THE USE OF GENETIC TAGGING TO ASSESS INSHORE ROCKFISH POPULATIONS WITHIN A MARINE CONSERVATION AREA IN THE STRAIT OF GEORGIA

by

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ABSTRACT

Fisheries and Oceans Canada recently implemented a spatial management strategy to address conservation concerns regarding British Columbia's inshore rockfish (*Sebastes* spp.) stocks. Unfortunately, the unique habitat requirements and physiological characteristics of inshore rockfish inhibit the effectiveness of many traditional monitoring methods. I evaluated the use of a novel, *in situ* genetic tagging technique to examine the abundance, distribution and movement of copper (*S. caurinus*) and quillback (*S. maliger*) rockfish within a small marine conservation area. I recorded a 3.1% recapture rate and produced abundance estimates for four of six study sites. The data also suggest evidence of a proportional relationship between catch-per-unit-soak time and rockfish density. Simulation analyses demonstrate that genetic monitoring techniques may be cost-comparable to assessments such as submersible surveys. My results identify genetic tagging as a potential alternative to traditional marking techniques for rockfish monitoring.

Keywords: rockfish, genetic tagging, mark-recapture, marine protected areas, fisheries monitoring techniques

DEDICATION

For my parents, who raised me to become a REMmer.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1
GENERAL INTRODUCTION

Problem Statement

Inshore rockfish (*Sebastes* spp.) in the Strait of Georgia are harvested as target or incidental catch in every hook and line and trawl fishery along the coast of British Columbia (Yamanaka and Lacko 2001). Despite quota reductions and limited entry into the inshore fleet imposed by Fisheries and Oceans Canada (DFO), catch-per-unit-effort (CPUE) indices continue to reflect declining trends in most inshore rockfish stocks (Yamanaka 2000; Fisheries and Oceans Canada (DFO) 2002). Managers now hope to relieve fishing pressure and conserve rockfish populations through adoption of a spatial management strategy (DFO 2002). In 1999, DFO established the first Rockfish Conservation Areas (RCAs) in BC as no-take zones for groundfish (Yamanaka et al. 2000). To date, they have created 107 RCAs, moving towards an ultimate goal of closing 30% of the Strait of Georgia, recently decreased from an original target of 50%, and 20% of the outside coast to rockfish harvest (Yamanaka and Lacko 2001; DFO 2002; DFO 2005; K.L. Yamanaka, DFO, pers. comm. 2006).

Initially, managers selected closed areas through a public consultation process following discussion with recreational and commercial harvesters and other stakeholders. Scientific evaluation was limited as many of the currently employed assessment methods for rockfish are unreliable, destructive and/or costly. Managers now support future closures by combining public feedback with scientific data identifying preferred rockfish

habitat from substrate maps and habitat complexity analyses (K.L. Yamanaka, DFO, pers. comm. 2005). Researchers then estimate habitat-specific rockfish densities using absolute abundance counts generated from submersible and remote operated vehicle (ROV) surveys. However, these surveys are relatively small-scale and extremely expensive to conduct (O'Connell and Carlile 1994), and so far have produced highly variable results (J. Martin, K.L. Yamanaka, DFO, pers. comm. 2006). Therefore, monitoring of the rockfish populations within the expanding network of RCAs could greatly benefit from the development of an effective, cost-efficient and non-destructive sampling strategy.

Literature Review

Rockfish biology

There are over 100 species of rockfish found worldwide, and approximately 30 species inhabit BC coastal waters (Love et al. 2002). Most rockfish are long-lived and sedentary, and associate with rocky, high relief substrates (Richards 1987; Matthews 1990a; Matthews 1990b; Murie et al. 1994; Pacunski and Palsson 1998; Pacunski and Palsson 2001; Love et al. 2002). In the waters off British Columbia, quillback (*S. maliger*) and copper (*S. caurinus*) rockfish are the dominant *Sebastes* species at depths less than 60m (Murie et al. 1994; Love et al. 2002) and they were the primary species of interest within my study sites.

Quillback and copper rockfish prefer high relief substrates such as boulder fields or rocky escarpments but may be found in low-relief areas such as sandy or muddy bottoms if sufficient algal cover is present (Love et al. 2002). Not surprisingly, the two species may aggregate together in the same location (Murie et al. 1994). While small home

ranges are often associated with high relief habitats (10-30m^2), range size tends to expand within low complexity areas (400-4000m^2) (Matthews 1990a; Matthews 1990b). Evidence of homing from telemetry and tagging studies further confirms quillback and copper rockfish preference for complex habitats, as they return to high relief substrates following displacement into less-preferential areas (Matthews 1990a; Matthews 1990b).

Although some species may inhabit waters up to 1500m deep, inshore species, such as *S. maliger* and *S. caurinus*, live at depths shallower than 100m (Love et al. 2002). Quillback rockfish inhabit waters ranging from subtidal to > 270m, while copper rockfish habitat extends from subtidal to depths of up to > 180m (Love et al. 2002). Rockfish control buoyancy through a closed swimbladder and often suffer severe barotrauma when brought to the surface from even relatively shallow depths (Starr et al. 2000).

Rockfish are long-lived and slow to mature. Some species do not reach 50% sexual maturity until 20 years of age (Parker et al. 2000), and may live for over 100 years (Love et al. 2002). Quillback and copper rockfish can live to ages of 95 and 50 years and, on average, reach 50% maturity by ages 11 and 7, respectively (Love et al. 2002). Recruitment is typically irregular and infrequent, and suitable conditions for strong year class formation may only occur once every 20 years (Love et al. 2002).

The Strait of Georgia hook-and-line rockfish fishery

Most of the Strait of Georgia rockfish harvest consists of quillback, copper and yelloweye (*S. ruberrimus*; red snapper) rockfish (Kronlund 1997; Kronlund and Yamanaka 1997). The commercial hook-and-line fishery landed catch peaked in 1995 with landings of 2640 tonnes, but has since shown consistent declines (Yamanaka 2000).

In 2002, DFO decreased the total allowable catch for the Strait of Georgia fishery by 75% from 125 tonnes to the current 32 tonnes (DFO 2002; DFO 2004). The full extent of recreational and subsistence catches, as well as levels of by-catch from other commercial fisheries, are difficult to accurately quantify, and therefore are largely unknown (Yamanaka et al. 2000).

Since the early 1990's the inshore fishery has been managed primarily through total allowable catch and limited entry (Kronlund and Yamanaka 1997; Yamanaka 2000), and additionally through area closures introduced in the late 1990s (DFO 2002). The recent Pilot Integration Proposal compiled by the Commercial Industry Caucus (CIC; a group of groundfish industry representatives) suggests integrating management of all groundfish fisheries and introducing an individual transferable quota system (V. deLeeuw, commercial rockfish fisherman, pers. comm. 2004; McGuigan and McMechan 2005). The CIC hopes the suggested changes will address conservation concerns regarding inshore rockfish, improvements to at-sea monitoring, and reduction of by-catch within the commercial fleets (McGuigan and McMechan 2005).

Despite increasingly restrictive regulations, fishery data continues to indicate low abundances of Strait of Georgia rockfish populations. Prior to 2002, fishing mortality was estimated to be approximately 6% (DFO 2002), which suggested that most rockfish were being harvested at above sustainable levels (Kronlund 1997). To promote sustainable harvest, DFO aims to reduce fishing mortality to levels less than natural mortality, estimated to be around 2% (DFO 2002).

Monitoring strategies

Researchers have only recently begun to perform scientific assessments of proposed RCAs, primarily using submersible surveys and habitat complexity analyses (K.L. Yamanaka, DFO, pers. comm. 2005). They require an appropriate method both to assess the suitability of current and future closed areas, and to monitor the status of rockfish in RCAs over time. Assessments need to determine the spatial distribution and absolute abundance of rockfish populations within closed areas. As conservation is the primary goal of RCAs, there is a need for non-destructive survey methods. Stock status information will help managers identify candidate closure locations, and assess the conservation success of a spatial management strategy.

Longline surveys

Fisheries and Oceans Canada currently conducts annual longline surveys to estimate levels of inshore rockfish abundance and fishing mortality (DFO 2004). Longline indices are proportional to relative abundance for some species (e.g. sablefish; Sigler 2000). However, inshore rockfish survey results may be difficult to interpret due to possible effects of line saturation (K.L. Yamanaka, DFO, pers. comm. 2003) which may produce a non-linear relationship between catch-per-unit-effort and relative abundance (Somerton and Kikkawa 1995). To avoid line saturation, Sigler (2000) suggests that: "A well-designed longline survey will have enough soak time to include most captures and enough baits for some to remain at the end". In addition, lethal sampling by longline conflicts with the conservation objective of the closed areas. However, longline surveys are still the least-expensive and most time efficient technique for assessing inshore rockfish populations.

Swept area analysis

Researchers often use swept area methods to analyse data from groundfish trawl fisheries and research trawl surveys. Although trawl surveys have been used to generate estimates of relative abundance for some rockfish species (e.g. Pacific ocean perch, *S. alutus*), they are not appropriate for surveys of inshore rockfish populations that inhabit high relief, untrawlable substrates (Jagielo et al. 2003).

Swept area estimates can be biased for a variety of reasons. Combined submersible-trawl studies have demonstrated that catchability may be highly age-dependent – as large rockfish are often "herded" towards the trawl gear while small fish escape through net meshes (Krieger 1993; Krieger and Sigler 1996; Miller and Methot 2002). In addition, rockfish density in untrawlable habitat cannot be accurately measured using trawl gear, and is likely to differ from the distribution and density of fish in trawled areas (Krieger 1993; Kirchner and McAllister 2002; Jagielo et al. 2003). Finally, trawl surveys often produce imprecise abundance estimates, making it difficult to track population trends unless changes in abundance are very large (Adams et al. 1995).

Visual surveys

DFO currently produces non-destructive visual estimates of quillback rockfish density from submersible and ROV surveys. Scientists extrapolate habitat-specific densities to larger-scale biomass estimates using habitat classifications produced from geophysical data (DFO 2004; K.L. Yamanaka, DFO, pers. comm. 2005). Although submersible surveys provide valuable information on species abundance, distribution, behaviour and habitat requirements, operating a submersible is expensive (O'Connell and Carlile 1994; K.L. Yamanaka, DFO, pers. comm. 2005) and such studies in Canada are

small in scale. For example, in the 2004 submersible surveys, only 41 individual quillback rockfish were observed, producing imprecise expanded biomass estimates (K.L. Yamanaka, DFO, pers. comm. 2005).

Underwater camera surveys are a less expensive visual survey option employed by DFO. Several authors have evaluated the use of ROVs for estimating groundfish abundance (O'Connell and Carlile 1994; Adams et al. 1995). Although potentially useful, and less expensive than performing a submersible survey, ROV studies are still costly (~$2000 US/day), and have the additional disadvantages of a small field of view, slower survey time, and tendencies for the ROV to become snagged on rocky terrain (O'Connell and Carlile 1994). Diver strip-transects are another visual survey option, but are constrained by the depth and time limits of the divers (Tuya et al. 2000).

Visual surveys have several potential sources of bias. For example, strong habitat associations may not exist in areas where stocks have yet to recover from fishing pressure (Tuya et al. 2000). In addition, underestimates of fish abundance may arise if observers miss fish hiding in crevices (Krieger 1993; Murie et al. 1994) or fish cannot be identified to species (Krieger and Sigler 1996). Finally, visual surveys are potentially biased due to fish attraction or avoidance behaviour, although there is still debate regarding the behavioural response of rockfish to submersible and ROV equipment (Murie et al. 1994; O'Connell and Carlile 1994; Starr et al. 1995; Krieger and Ito 1998).

Catch-per-unit-effort indices

Fishery-dependent catch-per-unit-effort (CPUE) indices are the only long-term time series available for most inshore rockfish species (Kronlund and Yamanaka 1997) and

CPUE trends indicate declining abundance of Strait of Georgia rockfish populations since the early 1990s (Yamanaka 2000; Yamanaka and Lacko 2001). However, the use of CPUE is often criticised, and it remains unclear whether CPUE indices accurately reflect the true magnitude of the inshore rockfish stock decline (Richards 1994; Kronlund and Yamanaka 1997; Yamanaka 2000; Yamanaka and Lacko 2001).

The possible lack of linearity in the relationship between CPUE and abundance is a well-recognised problem (e.g. Gulland 1964). It is often assumed that there is a linear relationship between CPUE and abundance (or density), implying abundance or density-independent catchability (e.g. Rose and Kulka 1999; Harley et al. 2001). However, a number of different factors may cause catchability to depend on abundance, including changes in gear selectivity or swept area (Peterman and Steer 1981), changes to fishery regulations (Hilborn and Walters 1992), technological improvements to fishing efficiency, interactions among fishermen (Cooke 1985; Hilborn and Walters 1992), individual skipper behaviour (Richards 1994), and incorporation of handling time into estimates of effort (Beddington 1979; Cooke 1985; Deriso and Parma 1987; Hilborn and Walters 1992). Biotic and environmental factors may also cause density-dependent catchability, including changes in the spatial distribution of fish populations (Cooke 1985; Hilborn and Walters 1992; Rose and Kulka 1999), fish behaviour (Richards 1994), inverse relationships between catchability and stock area (Winters and Wheeler 1985; Hansen et al. 2000), and simple random variation in catchability (Cooke 1985; Hilborn and Walters 1992).

Mis-use or mis-interpretation of a CPUE index can have serious consequences for fisheries management, and may have contributed, in part, to the collapse of the northern

cod fishery (Rose and Kulka 1999). For instance, if catchability is density-dependent, decreases in population size will not be reflected by proportional decreases in CPUE (Peterman and Steer 1981; Cooke 1985). Increases in catchability at smaller population sizes imply increased fishing mortality on smaller stocks (Peterman and Steer 1981). Spatially aggregated fish populations, such as rockfish, cause hyperstability of CPUE as a fishery-dependent stock index (Rose and Kulka 1999; Harley et al. 2001). For example, a vessel may sequentially deplete small populations of fish throughout a management area, but reported fishery CPUE will remain high until almost all aggregations have been depleted to un-fishable levels (Kronlund 1997; Yamanaka 2000; Yamanaka et al. 2000). Despite its obvious disadvantages, CPUE data is relatively easy to collect and, as a result, CPUE indices are still used for fisheries stock assessment.

Handling time (defined as the retrieval and resetting of gear) leads to non-linearity in the relationship between abundance or density and traditional CPUE indices (Beddington 1979; Cooke 1985; Deriso and Parma 1987; Hilborn and Walters 1992). In cases of single-hook angling, similar to methods used in this study, handling time per set remains relatively constant and contributes proportionally more effort as density increases, causing anglers to experience an upper limit to CPUE, and producing the appearance of density-dependent catchability (Deriso and Parma 1987). In the whaling industry, CPUE may appear to decrease for larger catches if these catches are also associated with longer handling times (Cooke 1985). Therefore, the removal of handling time may produce a linear relationship between a catch-per-unit-soak time (CPUST) index and density and CPUST may be a more appropriate population index than CPUE (Beddington 1979; Cooke 1985; Deriso and Parma 1987; Hilborn and Walters 1992).

Unfortunately, removal of handling time effects may not fully correct for the non-linearity between effort and population size, as random variation in catchability can still produce the appearance of saturated CPUST associated with high levels of density (Cooke 1985; Hilborn and Walters 1992). Averaging of CPUST to account for temporal or spatial variation may still produce biased results, because handling time comprises a proportionally greater amount of the total fishing time at high densities, causing high catchability data to be under-represented (Cooke 1985; Hilborn and Walters 1992).

Mark-recapture surveys

Mark-recapture surveys provide another option for rockfish stock assessment, but high post-release barotrauma mortality causes low effective marking rates in most experiments (Stanley et al. 1994; Starr et al. 2000; Yamanaka and Lacko 2001). Some studies attempt to reduce barotraumas by using divers to conduct tagging underwater, but this technique is time-consuming, costly and restricted to the depth limits of the divers (Starr et al. 2000). Another method, used by researchers and fishermen alike, attempts to decrease post-capture mortality by deflating swimbladders with hypodermic needles after fish are brought to the surface (Starr et al. 2000; V. deLeeuw, commercial rockfish fisherman, pers. comm. 2004). However, a mark-recapture study for gag (*Mycteroperca microlepis*) estimated high levels of post-release mortality even after swimbladder deflation (14% mortality at 11-20m capture depths to 85% mortality at 71-80m capture depths) (McGovern et al. 2005), and the same may be true for inshore rockfish.

Genetic tagging

Genetic tagging mark-recapture techniques are well established for studies of elusive terrestrial and marine mammal species. Researchers can remotely gather tissue from the species of interest using hair traps, scat collections, or biopsy darts (Paetkau et al. 1995; Palsbøll et al. 1997; Woods et al. 1998; Mills et al. 2000). Individual identifications are made from the extracted DNA samples using microsatellite loci.

Microsatellites are short tandem-repeat segments of DNA (e.g. CACACACACA) with relatively high mutation rates (Cunningham and Meghen 2001) caused by addition or deletion mutations (e.g. Moxon and Wills 1999). Microsatellites are effective in detecting individual variation in even relatively homogenous populations (Paetkau et al. 1995) as they are highly variable, non-coding regions of the genome. Until recently, microsatellite use in fisheries has been restricted to studies of population structure (e.g. Yamanaka et al. 2000; Matala et al. 2004) or tag loss evaluation (Feldheim et al. 2002). Aside from a group of researchers working in Northern Australia (R. Buckworth, AU, pers. comm. 2003), genetic tagging has not been reported as a method for monitoring marine fishes.

Closed-form mark-recapture models operate under the following basic set of assumptions: 1) equal capture probabilities, 2) capture probabilities do not change over time, 3) no births, deaths, immigration or emigration, 4) zero tagging mortality and, 5) permanent tags (Seber 1982). Genetic tagging helps meet many mark-recapture assumptions otherwise violated when traditional tagging methods are applied to studies of inshore rockfish. Minute tissue samples are collected remotely using specially designed biopsy hooks (NT Tags, Darwin, Australia), which eliminate the need to bring

fish to the surface. Thus, post-release mortality is likely negligible compared to studies where rockfish are removed from the water for tagging. In addition, tags are never "lost" since an animal maintains its genotype throughout its lifetime.

Together with submersible and ROV surveys, genetic tagging presents a non-destructive sampling strategy for the assessment of fish abundance in no-take areas. However, start-up and analysis costs for a genetic tagging project are expensive (Hammer and Blankenship 2001) as are the methods I employed to collect tissue samples *in situ*. The feasibility of incorporating genetic tagging techniques into studies of fisheries monitoring is currently unknown. My study attempts to evaluate the application of genetic tagging techniques to estimate abundance of small populations of inshore rockfish within the Strait of Georgia.

Project Goals

The primary goal of my research was to evaluate the feasibility of monitoring inshore rockfish populations using a novel genetic tagging technique. In doing so, I addressed three major objectives:

1. Develop *in situ* genetic techniques for tagging inshore rockfish populations.
2. Evaluate the suitability of genetic tagging for the assessment of abundance, distribution and movement of inshore rockfish.
3. Compare the accuracy and cost of genetic tagging surveys to visual monitoring methods.

To address my first objective, I performed preliminary aquarium and field studies evaluating the success of collecting rockfish tissue samples *in situ* using biopsy hooks. To address the second objective, I collected genetic data from populations of quillback (*S.*

maliger) and copper rockfish (*S. caurinus*) within a small marine conservation area in the Strait of Georgia. I used individual identifications to estimate abundance using a closed-form capture-recapture model, and genetic rockfish designations to evaluate the relationship between catch-per-unit-soak time (CPUST) and rockfish density.

Finally, I addressed my third objective using a simulation modelling approach to examine trade-offs between survey cost and abundance estimate precision using three genetic monitoring methods: 1) CPUST-derived estimates of absolute density, 2) capture-recapture estimates of absolute density, and 3) a CPUST index of relative density. I then compared the expense and efficiency of my proposed genetic survey methods to those of visual surveys.

Results of my study may help researchers and fisheries managers determine if genetic tagging is a feasible alternative for monitoring rockfish populations in conservation areas along the BC coast.

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CHAPTER 2
APPLICATION OF A GENETIC TAGGING TECHNIQUE TO THE MONITORING OF INSHORE ROCKFISH ABUNDANCE

Abstract

Fisheries and Oceans Canada (DFO) recently implemented a spatial management strategy for the conservation and management of British Columbia's inshore rockfish stocks (*Sebastes* spp). To date, DFO has closed groundfish harvest in 107 rockfish conservation areas (RCAs). The unique habitat requirements and physiological characteristics of inshore rockfish inhibit the effectiveness of many traditional stock monitoring methods. In my study, I evaluated the use of a novel, *in situ* genetic tagging technique to estimate the abundance, distribution and movement of copper (*S. caurinus*) and quillback (*S. maliger*) rockfish within a small RCA in the Strait of Georgia. Site-specific capture-recapture abundance estimates were calculated from a subset of individually identifiable genetic samples. Of the 351 samples analysed, I recorded 11 recaptures (3.1% recapture rate) and observed limited within-site movements. A proportional relationship between catch-per-unit-soak time (CPUST) and density suggests that a non-destructive CPUST index may be an alternative means of monitoring rockfish density. My results identify genetic tagging as a potential alternative to traditional marking techniques for evaluating rockfish abundance and distribution.

Introduction

Despite the application of increasingly restrictive regulations in the directed rockfish (*Sebastes* spp.) fishery in the Strait of Georgia, fisheries surveys continue to indicate low abundances of inshore rockfish populations (Yamanaka 2000; Fisheries and Oceans Canada (DFO) 2002). The Canadian government has recently adopted a spatial management strategy for rockfish conservation in response to these declining population trends (DFO 2002). To date, Fisheries and Oceans Canada (DFO) has created 107 rockfish conservation areas (RCAs), with a goal of closing 30% of the Strait of Georgia and 20% of the outside coast to rockfish harvest (Yamanaka and Lacko 2001; DFO 2002; DFO 2005; K.L. Yamanaka, DFO, pers. comm. 2006). Managers now require effective, non-destructive survey techniques to evaluate the suitability of current and future closed areas, and to monitor rockfish abundance within the RCAs.

Current RCA assessments use information on rockfish habitat preferences, substrate maps, and habitat complexity analyses to produce expanded biomass estimates from density data collected during small-scale submersible surveys (K.L. Yamanaka, DFO, pers. comm. 2005). However, large operating costs limit the extent of submersible surveys (O'Connell and Carlile 1994), and current biomass expansions are imprecise (K.L. Yamanaka, DFO, pers. comm. 2005). Other approaches, such as mark-recapture experiments, are unreliable because rockfish suffer severe barotraumas when brought to the surface (Starr et al. 2000).

My research examined the application of a novel genetic tagging technique to estimate inshore rockfish abundance. Genetic tagging mark-recapture methods are

commonly used to study terrestrial and marine mammals and involve the remote collection of tissue samples from the species of interest (Paetkau et al. 1995; Palsbøll et al. 1997; Woods et al. 1999; Mills et al. 2000). For example, scientists have analysed DNA to study the abundance, movement and mating characteristics of humpback whale populations (Palsbøll et al. 1997). Several studies have also used genetic tagging to investigate population variability (Paetkau and Strobeck 1994; Paetkau et al. 1995; Paetkau et al. 1998; Woods et al. 1999), home range analysis (Taberlet et al. 1997), parental relationships (Mowat and Strobeck 2000) and abundance of bear populations (Woods et al. 1999; Mowat and Strobeck 2000).

Genetic analysis is regularly utilised in studies of fish population structure (e.g. Yamanaka et al. 2000, Matala et al. 2004), however, few researchers have reported using genetic tagging to estimate fish abundance. In this paper, I describe the methods used to collect and analyse genetic tagging capture-recapture data to determine the abundance and distribution of quillback (*S. maliger*) and copper (*S. caurinus*) rockfish within a small marine conservation area in the Strait of Georgia, British Columbia. I then use the results from the capture-recapture analysis to evaluate a non-destructive catch-per-unit-soak time (CPUST) population index using genetic rockfish identifications. This study is the initial phase in evaluating the feasibility of monitoring BC inshore rockfish populations using genetic tagging techniques.

Methods

Site selection

Genetic tagging field trials were completed during the spring and summer of 2004 in the Trincomali Channel RCA in the Strait of Georgia, British Columbia (Fig. 2.1). Global Positioning System (GPS) coordinates for potential study locations were identified using echosounder transects (Echotec 212 CS; ICOM Colour Video Sounder FF-88) conducted in April 2004. Two sets of 700m long transects were spaced 500m – 700m apart, traversing areas within, and adjacent to, the RCA. The vessel captain (V. deLeeuw) identified prospective sites through recognition of rockfish habitat or schools of rockfish (Appendix A, Fig. A1). Five sets of GPS coordinates (RF9, RF15, RF26, RF31 and RF37) were randomly selected from the potential locations. A sixth location (RF2003), identified during preliminary studies in the summer of 2003, was then added during the third sampling trip. Site boundaries were roughly delimited using initial exploratory searches and following trips used saved vessel tracklines (NobelTec navigation system) to constrain sampling effort within the same region (Fig. 2.1). Boundaries in Fig. 2.1 outline the regions in which the majority of sampling was conducted (see Appendix D, Fig. D2).

A combination of bathymetric and backscatter data from multibeam sonar, high resolution seismic profiling, and sidescan sonar was used to classify bottom habitat into five different substrates: 1) sandy mud – mud, 2) sand – muddy sand, 3) gravel – sandy gravel, 4) boulder gravel – sandy gravel, and 5) bedrock (habitat classification courtesy of K. Picard and K. Conway, Natural Resources Canada; Pacific Geological Station; Sidney, B.C.). Sites were further characterised using mean depth estimated from the

geophysical data, percent habitat type and total site area (Appendix A, Table A1). Unless otherwise noted, maps of the study area were created using the PBSmapping Library (Schnute et al. 2004; R statistical software).

Biopsy hook design

Biopsy hooks (NT Tags, in Darwin, Australia) consisted of a single 89mm length of 12-gauge copper wire fitted with a biopsy tip. Tips were constructed by embedding two dental broaches inside a 12-gauge hypodermic needle tip with epoxy resin. Small pressure release vents were drilled along the sides of the hypodermic tip to increase the probability of tissue retention following a strike. The most effective hook style (Fig. 2.2) was identified during preliminary aquarium and field sampling in 2003.

Data collection

Sampling was conducted from the *Mariko*, a 13m commercial inshore rockfish vessel. All samples were obtained by trolling a single hook from a hydraulic-operated downrigger consisting of 90lb 7-strand steel mainline, 5m of 80lb monofilament, and 0.6m of 45lb Yamaline monofilament leader. Biopsy hooks were baited with 100 – 150mm strips of squid (1/4 squid per hook) attached to the hook shank by means of small elastic bands. Hooks were raised immediately following a strike and hypodermic tips were removed and placed in 95% ethanol for DNA preservation. A fish strike on the line was identified by the vessel captain, based on years of fishing experience.

Primary data recorded for each strike consisted of location (latitude, longitude), vessel track length (m), and depth (fa). GPS strike locations were recorded to the nearest 0.001 sec (approximately 3m) using either a Magellan 5200 Plus Differential Beacon

GPS (May and June 2004 trips) or a Furuno Navigator GP-32 WAAS/GPS (July and August 2004 trips). Vessel track lines were recorded using a NobelTec navigation system and were used to estimate the area swept by the gear. Strikes occurred just off-bottom, and the depth of the striking fish was recorded to the nearest tenth of a fathom (~0.2m) using the vessel's echosounder.

I also recorded the soak and handling times (hours) for every strike. Soak time was calculated as the elapsed time between the downrigger ball contacting bottom and the time of a strike. Handling time was calculated as the time between the previous strike and the time at which the downrigger ball was reset on the bottom. I used the ratio-of-means estimation (Pollock et al. 1997) to compute CPUST for each site as

$$C = \frac{\sum_t E_t}{\sum_t S_t} \quad (1)$$

where E_t and S_t are the total rockfish strikes and total soak times, respectively, during trip t, and C is the site-specific CPUST.

I allocated 150 biopsy hooks to each of six sampling trips. Sites were visited in a random order over the course of 2-3 day sampling trips, at roughly 2-week intervals from May to August 2004. As I had no prior knowledge of fish abundance in each site, I allocated tagging effort in proportion to the inverse of the mean site-specific soak time recorded over all rockfish strikes during the previous trip. Proportional sampling generally increases precision of final estimates due to the incorporation of previously known sources of variance (Som 1996).

Genetic analysis

Lab protocol

Genetic analysis was performed at the Pacific Biological Station Molecular Genetics Laboratory in Nanaimo, BC. DNA was extracted from biopsy hooks using DNAeasy kits (Qiagen, Valencia, CA). Removing all rockfish tissue from the biopsy hooks proved difficult. Therefore, following centrifugation and removal of ethanol, the hook tips were retained in ependorf tubes for the initial tissue digest using proteinase K. Alleles were amplified using polymerase chain reaction (PCR) and allele size was measured using standard electrophorectic techniques on an ABI 377 automated DNA sequencer (K. Miller, DFO, pers. comm. 2004).

Recapture identification

When employing genetic tagging techniques, there always remains some possibility that a recapture is incorrectly identified, because some individuals may share the amplified loci, while differing at other regions in the genome (Woods et al. 1999; Cunningham and Meghen 2001). Such a "shadow effect", or false positive, results in an over-abundance of recaptures and leads to an underestimation of population size (Mills et al. 2000). The relative strength of the shadow effect is quantified by calculating the probability of identity (P_{ID}) of a genotype (Paetkau et al. 1998; Waits et al. 2001). See Appendix B for further details.

Ten microsatellite loci for individual identification were selected after considering trade-offs between high amplification rates (Appendix B, Fig. B1) and high levels of heterozygosity (Appendix B, Fig. B2) (K. Miller, DFO, pers. comm. 2004). This careful

microsatellite selection reduced shadow effects while maximising hook efficiency (the percentage of hooks collecting sufficient DNA for individual identification). As the majority of tissue samples did not amplify at all markers, I calculated the P_{ID} for recapture pairs matching at three or more loci (Appendix B, Eq. B1). Using an upper estimate of 10 000 fish per site and assuming Hardy-Weinberg equilibrium, I determined it would be highly unlikely that DNA samples matching at four or more loci would come from two separate fish (Table 2.1). Therefore, I identified recaptures as samples amplifying and matching at a minimum of four loci. The spatial proximity of matches served as a secondary source of evidence for identifying "true" recaptures because inshore rockfish typically demonstrate limited movement (Matthews 1990a; Matthews 1990b).

Attempting to minimise the probability of false positive matches in the data inevitably increases the probability of incorrectly identifying true recaptures as different fish. I reduced the bias introduced by false negatives by culling all samples amplifying at fewer than four loci. These samples were no longer considered part of my marked population and were not included in the capture-recapture analysis (Appendix B).

The CPUST analysis does not require individual identification. Because the selected markers were specific to rockfish, the dataset for this analysis was comprised of all tissue samples amplifying at any number of microsatellites.

Capture-recapture analysis

I treated my study sites as closed populations due to the long-lived nature of rockfish (Love et al. 2002), their limited movement in high relief substrates (Matthews 1990a;

Matthews 1990b), and the RCA fishing closure. I analysed recapture data using a Schnabel census estimate adjusted for low recapture sizes (Krebs 1999). Site-specific abundances were calculated as (site index is ignored to simplify notation)

$$N = \frac{\sum_{t=2}^{T=6} (m_{t-1} * E_t)}{\sum_{t=2}^{T=6} r_t + 1} \quad (2)$$

where N is the site-specific estimates of rockfish abundance, m_{t-1} is the cumulative number of marks present prior to trip t, E_t is the number of individually identifiable rockfish samples collected during trip t; and r_t is the number of recaptures observed during trip t, and $T = 6$ is the total number of trips. The adjusted model produces unbiased estimates of abundance when E_t/N and m_{t-1}/N are less than 0.10 (Krebs 1999). Variances of $1/N$ were calculated using

$$V = \frac{\sum_{t=2}^{T=6} r_t}{\sum_{t=2}^{T=6} (m_{t-1} * E_t)^2}. \quad (3)$$

(Krebs 1999).

Catch-per-unit-soak time analysis

Several authors have proposed removing handling time from CPUE indices to derive a more appropriate index of abundance (Beddington 1977; Cooke 1985; Deriso and Parma 1987; Hilborn and Walters 1992). Previous studies demonstrate there may be a proportional relationship between population density and CPUST under the assumptions

of single-hook fishing and random fish distribution (Deriso and Parma 1987). Therefore, in addition to the capture-recapture model, I investigated a potentially less expensive, non-destructive survey technique using CPUST as an index of inshore rockfish relative density. To confirm whether CPUST is an appropriate indexing tool, I examined the relationship between CPUST and density using an inverse-variance weighted least squares linear model.

Density was defined as abundance divided by total area swept. Although the width swept by the hook will vary depending on fish behaviour, weather and oceanographic conditions, for the purpose of my analyses I assumed a constant track width of 3m (Appendix D, Table D2). Track length was measured as the distance travelled during the elapsed soak time. To avoid repeated counting of regions swept more than once, overlapping segments of track were combined into complex polygons for each site (Appendix D, Fig. D1). Although there is still uncertainty in my swept area estimate, I believe it provides a more accurate reflection of the true stock area than my arbitrary site boundaries.

I tested the hypothesis that the relationship between CPUST and density is linear with an intercept of zero. There are two obvious ways to test this hypothesis. The first is by fitting a linear model to log-transformed CPUST and density data (Peterman and Steer 1981; Hansen et al. 2000)

$$\ln C_i = \ln q + \beta \ln D_i \tag{4}$$

where q is the overall catchability of the sampling process, β is a non-linearity parameter, and D_i is rockfish density in site i. In this case, a β value that is significantly less than

one is evidence for a non-linear, hyperstable, relationship between the abundance index (CPUST) and density.

A simpler method that does not involve data transformation applies an ordinary linear regression model of the form

$$C_i = \alpha + qD_i . \tag{5}$$

If there is hyperstability in the relationship between CPUST and density, then the value of α will be > 0. In this case, the hypothesis test is whether the intercept term α is significantly greater than zero. A value of $\alpha > 0$ implies that catchability increases at low rockfish density. Although the Eq. 4 method is more common (e.g. Peterman and Steer 1981; Hansen et al. 2000), I chose Eq. 5 because it requires fewer assumptions about the underlying distribution of CPUST, which displayed high temporal and spatial variability (Fig. 2.3). I accounted for sampling variability by bootstrapping from the raw soak times and site-specific density estimates. I generated CPUST using a non-parametric bootstrap of site-by-trip soak time data, and generated rockfish density using a parametric bootstrap assuming a lognormal error distribution. For each of 500 replicate bootstrap data sets, I calculated a weighted least squares regression in which the weights were inversely proportional to the bootstrap standard errors of CPUST for each site (pooled over trips). I tested the null hypothesis H_0: $\alpha > 0$ by computing the proportion of bootstrap regression intercepts that were less than or equal to zero.

Results

Genetic analysis

Almost 80% of tissue samples collected during 2003 (preliminary study) and 2004 genetic tagging trips contained inshore rockfish DNA (952/1222). Remaining samples contained either no tissue, or tissue from an unidentified species (K. Miller, DFO, pers. comm. 2004). Hook efficiency, measured as the proportion of samples amplifying at a minimum of four rockfish microsatellite loci, was 44% (537/1222).

The Hardy-Weinberg P_{ID} for observed recaptures amplifying at a minimum of four markers ranged from 4.95×10^{-6} to 5.97×10^{-14} (Table 2.1), which translates into a 1:22 000 to $1:1.67 \times 10^{13}$ chance that two different fish drawn at random from each site would match at the observed loci. Therefore, I concluded that the probability of false positives in the observed recapture data was negligible given the four-loci minimum. The close proximity of recaptures to the location of the original samples provided further evidence that false positives were low (Appendix C, Table C3; Fig. 2.4 – Fig. 2.7).

Capture-recapture analysis

Capture-recapture analysis was performed using 351 individually identifiable rockfish samples collected during the 2004 surveys (Appendix C, Table C1). I detected 11 recaptures matching at four or more loci, in four of six study sites, with an overall recapture rate of 3.1% (Table 2.2). Recaptures occurred between two to eight weeks from the time of initial tissue collection and one fish was recaptured on two separate occasions in site RF31 (Appendix C, Table C3). I did not include two recaptures that were collected within sampling trips, as they were attributed to attraction behaviour of the fish.

Abundance estimates in each site ranged from 325 (density=0.028 fish/m^2) in RF15 to 798 (density=0.101 fish/m^2) in RF26 (Table 2.2); however, there is a high degree of variability in all estimates (Table 2.2). Rockfish were observed over all bottom habitats types with the exception of sandy mud – mud (Fig. 2.4 – Fig. 2.7). There was insufficient data to calculate density estimates associated with each substrate; therefore, I was unable to expand abundance estimates throughout the entire RCA based on habitat association.

All recaptures occurred within the original capture site. The distance travelled between captures ranged from 4m to 103m (Fig. 2.4 – Fig. 2.7) with the two largest movements (103m and 56m) occurring in low relief substrates, gravel sandy – gravel and sand – muddy sand, respectively. In two cases, recaptures were found on different substrates than the original marks (Appendix C, Table C3).

Catch-per-unit-soak time analysis

I assessed inshore rockfish catchability by testing the hypothesis that the relationship between CPUST and density passed through a zero intercept, which indirectly provides a test of relationship linearity. The intercept was ≤ 0 in only 5.6% of 500 bootstrap linear regressions (Fig. 2.8), but the value of a zero intercept fell within the 95% bootstrap confidence intervals of -7.7 to 48.6 (Table 2.3). Because an intercept of zero falls within my confidence intervals, the null hypothesis H_0: $\alpha > 0$ (density-dependent q) was rejected. However, the wide confidence intervals caused by the lack of contrast and high uncertainty in my paired data points provide only limited information regarding the true nature of the relationship (Fig. 2.9). The mean and standard error of the bootstrap intercepts and slopes also differed from the estimates produced using the original data (Table 2.3).

Discussion

Current rockfish survey methods are often expensive, inaccurate or destructive. However, there is a need to develop an effective, cost-efficient and non-invasive sampling strategy to assess and monitor protected rockfish populations within the developing network of RCAs along the BC coast. The objective of this study was to evaluate the use of genetic tagging as a potential survey tool for studies of inshore rockfish. This is the first time genetic tagging has been applied to a temperate fish population for the purpose of assessing fish abundance, movement and distribution.

Density estimates

My research demonstrated the application of a genetic tagging technique to a capture-recapture study of inshore rockfish. Identification of 11 recaptured rockfish samples was supported by low probability of identity values and proximity to original samples. The recapture data was sufficient to provide rockfish abundance estimates in four of my six study sites. The results of this study demonstrate that genetic tagging may be a useful tool for non-destructive, fisheries independent monitoring of inshore rockfish.

Conversion of capture-recapture abundances to corresponding measures of density using swept area presented several challenges. Despite the adjustments to swept area described in the methods, my estimated densities are, in some cases, approximately double the median copper and quillback densities reported in the literature (~0.04 fish/m^2; Richards 1987; Murie et al. 1994; Pacunski and Palsson 2001). My estimates are still within previously observed ranges (0-0.31 fish/m^2; Murie et al. 1994), suggesting it is possible that I simply surveyed several high-density populations. However, I had no way to approximate the mean distance from which a fish would approach a baited hook;

therefore, the estimated 3m track width was arbitrary. Bias in total swept area produced by an incorrectly assumed track width would also bias swept area densities (Appendix D, Table D2).

Movement patterns

Short-distance movements may be a result of (a) the fish following the baited hook, or (b) random GIS positioning error. In reality, the recaptured fish may not have moved from the original capture location. However, longer movements (e.g. > 10m) are unlikely to be caused by either (a) or (b).

The close proximity of recaptures observed in my study is consistent with other reports on quillback and copper rockfish movement (Matthews 1990a; Matthews 1990b). The greatest distances between marked and recaptured fish (56m and 103m) were observed over low relief habitat (gravel – sandy gravel and sand – muddy sand), respectively, while movements over strictly high relief substrates (boulder gravel – sandy gravel and bedrock) were always less than 30m. These results are again consistent with observations of variable home range size across different substrate types. Quillback and copper rockfish typically exhibit 10-30m^2 home ranges in high relief rocky habitat, while ranges over low relief bottoms vary from 400-4000m^2 (Matthews 1990a; Matthews 1990b). The restricted movement of tagged fish in this study suggests that even small marine protected areas may be sufficient to protect adult rockfish breeding stocks.

Rockfish distribution

I observed rockfish within all substrates classified by the multibeam survey with the exception of sandy mud – mud. In addition, I recorded comparatively low densities of

rockfish within the boulder habitat in the middle of the reserve (~0.03 fish/m^2). The presence of rockfish associated with low relief bottom types, and the comparatively low densities of rockfish within the boulder region, were unexpected given the affinity of inshore rockfish for high relief habitat commonly reported in the literature (Richards 1987; Matthews 1990a; Matthews 1990b; Murie et al. 1994; Yamanaka 2000, Pacunski and Palsson 2001; Love et al. 2002).

Other studies have noted weak associations between rockfish density and abiotic factors (Krieger 1998; Tuya et al. 2000). Some authors provide evidence that weak density-habitat correlations may be caused by over-fishing (Palsson 1998; Tuya et al. 2000; Pacunski and Palsson 2001). Low productivity and sporadic recruitment suggest that rockfish recovery from over-fishing should be slow (Kronlund 1997; Love et al. 2002), and significant increases in mean size or density may not be apparent until areas have been closed for at least 20 years (Palsson 1998; Tuya et al. 2000; Pacunski and Palsson 2001). For example, the nine-year closure to commercial harvest in Trincomali Channel may not have been sufficient time for rockfish densities within the boulder habitat to recover to concentrations similar to those observed in my other study sites.

Finer-scale habitat data may provide further clarification for the reasons behind the observed inshore rockfish distribution in the Trincomali Channel RCA. Inshore rockfish often associate with micro-habitat features (e.g. individual rockpiles) (Pacunski and Palsson 2001; K.L. Yamanaka, DFO, pers. comm. 2005), which were not identified in this study given the relatively low resolution of the geophysical data. For example, fish recorded in sand – muddy sand habitat could actually be closely associated with small boulder piles that were not detected in the current habitat assessment.

Survey improvements

Improvements to hook design should be the primary focus for future research evaluating the application of genetic tagging to surveys of fish populations. The low percentage of hooks collecting sufficient tissue for individual identification reduced the useable sample size, and unnecessary resources were spent collecting and analysing samples containing insufficient rockfish DNA. As hook and individual identification costs are non-negligible ($11Cnd and $20Cnd each, respectively), improving hook efficiency will greatly improve results for a given field and laboratory budget. Single-hook trolling and the vessel captain's accurate recognition of rockfish strikes prevented tissue contamination from multiple strikes, which is a significant concern in other genetic tagging studies (e.g. Woods et al. 1999; J. Harms, NOAA, pers. comm. 2005; J. Hempelmann, NOAA, pers. comm. 2005). However, setting several hooks at once would greatly improve sampling efficiency. An ideal hook design should improve tissue retention, while restricting the ability of multiple fish to strike the same hook. Further research is required to develop such a design.

Improvements to field study design would increase both data quality and survey efficiency. For example, random start points for fishing transects would ensure more thorough coverage of the study area, as opposed to the *ad hoc* randomisation employed during this study. I also recommend studies to estimate average track width, which will be influenced by fish attraction and avoidance behaviour. An underwater camera attached to the hook leader could be used to determine the distance from which fish, on average, will approach a baited biopsy hook, producing a more accurate estimate of total swept area and reducing one source of potential bias in the density estimates.

Even with such improvements, the high cost of biopsy hooks and individual genetic identification will contribute greatly to the costs of future genetic tagging capture-recapture studies (see Ch. 3). A major advantage of a CPUST survey is the reduced analysis costs associated with identification of species rather than individuals. Genetic analysis costs for species identification are approximately half the cost of identifying samples to the individual level ($10Cnd; K. Miller, DFO, pers. comm. 2005). In addition, less DNA may be required to make a species ID, which would increase the number of useable tissue samples collected by the current hook design. For example, only 44% of samples contained sufficient DNA for individual identification, but 78% of samples contained identifiable rockfish DNA.

The effectiveness a CPUST density index relies heavily on the assumption of density-independent catchability (Gulland 1964; Peterman and Steer 1981; Hilborn and Walters 1992; Rose and Kulka 1999). I attempted to account for known non-linearities by removing handling time from estimates of survey effort (Cooke 1985; Deriso and Parma 1987; Hilborn and Walters 1992). Although the 95% confidence limits generated by the bootstrap regression included a zero intercept, the wide intervals provide little information regarding the intercept's true value. Prior to the application of a CPUST index, the CPUST-density relationship should be more reliably calibrated with higher precision and higher contrast densities (Harley et al. 2001). If strong evidence of a linear relationship with a zero-intercept can be defined, a CPUST index may provide a more efficient, less costly application of genetic monitoring techniques (see Ch. 3).

Project feasibility

Fishery scientists could benefit from the development of affordable, non-destructive methods to assess and monitor the status of inshore rockfish populations within the rapidly expanding network of RCAs developing along the BC coast. Results of my study suggest that genetic tagging could provide an alternative means for evaluating the abundance, distribution and movement of quillback and copper rockfish populations.

The feasibility of conducting a genetic monitoring program for inshore rockfish hinges primarily on the cost and precision of genetic surveys compared to other non-destructive methods such as submersible and remote operated vehicle surveys. However, the most appropriate method will also depend on the needs of the fishery scientist or manager. For example, in instances where information on length and weight distributions or species behaviour is required, genetic methods alone will be insufficient.

In conclusion, scientists must first improve current genetic sampling methodology and compare the costs of visual and genetic monitoring programs before they can consider the applicability of genetic surveys for monitoring the status of BC inshore rockfish stocks.

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Tables

Table 2.1 Hardy-Weinberg probability of identity for each pair of recaptures matching at a minimum of four loci.

	Locus											
Recapture Genotype	*sal2*	*sal4*	*seb9*	*seb33*	*sme2*	*sme3*	*sme8*	*sra15*	*sar16*	*sru9*	P_{ID}	$1/P_{ID}$
1			X	X			X	X			4.95×10^{-6}	2.02×10^{5}
2	X		X	X			X	X			4.74×10^{-7}	2.11×10^{6}
3	X	X		X				X			4.26×10^{-6}	2.34×10^{5}
4	X			X				X	X		3.00×10^{-7}	3.33×10^{6}
5	X		X	X			X		X	X	2.44×10^{-9}	4.10×10^{8}
6	X		X	X	X	X		X	X	X	5.97×10^{-14}	1.67×10^{13}
7	X	X	X	X	X	X			X	X	1.35×10^{-11}	7.40×10^{10}
8	X		X	X			X		X		8.16×10^{-8}	1.23×10^{7}
9	X		X	X	X	X			X	X	2.24×10^{-12}	4.47×10^{11}
10			X	X	X		X		X	X	1.89×10^{-10}	5.30×10^{9}
11	X			X	X	X		X		X	1.39×10^{-10}	7.16×10^{9}

Note: Note that numbers 6 and 7 are the same fish, recaptured twice. On average, one in $1/P_{ID}$ fish drawn at random from the population will share the given genotype.

Table 2.2 Summary of recapture results and Schnabel census abundance estimates.

Trip Start Date	*June 8*	*June 28*	*July 7*	*July 22*	*Aug 8*			
Site	**Recaptures**					**Abundance**	**Density (no./m^2)**	**CV**
RF15	0	0	0	1	0	325	0.029	0.500
RF26	0	1	1	0	0	798	0.102	0.471
RF31	0	3	1	0	0	570	0.085	0.400
RF37	0	1	1	0	2	522	0.072	0.400

Note: CV = coefficient of variation. No recaptures were found in sites RF9 or RF2003.

Table 2.3 Linear regression summary results for the observed and bootstrapped mean intercept and slope values calculated for the CPUST-inshore rockfish density relationship.

Data Type	α	α SE	q	q SE	% of α ≤ 0	% of q ≤ 0	95% α CIs	95% q CIs
Observed means	13.5	9.3	317.9	138.9	---	---	-4.7 to 31.7	45.6 to 590.1
Bootstrap means	20.2	10.1	247.8	155.2	5.6	9.8	-7.7 to 48.6	-307.5 to 864.7

Note: Density standard errors are generated from a parametric bootstrap; CPUST estimates and standard errors are generated through a non-parametric bootstrap of soak times. α = intercept; q = slope. 95% confidence intervals for bootstrap means are derived from the bootstrapped data; confidence intervals for the observed means are calculated assuming a normal distribution.

Figures

Fig. 2.1 **Location of the Trincomali Channel Rockfish Conservation Area study area for developing the genetic tagging method. Inset shows a close-up of the conservation area and study sites. Reserve boundaries extend from Spotlight Cove in the northeast (48.980°N 236.432°W), south to Retreat Cove (48.943°N 236.491°W) both on Galiano Island, and then west to Panther Point (48.932°N 236.465°W) and Chivers Point (48.957°N 236.426°W) on Wallace Island.**

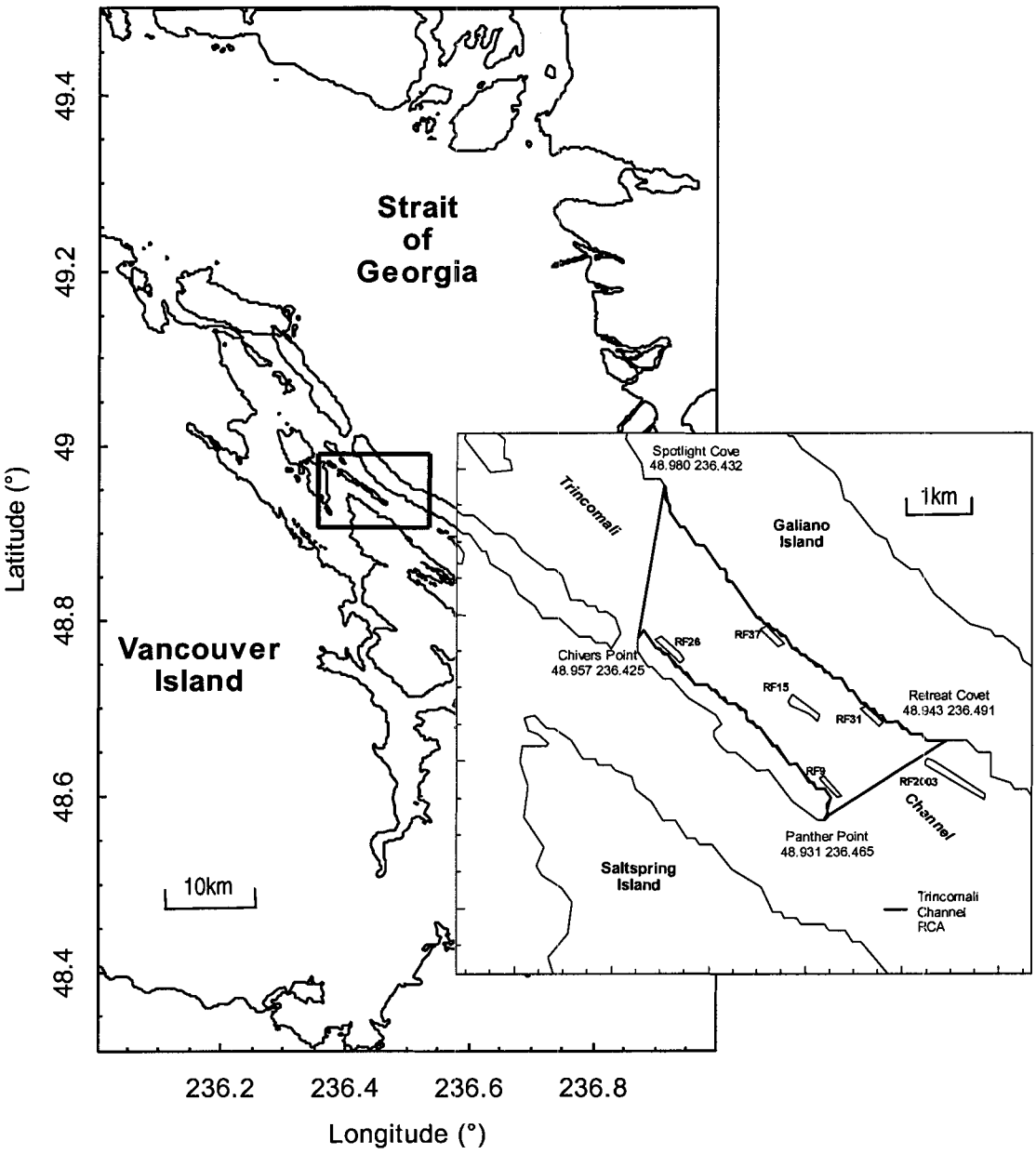


Fig. 2.2 **The primary biopsy hook style used in the genetic tagging field trials. U-shaped with a slight offset and baited with a 100-150mm strip of squid. Hooks were manufactured by NT Tags, in Darwin, AU.**

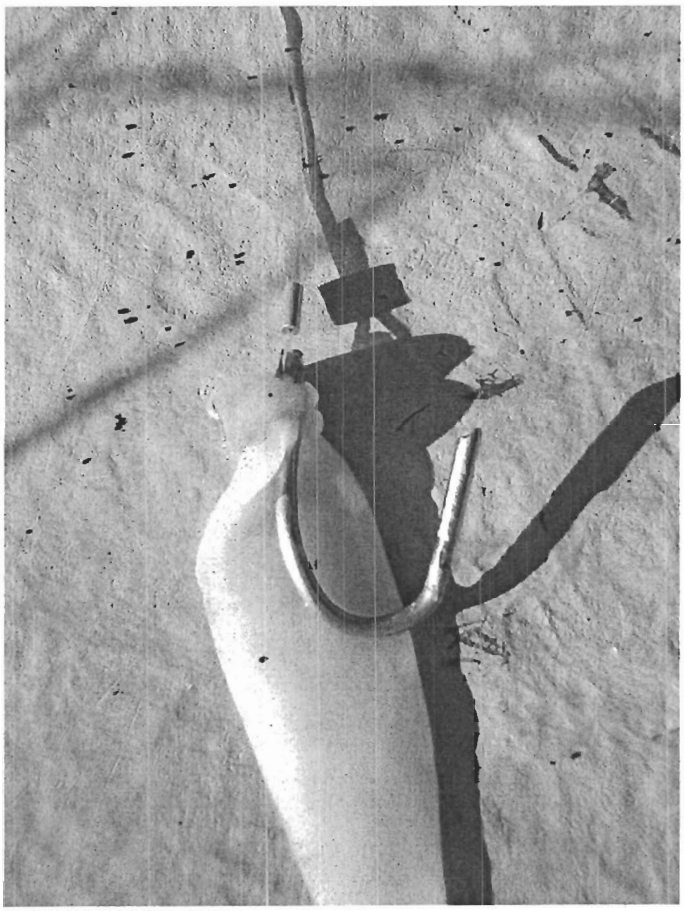


Fig. 2.3 Mean soak time (time elapsed between downrigger ball touching bottom and a subsequent rockfish strike) pooled across all trips (left panel), and across all sites (right panel). Error bars show +/- 2 SE.

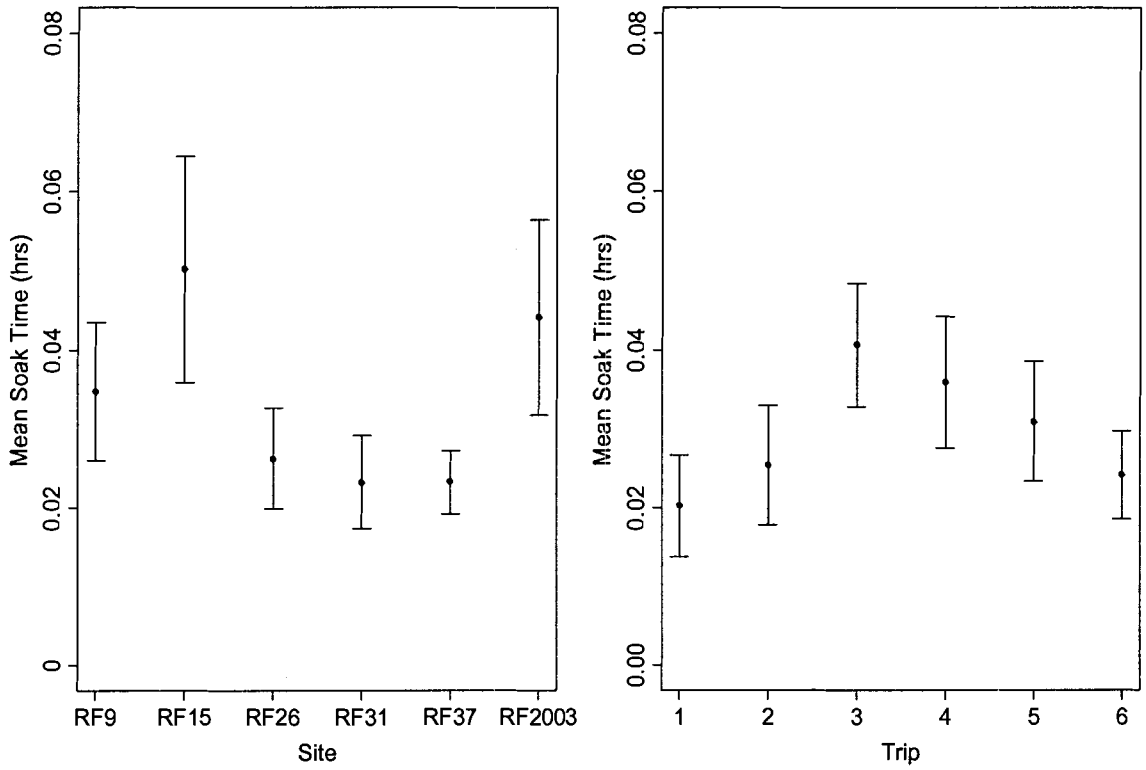


Fig. 2.4 **Capture-recapture strike locations overlaid on bottom habitat. Bathymetric and backscatter data were used to classify habitat as: 1) sandy mud – mud, 2) sand – muddy sand, 3) gravel – sandy gravel, 4) boulder gravel – sandy gravel and 5) bedrock. Recaptures are shown as black rockfish. Yellow lines indicate the distance travelled between mark and recapture. Habitat classification courtesy of K. Picard and K. Conway, NRCan; GIS mapping courtesy of L. Lacko, DFO.**

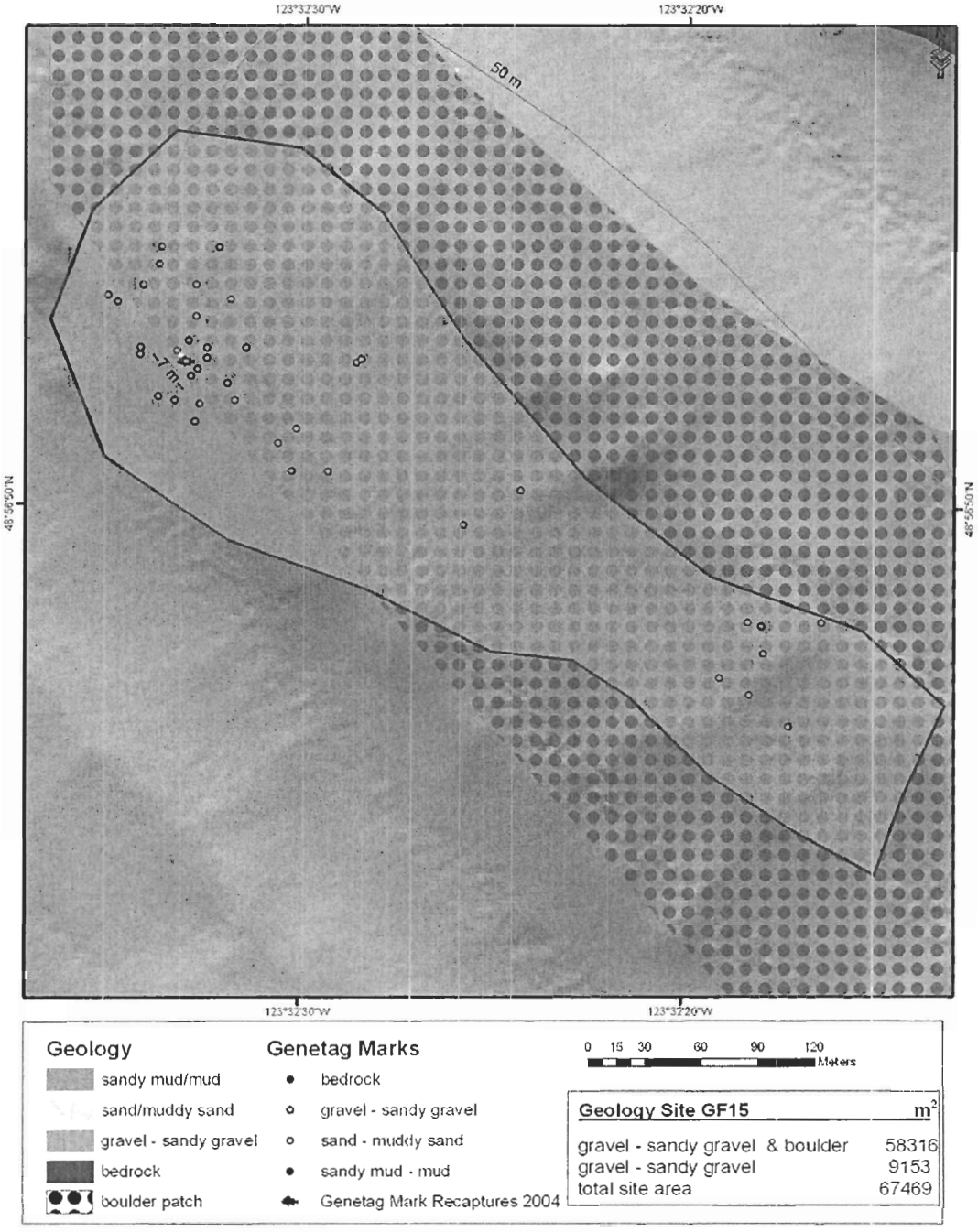


Fig. 2.5 Capture-recapture strike locations overlaid bottom habitat for site RF 26. For full explanation see Fig. 2.4.

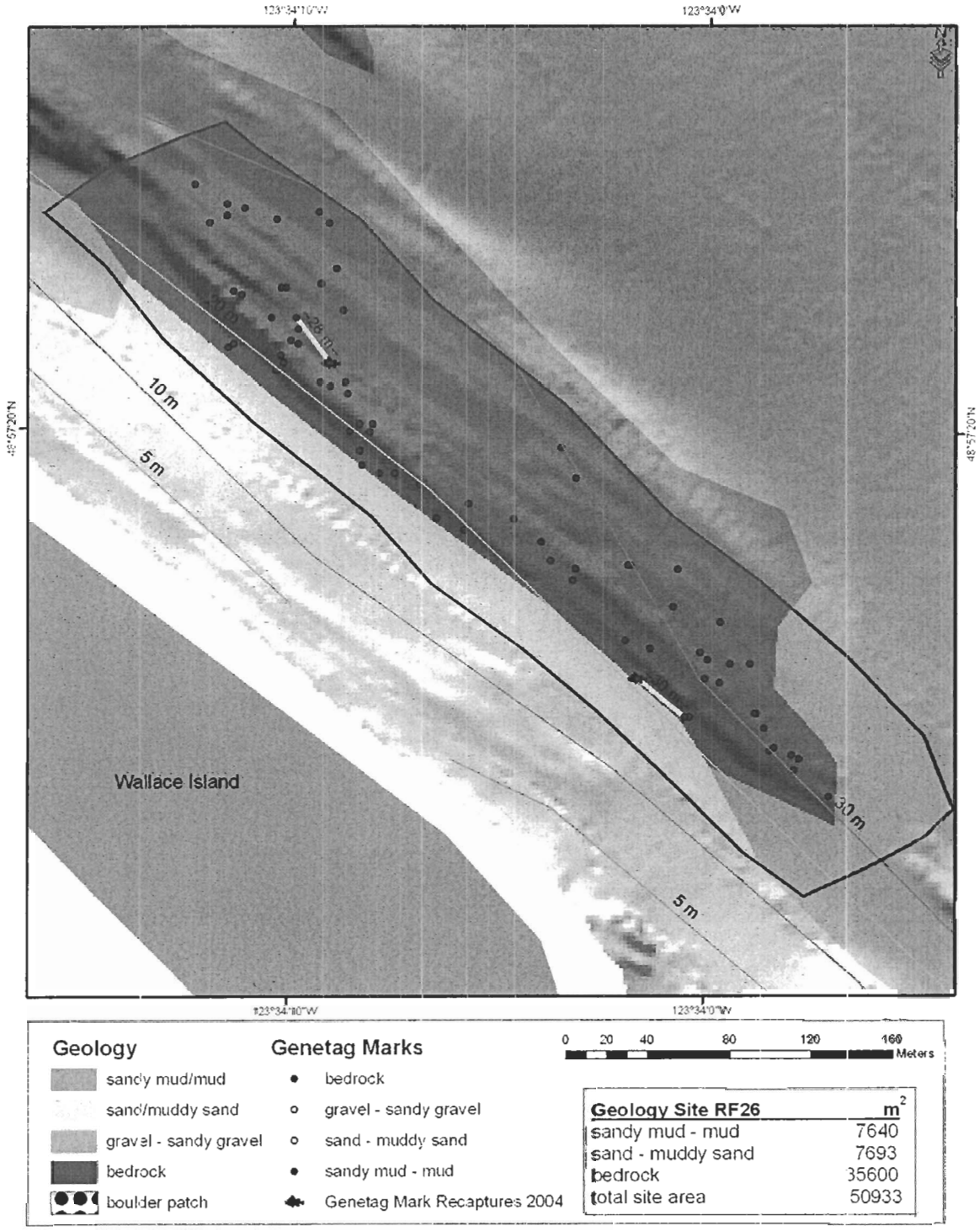


Fig. 2.6 Capture-recapture strike locations for site RF31. For full explanation see Fig. 2.4.

Fig. 2.7 Capture-recapture strike locations for site RF37. For full explanation see Fig. 2.4.

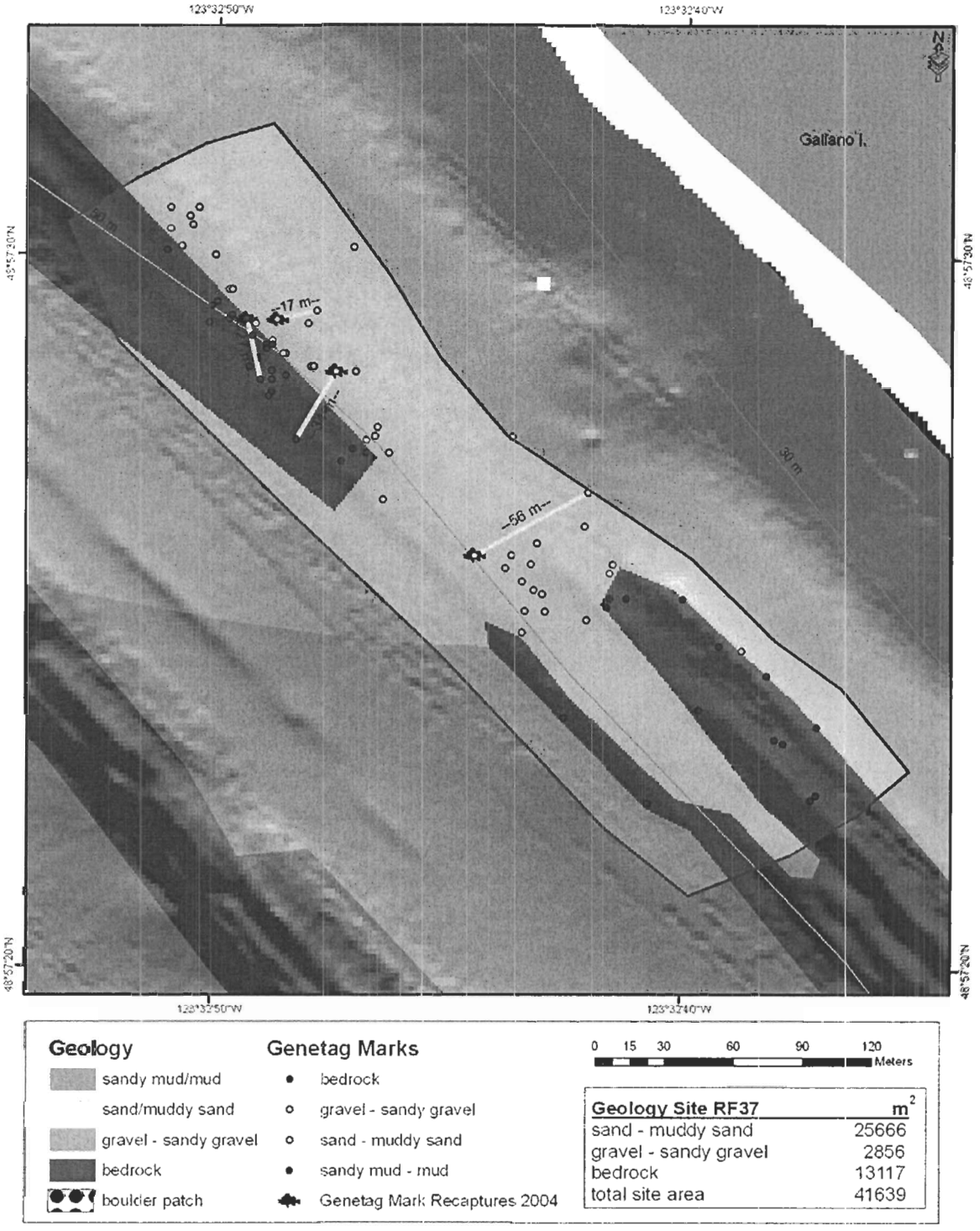


Fig. 2.8 **Frequency histogram of intercept values from 500 simulations for a weighted least squares linear regression between parametric bootstrap estimates of density assuming log-normal distributions, and non-parametric site-specific CPUST estimates. The vertical line indicates the intercept estimate from the best-fit weighted least squares regression (~13.48). The histogram illustrates that the majority of estimated intercepts were greater than zero, suggesting a potential non-linear relationship between density and CPUST.**

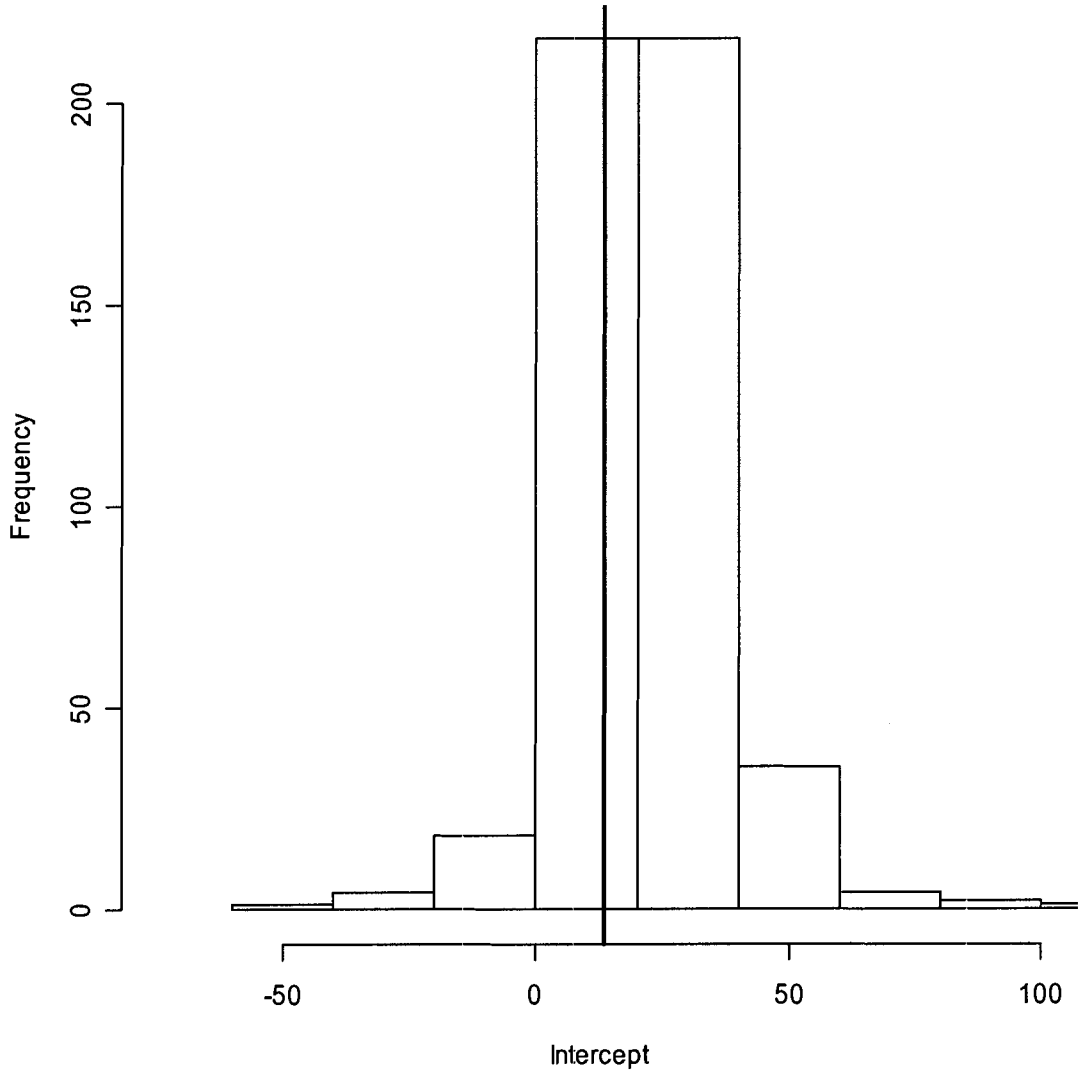
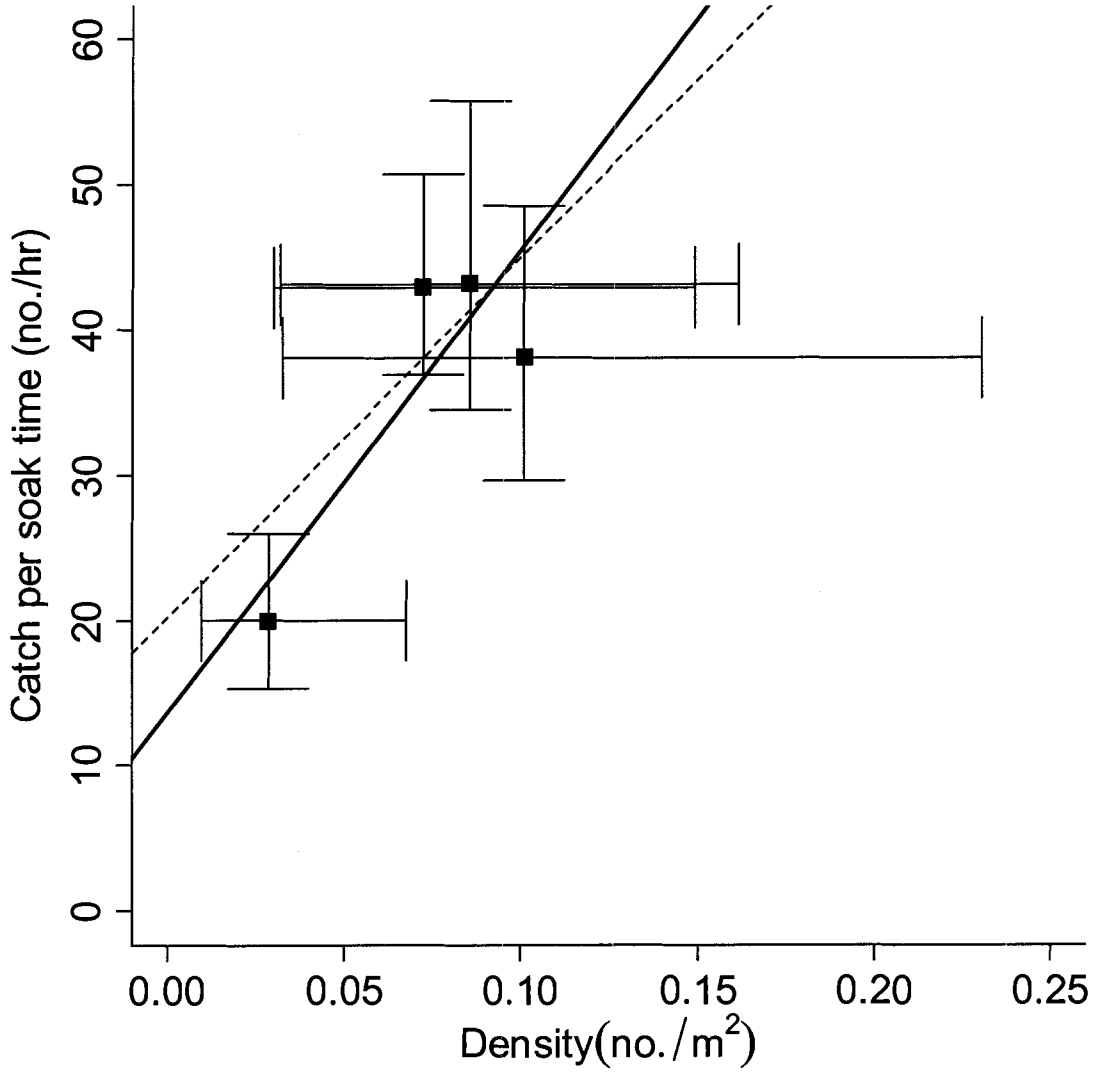


Fig. 2.9 **Observed relationship between rockfish density (no./m^2) measured by genetic tagging and catch-per-unit-soak time (CPUST; no./hr). Solid line shows the best-fitting weighted least squares regression. Dashed line shows the bootstrap regression. Error bars for CPUST represent 95% confidence limits from non-parametric bootstrapping. Error bars for density are 95% confidence limits from a parametric bootstrap assuming log-normal distributions.**



CHAPTER 3
FEASIBILITY OF GENETIC METHODS FOR INSHORE ROCKFISH MONITORING

Abstract

Management of inshore rockfish (*Sebastes* spp.) populations off the coast of British Columbia has been complicated by inadequacies in fisheries-independent surveys. In this study, I used simulation modelling to evaluate three potential genetic monitoring techniques: 1) converting catch-per-unit-soak time (CPUST) data to estimates of absolute rockfish density using a calibrated model, 2) estimating absolute density from a capture-recapture study, and 3) using CPUST as a relative density index. The *in situ* collection of rockfish tissue for analysis of individual or species identifications allows researchers to perform non-destructive capture-recapture or CPUST surveys, respectively. Assuming a high coefficient of variation (CV) ($CV \leq 0.8$) in density estimates, the genetic methods were similar, or lower, in cost to traditional submersible and remote operated vehicle surveys. Sampling times for the CPUST - derived density surveys and capture-recapture experiments could be improved with increases in tissue capture rates. The use of a CPUST relative density index is the least expensive and most time efficient survey option, and may be adequate for monitoring population trends. The results of this study suggest that non-destructive genetic sampling could be a viable alternative for monitoring rockfish populations within marine conservation areas, and for producing estimates of absolute rockfish abundance.

Introduction

The expanding network of rockfish conservation areas (RCAs) in the Strait of Georgia introduced a requirement for non-destructive, fisheries-independent monitoring techniques (Yamanaka and Lacko 2001; Fisheries and Oceans Canada (DFO) 2002; DFO 2005). Government scientists currently use a combination of manned submersible and remote operated vehicle (ROV) surveys to conduct annual inshore rockfish (*Sebastes* spp.) population assessments and to evaluate potential area closures (DFO 2004; K.L. Yamanaka, DFO, pers. comm. 2005). However, the high expense associated with visual monitoring programs (O'Connell and Carlile 1994; K.L. Yamanaka, DFO, pers. comm. 2005) has encouraged the exploration of alternative non-destructive survey methods.

In Chapter 2, I described the application of a novel *in situ* genetic tagging technique to a capture-recapture study of quillback (*S. maliger*) and copper (*S. caurinus*) rockfish within a small RCA in the Strait of Georgia. Biopsy hooks (NT Tags, Darwin, Australia) eliminated the need to bring fish to the surface, thereby reducing the high post-release mortality rates typically observed in rockfish tagging studies (Stanley et al. 1994; Starr et al. 2000; Yamanaka and Lacko 2001). My results demonstrated that genetic tagging could be used to produce capture-recapture estimates of absolute rockfish abundance. However, these studies may not be appropriate for wide-scale monitoring, as they are limited by the high costs of individual DNA identification, and by the intense sampling effort required to produce precise density estimates, even on a small scale.

An alternative application of genetic sampling is the use of a catch-per-unit-soak time (CPUST) index. Previous studies have shown that CPUST is proportional to fish density under assumptions of single hook angling and random fish distribution (Deriso and

Parma 1987). Evidence provided in Chapter 2 suggests a proportional relationship between CPUST and rockfish density field observations. Non-destructive CPUST surveys are completed by identifying rockfish DNA from biopsy hook tissue. A species identification is half the price of an individual analysis (K. Miller, DFO, pers. comm. 2005), thus reducing the laboratory costs compared to capture-recapture studies. In addition, while marks can only be recorded for individually identifiable tissue samples (44% of hooks from field study), CPUST data can be collected for all hooks containing rockfish DNA (80% of hooks from field study). Field observations also indicate that relatively few soak time values are necessary to produce precise CPUST indices, allowing for rapid survey completion. As a result, CPUST surveys may be a feasible method for estimating relative rockfish density and completing non-destructive inshore rockfish monitoring programs. However, a relative density index does not provide the necessary information for sustainably managing the inshore fishery using total allowable catch (Kronlund and Yamanaka 1997; Yamanaka 2000).

Estimating absolute rockfish density from CPUST surveys requires the initial calibration of a CPUST-density model compiled using capture-recapture density estimates and associated CPUST values. Subsequent surveys need only collect CPUST data, which can then be converted to density estimates using the fitted calibration model. The cost of estimating absolute density from CPUST will be influenced by not only the precision and contrast of the density estimates used for model calibration (Hansen et al. 2000; Harley et al. 2001), but also by the biopsy hook efficiency rates and the precision of the CPUST data. However, prior to investing in the collection of genetic information,

scientists and managers will need to know the conditions, if any, under which the costs of CPUST and visual surveys become comparable.

The objective of this paper is to determine the relationship between total survey cost and the precision of absolute rockfish density estimates derived from a CPUST index. Simulation models are applied to evaluate the influence of the quantity and precision of capture-recapture density estimates, the tissue capture efficiency of the biopsy hooks, and the precision of the CPUST survey, on the total cost and precision of the derived densities. Finally, I use data from my original field sites as a case study, to compare the cost and sampling efficiency of traditional visual survey methods to those of three genetic monitoring techniques: 1) deriving absolute densities from genetic CPUST surveys, 2) estimating absolute densities from genetic tagging capture-recapture surveys and, 3) using genetic CPUST data as a relative density index.

I demonstrate that, under certain conditions of required precision and biopsy hook efficiency, non-destructive genetic monitoring programs may be comparable, or even lower in cost, to submersible and ROV surveys. The results of this feasibility analysis will enable fisheries scientists and managers to determine whether genetic tagging is an appropriate monitoring tool for studying inshore rockfish.

Methods

Due to a lack of available field data, I used a simulation model to evaluate the trade-offs between survey costs and the resulting precision in CPUST - derived absolute densities ($\hat{D}$). The coefficient of variation of the derived densities, $\hat{\tau}$, and the total survey cost, θ, will be influenced by the precision of both the calibrated CPUST-density model

and the CPUST survey data. Of key interest, is the level of precision in $\hat{D}$ that can be achieved for comparable, or lower, costs than the costs of submersible or ROV surveys. In the following sections I describe the contributing survey cost components, and the methods used to generate the simulated data. In doing so, I identify the critical information researchers will need to collect and analyse before investing in a non-destructive genetic monitoring program.

Survey cost components

The total sampling effort ($\tilde{H}$, defined as the number of biopsy hooks) required to generate CPUST-derived densities, $\hat{D}$, is related to: 1) the coefficient of variation of capture-recapture density estimates ($\tilde{\sigma}$), 2) the number of density estimates used for model calibration (sites surveyed, *S*), and 3) the coefficient of variation of mean catchability ($\hat{\kappa}$) from the CPUST survey. In addition, effort is affected by, 4) the biopsy hook tissue capture rates (P_{DNA}), defined in the capture-recapture model as the observed percentage of hooks collecting enough tissue for individual analysis ($P_{DNA} = 0.44$), and in the CPUST survey as the observed percentage of hooks collecting rockfish DNA ($P_{DNA} = 0.8$) (see Ch. 2).

To translate sampling effort into cost, I separated expenses into five distinct components: supplies (c_1), boat charter (c_2), staffing (c_3), and individual (c_4) and species (c_5) genetic identification. Equation 1 represents the relationship between the four effort parameters ($\tilde{\sigma}, \hat{\kappa}, S, P_{DNA}$) and total survey cost (θ)

$$\theta(\tilde{\sigma}, \hat{\kappa}, S, P_{DNA}) = c_1 * \tilde{H} + c_2 * \hat{B} + c_3 * \hat{B} + c_4 * \tilde{H}_1 + c_5 * \tilde{H}_2 \quad (1)$$

where $\tilde{H}_1$ is the sampling effort for the CPUST survey, $\tilde{H}_2$ is the effort for the capture-recapture survey ($\tilde{H} = \tilde{H}_1 + \tilde{H}_2$), and $\hat{B}$ is the total sampling time. Simulated data is represented by "~" and calculated data is represented by "^". A complete notation list is provided in Table 3.1. Equation 1 was computed for data from a number of sites and then summed to give the total survey cost.

For illustrative purposes, cost components (c) were based on observed expenses experienced during my field season. However, expenses will vary across research programs depending on the personnel and contractors used to complete the study. All costs are presented in Canadian dollars.

With the exception of the biopsy hooks, supply costs were minimal. Therefore, I simply multiplied $\tilde{H}$ by the price per biopsy hook (c_1=\$11) to estimate the total cost of research supplies.

Boat charter costs were estimated by calculating the total fishing time (hours to complete both the capture-recapture and soak time surveys; $\hat{B}$ in Eq. 1 and 2) using

$$\hat{B} = (b * \tilde{H}_1) + (b_{\max} * \tilde{H}_2) \quad (2)$$

where b is the observed site-specific mean total time fished per hook (handling time + soak time; Table 3.2). The time to complete the CPUST survey was calculated by multiplying the number of hooks used in each site, $\tilde{H}_1$, by the site-specific b. I conservatively estimated the capture-recapture survey time by multiplying fishing

effort, $\tilde{H}_2$, by the greatest observed total time fished per hook, b_{max}. I then divided $\hat{B}$ by an eight-hour workday, and applied a boat charter charge of \$550 (c_2) per day.

Staffing salaries were also related to sampling time. A wage of \$8.50/hour (c_3) for a single research assistant was set using the Natural Sciences and Engineering Research Council of Canada (NSERC) Canada Graduate Scholarship as a standard (\$17 500 per annum; NSERC 2005).

To compute the total costs for genetic analysis, effort was again segregated into separate CPUST ($\tilde{H}_1$) and capture-recapture ($\tilde{H}_2$) components. Laboratory costs for the CPUST survey were estimated by multiplying $\tilde{H}_1$ by the per sample cost for species identification (c_4 = \$10), while the capture-recapture effort, $\tilde{H}_2$, was multiplied by the cost for an individual analysis (c_5 = \$20 per sample) (K. Miller, DFO, pers. comm. 2005).

Cost comparisons

Using my field data (see Ch. 2) as a case study, I applied a simulation framework to conduct sensitivity analyses evaluating the effect of the $\tilde{\sigma}$, S, $\hat{\kappa}$ and P_{DNA} parameters on the cost of deriving absolute rockfish densities from my observed CPUST values (Table 3.3). I then compared the simulated costs to the estimated costs of conducting submersible and ROV surveys in the same areas. Submersible and ROV data were estimated by adjusting costs and sampling times from larger-scale surveys to the approximate number of sampling days for my study sites (K.L. Yamanaka, DFO, pers. comm. 2005; Table 3.4). As well as estimating the cost of generating absolute densities from CPUST data, I used the capture-recapture simulation model to evaluate the cost of directly estimating absolute density from a genetic tagging study. The capture-recapture

data was generated using observed site densities (Table 3.5), and expanded swept area indices were used to approximate site abundance in cases where no recapture data was available (Appendix D, Table D1). In addition, I isolated the cost of the CPUST survey to evaluate the feasibility of monitoring RCAs using a CPUST index of relative density.

For the purpose of cost comparison, simulations were performed assuming a target density (or CPUST) coefficient of variation of 0.8 or 0.4. Results of the sensitivity analyses were used to determine precision and biopsy hook efficiency conditions under which genetic survey techniques become economically feasible compared to visual survey methods.

Simulation model overview

The basic model components needed to simulate the calibration process are illustrated in Figures 3.1 and 3.2. I first simulated a capture-recapture survey (Fig. 3.2.B, and Fig. 3) to estimate density ($\tilde{D}$) and calculate coefficients of variation in mean catchability ($\hat{\kappa}$). I then generated CPUST ($\hat{C}$) from $\tilde{D}$ and $\hat{\kappa}$ values using a stochastic model (Fig. 3.2.C) parameterised with observed catchability data (Fig. 3.2.A). Finally, a linear regression fit to $\tilde{D}$ and $\hat{C}$ (Fig. 3.2.D), was used to derive densities ($\hat{D}$) corresponding to my observed CPUST data (C) (Fig. 3.2.E).

Simulated capture-recapture survey

In order to calibrate a CPUST-density model, densities first need to be estimated from a capture-recapture survey. The precision of the model calibration depends on the contrast, precision and number of differing densities used to fit the relationship (Hansen et al. 2000; Harley et al. 2001). The capture-recapture simulation procedure was designed

to examine the influence of: 1) density precision, 2) number of site estimates, and 3) biopsy hook sampling efficiency, on the cost of model calibration. The simulation model structure is summarised by the flow diagram in Figure 3.3.

Data generation

For each simulated site, I generated "true" site abundances (N) by separating my widest observed 95% density confidence limits into four equally spaced categories (Table 3.6). I then drew random densities from each category, and multiplied the values by a constant 7 800m^2 area (the mean area swept across my study sites; Appendix D, Fig. D1). Increases in the number of contrasting densities used to fit the calibration model was simulated by increasing the total number of sites surveyed ($S = 4, 8, 12$).

I randomly generated recaptures ($\tilde{r}_t$) using a binomial distribution with site-specific capture probabilities (P_{cap}) and trials equal to the cumulative number of marks in a site at the end of the previous trip ($\tilde{m}_{t-1}$). Probability of capture was modelled as a function of fishing effort and catchability

$$P_{cap} = 1 - e^{-q\tilde{E}} \tag{3}$$

where q is the proportion of the population caught with a single unit of effort (a single hook) and $\tilde{E}$ is the number of individually identifiable DNA samples. In this case, $q = 1/N$, where N is the "true" number of rockfish within an individual site. I randomly generated trip-specific $\tilde{E}$ values from the total number of hooks, $\tilde{H}_2$, using a binomial

distribution with $P_{DNA} = 0.44$. Equation 3 was used instead of $P_{cap} = \tilde{E}/N$, to account for the possibility of multiple recaptures.

Estimates of population abundance and variance were calculated using a Schnabel census adjusted for low sampling effort (Krebs 1999). For each site, estimates of population size were calculated as

$$\tilde{N} = \frac{\sum_{t=2}^{T=5}\left(\tilde{m}_{t-1} * \tilde{E}_t\right)}{\sum_{t=2}^{T=5}\tilde{r}_t + 1} \quad (4)$$

where $T = 5$ is the total number of sampling trips, $\tilde{m}_{t-1}$ is the cumulative number of marks present at the start of trip t, $\tilde{E}_t$ is the number of individually identifiable rockfish samples collected during trip t; and $\tilde{r}_t$ is the number of recaptures observed during trip t.

The variance of $1/\tilde{N}$ was then calculated using

$$\tilde{V} = \frac{\sum_{t=2}^{T=5}\tilde{r}_t}{\sum_{t=2}^{T=5}\left(\tilde{m}_{t-1} * \tilde{E}_t\right)^2} \quad (5)$$

(Krebs 1999).

Monte Carlo simulations

Sampling effort was initialised at 10 hooks per trip per site, over five separate trips. For each of 500 simulations, I repeated the randomised data generation process and

calculated the site-specific coefficient of variation ($\tilde{\sigma}$) in abundance from the capture-recapture model (see Fig. 3.3)

(6) $$\tilde{\sigma} = \tilde{N}\sqrt{\tilde{V}}\,.$$

I then noted whether $\tilde{\sigma}$ fell below a desired precision value (σ'). If the percentage of simulations in which $\tilde{\sigma} \le \sigma'$ was $\ge 80\%$, I considered the current effort level sufficient for meeting precision requirements, recorded the critical hook number, $\tilde{H}_2$, and repeated the process for the next site. If $\tilde{\sigma} \le \sigma'$ in less than 80% of simulations, I increased effort by two hooks per trip and repeated the simulation procedure. Once $\tilde{H}_2$ was determined for each site, this required effort was entered into the survey cost model (Eqs. 1 and 2).

Generating CPUST

Observed field results were combined with simulated data to generate CPUST values ($\hat{C}$) corresponding to the site-specific capture-recapture density estimates ($\tilde{D}$) using the following linear model with lognormal errors

(7) $$\hat{C} = \bar{q}\tilde{D}e^{\hat{\kappa}w - \frac{\hat{\kappa}^2}{2}}$$

where $\bar{q}$ is the observed mean catchability coefficient, w is a normally distributed random variate (~N(0,1)) and $\hat{\kappa}$ is the site-specific coefficient of variation of mean catchability (Fig. 3.2.C).

Values of $\hat{\kappa}$ were calculated from the mean number of hooks per trip collecting rockfish DNA ($\tilde{\bar{E}}$) according to

(8) $$\bar{\tilde{E}} = \frac{\tilde{H}_2 * P_{DNA}}{5}, \text{ and}$$

(9) $$\hat{\kappa} = d * e^{-b\bar{\tilde{E}}}$$

where $P_{DNA} = 0.8$ and $\tilde{H}_2$ is generated by the capture-recapture simulation. Model parameters (b and d) were estimated by fitting a robust log linear model to observed trip-specific effort and catchability data (see Fig. 3.4).

CPUST-density model calibration

I fit a weighted least squares linear regression forced through a zero intercept to the estimated capture-recapture densities and corresponding CPUST values (Fig. 3.2.D) and solved for the slope, $1/\hat{q}$

(10) $$\tilde{D} = \frac{1}{\hat{q}}\hat{C}.$$

I accounted for uncertainty in $\hat{C}$ and $\tilde{D}$ using parametric bootstraps assuming lognormal error distributions, with the standard deviations set to $\hat{\kappa}$ and $\tilde{\sigma}$, respectively. For each of 500 replicate bootstrap data sets, I calculated density from each $\hat{C}$ value using the calibrated model. Finally, I divided the standard deviation of the bootstrapped replicate densities by the mean density over all replicates, and recorded the mean coefficient of variation ($\hat{\varepsilon}$) over all sites as a measure of calibration model precision.

Estimating density from CPUST

To provide an example application of the calibration model, I calculated the cost of estimating density from observed CPUST collected within my original study areas. Changes to the coefficient of variation in the calibrated model ($\hat{\varepsilon}$) and the CPUST survey ($\hat{\kappa}$) will affect both survey cost (θ) and the coefficient of variation of the derived densities ($\hat{\tau}$). Again, $\hat{\varepsilon}$ will be influenced by changes to the precision ($\tilde{\sigma}$) and number of density estimates (S) used to produce the calibration model. However, the interaction between $\hat{\varepsilon}$ and $\hat{\kappa}$ is unlikely to be strictly additive (W. de la Mare, SFU, pers. comm. 2005). Therefore, I iteratively varied the $\tilde{\sigma}$, S and $\hat{\kappa}$ values in my models to analyse the tradeoffs between $\hat{\tau}$ and the combined capture-recapture and CPUST survey costs.

Data generation

Variability in observed CPUST (C) was incorporated into density estimates by multiplying each C (Table 3.3) by a lognormal error term with a standard deviation equal to the target mean catchability coefficient of variation, $\hat{\kappa}$. I calculated the CPUST survey effort corresponding to each level of required precision using

$$\tilde{H}_1 = \frac{\log d - \log \hat{\kappa}}{bP_{DNA}} \tag{11}$$

where $P_{DNA} = 0.8$, and b and d are the same parameters as in Eq. 9. I then used the calibrated CPUST-density model to estimate densities from the random site-specific CPUST values.

Monte Carlo simulations

For each of 500 simulations, I generated site-specific densities, $\hat{D}$, from the bootstrapped CPUST-density model and the randomised observed CPUST values (C) (Fig. 3.2.E). The density coefficient of variation, $\hat{\tau}$, was calculated from the mean and standard deviation of densities recorded over all simulations. I then varied the coefficient of variation ($\tilde{\sigma}$) and quantity (S) of estimated capture-recapture densities, and the coefficient of variation in mean catchability ($\hat{\kappa}$) and evaluated the resulting impact on the mean $\hat{\tau}$ averaged over all sites, and the total survey cost, θ. Finally, the $\hat{\tau}$ from each set of simulations was compared to a target coefficient of variation, τ', and I recorded the combined cost for the capture-recapture and CPUST surveys that used the smallest combined critical effort ($\tilde{H}$; i.e. the least expensive surveys) to meet the target coefficient of variation.

Results

Simulated capture-recapture survey

As anticipated, increases in the precision of the capture-recapture density estimates, $\tilde{\sigma}$, corresponded to increases in required sampling effort (Fig. 3.5). However, increases in estimated precision did not always correspond to improvements in percent bias (Fig. 3.6). Abundance was negatively biased at high coefficients of variation and, to a degree, improved with increasing precision. However, a positive bias was observed at small $\tilde{\sigma}$ values (e.g. $\tilde{\sigma} \leq 0.2$). Improvements to tissue capture rates caused a nearly linear decrease in the estimated capture-recapture sampling expenses (Fig. 3.7).

CPUST-density model calibration

The precision of the calibrated model was more strongly influenced by improvements to the coefficient of variation of capture-recapture densities ($\tilde{\sigma}$) than by increases in the number of estimates (number of sites surveyed) used to fit the CPUST-density relationship (Fig. 3.8). As a result, to achieve a given coefficient of variation for the calibration model, $\hat{\varepsilon}$, it was less expensive to survey fewer sites with higher precision, than more sites with lower precision (Fig. 3.9). Therefore, remaining analyses evaluated a CPUST-density model calibrated using only four densities.

Estimating density from CPUST

The contour plot in Figure 3.10 illustrates the changes in the coefficient of variation of CPUST-derived densities, $\hat{\tau}$, as a function of changes in the coefficient of variation of mean catchability, $\hat{\kappa}$, used to generate the CPUST survey, and in the coefficient of variation of the calibration model, $\hat{\varepsilon}$. The results illustrate that $\hat{\tau} < \hat{\kappa} + \hat{\varepsilon}$, in other words, the variability in the derived density was typically less than the sum of the contributing coefficients. Results appear to be more sensitive to changes in the precision of the CPUST survey than in the calibration model.

The total cost of calibrating the CPUST-density model and performing a CPUST survey, θ, was also influenced by $\hat{\kappa}$ and $\hat{\varepsilon}$, as illustrated in Figure 3.11. Total survey cost was more sensitive to changes in the precision of the calibration model than changes in the precision of the CPUST survey, particularly at small values of $\hat{\varepsilon}$. Figure 3.12 illustrates the relative contribution of the various cost components to the total survey cost.

The genetic analysis and biopsy hooks produced the greatest relative expense, while staffing salaries were nearly negligible given the assumed low hourly rate.

Survey Costs

Current visual surveys are known to produce results with low levels of precision (K.L. Yamanaka, DFO, pers. comm. 2005; J. Martin, DFO, pers. comm. 2006). Therefore, the estimated costs and sampling times for surveying my field sites using submersible and ROV surveys were compared to the costs and sampling times for simulated CPUST-derived density ($\hat{D}$), capture-recapture ($\tilde{D}$), and CPUST index (C) surveys, assuming a relatively high target coefficient of variation = 0.8 (upper plot in Fig. 3.13). Under the low precision sampling scenarios, genetic survey costs were typically less than the costs of the visual surveys. However, given the current average tissue capture rates ($P_{DNA} = 0.44$), the sampling times for the CPUST-derived density and capture-recapture surveys were up to three times as long as the submersible option. Increases to P_{DNA} in the capture-recapture model ($P_{DNA} = 0.8$) led to decreases in expected costs and sampling times. In all scenarios, a simple CPUST index, C, was the least expensive, and most time efficient, sampling strategy.

The lower plot in Figure 3.13 presents simulated data generated with a more precise target coefficient of variation = 0.4. Increasing precision requirements corresponded to large increases in simulated sampling cost and time. However, increases to tissue capture-efficiency still succeeded in reducing the costs of the precise CPUST-derived density and capture-recapture surveys below those of the current submersible alternative.

Discussion

Feasibility analysis

Although genetic tagging techniques had already been applied to a small-scale inshore rockfish experiment (see Ch. 2), the applicability of genetic methods to a wider-scale monitoring program remained to be evaluated. In this paper, I utilised a simulation modelling approach to assess the trade-offs between sampling cost and estimated precision of three non-destructive genetic survey methods: 1) CPUST-derived absolute densities, 2) capture-recapture tagging studies, and 3) a CPUST relative density index. I assessed the feasibility of these methods for inshore rockfish monitoring by comparing simulated results to the estimated cost and sampling time for visually surveying my original study area.

Given the high cost and low precision associated with current submersible and ROV surveys (O'Connell and Carlile 1994; K.L. Yamanaka, DFO, pers. comm. 2005; J. Lochead and J. Martin, DFO, pers. comm. 2006), genetic monitoring programs appear to be a viable alternative. Assuming current tissue capture rates ($P_{DNA} = 0.44$), most low-precision genetic surveys (target coefficient of variation ≤ 0.8), are less expensive than either of the submersible or ROV alternatives. Increases in desired precision (coefficient of variation ≤ 0.4) corresponded to large increases in associated sampling cost. Because the current precision of DFO visual surveys is poor, it is impossible to determine whether a genetic program remains cost-comparable under scenarios of increased target density precision.

Although less costly, the sampling times for CPUST-derived densities or capture-recapture studies are triple the length of sampling times for submersible surveys. Changes

to biopsy hook design, producing increases in tissue capture rates, could allow researchers to precisely estimate relative or absolute rockfish densities while further decreasing cost and increasing sampling efficiency.

Bias and precision

My capture-recapture simulation model revealed a negative relationship between percent bias and the coefficient of variation in density estimates (Fig. 3.6). At small coefficients of variation, $\tilde{\sigma}$, corresponding to high levels of effort (Fig. 3.5), the use of the adjusted Schnabel model for small recapture sizes (Eq. 4) decreased the positive bias associated with the unadjusted Schnabel model, which omits the "+ 1" term in the denominator of the population equation (Seber 1982; Krebs 1999; Borchers et al. 2002) (Appendix C, Fig. C2). The negative bias correlated with large $\tilde{\sigma}$ (small effort) occurs because the maximum population size estimated by the recapture model (population estimate under a single recapture scenario) is limited by the amount of effort applied to the survey. When the effort is small, this maximum threshold may fall below the "true" population size. As the target $\tilde{\sigma}$ is further increased, the maximum estimable population size continues to decrease, producing the appearance of increased negative bias. A negative bias may also arise when analysing field data, if variations in fish behaviour lead to the failure of the model assumption of capture homogeneity (e.g. Seber 1982; Borchers et al. 2002).

Biased abundance estimates could have serious implications if managers plan to re-open closed areas once protected populations meet a target level of recovery. A negative bias could result in lost fishing opportunity if areas are unnecessarily closed, while an overestimate could lead to harvesting from depleted stocks. As demonstrated here, a

simulation modelling approach may help scientists determine if their recapture data and choice of recapture model is likely to produce biased density estimates, and allow them to correct for expected biases.

Other sources of simulation bias relate to the underlying parameterisations of my cost model. For illustrative purposes, the cost criteria used in this study were particular to my research design and researchers should repeat this feasibility analysis with their own specifications prior to conducting a genetic monitoring program.

Genetic survey costs

The cost and sampling time associated with calculating CPUST-derived densities were greater than those for completing either a genetic tagging capture-recapture study or a CPUST index survey, due to the additional expense of the one-time calibration procedure. By far the cheapest and most efficient alternative was the use of a CPUST index of relative density. However, the relative cost of these three methods will vary depending on the scale of the study.

The total cost of estimating absolute densities from CPUST data includes both the initial expense of calibrating the CPUST-density model, and the cost of subsequent CPUST surveys. Trade-offs between the precision of the two survey components will vary depending on the scale of the research project. Researchers could potentially reduce costs of a small-scale study by compensating for a relatively imprecise predictive model by collecting highly precise CPUST data, as illustrated by the results of this paper. However, greater savings may be incurred for larger-scale projects by focusing efforts on increasing the precision of the one-time calibration model.

The major expense associated with the model calibration, is the cost of the individual DNA analysis. Costs reported in the literature range from $20-40 (Hammer and Blankenship 2000) to $55-105 (Woods et al. 1999) per sample, depending on the laboratory and the number of identifying loci (Waits and Leberg 2000). My simulations also ignored the substantial initial expense of equipping a laboratory ($100 000 – $300 000; Hammer and Blankenship 2000) or producing baseline genetic information for the species of interest, if not otherwise available.

The substantial decrease in the cost of a CPUST compared to a genetic tagging survey results from both the low cost of the species identification analysis, and the efficiency with which precise CPUST estimates are obtained. Because the CPUST survey costs are almost negligible compared to the costs of a genetic tagging study, wide-scale monitoring programs could be conducted more efficiently with CPUST surveys and a one-time calibration model, than with multiple capture-recapture experiments. However, the presence of a linear relationship between CPUST and inshore rockfish density must first be confirmed, otherwise mis-interpretation of the CPUST data could lead to serious management concerns (e.g. Peterman and Steer 1981; Cooke 1985; Kronlund 1997; Rose and Kulka 1999; Yamanaka 2000; Yamanaka et al. 2000; Harley et al. 2001).

The cost of capture-recapture studies could be substantially decreased by improving biopsy hook tissue capture rates. Although a 100% capture rate is unrealistic, early stages of genetic tagging research on Spanish mackerel (*Scomberomorus commerson*) in northern Australia achieved mean rates of 70% (R. Buckworth, AU, pers. comm. 2005). Although the aggressive striking behaviour of mackerel is partially responsible for the

improvement, changes to hook design could produce similar results for studies of inshore rockfish.

Selecting monitoring programs

Despite the potential for cost-comparability, visual survey methods remain superior to genetic sampling in several ways. Even with biopsy hook improvements, submersible surveys remain the most time efficient option for assessing absolute rockfish density. Ultimately, time-pressured scientists may be willing to invest more money for the advantage of completing a more efficient study. The value of visual surveys is enhanced by the opportunity to collect additional biological and behavioural information regarding the species of interest. In addition, there remain several sources of un-quantified bias in genetic tagging surveys, such as the effects of attraction or avoidance behaviour and uncertainty in the area swept by the biopsy hooks (see Ch. 2). Finally, DNA marks introduce a unique issue, as one can never identify recaptures with 100% certainty (Woods et al. 1999; Cunningham and Meghen 2001) which may lead to additional biases in abundance estimates (Waits and Leberg 2000).

Alternatively, there are several reasons why research scientists may want to consider a genetic monitoring method. Submersible surveys are expensive and contain their own set of problematic biases, such as underestimating density because of missed observations or because fish cannot be identified to species (Krieger 1993; Murie et al. 1994; Krieger and Sigler 1996). Although ROVs are a cheaper visual survey option, they are less time-efficient and are restricted by a smaller field of view. In addition, there are several benefits to completing a genetic tagging study. The method is non-destructive and completed *in situ*, minimising the impact on depleted populations. Collected DNA can

also be analysed for studies of population structure (e.g. Yamanaka 2000), or parental analysis for the tracking of juveniles (L. Hauser, University of Washington, pers. comm. 2005). Unlike visual surveys, a capture-recapture study can also provide information on long-term or seasonal movements of rockfish. Finally, genetic monitoring experiments are logistically simple and require fewer staffing and equipment demands than submersible surveys.

Scientists may also want to consider whether a relative density index is suitable for their purposes. As previously discussed, collection of CPUST data is both less expensive and more time efficient than the other alternatives explored in this feasibility analysis. If the presence of a linear relationship between CPUST and inshore rockfish density is confirmed, then CPUST surveys could be a useful tool for monitoring population trends within DFO's expanding network of rockfish conservation areas. Ultimately, the selection of an appropriate technique will depend on the requirements and the scale associated with a particular study.

Future directions

Given the expanding network of rockfish conservation areas, the addition of an alternative non-destructive sampling technique could benefit DFO inshore rockfish scientists. However, improvements to the approach are necessary before precise absolute density estimates can be obtained for a reasonable cost. Future research should focus on the development of an improved biopsy hook design and the collection of sufficient data to clarify the CPUST-density relationship. Pending further research, I believe genetic monitoring techniques could provide a valuable alternative as non-destructive sampling methods for inshore rockfish species.

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Tables

Table 3.1 Notation list. Symbols are listed in the order they appear in the text.

Symbol	Definition
σ	Capture-recapture coefficient of variation of estimated density
S	Total number of capture-recapture density estimates
κ	Coefficient of variation of mean catchability
P_{DNA}	Probability a hook will collect sufficient DNA to make an ID
θ	Total survey cost for deriving density from observed CPUST
H	Critical effort (total number of hooks) for meeting CV requirements
B	Total fishing time
b	Observed site-specific mean total time fished per biopsy hook
D	Rockfish density
C	Catch-per-unit-soak time (CPUST)
N	Rockfish abundance
P_{cap}	Probability of capture
m	Cumulative number of marked rockfish
q	Catchability coefficient
E	Effort (number of hooks collecting tissue to make individual IDs)
T	Capture-recapture trips
r	Recaptures
V	Schnabel census estimated variance of 1/N
σ'	Required coefficient of variation of estimated density
ω	Normally distributed random variate (~N(0,1))
b, d	Negative exponential distribution parameters
ε	Coefficient of variation for calibration model
τ	Coefficient of variation of density derived from observed CPUST
Superscripts	
~	Simulated value notation
^	Estimated value notation

Table 3.2 Mean total fishing time per hook for each study site over six sampling trips.

Site	Mean total time (hrs)
RF9	0.042
RF15	0.058*
RF26	0.047
RF31	0.043
RF37	0.047
RF2003	0.044

Note: Total time is the time elapsed from when the downrigger touches bottom, until the time the subsequent downrigger touches bottom. *Largest total time fished per hook used to calculate sampling time for the simulated capture-recapture data used to calibrate the CPUST-density model.

Table 3.3 Observed catch-per-unit-soak time (CPUST) in each study site.

Site	Mean CPUST (no. rockfish/hr)
RF9	28.72427
RF15	19.94928
RF26	38.11167
RF31	42.94107
RF37	42.92812
RF2003	22.70227

Note: CPUST was calculated as the pooled rockfish catches over all six field sampling trips over the total pooled soak time.

Table 3.4 Estimates of sampling cost and efficiency for submersible, and ROV surveys within my original Trincomali RCA study sites. Costs are in Canadian dollars.

Survey technique	Actual sampling time (days)	Actual sampling cost	Estimated RCA sampling time (days)	Approximate RCA sampling cost
Submersible	14	$320 000	2	$50 000
ROV	28	$180 000	7	$6 500

Note: Estimates of sampling time and cost for large-scale surveys provided by K.L. Yamanaka, DFO, pers. comm. 2005.

Table 3.5 Estimates of true abundance used to generate recapture data in the capture-recapture simulation model.

Site	Abundance estimate
RF9	366^{SA}
RF15	325^{CR}
RF26	798^{CR}
RF31	570^{CR}
RF37	522^{CR}
RF2003	317^{SA}

Note: ^{SA} denotes abundances approximated using an expansion of swept area indices (see Appendix D, Table D1) as no recapture data was available for the site. ^{CR} denotes abundances calculated from the Schnabel census capture-recapture model.

Table 3.6 Upper and lower bounds of density confidence intervals used for the simulated calibration of the CPUST-density model.

Category	Lower limit	Upper limit
1	0.008*	0.069
2	0.069	0.130
3	0.130	0.191
4	0.191	0.252**

Note: To stay within the range of the observed data but introduce increased contrast, I divided the difference between the observed minimum and maximum density CLs, and set random values from each category as "true" density conditions for the model. *minimum observed abundance CL **maximum observed abundance CL

Figures

Fig. 3.1 **A general schematic representation of the main data components required to compute derive the coefficient of variation of density ($\hat{\tau}$) from the calibrated CPUST-density model.**

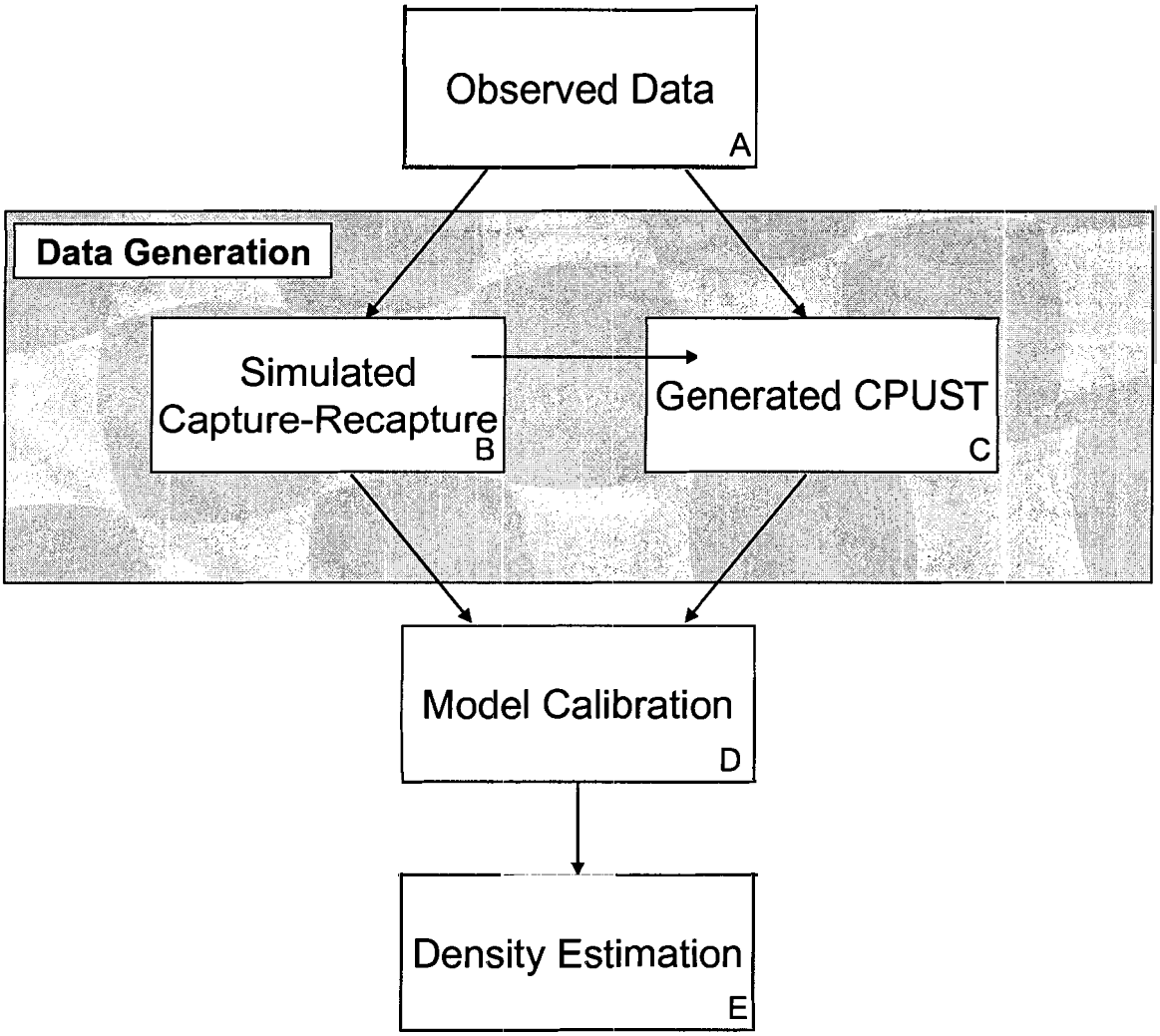


Fig. 3.2 **A detailed schematic representation of the information and steps required to simulate the derivation of density ($\hat{D}$) from observed CPUST (C) values using the calibrated CPUST-density model. Notations defined in Table 3.1.**

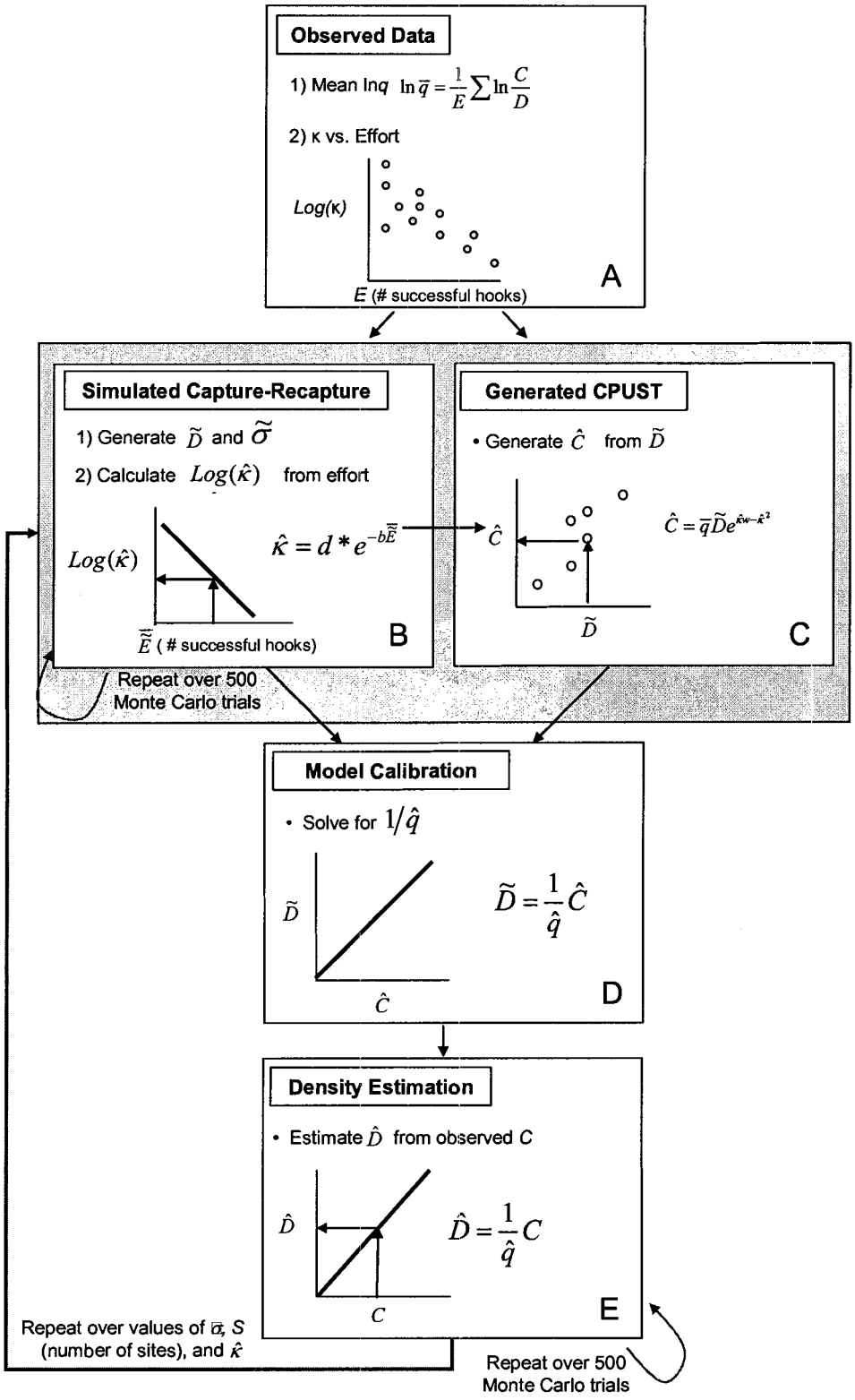


Fig. 3.3 A simulation flow diagram for the capture-recapture model used to generate the level of effort ($\widetilde{H}$, number of biopsy hooks) necessary to achieve a target density coefficient of variation ($\widetilde{\sigma}$).

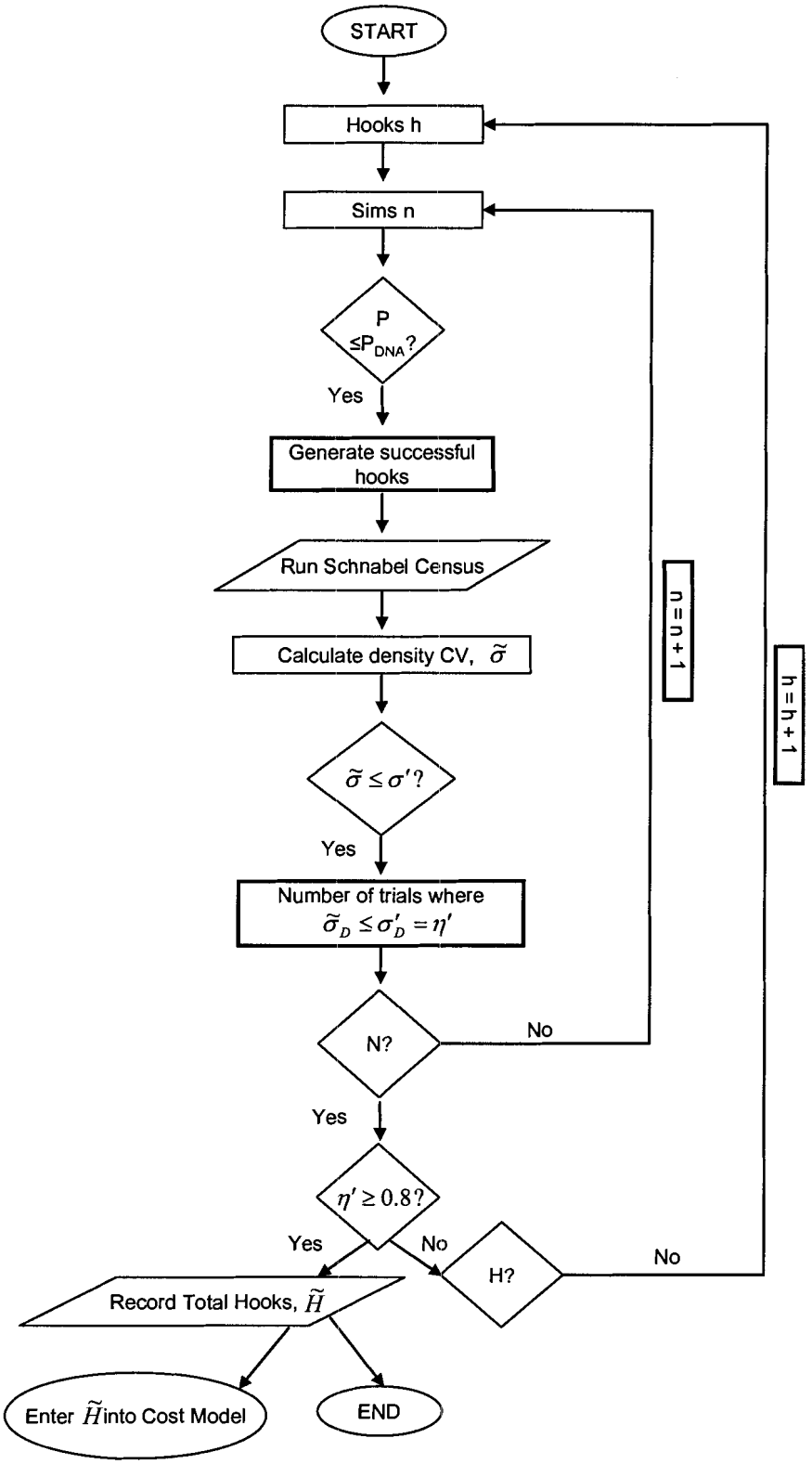


Fig. 3.4 **Best-fit robust linear relationship (square points) between observed (circle points) log coefficients of variation in mean catchability (κ) and the number of biopsy hooks collecting rockfish tissue (E).**

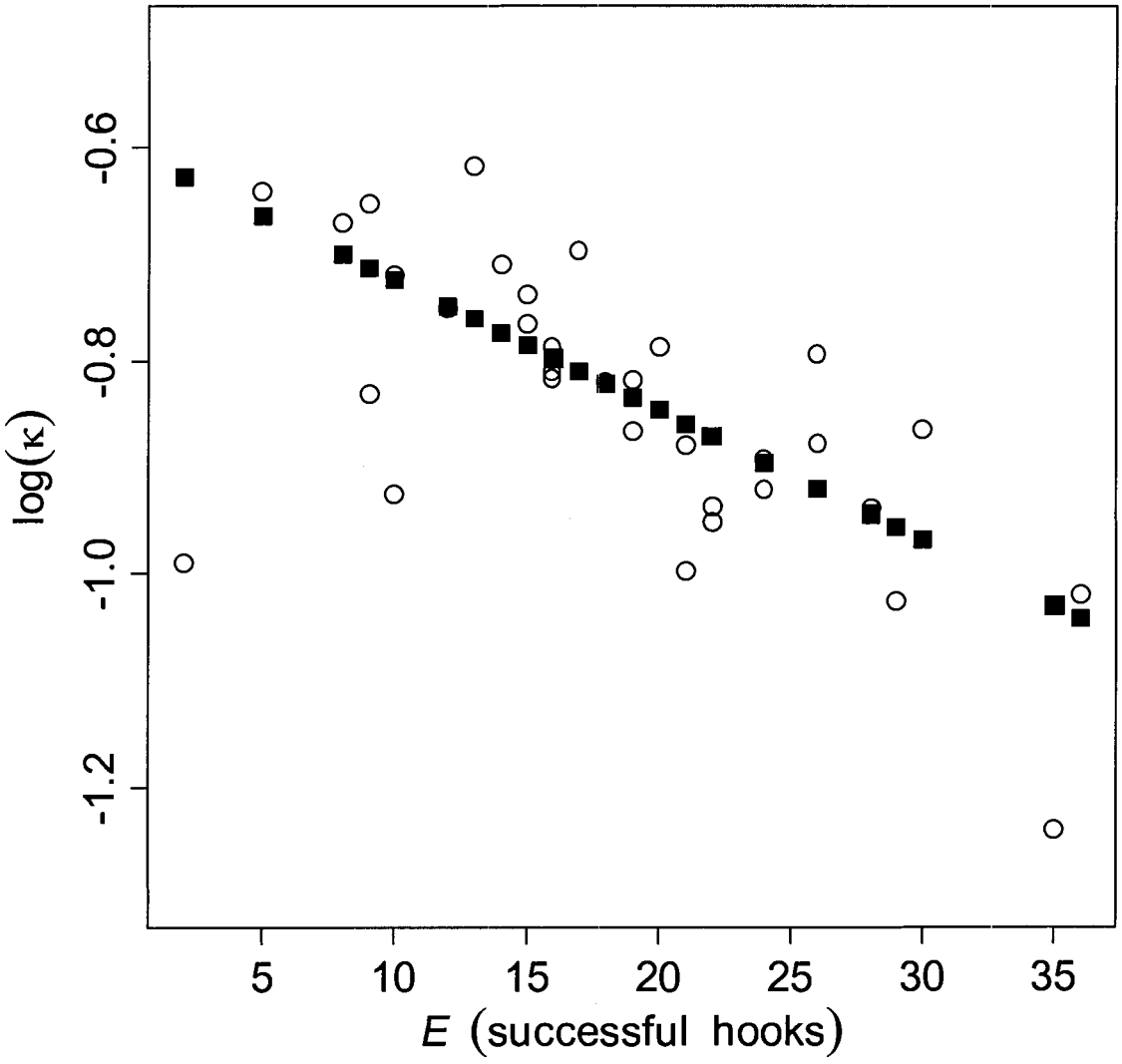


Fig. 3.5 Relationship between total sampling effort ($\widetilde{H}$) and the estimated coefficient of variation of density from the capture-recapture model ($\widetilde{\sigma}$).

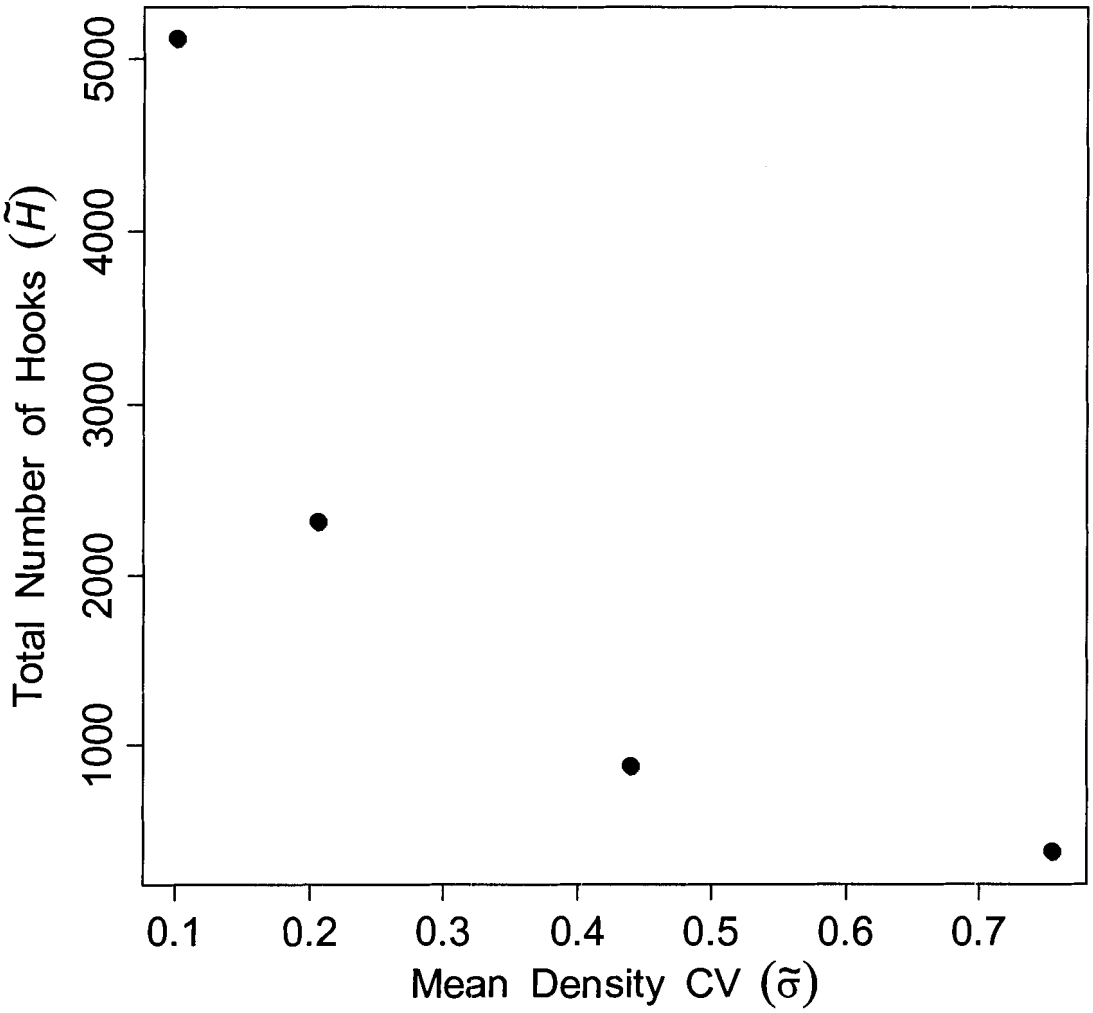


Fig. 3.6 **Relationship between percent bias in Schnabel census density estimates ($\widetilde{D}$) and the coefficient of variation of density ($\widetilde{\sigma}$). Mean $\widetilde{\sigma}$ are averaged over all simulations and sites. A negative bias is associated with imprecise estimates (large $\widetilde{\sigma}$), while positive biases correspond to highly precise estimates.**

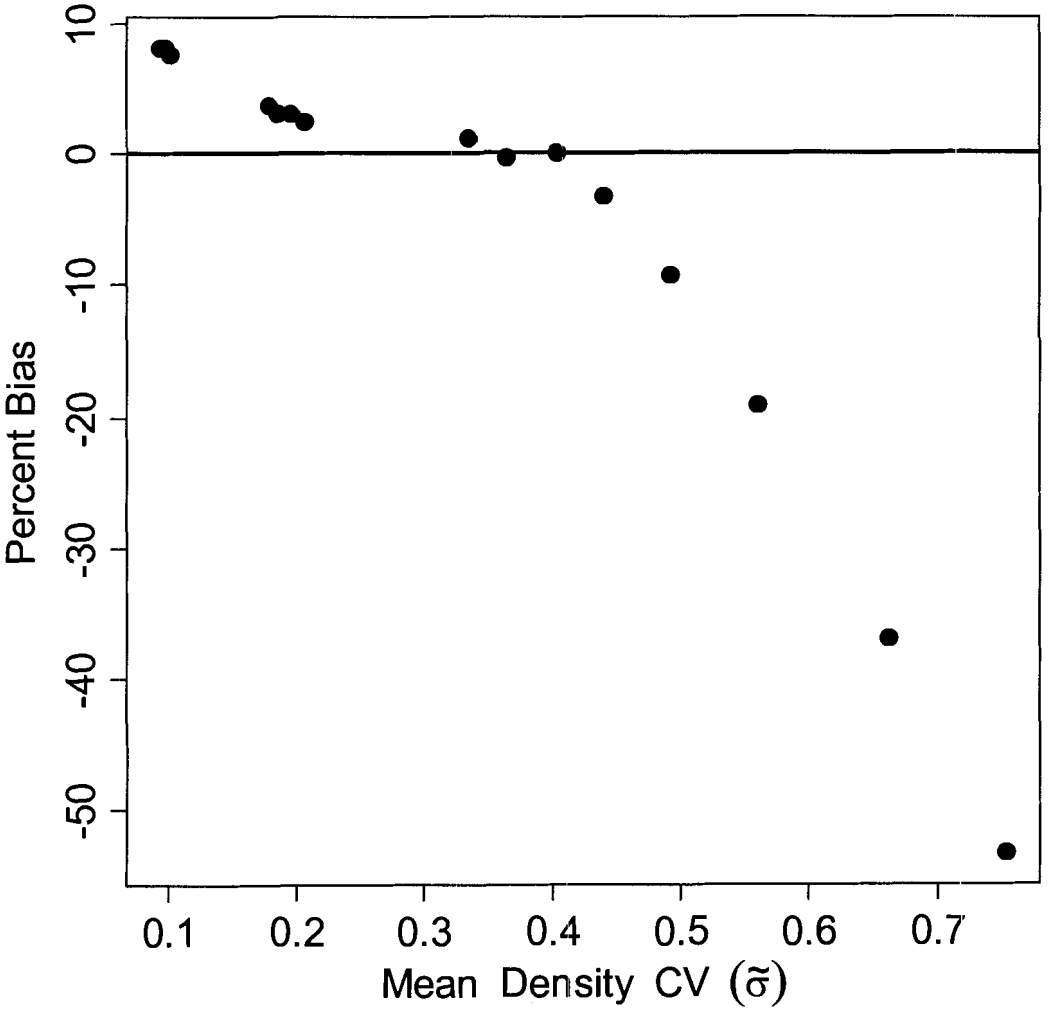


Fig. 3.7 Relationship between improvements to biopsy hook tissue capture rates (P_{DNA}) and the estimated cost of a genetic tagging capture-recapture survey. Assuming a target density coefficient of variation = 0.2.

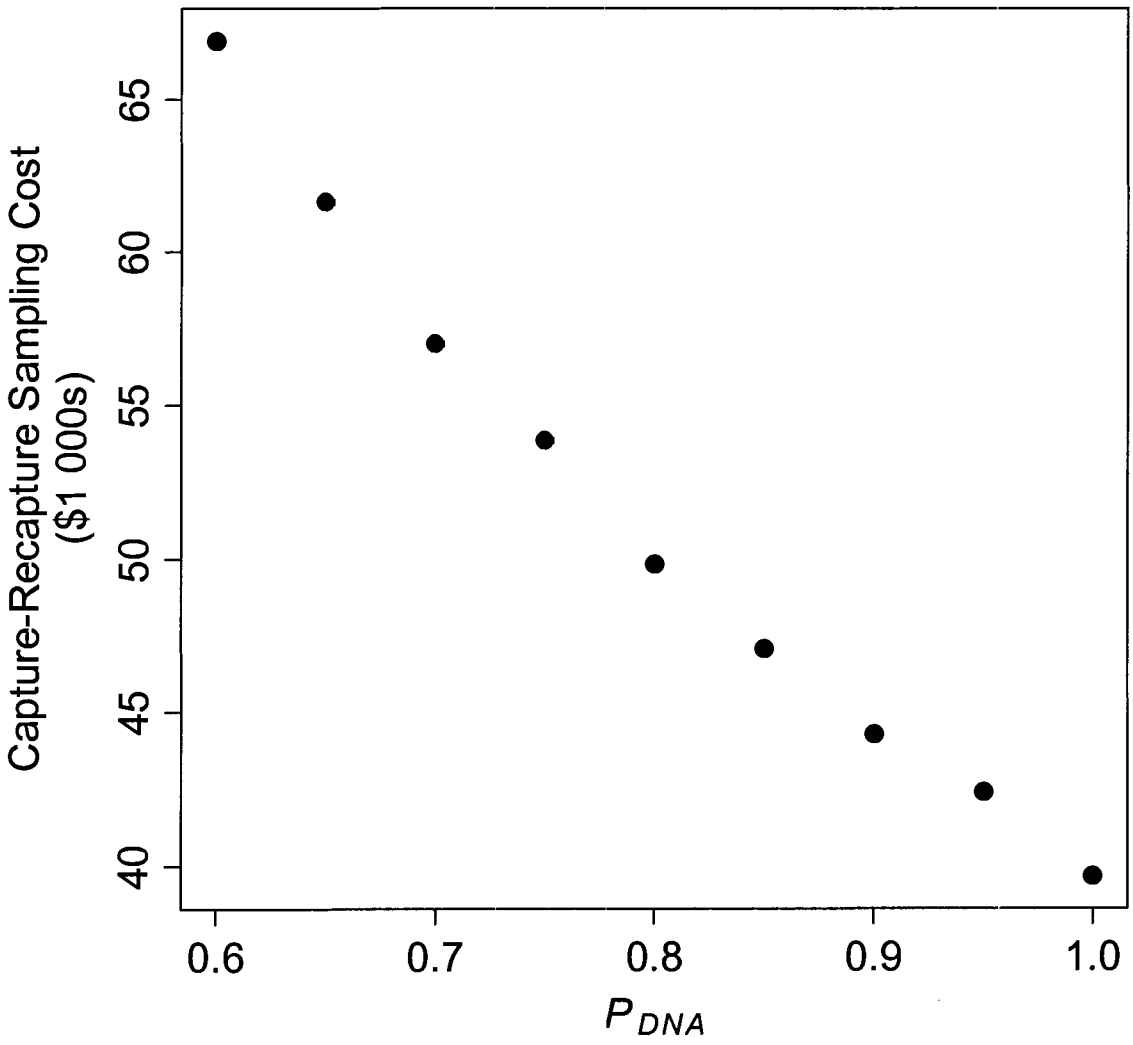


Fig. 3.8 **The relationship between the precision ($\tilde{\sigma}$) and number of density estimates (sites) from the simulated capture-recapture survey on the coefficient of variation of the calibrate CPUST-density model ($\hat{\varepsilon}$).**

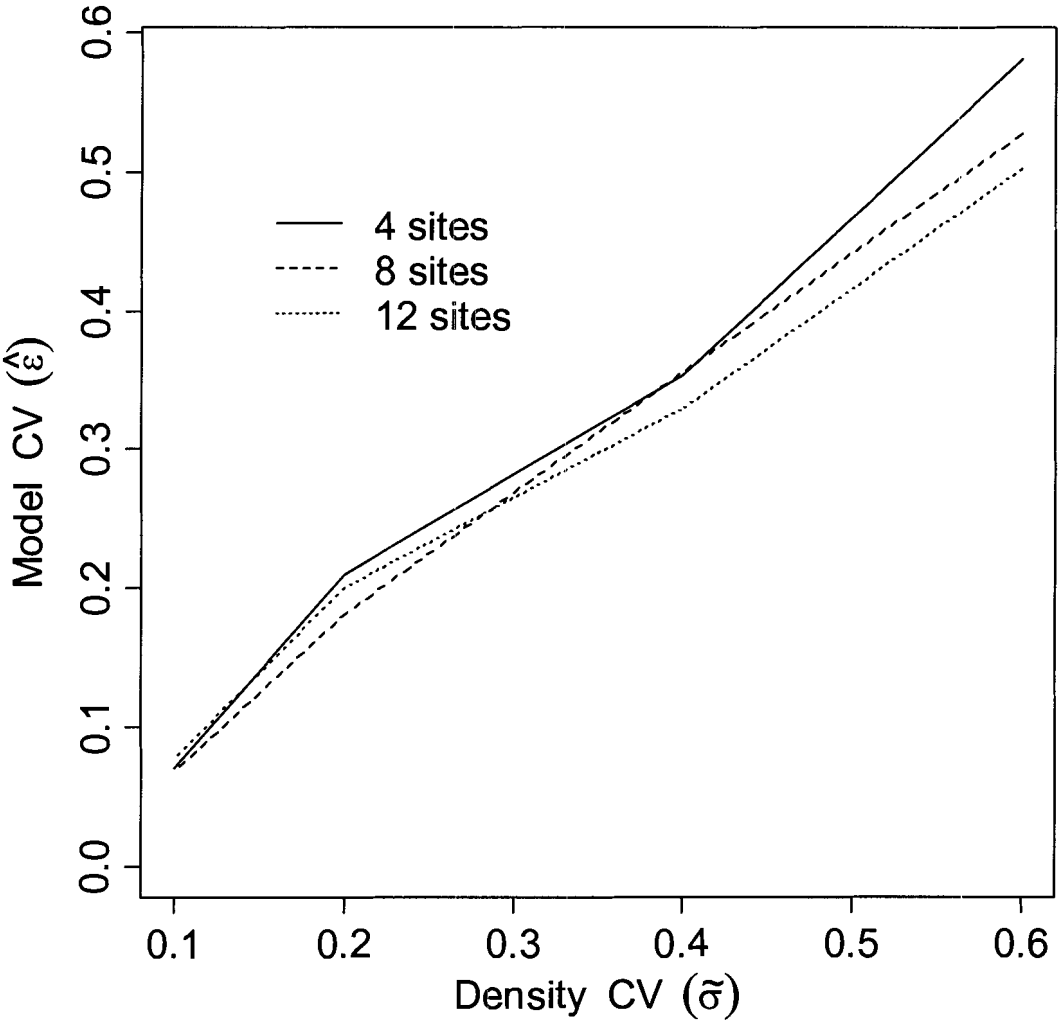


Fig. 3.9 **Changes to model calibration cost due to changes in required model precision ($\hat{\varepsilon}$) and the number of capture-recapture site density estimates used to calibrate the model.**

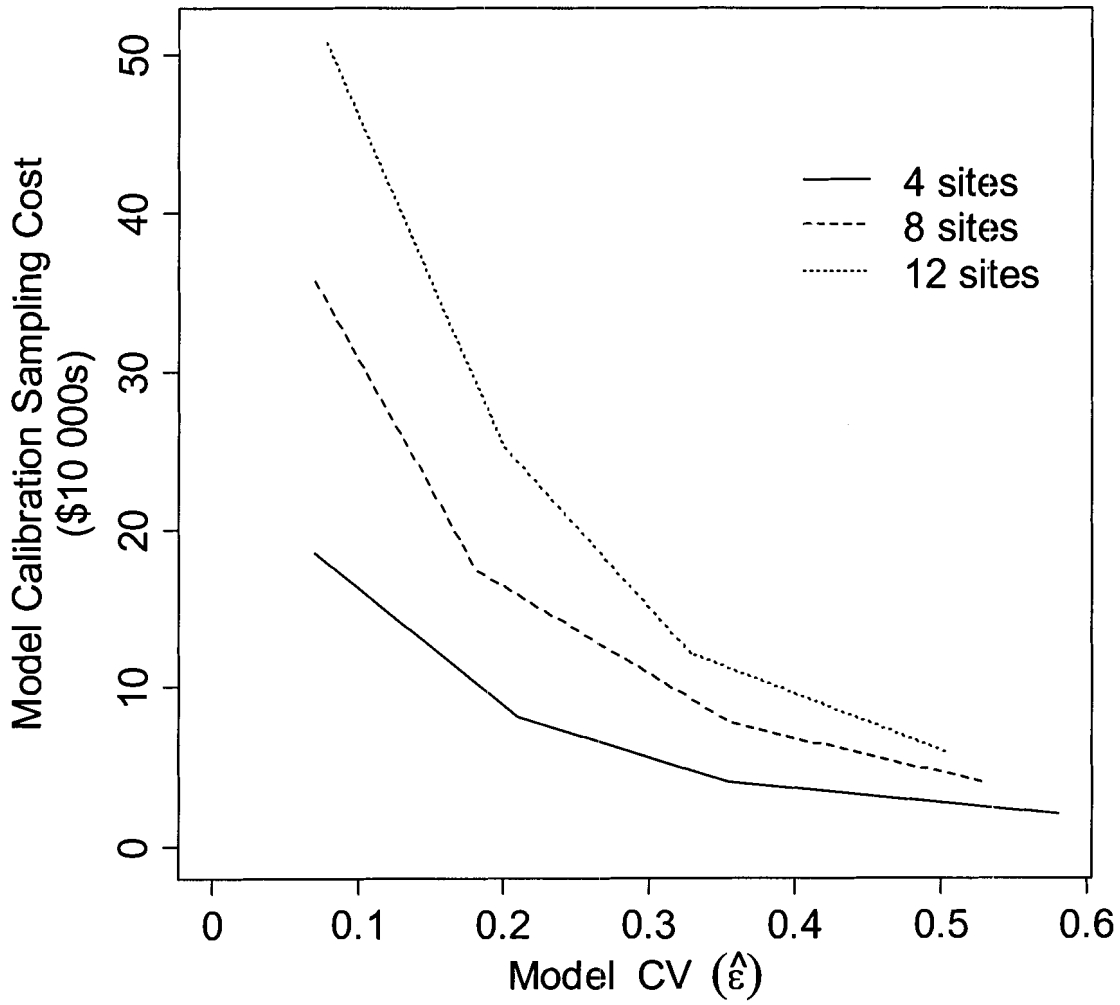


Fig. 3.10 **A contour plot illustrating the contribution of calibration model ($\hat{\varepsilon}$) and CPUST ($\hat{\kappa}$) variability to the final value of the mean coefficient of variation of density ($\hat{\tau}$) derived from observed CPUSTs. Calibration error is from a 4-density model, with estimated capture-recapture density CVs ($\tilde{\sigma}$) of 0.1, 0.2, 0.4, and 0.6, respectively.**

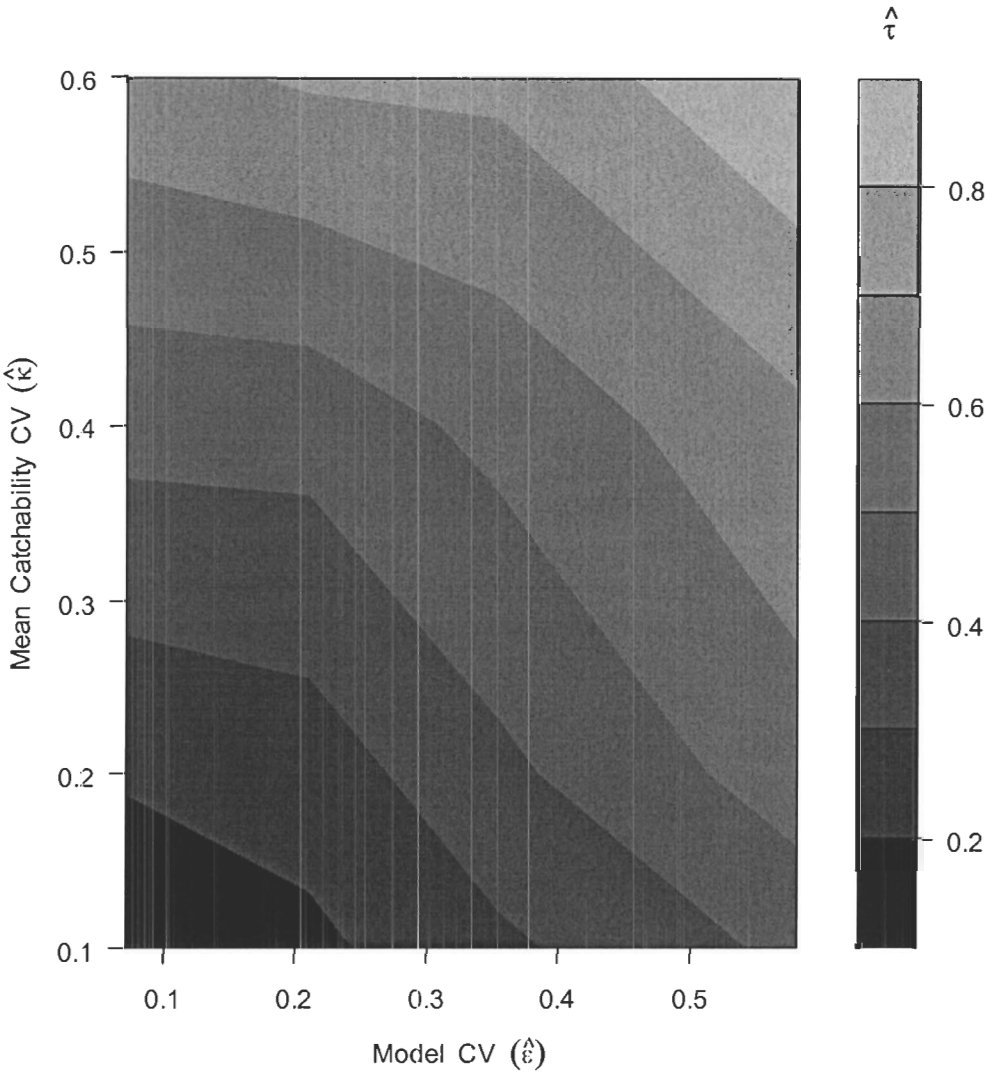


Fig. 3.11 A contour plot illustrating the impact of calibration model ($\hat{\varepsilon}$) and CPUST ($\hat{\kappa}$) variability on the total survey cost (θ) associated with deriving density from a CPUST survey. Model calibration error and costs are from a 4-site model.

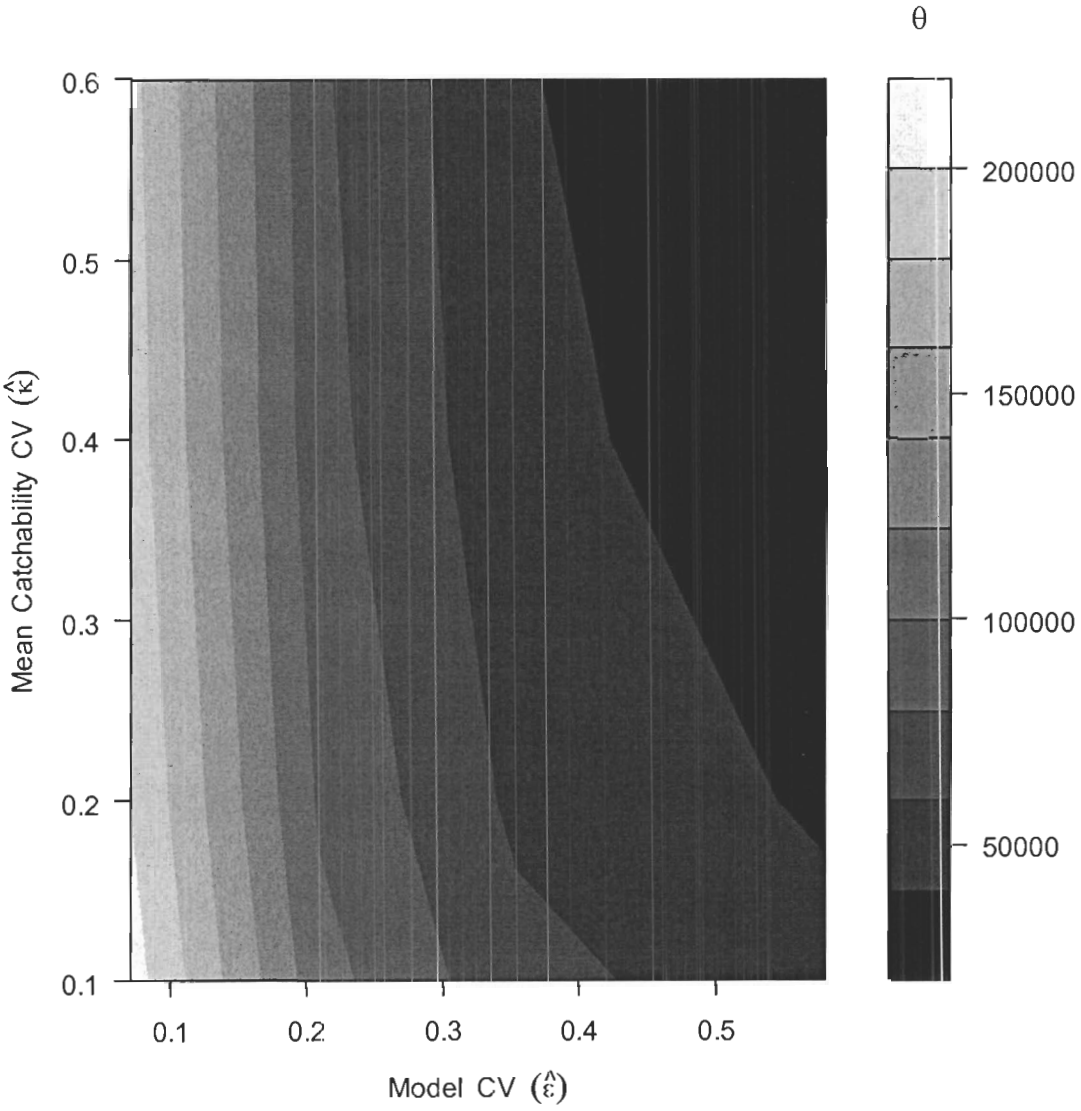


Fig. 3.12 The mean fraction of total survey cost comprised by each individual element included in the cost analysis. Percent cost was averaged over all simulated results and across all precision combinations for the calibration model and CPUST survey. Calibration costs were for a 4-site model.

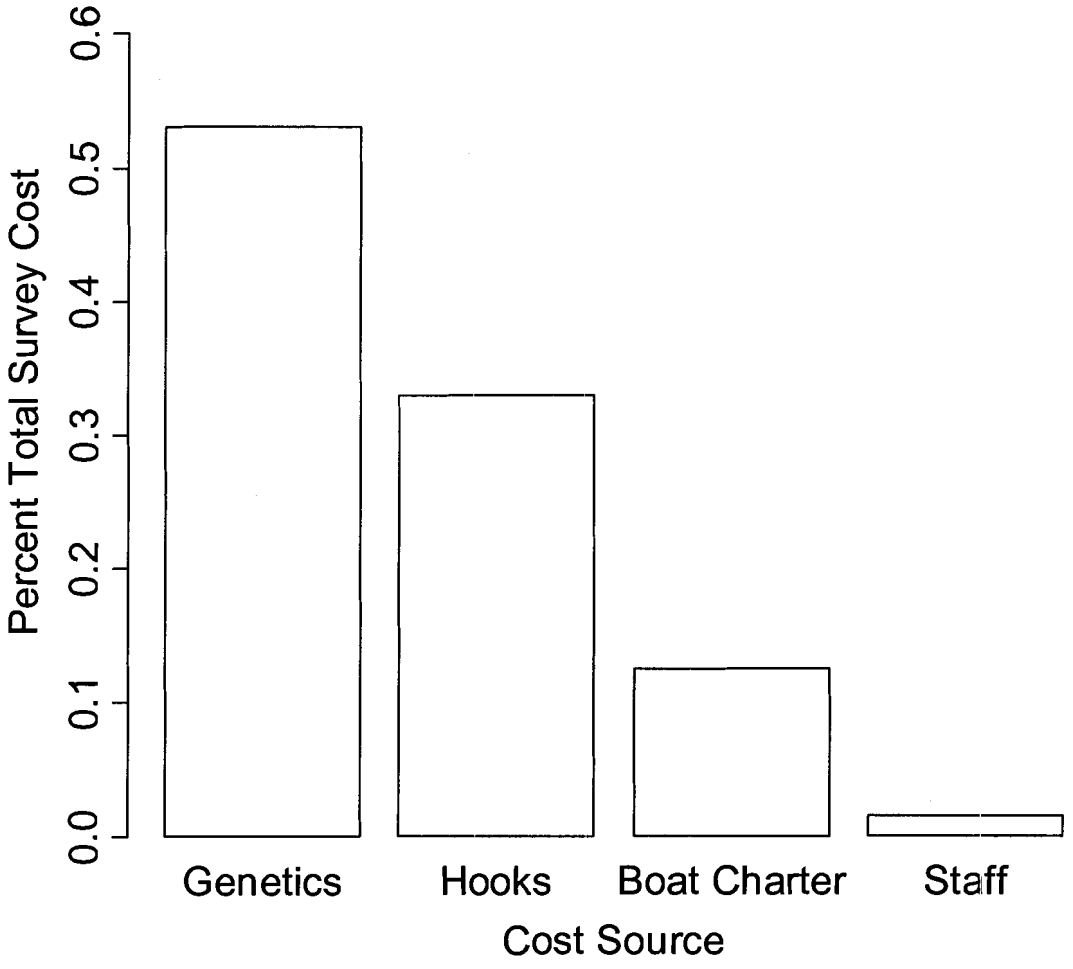
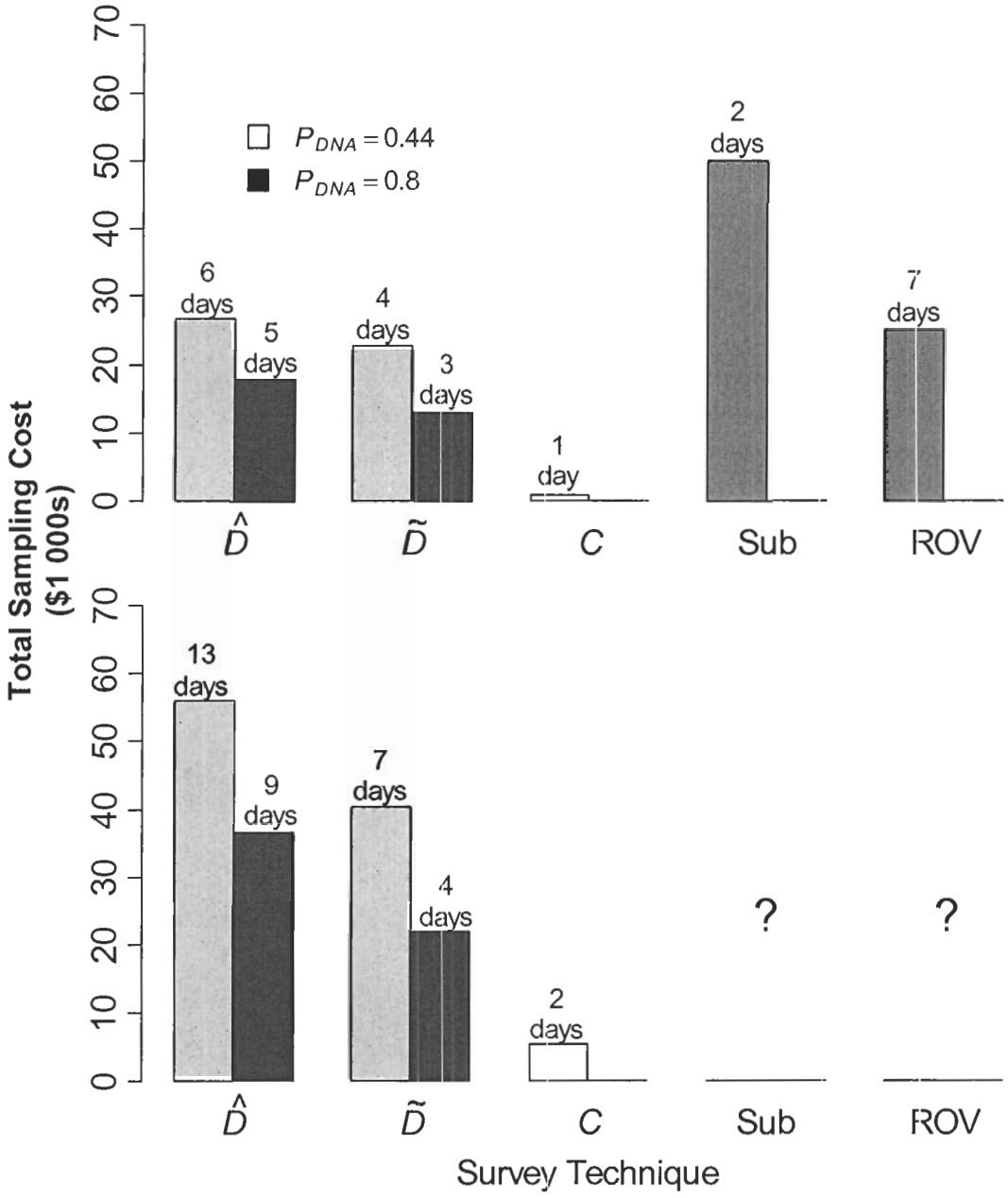


Fig. 3.13 **Cost comparison for five non-destructive survey methods: CPUST-derived density ($\hat{D}$), capture-recapture ($\tilde{D}$), CPUST index (C), submersible (Sub) and ROV, within my original study sites. The upper plot simulates data assuming a target coefficient of variation = 0.8, while the lower plot assumes a target CV = 0.4. P_{DNA} = proportion of biopsy hooks collecting individually identifiable rockfish tissue samples. Submersible and ROV estimates provided by K.L. Yamanaka, Pacific Biological Station, Nanaimo, B.C.**



CHAPTER 4
CONCLUSIONS

Overview

A combination of physiological constraints and specific habitat requirements limits the effectiveness of traditional monitoring tools for assessing populations of inshore rockfish. Given the rapidly expanding network of RCAs, an effective, cost-efficient and non-destructive sampling technique could be extremely valuable for continued assessment and monitoring of rockfish populations within closed areas. The goal of my master's project was to evaluate the use of one potential alternative technique in the form of genetic tagging. Although genetic tagging is commonly applied to studies of elusive terrestrial and marine mammals (Paetkau et al. 1995; Palsbøll et al. 1997; Woods et al. 1998; Mills et al. 2000), little research has explored its applicability in terms of fisheries monitoring.

To address my goal, I collected rockfish DNA samples *in situ* using biopsy hooks designed by a group of Australian researchers. The use of the biopsy hooks removed the need to bring rockfish to the surface and thus greatly minimised rates of post-marking mortality (Stanley et al. 1994; Starr et al. 2000; Yamanaka and Lacko 2001). Over the course of several weeks during the summer of 2004, I performed a capture-recapture genetic tagging study within a small rockfish conservation area in the Strait of Georgia. Eleven recaptures, resulting in a 3.1% recapture rate, provided abundance estimates in four of my six study sites. Subsequent analyses revealed a proportional relationship

between catch-per-unit-soak time (CPUST) and density, although poor data contrast and precision made it statistically difficult to interpret the final results.

Although Chapter 2 describes the successful application of genetic tagging in the field, feasibility will ultimately depend on the cost and efficiency of this method. Therefore, in Chapter 3, I applied a simulation modelling approach to evaluate trade-offs between sampling cost and estimate precision for several different applications of genetic sampling. Even with low tissue capture rates, the cost of estimating absolute rockfish abundance from CPUST or capture-recapture data may be comparable to the cost of current visual surveys. If additional data confirms the presence of a linear relationship between rockfish density and CPUST, a CPUST relative density index could provide an inexpensive tool for monitoring rockfish population trends within closed areas.

Future work

As my research only provides an initial exploration of genetic survey methods for assessment of inshore rockfish populations, there are several avenues for future research. Prior to further field studies, additional time should be invested in developing more efficient biopsy hooks. Not only will improved hook efficiency lead to decreases in cost of genetic analysis and field supplies, it will also decrease sampling time.

Following improvements to hook design, a major challenge I faced in this project was the quantification of rockfish density. Given the high degree of uncertainty in the estimate of the biopsy hook track width, I suggest the use of underwater camera surveys to estimate the mean distance from which a fish will approach a baited hook.

Finally, I recommend performing further small-scale studies to collect high contrast, high precision density and soak time data to resolve the true nature of the relationship between CPUST and density. If catchability proves to be density-independent, then use of CPUST data may be a less expensive application of genetic tagging than a capture-recapture survey.

Conclusions

My results demonstrate the first application of genetic tagging to capture-recapture studies of quillback and copper rockfish, and provide evidence of a proportional relationship between catch-per-unit-soak time and rockfish density. Simulation modelling shows that genetic and visual surveys may be cost comparable. I believe my results demonstrate that there is a warrant for further exploration into the application of genetic sampling as a feasible alternative to rockfish monitoring. Taking into account future modifications, genetic monitoring programs may serve as a viable technique for studies of inshore rockfish.

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APPENDICES

APPENDIX A: SITE CHARACTERISTICS

Tables

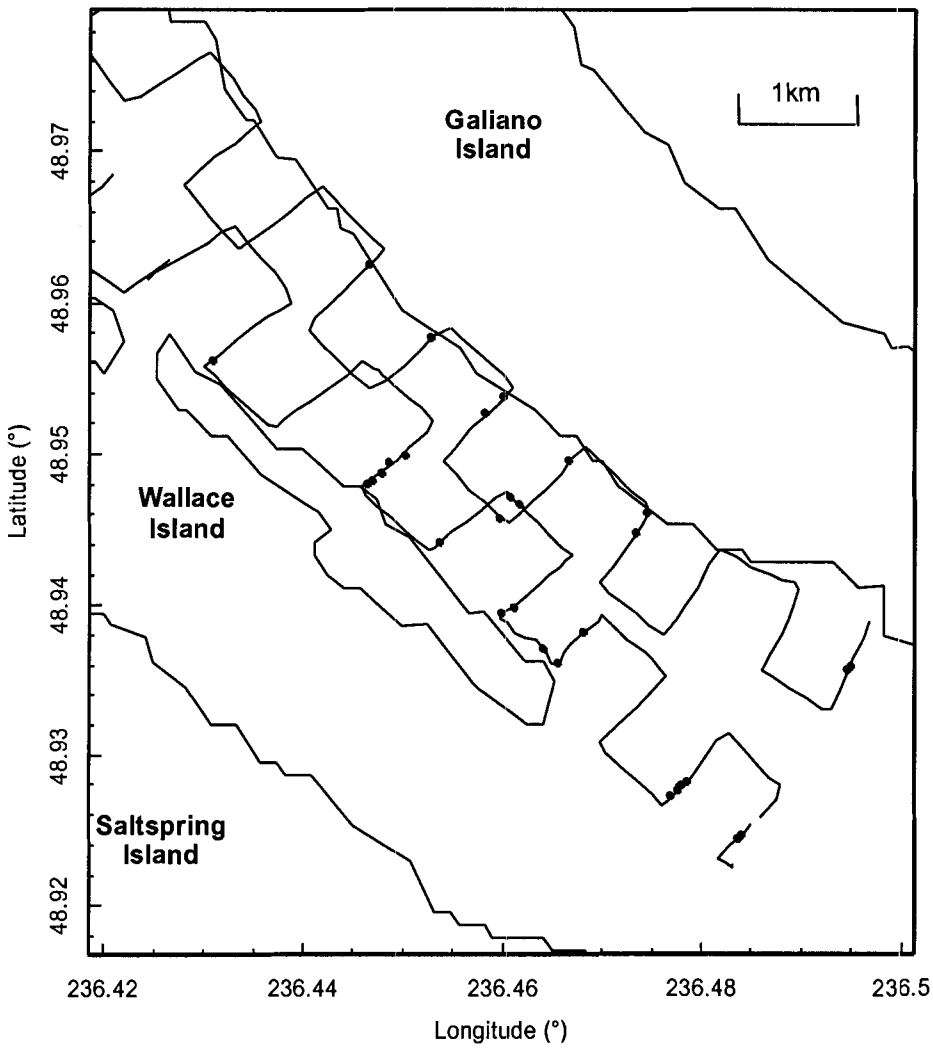
Table A1 General description of genetic tagging study sites sampled within the Trincomali Channel RCA.

Site Characteristic	RF9	RF15	RF26	RF31	RF37	RF2003
Mean Strike Depth (m)	22.31	43.18	29.08	24.49	41.42	36.45
% Bottom Type						
B	47.5	0	69.9	19.2	31.5	49.4
SMM	16.7	0	15.0	0	0	46.9
SM	35.9	0	15.1	0	61.6	3.7
GS	0	13.6	0	80.8	6.9	0
BGS	0	86.4	0	0	0	0
Site Area (m^2)	$2.55x10^4$	$6.75x10^4$	$5.09x10^4$	$3.19x10^4$	$4.16x10^5$	$1.09x10^5$

Note: B = bedrock, SMM = sandy mud – mud, SM = sand – muddy sand, GS = gravel – sandy gravel, BGS = boulder gravel – sandy gravel.

Figures

Fig. A1 **Echosounder transect lines used to identify potential genetic tagging sites. Points indicate sites with either suitable habitat (bedrock or boulder) or apparent concentrations of inshore rockfish identified by the vessel skipper (V. deLeeuw).**



APPENDIX B: GENETIC ANALYSIS

Probability of identity

Probability of identity (P_{ID}) calculations are employed in the fields of criminology (e.g. Foreman et al. 1997; Evett and Weir 1998) and ecology (e.g. Paetkau and Strobeck 1994; Paetkau et al. 1995; Palsbøll et al. 1997; Paetkau et al. 1998) to provide evidence regarding the identification of an offender, or a recapture.

Waits et al. (2001) describe a "reasonably low" P_{ID} as ranging from 0.01 – 0.0001, while other authors have selected a sibling match probability lower than 0.05 (Woods et al. 1999). In other words, the selection of a critical match criterion is at the discretion of the researcher, and preferred values will vary depending on the population size and structure of the species of interest (Waits and Leberg 2000).

Researchers aim to minimise P_{ID} in a variety of ways. Possible options are: (1) selecting highly heterozygous microsatellites, and/or (2) screening as many loci as possible. However, in addition to escalating costs, screening additional loci increases the probability of laboratory error in the form of false negatives, leading to possible upwards bias in abundance estimates (Waits and Leberg 2000). My ability to minimise recapture P_{ID} was further restricted due to biopsy hook inefficiency, which resulted in reduced amplification rates of highly variable markers and produced tissue samples with fewer than 10 detectable loci.

I determined the minimum number of loci required to obtain a high level of confidence in my recapture observations assuming a Hardy-Weinberg P_{ID} (Eq. B1) (unbiased for small sample sizes; Paetkau et al. 1998) for each recaptured fish

(B1) $$P_{ID_{unbiased}} = \frac{n^3\left(2a_2^2 - a_4\right) - 2n^2\left(a_3 + 2a_2\right) + n\left(9a_2 + 2\right) - 6}{\left(n-1\right)\left(n-2\right)\left(n-3\right)}$$

where n is the size of the population from which the allele frequency information is drawn and

$$a_i = \sum_j p_j^i$$

(Paetkau and Strobeck 1994)

where p_j is the frequency of the j^{th} allele. Assuming independence among loci, the total P_{ID} is calculated by multiplying across all individual locus P_{ID}s (Ayres and Overall 2004).

However, standard P_{ID} equations assume populations are in Hardy-Weinberg equilibrium (i.e. random mating and no linkage among alleles; Paetkau et al. 1995), and may produce negatively biased results if there is a high degree of relatedness among samples (Donnelly 1995; Mills et al. 2000; Wilberg and Dreher 2004). A conservative upper bound can be calculated using the sibling probability of identity (P_{IDSib}), the probability two individuals share the same genotype given they are siblings (Woods et al. 1999; Wilberg and Dreher 2004).

Therefore, in addition to calculating the Hardy-Weinberg P_{ID} for four-loci (Ch. 2, Table 1) and three-loci matches (Table B1), I also calculated P_{IDSib} (Eq. B2) (Evett and Weir 1998)

(B2) $$P_{IDSib} = 0.25 + 0.5 * \sum_i p_i^2 + 0.5 * \left(\sum_i p_i^2 \right)^2 - 0.25 * \sum_i p_i^4$$

(Evett and Weir 1998, p. 116)

where p_i is the frequency of the i^{th} allele. However, geneticists recognise P_{IDSib} to be overly conservative unless the population is dominated by sibling relationships (Ayres and Overall 2004). Therefore, I simply present the P_{IDSib} results for the sake of comparison.

However, minimising P_{ID} to reduce the probability of false *positives* produces a danger of increasing the presence of false *negatives* – treating two genotypes separately when they actually come from the same fish (Stevick et al. 2001). In an effort to minimize possible shadow effects by ignoring samples matching at ≤4 loci, I may have mistakenly identified some recaptures as different fish. As a result, the original issue is reversed, and without correction, an *underestimate* of recaptures may lead to a population *overestimate* (Waits and Leberg 2000; Luckacs and Burnham 2005).

There are several possible approaches to reducing the bias introduced by false negatives. In the past, researchers have both rejected the data from which accurate identifications could not be made (Stevick et al. 2001; Luckacs and Burnham 2005) and employed meticulous laboratory practices to avoid genotyping error (Waits and Leberg 2000). More recently, some authors have produced models that attempt to account for

mis-identifications, allowing the researcher to reject fewer samples without producing biased results (e.g. Mills et al. 2000; Stevick et al. 2001; Luckacs and Burnham 2005). However, current models assume that enough loci are detectable to identify individuals with high probability of success if genotyping is without error. Given the low amplification rate of many of my loci, this assumption could not be met for my rockfish data.

I instead compared two simple approaches for reducing the presence of false negatives in my samples. First, I employed the traditional method of including only those samples I could confidently identify as individuals (see Ch. 2). However, while this reduces the probability of false negatives, it also greatly reduces the amount of useable data (Stevick et al. 2001; Luckacs and Burnham 2005). In an attempt to increase the number of useable samples, my second method expanded the number of four-loci recaptures by the percentage of all rockfish samples with a minimum of four loci (368/667; 55%). The expansion allowed me to roughly correct for missed recaptures, which otherwise would go unobserved because low DNA concentrations led to the detection of fewer than four loci. In the second approach, the marked population consisted of all samples containing identifiable rockfish DNA. However, the expansion may not entirely correct for false negatives and, therefore, may still overestimate of population size.

Tables

Table B2 Sibling probability of identity for the minimum four loci match recaptures displayed in Ch. 2, Table 1.

	Locus											
Recapture Genotype	*sal2*	*sal4*	*seb9*	*seb33*	*sme2*	*sme3*	*sme8*	*sra15*	*sar16*	*sru9*	P_{IDSib}	$1/P_{IDSib}$
1			X	X			X	X			$1.52x10^{-2}$	65.94
2	X		X	X			X	X			$5.95x10^{-3}$	168.08
3	X	X		X				X			$1.46x10^{-2}$	68.48
4	X			X				X	X		$1.09x10^{-2}$	91.53
5	X		X	X			X		X	X	$1.66x10^{-3}$	602.77
6	X		X	X	X	X		X	X	X	$1.13x10^{-4}$	$8.82x10^{3}$
7	X	X	X	X	X	X			X	X	$1.33x10^{-4}$	$7.54x10^{3}$
8	X		X	X			X		X		$5.11x10^{-3}$	195.43
9	X		X	X	X	X			X	X	$3.56x10^{-4}$	$2.81x10^{3}$
10			X	X	X		X		X	X	$1.22x10^{-3}$	821.66
11	X			X	X	X		X		X	$1.06x10^{-3}$	941.70

Table B3 Hardy-Weinberg probability of identity for each pair of recaptures matching at only three loci.

	Locus											
Recapture Genotype	*sal2*	*sal4*	*seb9*	*seb33*	*sme2*	*sme3*	*sme8*	*sra15*	*sar16*	*sru9*	**P_{ID}**	**$1/P_{ID}$**
1			X	X						X	$6.35X10^{-5}$	$1.58X10^{4}$
2	X		X							X	$6.53X10^{-5}$	$1.53X10^{4}$
3	X	X		X							$5.21X10^{-4}$	$1.92X10^{3}$
4	X		X					X			$5.84X10^{-5}$	$1.71X10^{4}$
5	X		X							X	$6.53X10^{-5}$	$1.53X10^{4}$
6	X	X		X							$5.21X10^{-4}$	$1.92X10^{3}$

Note: I considered the risk of a shadow effect to be too high using three-loci matches as it is probable that more than one fish in a single site shares a three-locus genotype.

Figures

Fig. B1 **Amplification success (% of rockfish samples containing amplified loci) of each locus used to identify my rockfish samples. Frequency often decreases with increased locus size, as small DNA samples may not contain the larger loci.**

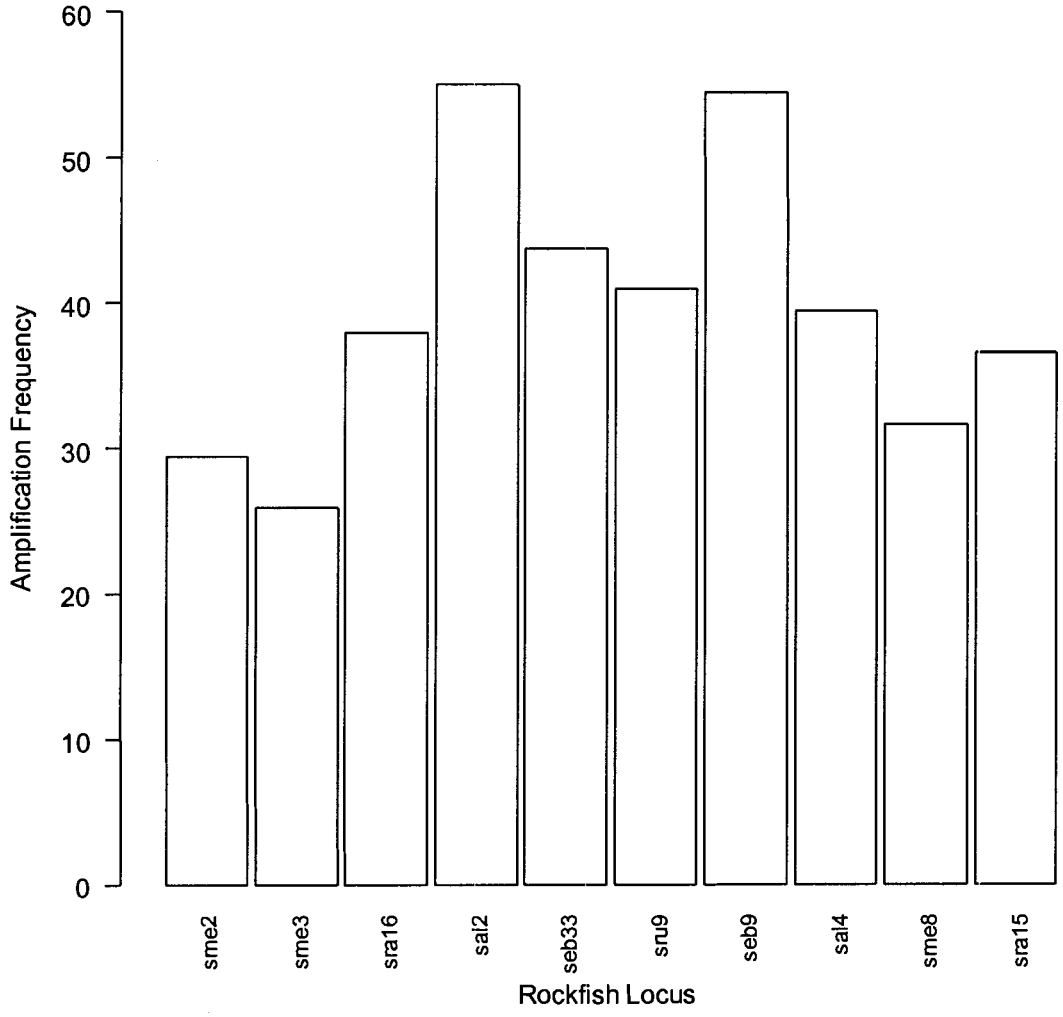
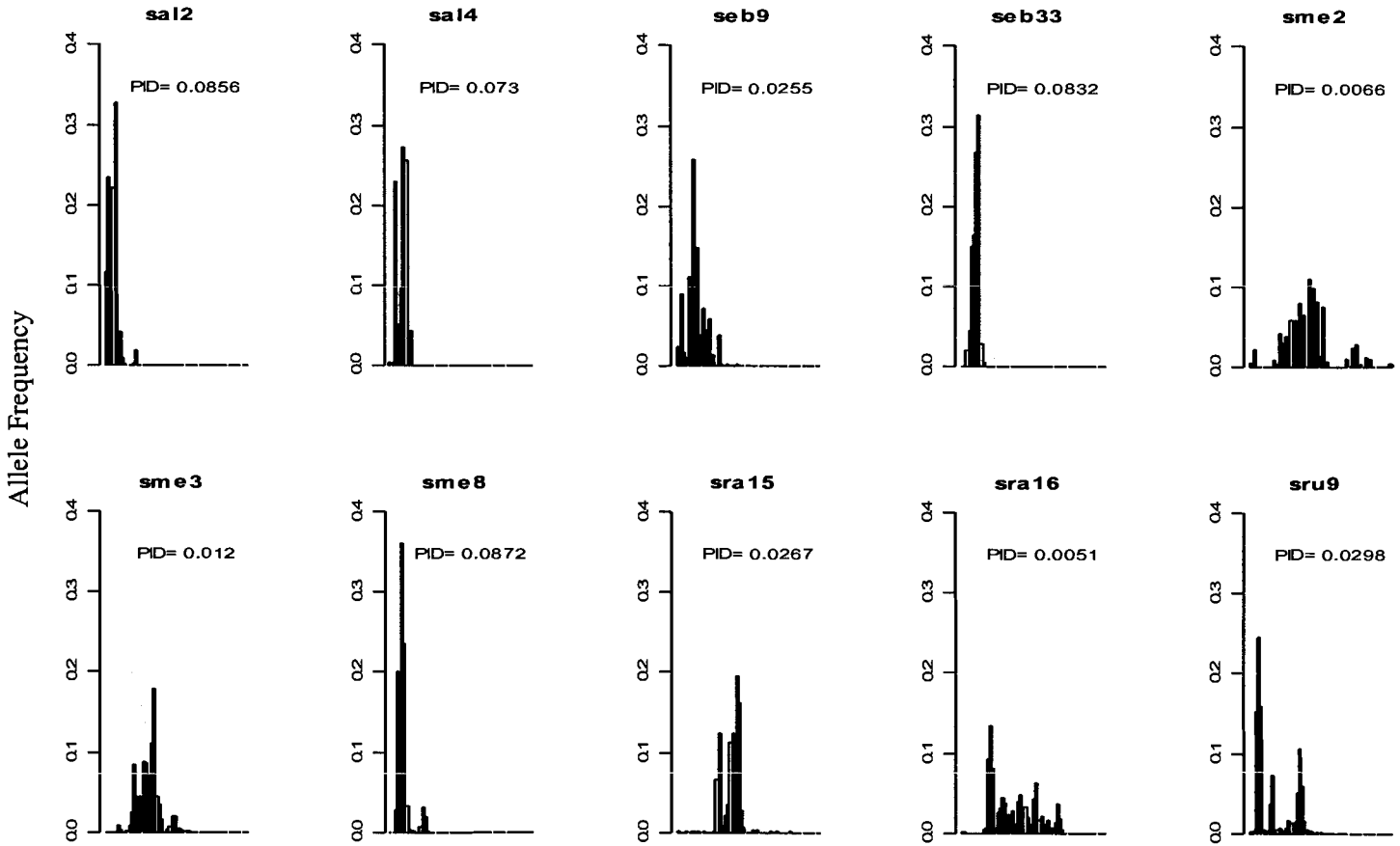


Fig. B2 **Allele frequency distribution in my amplified samples, and associated probability of identity at each locus (2003/2004 samples combined). Highly heterogenous loci (e.g. sme2, sme3, sra16, sru9) are associated with lower P_{ID}s and are more useful for individual identification, but tend to have lower amplification rates due to their large size.**



APPENDIX C: CAPTURE-RECAPTURE ANALYSIS

Model comparison

There are numerous methods for analysing closed-population multiple-marking session data (e.g. Seber 1982). I used simulation modelling to compare two well-known approaches: the mean Peterson estimate (Eqs. C1 and C2) and the Schnabel census (Ch. 2, Eqs. 2 and 3). I selected the Schnabel and Peterson methods because they are quite commonly used, and are relatively straightforward to program in R.

A mean Peterson estimate of abundance is simply a variation of the standard Peterson estimate for two sampling trips. However, a simple expansion of the Peterson model tends to produce overestimates in population size (Krebs 1999; Fig. C3). Therefore, as with the Schnabel Census, I applied a correction factor to the denominator of Eq. C1 to reduce the bias caused by low numbers of recaptures (Seber 1982)

(C1) $$N_t = \left(\frac{(m_{t-1}+1)*(E_t+1)}{r_t+1} \right) - 1$$

Variance of N_t was directly estimated for the Peterson method using

(C2) $$V_t = \frac{(m_{t-1}+1)*(E_t+1)*(m_{t-1}-r_t)*(E_t-r_t)}{(r_t+1)^2*(r_t+2)}$$

where N_t and V_t are the estimates of rockfish abundance and variance, respectively, in each site after trip t; m_{t-1} is the cumulative number of marks present prior to trip t; E_t is

the number of rockfish tissue samples collected during trip t; and r_t is the number of site-specific recaptures observed during trip t. The adjusted model produces unbiased estimates of abundance if $N < m + E$ and $r > 7$ (Krebs 1999).

I evaluated the two models by simulating a capture-recapture experiment over a range of "true" population sizes. The first evaluation used site-specific abundances proportional to estimates obtained using the Schnabel census in Ch. 2, and held effort constant at the number of individually identifiable samples collected during my field studies (Fig. C1). The second evaluation input site-specific abundances using the recapture expansion data described below and held effort at the number of collected rockfish tissue samples (Fig. C2). Recaptures were generated using the same model framework described in Chapter 3.

The Schnabel census consistently produced more precise and less biased estimates than the mean Peterson method (Fig. C1 – C3). However, both models showed a strong tendency towards a negative bias. Negative biases likely arise because the "true" population size surpasses the maximum abundance estimate achievable from the recapture model given the current level of effort (occurs when $r = 1$). The adjusted Schnabel and Peterson models are both relatively effective at reducing the positive bias normally associated with the simple estimators (no "+ 1" term in the denominator of the population estimator; compare Fig. C1 and Fig. C2). The Schnabel census appears relatively unbiased given the current level of sampling effort and estimated population sizes and consistently performs better than the mean Peterson model; therefore, I completed my final recapture analysis using the Schnabel estimator.

Recapture expansion

As described in Appendix B, I also attempted to adjust my data for potential negative bias by including all rockfish tissue samples in my recapture analysis and expanding the number of observed recaptures by the percentage of samples amplifying at ≥ 4 loci. This data consisted of 653 identifiable rockfish samples (Table C1), and an expanded estimate of 22 recaptures (Table C2), producing an estimated recapture rate of 3.4%. Using the adjusted data I produced slightly higher abundance estimates ranging from 540 in RF15 to 1477 in RF26 (Table C4). However, the density estimates produced were more comparable to those generated from the culled data set (Ch. 2, Table 2), as the swept area calculated for the un-culled data increased accordingly to the increase in the size of the dataset used for the analysis (Appendix D, Fig. D1 and D2). As more recaptures were available to compute the expanded abundance estimates, precision also increased slightly compared to the estimates produced from the culled dataset.

Tables

Table C1 Cumulative number of marked rockfish present in each site at the end of each sampling trip (m_t in Eqs. 2-3 and Eq. C1-C2).

Trip	1		2		3		4		5		6	
Site	*C*	*UC*	*C*	*UC*	*C*	*UC*	*C*	*UC*	*C*	*UC*	*C*	*UC*
RF9	3	10	11	21	23	37	33	53	38	69	48	91
RF15	6	9	16	23	23	35	31	47	35	55	39	61
RF26	14	18	28	36	41	56	49	79	64	110	75	132
RF31	27	38	55	77	68	99	79	116	82	132	84	135
RF37	22	28	35	52	45	66	51	86	65	109	77	137
RF2003	NA	NA	NA	NA	4	12	11	28	17	48	17	48

Note: Values are presented for both culled (C) and un-culled (UC) data and are equivalent to the total number of tags, less the recaptures and outliers in each site. RF2003 was not sampled until the third sampling trip, and full results from the final trip were not available for some sites. Trips: 1 = May 10, 2 = June 8, 3 = June 28, 4 = July 7, 5 = July 22, 6 = August 8.

Table C2 Summary of expanded recaptures within a given site and sampling trip (r_t in Eqs. 2-3 and Eq. C1-C2) for sites in which fish were recaptured.

Start Date	June 8	June 28	July 7	July 22	Aug 8	RR %
Trip	2	3	4	5	6	
Site						
RF15	0	0	0	2	0	2.5
RF26	0	2	2	0	0	2.6
RF31	0	6	2	0	0	4.5
RF37	0	2	2	0	4	4.9

Note: Values are presented for expanded recaptures used in the analysis of the un-culled dataset. Recapture Rate (RR) = total recaptures/total marks.

Table C3 General information for the 11 observed recaptures collected during the 2004 sampling trips.

Match	Start Date	Trip	Site	Bottom Habitat	Distance moved (m)	# loci matched
1	May 10	1	RF31	GS		
1	June 28	3	RF31	GS	102.5	4
2	May 10	1	RF15	BGS		
2	July 22	5	RF15	BGS	7.4	5
3	May 10	1	RF37	B		
3	July 7	4	RF37	SM	34.2	4
4	May 10	1	RF37	SM		
4	August 8	6	RF37	SM	17.4	4
5	June 8	2	RF26	B		
5	June 28	3	RF26	B	28.0	6
6	June 28	3	RF31	B		
6	July 7	4	RF31	GS	12.2	7
6	July 22	5	RF31	B	4.4	
7	May 10	1	RF31	GS		
7	June 8	2	RF31	GS	24.6	5
8	June 8	2	RF37	SM		
8	Aug 8	6	RF37	SM	56.0	7
9	June 28	3	RF26	B		
9	July 7	4	RF26	B	29.6	6
10	June 8	2	RF37	B		
10	June 28	3	RF37	SM	26.6	6

Note: Trips: 1 = May 10, 2 = June 8, 3 = June 28, 4 = July 7, 5 = July 22, 6 = August 8. Bottom habitat codes: B = bedrock, SM = sand muddy – sand, GS = gravel sandy – gravel, BGS = boulder gravel – sandy gravel.

Table C4 Summary of Schnabel census capture-recapture results for the un-culled dataset.

Site	Abundance Estimate	Density Estimate	CV
RF9	---	---	---
RF15	540	0.0338	0.4714
RF26	1477	0.0983	0.4000
RF31	961	0.0849	0.3307
RF37	935	0.0771	0.3143
RF2003	---	---	---

Note: Density = abundance/swept area. CV = coefficient of variation. Marks include all samples amplifying at rockfish DNA. Recaptures are estimated by expanding observed recaptures by the percentage of samples amplifying at four or more loci (55%). There were no recaptures in sites RF9 or RF2003.

Figures

Fig. C1 **Comparison of Schnabel Census and Mean Peterson simulation results. True population size is proportional to abundances calculated for sites RF15, RF26, RF31 and RF37 using the Schnabel Census for marks amplifying at ≥ 4 loci.**

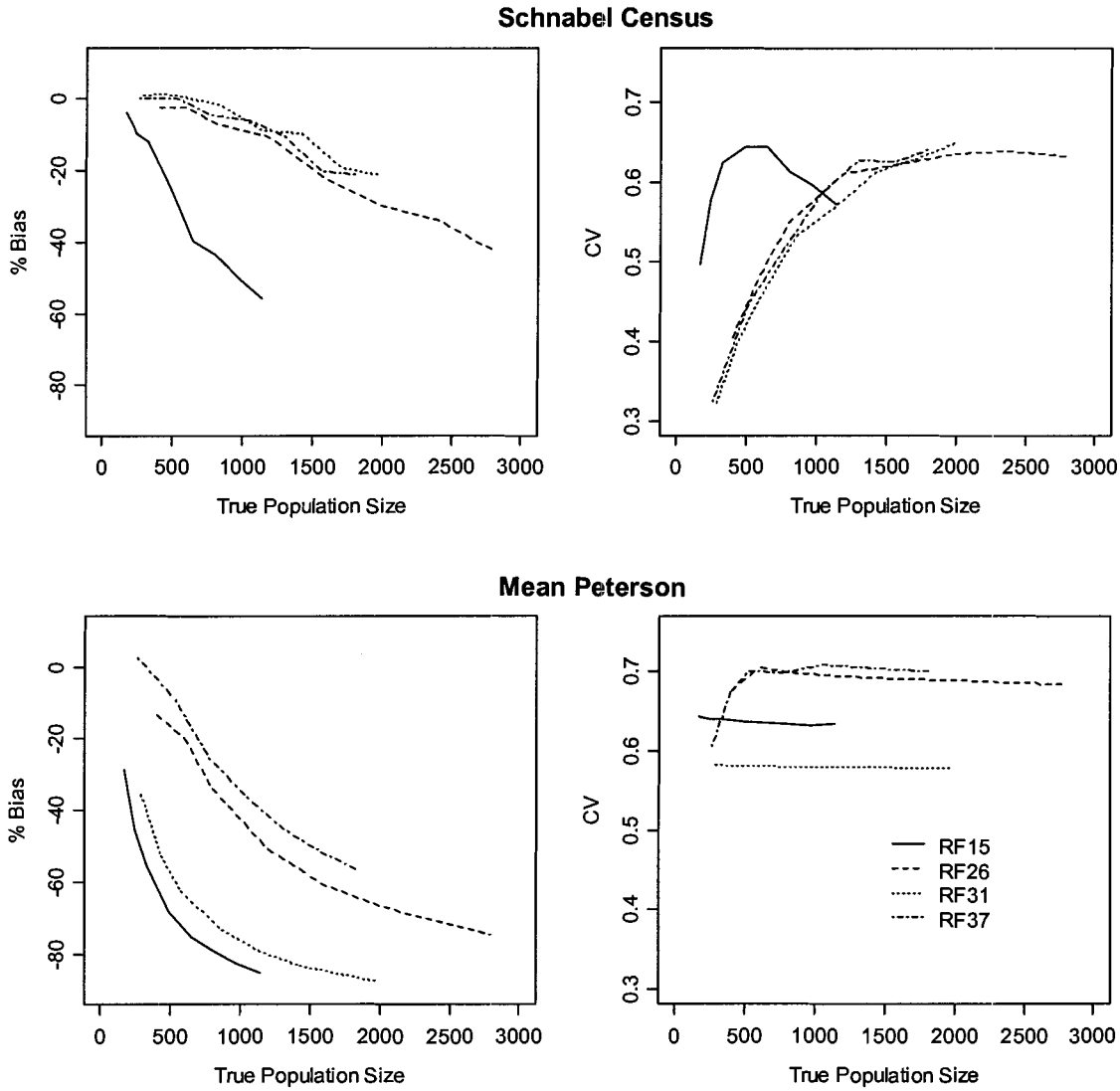


Fig. C2 **Comparison of Schnabel Census and Mean Peterson simulation results for models un-adjusted for small sample size (no "+ 1" term in the denominator of the population equations). True population size is proportional to abundances calculated for sites RF15, RF26, RF31 and RF37 using the Schnabel Census for marks amplifying at ≥ 4 loci.**

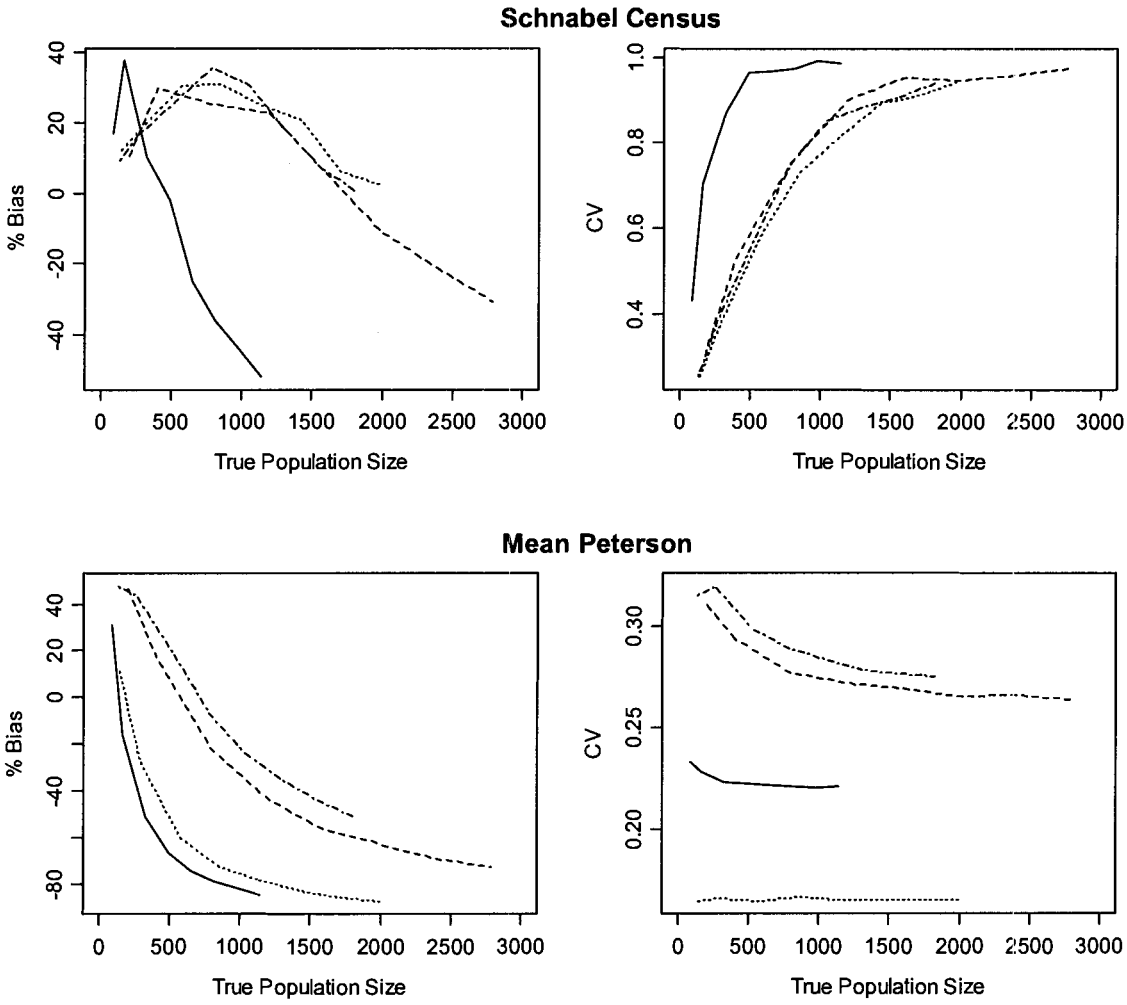
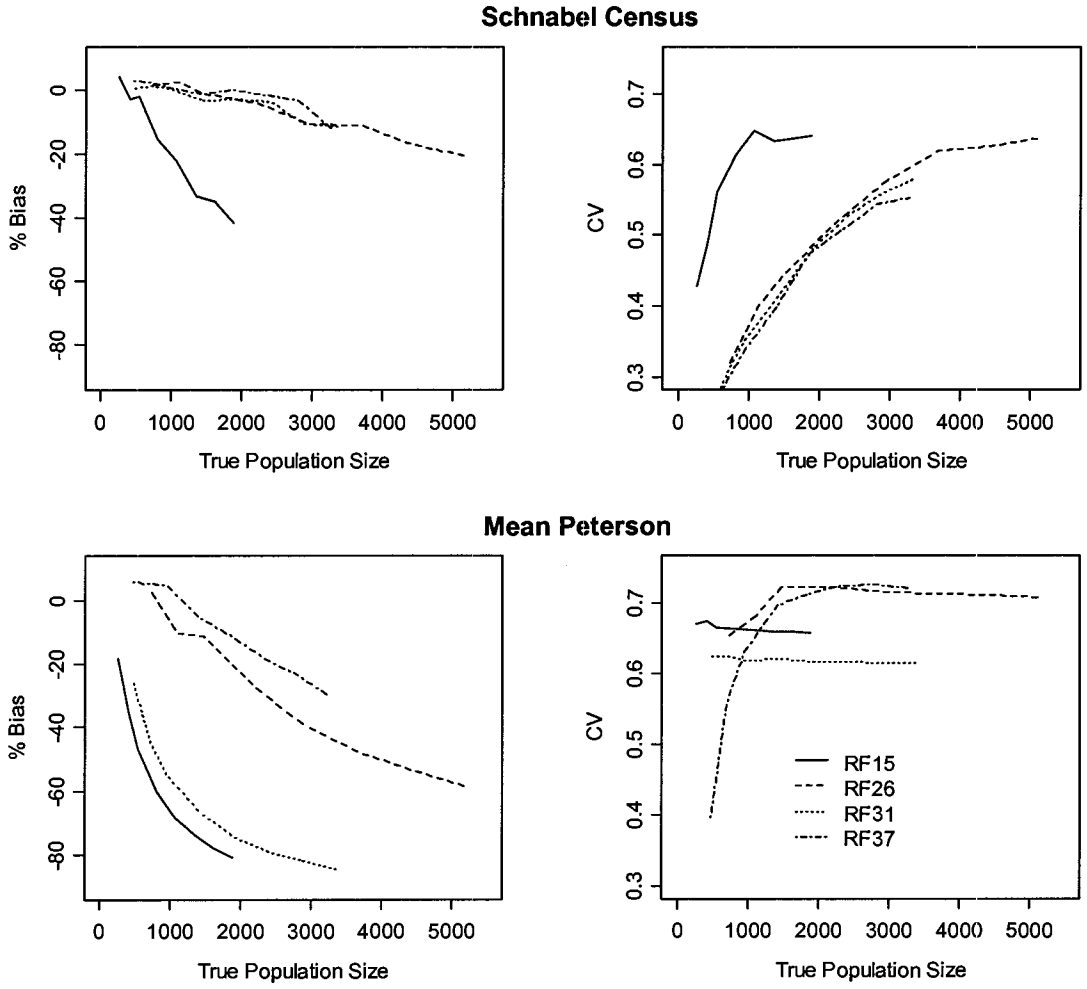


Fig. C3 Comparison of Schnabel Census and Mean Peterson simulation results. True population size is proportional to abundances calculated for sites RF15, RF26, RF31 and RF37 using the Schnabel Census for the recapture expansion dataset.



APPENDIX D: SWEPT AREA

Tables

Table D1 Estimated abundances for RF9 and RF2003 using expanded relative swept area indices.

Site	Mean swept area	Relative swept area	Expanded abundance estimate
RF9	59.201	0.165	365.628
RF2003	51.308	0.143	316.882

Note: Mean swept area computed using q=1, and track width = 3m. Relative swept area equals mean site swept area divided by sum of swept area for four sites with known capture-recapture abundances. Expanded estimate equals relative site swept area multiplied by total capture-recapture abundance estimate.

Table D2 Change in area swept and estimated density for three different assumed track widths: 1m, 3m (used in Ch 2 analyses), and 10m.

	Track width					
	1m		3m		10m	
Site	*Area (m^2)*	*Density (no./m^2)*	*Area (m^2)*	*Density (no./m^2)*	*Area (m^2)*	*Density (no./m^2)*
RF15	4188	0.078	11424	0.029	28429	0.011
RF26	2879	0.277	7908	0.102	19947	0.040
RF31	2729	0.209	6682	0.085	14482	0.039
RF37	2802	0.186	7230	0.072	16433	0.032

Note: Increases in area are not directly proportional to changes in track width, because overlapping sections of tracks are only counted once.

Figures

Fig. D1 **Total area swept during the collection of tissue samples amplifying at ≥ 4 loci pooled across all trips. Sections of track overlap were removed by combining track segments into complex polygons. Dashed lines indicate site boundaries.**

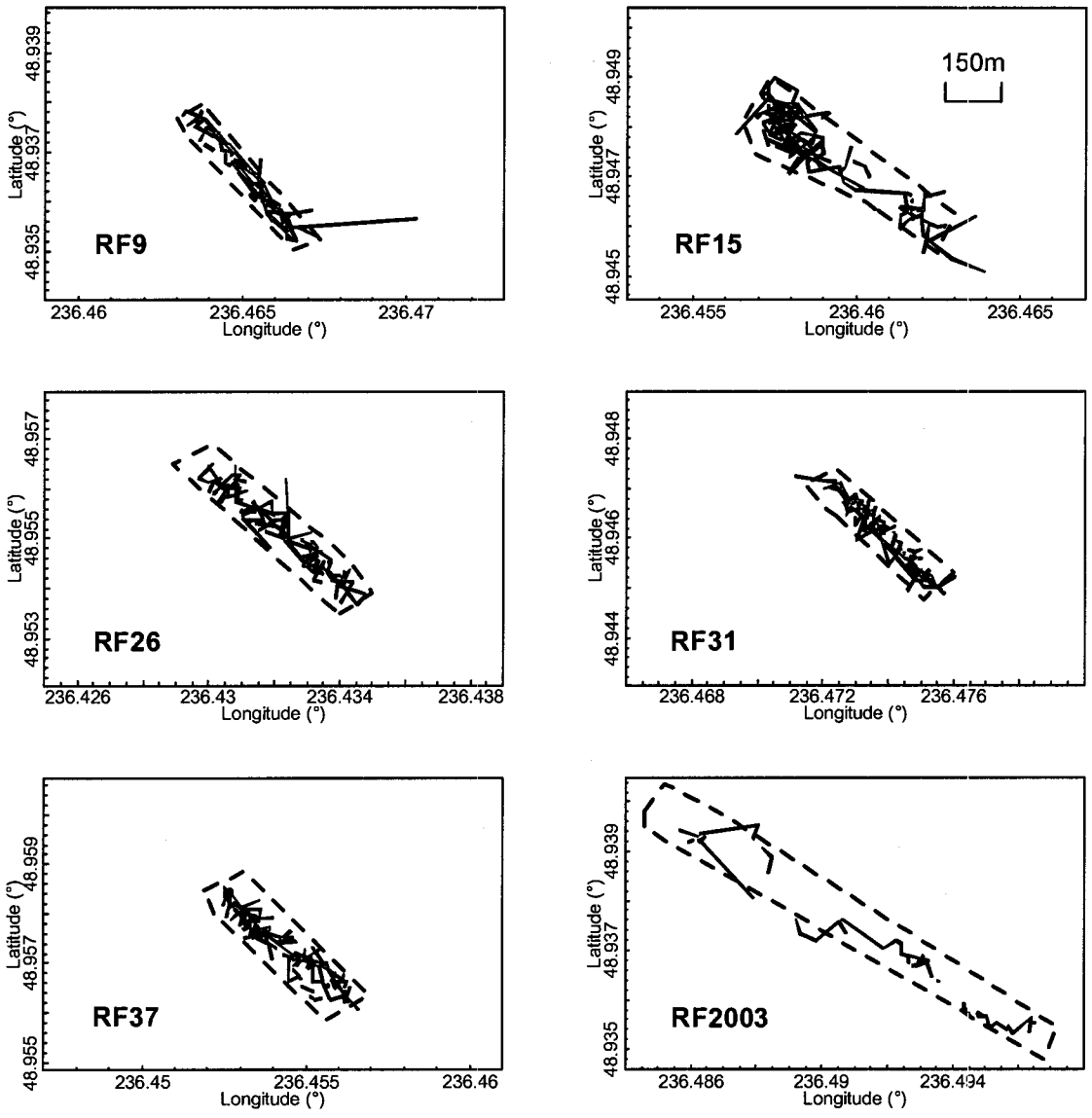


Fig. D2 Total area swept during the collection of all rockfish tissue samples pooled over all trips. Sections of overlap were removed by combining track segments into complex polygons. Dashed lines indicate site boundaries.

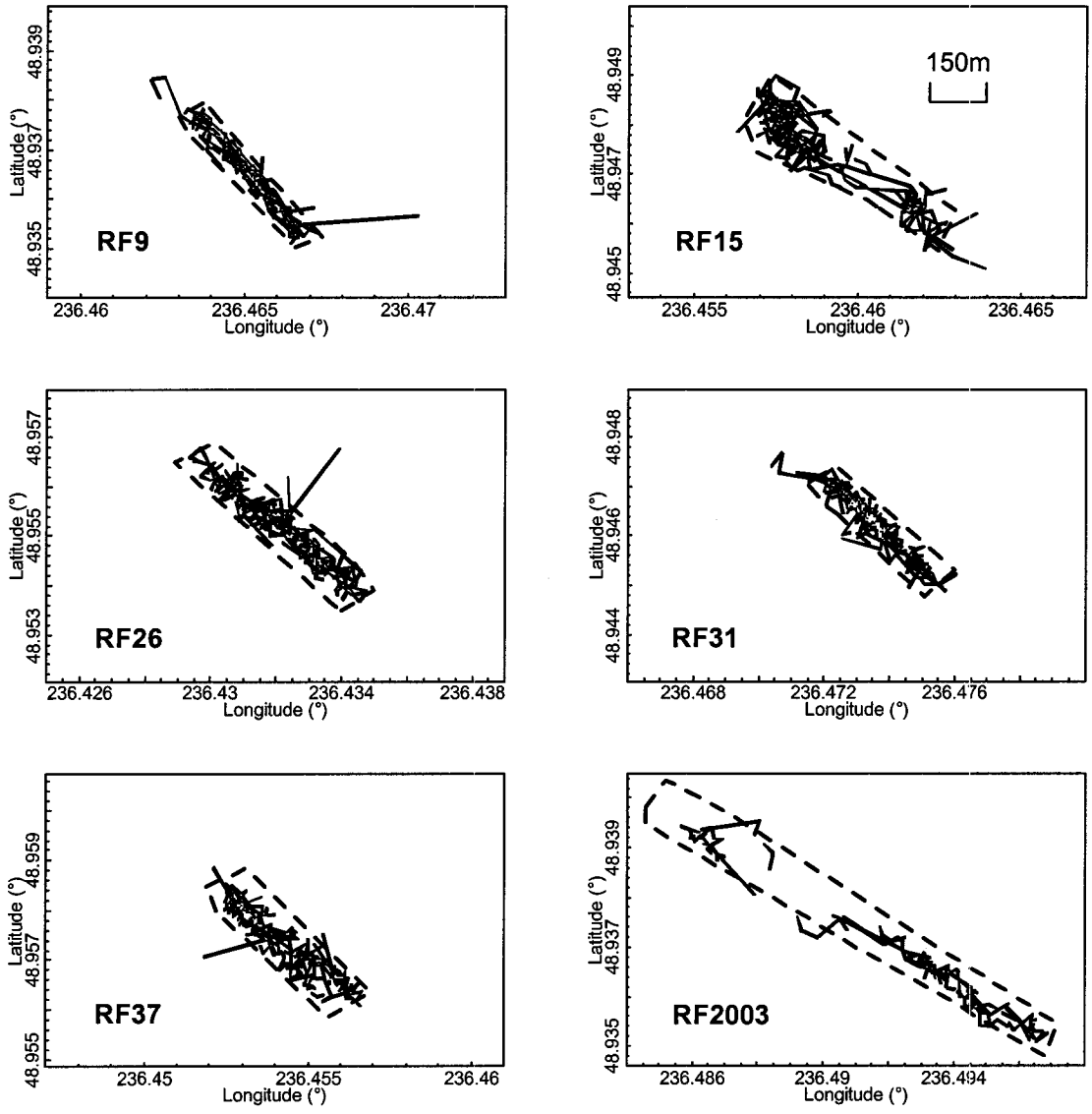
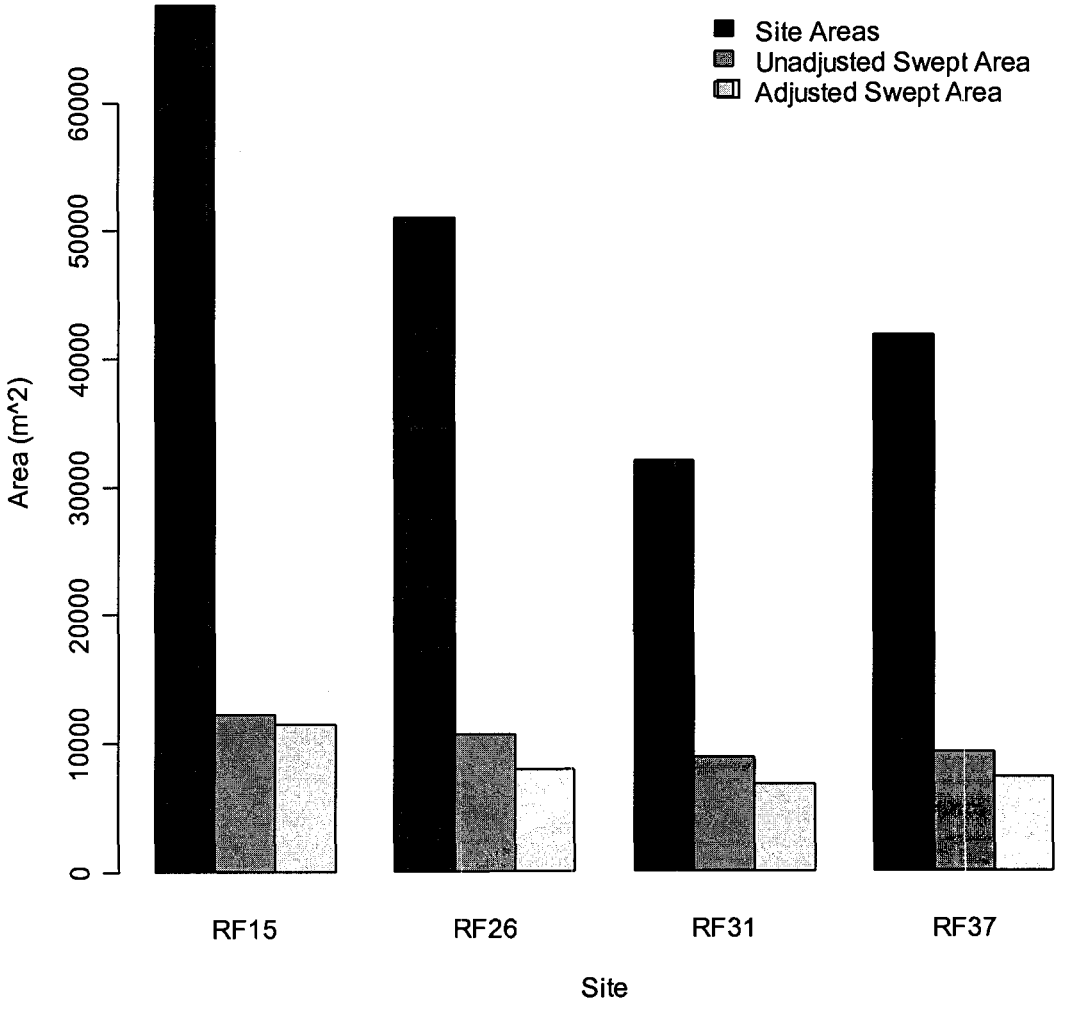


Fig. D3 **Comparison of full site areas, unadjusted swept areas (area = Σlength*width for *n* tracks in each site; i.e. overlapping sections not removed), and adjusted swept areas (complex polygons with overlapping sections removed).**



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