{ts})}$$

where z is the upper α/2 point of the standard Normal distribution (*e.g.*, $z_{0.025} = 1.96$).

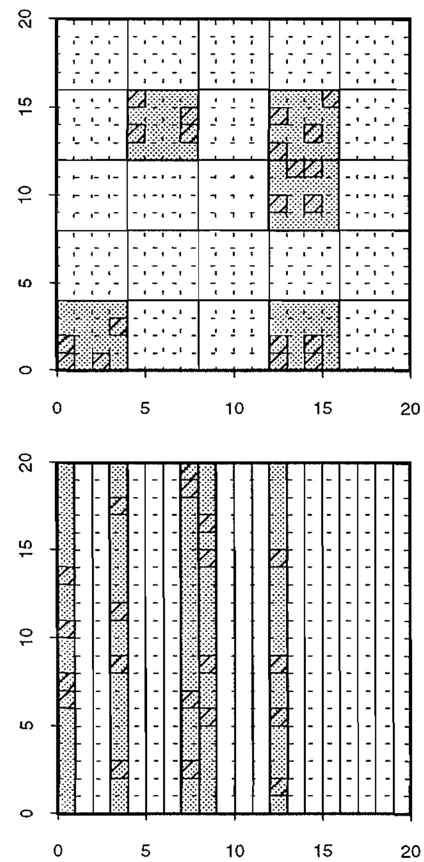


Figure 14.1 Two-stage random samples with n=5 first stage units and m=4 quadrats in each selected FSU. The lower diagram uses strips of quadrats as the first stage units.

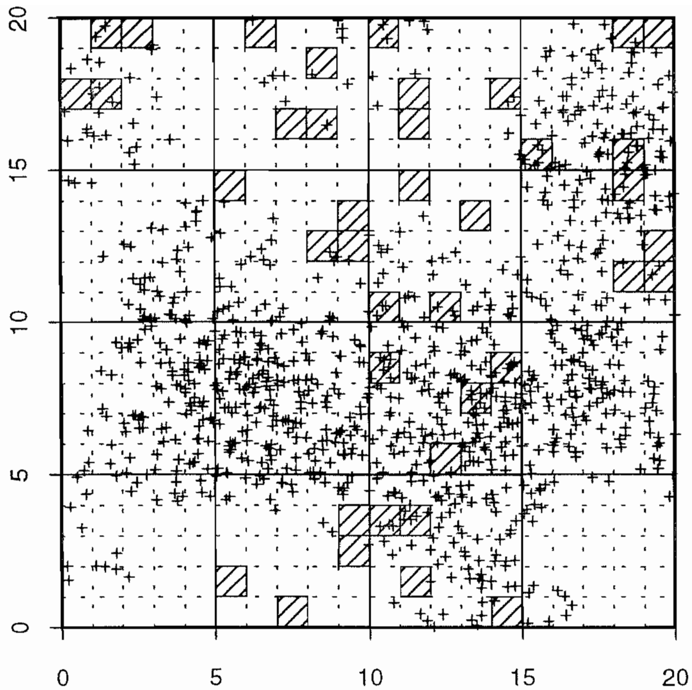


Figure 14.2 A two-stage random sample of n=10 first stage units with m=4 quadrats selected from each FSU. The positions of 1000 clams are indicated by the "+" symbols.

Chapter 15 - Case Study 4

This case study reports the results of a clam survey on the Goldstream Estuary as a prerequisite of proposed depuration harvests. For brevity, this case study presents the results of the survey and population estimates for Manila clams only. Reports for actual surveys would also include population estimates of littleneck clams, presentation of biological information and electronic and hard copies of original number and weight data and biological data. [Note: Because we have not used two-stage sampling in a single stratum survey, this case study we will examine one stratum from the 1996 Goldstream survey.]

Personnel and Contacts

The survey was designed and supervised by G. Gillespie (DFO PBS). Samples were collected by J. Bond, G. Gillespie, S. Head, G. Horonowitch and G. Jorgenson (DFO PBS).

Site Description

Goldstream Estuary lies at the head of Finlayson Arm, Saanich Inlet (48°29.7'N, 123°33.0'W). The harvest area is a large, gently sloping estuary cut by several active stream channels. The substrate is a thick layer of silt, except in and near the stream channels, where it is primarily gravel/sand. The harvest permit area extends from the overhead power lines adjacent to the mouth of Arbutus Creek to the southern tip of Sawluctus Island (Figure 15.1). [Note: The stratum used in this case study was closest to the head of the estuary.]

Survey Design and Randomization

The survey area was 80 m from high to low tide boundaries, and 150 m along the baseline. The reference line for first stage selection ran perpendicular to the tide line (Figure 15.1).

Distances along the reference line were selected in the first stage. Each distance represents a strip cluster of quadrats across the stratum. Because the reference line is 80 m long, and there are 2 possible quadrats per m, 5 random numbers were selected between 0 and 159. The selected numbers were then divided by 2 to convert to meters. The second stage of selection involved choosing 6 quadrats from all possible quadrats within each first stage unit (FSU). Six random numbers were chosen between 0 and 299 (as there are 300 possible samples in a 150 m strip cluster) and divided by 2 to convert to meters (Table 15.1).

Quadrat labels were composed of an FSU code and a quadrat number within each FSU (Table 15.2). Quadrat numbers follow the FSU number, separated by a dash. Thus, 1-1 is the first quadrat in FSU 1, 1-2 is the second, etc.

Field Results

The field survey was completed on August 26 and 27, 1996. Tides were 0.6 m at 0930 PDT on the 26th, and 0.5 m at 1015 PDT on the 27th. A total of 30 quadrats were sampled (Table 15.2). This was slightly less than the protocol requirement of 36 samples from a survey area this size. Samples were returned to PBS for sorting and processing while alive.

Biomass and Abundance Estimates

Density and total stock estimates for Manila clams are in Table 15.3 and Table 15.4. Mean weights of Manila clams were 22.72 g for legal-size and 8.53 g for sublegal-size. Precision of estimates was relatively good for Manila clams. Confidence intervals were 30% and 14% of the total estimated abundance and 35% and 20% of the total estimated biomass, for legal and sublegal size categories, respectively. M_{opt} values were 2.79 for biomass and 4.23 for abundance.

Biological Data

Biological samples were chosen by randomly selecting FSUs from all available FSUs and processing all of the clams in each quadrat for length, weight and age. Additional FSUs were selected until a total of at least 200 Manila and littleneck clams were measured, and the final FSU completed. [Note: The results are not included in this case study for brevity. Complete survey reports would be accompanied by figures summarizing length and age frequencies of Manila and littleneck clams.]

Original Data

[Note: Summaries of the original data are not included in this case study for brevity. Complete survey reports would be accompanied by electronic and hard copies of the both the number and weight and biological data.]

Table 15.1 Survey area (m^2), number of potential (N) and selected (n) FSUs, number of potential and selected quadrats per FSU, and overall number of potential (M*N) and selected (m*n) quadrats for stratum 1 of the 1996 Goldstream Estuary clam survey.

Area (m^2)	N	n	M	m	M*N	m*n
12,000	160	5	300	6	48,000	30

Table 15.2 Quadrat location and number and weight data for Manila clams from the 1996 Goldstream Estuary clam survey.

Quadrat	x (m)	y (m)	Quad. Size (m^2)	Species	# Legals	# Sublegals	Wt Legals (g)	Wt Sublegals (g)
1-1	6.5	7.0	0.25	82B	4	3	103	32
1-2	6.5	46.5	0.25	82B	0	0	0	0
1-3	6.5	87.0	0.25	82B	21	38	392	336
....	....	....	....	....	....	....	....	....
5-5	78.5	135.5	0.25	82B	12	8	337	59
5-6	78.5	141.5	0.25	82B	20	12	566	95

Table 15.3 Density and total abundance estimates of Manila clams from the 1996 Goldstream Estuary clam survey.

Legal Density (#/m^2)	SE	Sublegal Density (#/m^2)	SE	Total Legals ('000's)	95% CL ('000's)	Total Sublegals ('000's)	95% CL ('000's)
40.13	1.11	56.67	0.76	481.6	(338.3; 624.9)	680.0	(582.7; 777.3)

Table 15.4 Density and total biomass estimates of Manila clams from the 1996 Goldstream Estuary clam survey.

Legal Density (kg/m^2)	SE	Sublegal Density (kg/m^2)	SE	Total Legals (kg)	95% CL (kg)	Total Sublegals (kg)	95% CL (kg)
0.912	0.030	0.483	0.009	10,944	(7,136; 14,752)	5,800	(4,621; 6,979)

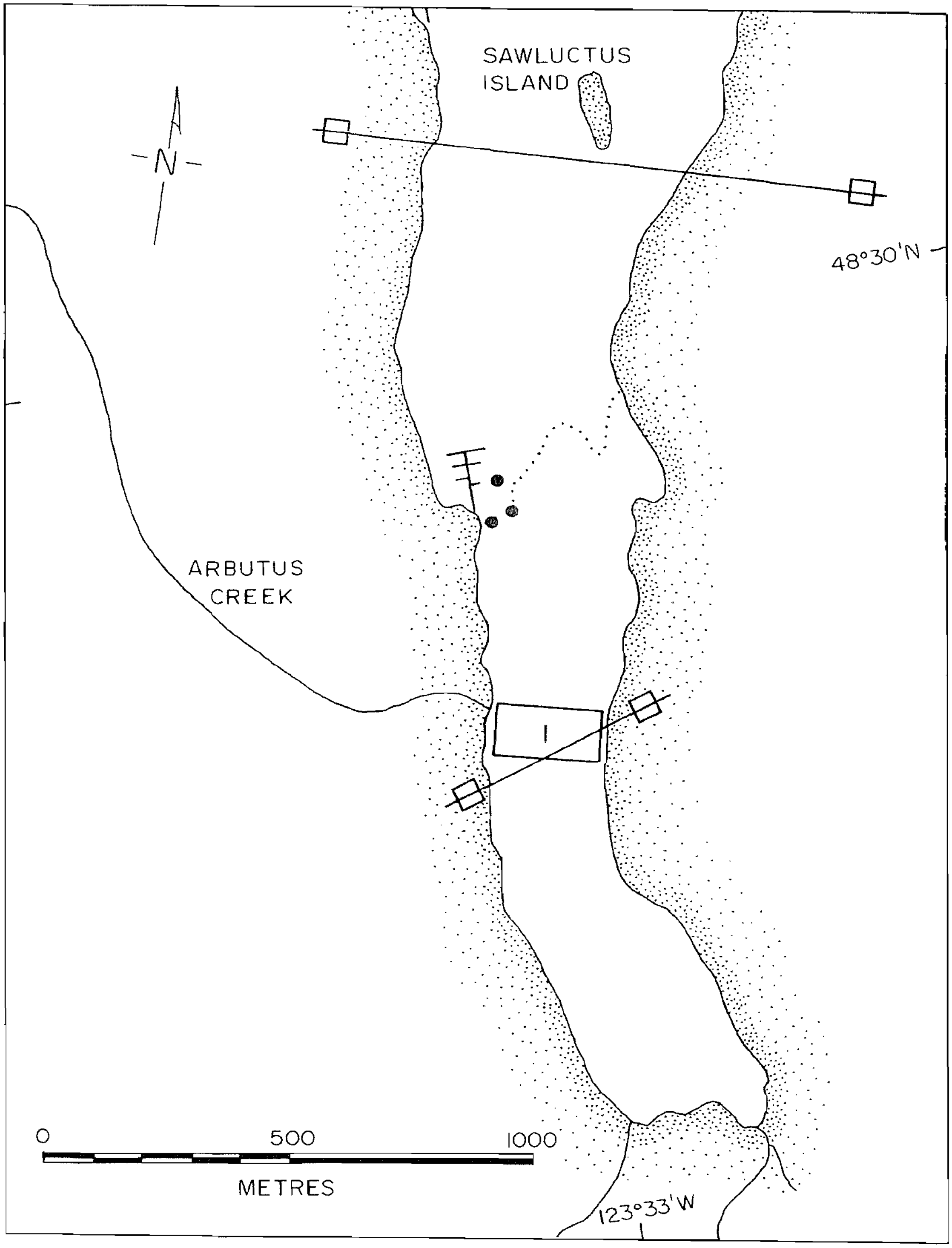


Figure 15.1 Layout of the 1996 Goldstream Estuary clam survey.

Chapter 16 - Stratified Two Stage Sampling

Stratified multi-stage sampling designs are commonly encountered in large-scale surveys. In this chapter, the analysis of a stratified two-stage design with the following characteristics is described (1) the first stage units are of equal size within each stratum, (2) the first stage units can vary in size among strata, and (3) second stage units are of equal size. No new design concepts are involved; the entire population of first stage units is partitioned into strata just as in stratified random sampling. First stage units are selected from each stratum independently, and a random sample of quadrats is chosen from each selected first stage unit as in two-stage sampling. For example, Figure 16.1 shows a diagram of a stratified two-stage sample.

As discussed in Chapter 14, the size and shape of strata and first stage units is very flexible, although rectangles are most convenient for randomization and physical layout. The design described in this chapter is restricted to the case where FSUs are the same size within a given stratum, but may vary in size among strata. Also, the number of quadrats selected from an FSU is the same within each stratum. The restriction that FSUs be of equal size within a stratum is not difficult to achieve in the field by carefully choosing the size and shape of each stratum.

Definition 4*: A stratified two-stage random sample requires that the first stage units be partitioned into non-overlapping strata. The sample is obtained by first selecting a simple random sample of first stage units within each stratum. Then, a simple random sample of second stage units (quadrats) is drawn from each of the previously selected first stage units. Randomization occurs independently among strata.*

In simple random sampling and stratified random sampling, the variability of the estimate of the mean or total occurs because different samples of quadrats (the first stage units) give different estimates. 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.

Advantages of stratified two-stage sampling

- survey effort can be spread over a large area using stratification and two randomization stages;
- stratification may increase the precision of estimates of the population mean or total;
- mistakes may be confined to a single stratum or single first stage unit;
- it is faster to locate quadrats within first stage units, relative to locating quadrats at random over a larger area;

- two variance components can be estimated to indicate whether more first stage units or more quadrats need to be sampled on subsequent surveys.

What you will learn

- how to select a stratified two-stage random sample;
- estimating the population mean and total;
- computing confidence intervals for a two-stage random sample.

Learning the material covered in this chapter requires that you have read and understood the information presented in Chapters 4, 5 and 14. Statistical notation required for this chapter is contained in Table 16.1.

Selecting a two-stage random sample

What to do

1. Partition the first stage units into H non-overlapping strata;
2. Determine the number of distinct first stage sampling units, N_h, within each stratum;
3. Select a simple random sample of n_h first stage units from each stratum;
4. Determine the number of distinct quadrats in each of the first stage units, M_h, selected in step (3);
5. Select m_h quadrats from each of the FSUs selected in step (3), using simple random sampling.

Example 16.1 A stratified two-stage random sample.

The simulated population of clams in Figure 5.2 *has been reproduced in* Figure 16.2. *A stratified two-stage sample has been selected. The strata contain 200, 150, and 50 quadrats. Sample sizes are 10, 5, and 2 FSUs, respectively. In this case, all first stage units contain 10 quadrats, with three quadrats selected at random from each FSU. The sampling data are shown in* Table 16.2.

Estimating the population mean

One objective of a survey is to estimate the mean density of clams 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 in Table 16.3.

Example 16.2 How to compute the mean and variance for stratified two-stage sampling.

For the simulated data in Figure 16.2, *the mean and variance can be estimated using the equations in* Table 16.3. *The sample mean* $\bar{y}_{sts}$ *is the average of the y-values in the sample:*

$$\begin{aligned}\bar{y}_{sts} &= W_1\bar{y}_1 + W_2\bar{y}_2 + W_3\bar{y}_3\\ &= \frac{(20)(10)}{400}1.8 + \frac{(15)(10)}{400}3.2 + \frac{(5)(10)}{400}4.0\\ &= 2.6\end{aligned}$$

Then, the estimated variance of the stratified two-stage mean is given by:

$$\begin{aligned}\hat{V}(\bar{y}_{sts}) &= 0.5^2\left[\frac{1-10/20}{10}4.92 + \frac{(10/20)(1-3/10)}{(10)(3)}2.2\right]\\ &+0.375^2\left[\frac{1-5/15}{5}1.37 + \frac{(5/15)(1-3/10)}{(5)(3)}5.6\right]\\ &+0.125^2\left[\frac{1-2/5}{2}5.56 + \frac{(2/5)(1-3/10)}{(2)(3)}9.3\right]\\ &= 0.1386\end{aligned}$$

Finally, the standard error of the stratified two-stage mean is given by:

$$SE(\bar{y}_{sts}) = \sqrt{\hat{V}(\bar{y}_{sts})} = \sqrt{0.1386} = 0.372$$

Estimating the population total

An estimate of the total number or weight of clams in the survey area can be obtained by estimating the population total, τ_{sts}. The required estimators are given in Table 16.4.

Example 16.3 How to compute the total and variance for a stratified two-stage design.

For the simulated data in Figure 16.2, *the estimated total and variance can be computed using the equations in* Table 16.4. *The population total can be estimated as follows:*

$$\begin{aligned}\hat{\tau}_{sts} &= [(20)(10) + (15)(10) + (5)(10)]2.6\\ &= 1040\end{aligned}$$

Then, the estimated variance of the stratified two-stage total is given by:

$$\begin{aligned}\hat{V}(\hat{\tau}_{sts}) &= (400)^2 0.1386\\ &= 22{,}192\end{aligned}$$

Confidence intervals*

Confidence intervals for population parameters can be computed using a standard normal approximation as shown in Table 16.5. For a 95% confidence interval, $z_{\alpha/2}$=1.96. The 95% confidence interval for the population mean is (1.87, 3.33) clams per quadrat for the data in Table 16.2.

Further Reading

The stratified two-stage sampling design presented in this chapter is part of a larger class of multi-stage sampling designs. More information can be found in the books by Cochran (1963), Schaeffer *et al.* (1996) and Thompson (1992).

Table 16.1 Notation required for stratified two-stage random sampling.

Symbol	Description
h	Index for the strata
i	Index for the first stage units
j	Index for the second stage units (quadrats)
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
μ	The population mean
τ	The population total
$\bar{y}_{sts}$	The estimated population mean
$\hat{V}(\bar{y}_{sts})$	The estimated variance of the population mean
$\hat{\tau}_{sts}$	The estimated population total
$\hat{V}(\hat{\tau}_{sts})$	The estimated variance of the population total
s_{1h}^2	The sample variance **among** first stage units in the stratum h
s_{2h}^2	The sample variance **within** the first stage units in stratum h

Table 16.2 Data from a stratified two-stage random sample.

Observation	Stratum	First Stage Unit	Clam Count
1	1	9	0
2	1	9	2
3	1	9	3
4	1	7	9
5	1	7	9
6	1	7	4
7	1	20	0
8	1	20	1
9	1	20	0
10	1	4	0
11	1	4	1
12	1	4	2
13	1	10	6
14	1	10	3
15	1	10	2
16	1	12	0
17	1	12	0
18	1	12	1
19	1	17	0
20	1	17	0
21	1	17	3
22	1	5	3
23	1	5	2
24	1	5	1
25	1	1	0
26	1	1	0
27	1	1	0
28	1	15	0
29	1	15	0
30	1	15	2
31	2	4	0
32	2	4	4
33	2	4	0
34	2	15	3
35	2	15	4
36	2	15	6
37	2	13	4
38	2	13	5
39	2	13	0
40	2	3	4
41	2	3	0
42	2	3	6
43	2	6	4
44	2	6	6
45	2	6	2
46	3	1	1
47	3	1	8
48	3	1	8
49	3	4	4
50	3	4	1
51	3	4	2

Table 16.3 Estimators of the population mean and variance for a stratified two-stage design.

Estimator of the population mean:

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

where

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

Estimator of the variance of the population mean:

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

where

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

and

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

Table 16.4 Estimators of the population total and variance for stratified two-stage sampling.

Estimator of the population total:

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

Estimator of the variance of the total:

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

Table 16.5 Confidence intervals for stratified two-stage random sampling.

Approximate 100(1-α)% confidence interval for the population mean:

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

Approximate 100(1-α)% confidence interval for the population total:

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

where z is the upper α/2 point of the standard Normal distribution.

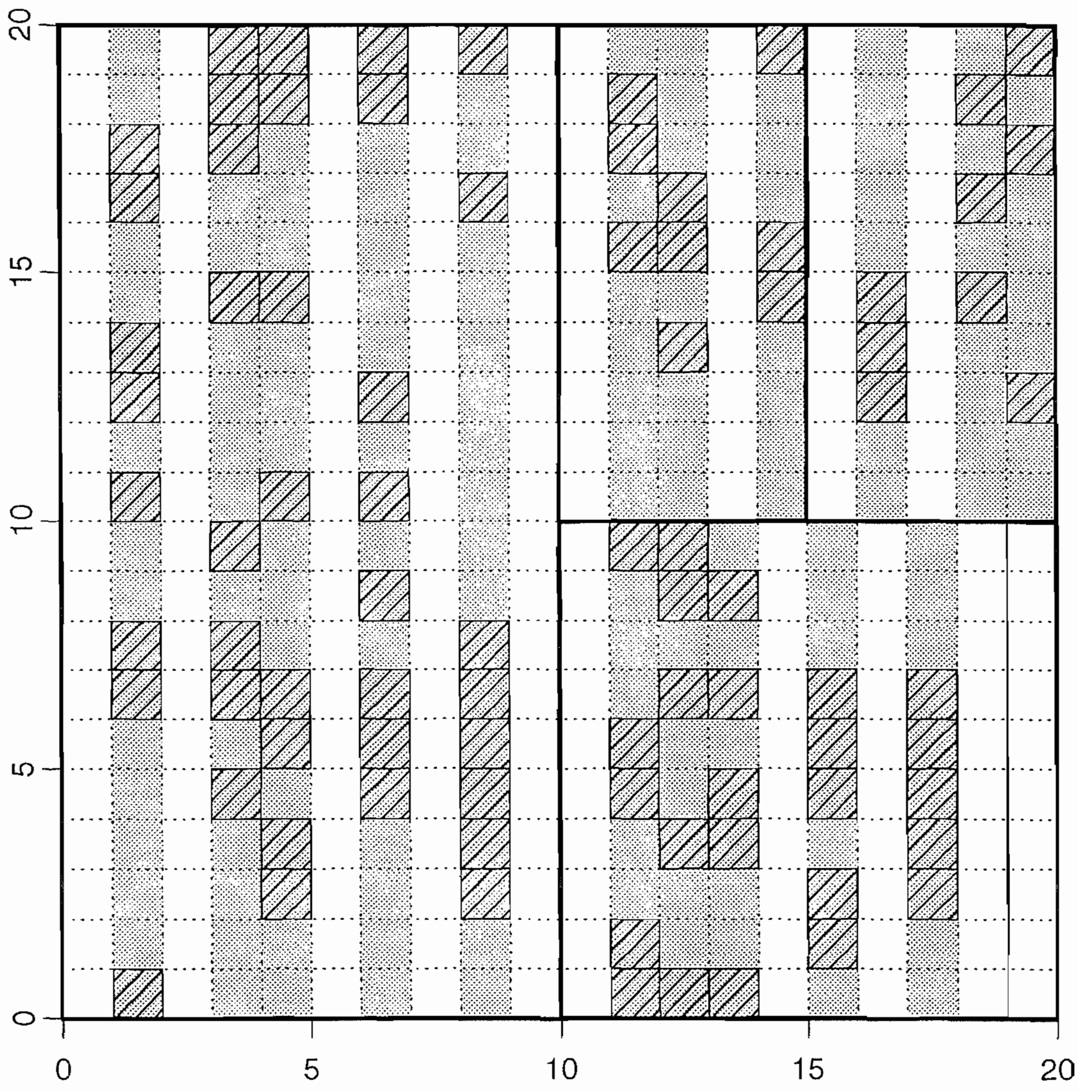


Figure 16.1 Diagram of a stratified two-stage sampling design. The four strata contain 5, 5, 3, and 3 first stage units. Within each stratum, the number of quadrats selected is the same within each first stage unit (8,5,3,3).

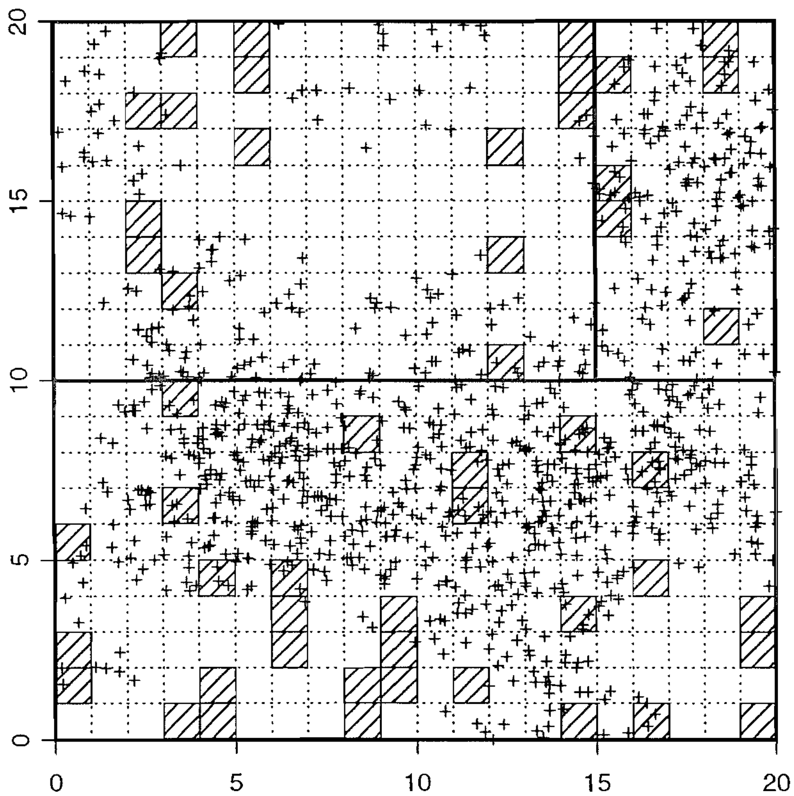


Figure 16.2 A stratified two-stage random sample. Three strata containing 200, 150, and 50 quadrats are bounded by thick lines. First stage units (10, 5, and 2) were selected at random without replacement independently from the three strata, with three quadrats selected at random without replacement from each FSU.

Chapter 17 - Case Study 5

This case study reports the results of a clam survey on the Goldstream Estuary as a prerequisite of proposed depuration harvests. For brevity, this case study presents the results of the survey and population estimates for Manila clams only. Reports for surveys submitted to DFO require population estimates of littleneck clams, presentation of biological information and electronic and hard copies of original number and weight data and biological data.

Personnel and Contacts

The survey was designed and supervised by G. Gillespie (DFO PBS). Samples were collected by J. Bond, G. Gillespie, S. Head, G. Horonowitch and G. Jorgenson (DFO PBS).

Site Description

Goldstream Estuary lies at the head of Finlayson Arm, Saanich Inlet (48°29.7'N, 123°33.0'W). The harvest area is a large, gently sloping estuary cut by several active stream channels. The substrate is a thick layer of silt, except in and near the stream channels, where it is primarily gravel/sand. The harvest permit area extends from the overhead power lines adjacent to the mouth of Arbutus Creek to the southern tip of Sawluctus Island.

Survey Design and Randomization

Each stratum was 80 m from high to low tide boundaries. The reference line for first stage selection ran perpendicular to the tide line from a point directly beneath the white marker on the overhead powerlines to Sawluctus Island, bearing 353.5° magnetic. The base lines for each stratum were aligned on a bearing of 83.5° magnetic, at 80 m intervals along the reference line. Strata were aligned with clam bearing area by moving the stratum boundaries east or west, and varying the width of strata to include all harvestable ground. The widths of strata 1-4 were 150, 132, 160 and 125 m along the baseline, respectively (Figure 17.1).

Distances along the reference line were selected in the first stage. Each distance represents a strip cluster of quadrats across the long axis of each stratum. Because the reference line is 80 m long, and there are 2 possible quadrats per m, there are 160 potential FSUs in each stratum. Five random numbers were selected between 0 and 159. The selected numbers were then divided by 2 to convert to meters. The second stage of selection involved choosing 6 quadrats from all possible quadrats within each FSU. In the first stratum, 6 random numbers were chosen between 0 and 299 (as there are 300 possible samples in a 150 m strip cluster) and divided by 2 to convert to meters (Table 17.1).

Quadrat labels were composed of a stratum code, an FSU code and a quadrat number within each FSU (Table 17.2). The stratum is reflected in the hundreds column of the label: stratum 1 is all "100s", stratum 2 is "200s", etc. Each FSU is documented in the ones column of the label: 101 represents the first FSU in stratum 1, 102 the second, etc. Quadrat numbers follow the stratum/FSU number, separated by a dash. Thus, 101-1 is the first quadrat in FSU 1 of stratum 1, 101-2 is the second, etc.

Field Results

The field survey was completed on August 26 and 27, 1996. Tides were 0.6 m at 0930 PDT on the 26th, and 0.5 m at 1015 PDT on the 27th. A total of 120 quadrats were sampled (Table 17.2). This was slightly less than the protocol requirement of 136 samples from a survey area this size. Samples were returned to PBS for sorting and processing while alive.

Biomass and Abundance Estimates

Density and total stock estimates for Manila clams are in Table 17.3 and Table 17.4. Mean weights of Manila clams were 23.33 g for legal-size and 7.28 g for sublegal-size. Precision of estimates was relatively good for Manila clams. Confidence intervals were 17 and 19% of the total estimated sublegal and legal abundance and 21 and 19% of the total estimated sublegal and legal biomass, respectively. M_{opt} values were generally less than 6, suggesting that future survey design may wish to utilize more FSUs with fewer quadrats per FSU.

Biological Data

Biological samples were chosen using a two-stage approach. In the first stage, strata were chosen at random without replacement. Within the chosen stratum, a single FSU was chosen at random without replacement from all available FSUs and processing all of the clams in each quadrat for length, weight and age. Once all strata had been selected, the process was repeated, until a total of at least 500 Manila and littleneck clams were measured, and the final selected FSU completed. [Note: The results are not included in this case study for brevity. Complete survey reports would be accompanied by figures summarizing length and age frequency distributions for Manila and littleneck clams.]

Original Data

[Note: Summaries of the original data are not included in this case study for brevity. Complete survey reports would be submitted with electronic and hard copies of both number and weight and biological data.]

Table 17.1 Stratum area (m^2), number of potential (N_h) and selected (n_h) FSUs, number of potential and selected quadrats per FSU, and overall number of potential (M_h*N_h) and selected (m_h*n_h) quadrats for the 1996 Goldstream Estuary clam survey.

Stratum	Area (m^2)	N_h	n_h	M_h	m_h	M_h*N_h	m_h*n_h
1	12,000	160	5	300	6	48,000	30
2	10,560	160	5	264	6	42,240	30
3	12,800	160	5	320	6	51,200	30
4	10,000	160	5	250	6	40,000	30
Total	45,360					181,440	120

Table 17.2 Quadrat location and number and weight data for Manila clams from the 1996 Goldstream Estuary clam survey.

Quadrat	x (m)	y (m)	Quad. Size (m^2)	Species	# Legals	# Sublegals	Wt Legals (g)	Wt Sublegals (g)
101-1	6.5	7.0	0.25	82B	4	3	103	32
101-2	6.5	46.5	0.25	82B	0	0	0	0
101-3	6.5	87.0	0.25	82B	21	38	392	336
....	....	....	....	....	....	....	....	....
405-5	76.0	88.5	0.25	82B	15	12	301	83
405-6	76.0	120.5	0.25	82B	9	6	207	58

Table 17.3 Density and total abundance estimates of Manila clams from the 1996 Goldstream Estuary clam survey.

Stratum	Legal Density (#/m^2)	SE	Sublegal Density (#/m^2)	SE	Total Legals ('000's)	95% CL ('000's)	Total Sublegals ('000's)	95% CL ('000's)
1	40.13	1.11	56.67	0.76	481.6	(338.3; 624.9)	680.0	(582.7; 777.3)
2	48.93	1.90	52.93	1.95	516.7	(301.5; 731.9)	559.0	(337.8; 780.2)
3	56.53	1.53	83.07	1.87	723.6	(513.8; 933.4)	1,063.3	(806.0; 1,320.6)
4	45.07	2.21	57.33	2.95	450.7	(213.6; 687.8)	573.3	(256.2; 891.6)
Total					2,172.6	(1,763.9; 2,581.3)	2,875.6	(2,401.1; 3,350.1)

Table 17.4 Density and total biomass estimates of Manila clams from the 1996 Goldstream Estuary clam survey.

Stratum	Legal Density (kg/m^2)	SE	Sublegal Density (kg/m^2)	SE	Total Legals (kg)	95% CL (kg)	Total Sublegals (kg)	95% CL (kg)
1	0.912	0.030	0.483	0.009	10,944	(7,136; 14,752)	5,800	(4,621; 6,979)
2	1.117	0.041	0.396	0.016	11,800	(7,131; 16,469)	4,183	(2,347; 6,019)
3	1.254	0.035	0.613	0.018	16,053	(11,265; 20,841)	7,847	(5,395; 10,299)
4	1.165	0.056	0.327	0.027	11,655	(5,667; 17,643)	3,267	(366; 6,168)
Total					50,452	(40,701; 60,203)	21,097	(16,716; 25,478)

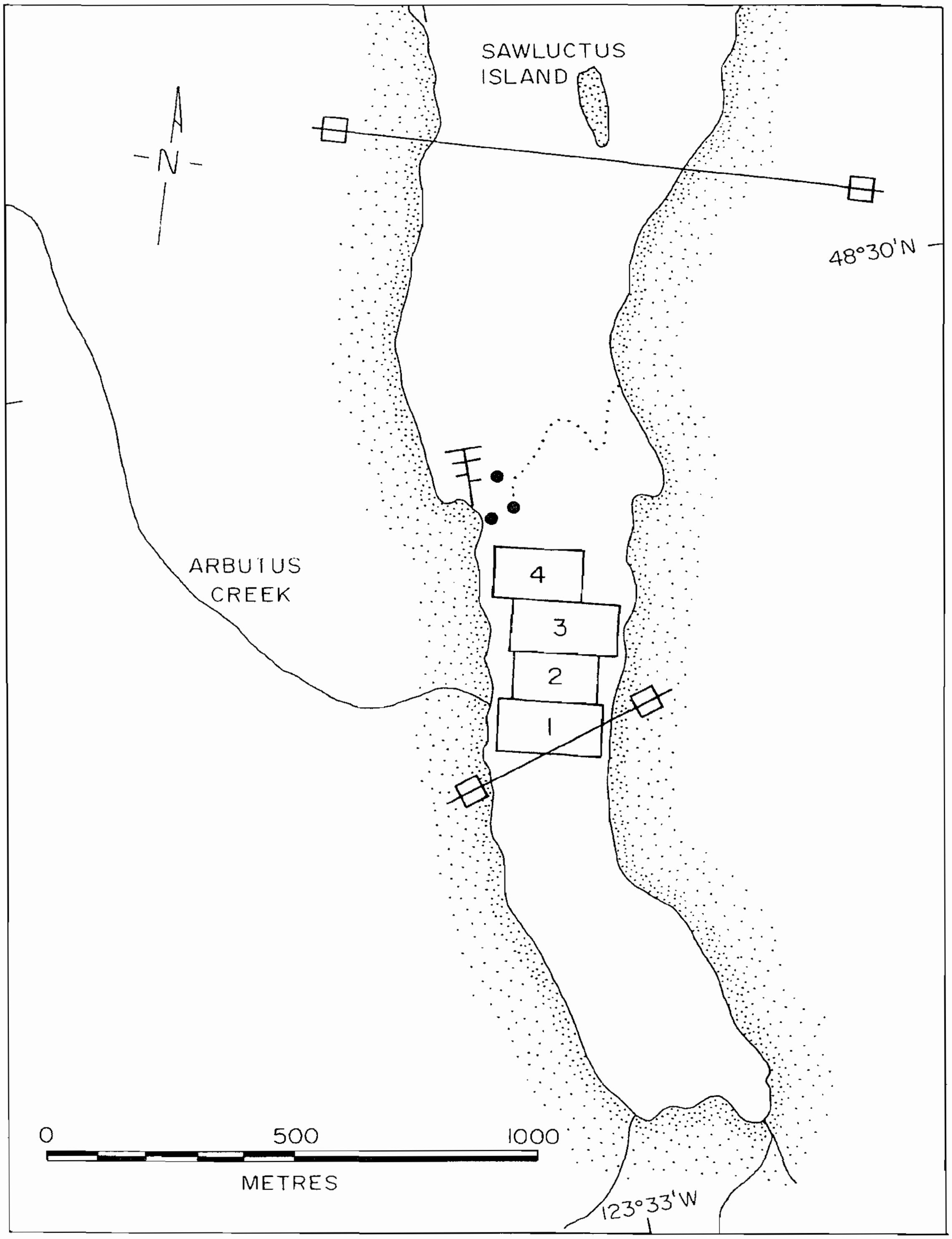


Figure 17.1 Layout of the 1996 Goldstream Estuary clam survey.

Acknowledgments

We thank the many people who assisted us in surveys at Goldstream Estuary, Dunsmuir Islands and Savary Island. Discussions with Carl Schwarz were extremely helpful in the formulation of sampling considerations. Thanks also to Tim Mulligan, who suggested using range finders on large beaches. We thank Bruce Adkins, Jim Boutillier and Kerry Marcus for their reviews of the manuscript.

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Glossary

ABUNDANCE - the number of organisms in a given area or volume of habitat. We refer to the abundance of a given species of clams within a survey area, measured in number of individuals.

ACCURACY – a measure of the closeness of a parameter estimate to the actual value.

ADDUCTOR MUSCLES - a pair of muscles, one forward and one to the rear, that draw the shells together when contracted.

AGE CLASS - a number of animals which have been assigned a similar age. Not the same as year class, since errors in ageing may assign animals one year class to more than one age group.

ANNULUS - any of the concentric checks on the outer surface of the shell of a bivalve mollusc which is deposited once per year during a seasonal period of slow growth, and is used to assign an age group to the animal. Plural: annuli.

BED - a concentration of clams, most often used to denote concentrations sufficient to support commercial harvesting activities. Also used for other commercially exploited invertebrates, *e.g.*, geoducs, sea cucumbers.

BIOMASS - the amount of living material in a given area or volume of habitat. We refer to the biomass of a given species of clams within a survey area, measured in kilograms or tonnes.

BIVALVE - having two valves or shells. Bivalve molluscs include clams, oysters, scallops and mussels.

CHECKS - marks, growth zones or parts of growth zones which do not form on an annual basis (as annuli do) but reflect environmental or physiological changes which affect the growth of the animal.

CIRCULUS - any of the concentric checks on the outer surface of the shell of a bivalve mollusc. Circuli may be deposited annually (see **ANNULUS**) or may be deposited as a result of other circumstances which decrease the growth rate of the animal (spawning checks, disturbance checks, temperature shocks, etc.).

CONCENTRIC SCULPTURE - fine lines on the outer surface of the shells of clams and scallops which represent historic positions of the outer margin of the shell.

CONFIDENCE COEFFICIENT - the probability that the confidence interval contains the true population value on repeated sampling.

Confidence Interval - a numerical range that has a specified probability of containing the true population value on repeated sampling.

Confidence Limits - the endpoints of confidence intervals. For example, if an estimated parameter is 2.0 and the confidence interval is +/- 0.5, the confidence limits are 1.5 and 2.5.

Density - the number or weight of organisms per unit area or volume of habitat. We refer to the density of a given species of clams within a survey area, measured in number of clams per square meter or weight of clams per square meter.

Design Effect - a statistic which compares the variance resulting from a stratified random estimator to the variance if stratification is disregarded, *i.e.*, the survey is analysed as a simple random sample to determine whether precision was increased by stratification.

Edge Effect - potential bias introduced to survey samples due to decisions regarding whether or not to include clams in the sample when they occur on the edge of the quadrat. Inclusion of too many clams (clams that do not stick more than halfway into the quadrat) will bias the resulting estimates upward.

Estimate - a number (statistic) calculated using the sample data, *e.g.* the mean.

Estimator - a mathematical equation for calculating an estimate.

Filter Feeding - a type of suspension feeding in which particles (plankton and detritus) are collected form a water current and ingested.

First Stage Units - the sampling units selected in the first randomization step of a multi-stage design.

Frequency Distribution – a tabulation of the number of times observations occur in specified intervals. For example, the number of clams determined to be age two, three, ..., seven and eight. The frequency distribution is often presented graphically as a histogram or bar chart.

Height - the greatest straight-line distance through a clam from the umbo to the ventral surface of the valves.

Independently - in a statistical sense, the concept that a sampling unit is selected in a way that is not influenced by previous selections.

Larva - a young stage of certain animals which occurs before the final adult form is acheived. Plural: larvae

LEGAL-SIZED - a clam which has grown larger than the minimum legal size, or has not attained the maximum legal size. See **SIZE LIMIT.**

LENGTH - the greatest straight-line distance through a clam from the anterior to posterior margins of the valves (see Figure 3.1).

LENGTH-AT ANNULUS - measurements of shell length at each visible annual check on a shell's surface, which provides a growth history for each individual animal.

LIGAMENT - a spring-like structure joining the dorsal edges of bivalve shells. The ligament forces the shells open when the adductor muscles are relaxed.

LUNULE - a heart-shaped depression in front of and close to the umbones.

MEAN - the arithmetic mean, or average of a set of data.

MEAN BIOMASS - the amount of living material per unit area or volume of habitat. For example, the mean weight (kg) per square meter of Manila clams.

MEAN DENSITY - the number of living organisms per unit area or volume of habitat. For example, the mean number per square meter of Manila clams.

MUSCLE SCARS - impressions on the inner surfaces of the shells which indicate the region of attachment of the adductor muscles.

PALLIAL LINE - a mark or depression on the inner surface of the shell which indicates the area of attachment of the mantle to the shell.

PALLIAL SINUS - a notch in the pallial line which indicates the area where the retractor muscles of the siphon are located.

PARAMETER - the attribute of the population being estimated; the true value of the quantity of interest, *e.g.* the density of clams, total number of clams, or total biomass of clams in the survey area.

PLANKTONIC - animals or life stages which drift in the upper layers of the water column, carried about passively by the currents.

PLUS GROWTH - the period of rapid growth following formation of the annulus; in temperate waters synonymous with summer growth zone.

POPULATION - in statistics, a collection of distinct units to be sampled.

PRECISION – a measure of variability of parameter estimates; low variability means high precision..

PROBABILITY SAMPLING - the process of selecting a collection of sampling units from a population, where the probability of selection is known for each unit.

QUADRAT - a sampling unit, generally the sampling unit selected at the last stage in a sampling design.

RADIAL SCULPTURE - fine lines or striae which extend from the umbone to the outer margin on the external surface of the shell.

RANDOMIZATION - a procedure for selecting sampling units with known probability.

REFERENCE LINE - the "line-of-best-fit" used to align strata and locate first stage units or quadrats during a survey.

SAMPLE - a collection of sampling units selected (at random) from a population.

SAMPLING ERROR - the difference between the true value of a population parameter and the estimate of the parameter. Sampling error arises from measuring only a subset of the population for a given sample.

SAMPLING UNIT - a distinct sub-unit of the population. The sampling units can be numbered and selected at random for inclusion in the sample.

SIMPLE RANDOM SAMPLE - a survey design where n distinct units are selected from the N units in the population such that every possible sample of n units has the same probability of selection.

SIPHON - a tube-like structure, modified from mantle tissue, which is specially adapted for taking in or discharging water. The siphonal currents deliver food items to the gills of clams, and purge wastes (and eggs or sperm) from their bodies.

SIZE LIMIT - the size at which clams (or other animals) may be legally harvested. Minimum size limits protect immature individuals and/or some minimum spawning population. Maximum size limits may protect large, mature individuals from harvest. Legal size for clams in commercial fisheries in British Columbia are: Manila and littleneck clams ≥38 mm; butter clams ≥63 mm; and razor clams ≥90 mm.

SIZE SELECTIVITY - The bias introduced into survey samples when a portion of the population is not collected due to difficulty in detecting them in the quadrat. Usually involves under-representation of very small clams in the collected samples.

SPAT - larval bivalves at the time of setting.

STANDARD DEVIATION – a statistic that measures the variability of a set of data, calculated as the square root of the sample variance of the set of data.

STANDARD ERROR - the measure of the variability of a statistic (*e.g.*, mean); in stratified sampling, it is calculated as the square root of the esitmated variance of the stratified mean.

STRATIFICATION – the division of a population of sampling units into non-overlapping partitions called strata.

STRATIFIED RANDOM SAMPLE - a survey design where the sampling units are divided into H non-overlapping groups (strata) and a simple random sample is drawn from each group.

STRIP CLUSTER – a collection of quadrats arranged in a strip one quadrat wide.

STUNTING - poor growth. Stunted clams appear to be rounded with blunt shell margins, and are usually thicker and heavier than would be expected for their length.

SUBLEGAL SIZE - a clam whose length is below the minimum legal size limit.

SURVEY SAMPLING - see Probability Sampling.

THICKNESS - the maximum straight-line distance measured across the closed valves of a clam (see Figure 3.1).

UMBONE - a projection of the shell close to the hinge on bivalve molluscs. The umbone represents the oldest part of the shell, with the newest growth at the distal margin of the shell. Often referred to as the "umbo" or "beak".

VARIABLE - the name of a measurement, *e.g.* the length or weight of a clam.

VARIANCE – a statistic proportional to the sum of squared deviations of each observation from the mean that measures the variability of the sampled data.

WEIGHT - (1) the mass of an object; or (2) the calibration of an estimated parameter relative to some other characteristic of the sampling design, *e.g.*, an overall estimate of the mean number of clams per unit area might be weighted by multiplying each stratum mean by the proportion of the total survey area represented by each stratum, and summing the weighted means.

WIDTH - the greatest straight-line distance across the valves of a clam measured from the dorsal margin to the ventral margin (see Figure 3.1).

WITH REPLACEMENT (SAMPLING) - selection of samples in which each sampling unit has known probability of being selected during each draw, and sampling units can be selected more than once in the sample (as they are "replaced" in the population after each draw).

WITHOUT REPLACEMENT (SAMPLING) - selection of samples in which each sampling unit has known probability of selection in each draw, but is removed from the population (*i.e.*, is not "replaced") upon being selected. The remaining sampling units have known probability of being selected in the next draw. No sampling unit can be selected twice.

Y-VALUE - the observed measurement, same as a variable.

YEAR CLASS - animals born in a single year (*i.e.* a cohort).