

Using genetic species identification and environmental data to distinguish historical catches of cryptic Blackspotted Rockfish (*Sebastes melanostictus*) and Rougheye Rockfish (*Sebastes aleutianus*) in British Columbia

by

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Approval

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Abstract

Visually classifying species is the most common method used in fisheries to estimate catch compositions for commercial and survey data, but catch records can be confounded when two or more morphologically similar species are classified as a single species (i.e., cryptic species)—as is the case for Blackspotted Rockfish and Rougheye Rockfish. To partition catches between the two species, I used genetic species identification data in regression models relating the proportion of Blackspotted Rockfish relative to the overall Rougheye/Blackspotted Rockfish catch to measures of set depth, location, and bottom ruggedness. The best model included a negative relationship with longitude and positive relationship with bottom ruggedness. I also used large-scale spatial predictors to estimate historical landings of each species, finding that the inclusion of trap longline commercial data after 2006 caused an increase in the relative proportion of Blackspotted Rockfish caught (out of the total Rougheye/Blackspotted Rockfish landings). Finally, I examined observer accuracy in distinguishing Blackspotted and Rougheye Rockfish and found that while 86% of fish were identified correctly, Blackspotted Rockfish were more likely to be misidentified, leading to a 55% overestimate of the actual Rougheye Rockfish catch and a 14% underestimate of the Blackspotted Rockfish catch. My results indicate that set-specific variables are most useful in estimating proportions of Blackspotted Rockfish and can be used to estimate how spatial shifts in fishing efforts will impact fishing mortality of Blackspotted Rockfish and Rougheye Rockfish.

Keywords: cryptic species; *Sebastes melanostictus*; *Sebastes aleutianus*; identification error

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1. Introduction

Fisheries scientists and managers often rely on data from observer programs to perform stock assessments, construct fishery management plans, develop bycatch reduction tactics, and identify the need for protective regulations for vulnerable species (Brooke 2012). However, misidentification of species by observers can reduce the quality of observer data (e.g., bycatch ratios), possibly lowering the likelihood of recognizing ecosystem consequences such as changes in species abundances due to fishing (Tillett et al. 2012). In particular, the presence of cryptic species (i.e., two or more species classified as a single species) has been shown to increase the probability of misidentification because the species cannot be visually distinguished via traditional morphological methods (Tillett et al. 2012). Cryptic species have been identified for many commercially targeted and bycatch species including crustaceans, gastropods, elasmobranchs, bony fishes, birds, and mammals (Quattro et al. 2006). In the absence of stock structure information, cryptic species are commonly managed as a species complex, which could be problematic when populations are disproportionately affected by changes in fishing effort (Berntson & Moran 2009, Rademeyer et al. 2008). Management challenges associated with cryptic are increasing as the number of identified cryptic species has increased dramatically over the past few decades.

Cryptic species are mainly identified via molecular genetic techniques (Bickford et al. 2007). Recent population genetic analyses have suggested cryptic speciation in many commercially important species, including 13 potential cryptic grey mullet species within the single *M. cephalus* (Acanthopterygii: *Mugilidae*) (Durand & Borsa 2015), Goldstripe Sardinella (*Sardinella gibbosa*) (Thomas et al. 2014), Baltic Sea Herring (*Clupea harengus*) (Corander et al. 2013), and Atlantic Cod (*Gadus morhua*) (Bradbury et al. 2014). Several rockfishes (*Sebastes* spp.) have been redescribed in light of molecular data indicating cryptic speciation in Vermilion Rockfish (*S. miniatus* and *S. crocotulus*) (Hyde et al. 2008), Rougheye Rockfish (*S. aleutianus* and *S. melanostictus*)

(Gharrett et al. 2005), Blue Rockfish (*S. mystinus* and *S. diaconus*) (Frable et al. 2015), and Dusky Rockfish (*S. ciliatus* and *S. variabilis*) (Orr & Blackburn 2004). Recent paradigm shifts in fisheries management, from managing solely for single-stock productivity to an ecosystem approach, have raised interest in conserving biodiversity (Stringer et al. 2009), but species composition of commercial catch must be accounted for to track changes in biodiversity.

While research into the genetic composition of fisheries grows, managing ocean fisheries via genetic species identification methods is limited by high cost and labour requirements. Ideally, cheaper alternatives can be developed to quantify fishing mortality for morphologically similar stocks that are commercially exploited. For fisheries such as Seabob Shrimp (*Xiphopenaeus kroyeri* and *X. riveti*), Goldstripe Sardinella, Cape Hake (*Merluccius capensis* and *M. paradoxus*), and Vermilion Rockfish, cryptic populations are spatially segregated and classified in reference to a spatial or depth boundary (Gusmão et al. 2006, Thomas et al. 2014, Rademeyer et al. 2008, Hyde et al. 2008). For cryptic species that exhibit overlapping distributions, differing habitat preferences may provide methods to distinguish species. Benoît & Rochet (2012) developed a harmonic regression model for southern Gulf of St. Lawrence flatfish (*Pleuronectidae*) and skate (*Rajidae*) species, predicting the relative species composition of fish assemblages based on habitat variables.

In this study, I investigated whether physical variables associated with the geographic locations of the catches could be used to predict the relative frequencies of cryptic Rougheye and Blackspotted Rockfish species in fishery-independent abundance surveys. First, genetics-based species identifications were used to estimate proportions of Blackspotted Rockfish (out of the total Rougheye and Blackspotted Rockfish catch per set) from research survey catches. Using linear regression, I explored the relationship of spatial and bathymetric variables to estimated proportions of Blackspotted Rockfish. I expected that spatial and depth variables affect catch proportions because prior studies from Gulf of Alaska (GOA) suggest compositions of Rougheye Rockfish and Blackspotted Rockfish differ throughout the Bering Sea/Aleutian Islands Area, and Blackspotted Rockfish tend to more commonly occur in deeper waters than do Rougheye (Hawkins et al. 2005, Gharrett et al. 2005, Orr & Hawkins 2008).

Ultimately, I aimed to estimate proportions of Blackspotted Rockfish in commercial catch data historically recorded simply as "Rougheye Rockfish", but the records were only available as spatially-aggregated catches. Therefore, I used a median polish nonparametric technique to estimate large-scale spatial trend for gridded data (Cressie 1993). I chose median polish because there was a clear spatial trend across the study area, but it was not clear that the trend would easily be modelled by some function of the row and column coordinates of the grid. I applied the final model to aggregated commercial catches of Rougheye and Blackspotted Rockfish from three B.C. fisheries and discuss potential applications for estimating species-specific fishing mortality for use in stock assessments.

1.1. The Rougheye/Blackspotted Rockfish Fishery

British Columbia commercial groundfish fisheries encounter Rougheye and Blackspotted Rockfish assemblages along the entire coast, most commonly between depths of 100 – 600 m. Based on catch records including discards from 2000 – 2009, groundfish trawl and hook-and-line fisheries catch an average of 57% and 23%, respectively, of the annual commercial Rougheye/Blackspotted catch (Fisheries and Oceans Canada 2012). The Pacific Halibut (*Hippoglossus stenolepis*) fishery and Sablefish (*Anoplopoma fimbria*) longline trap fishery catch an average of 16% and 4% of the annual commercial Rougheye/Blackspotted catch, while the combined catch from the North Pacific Spiny Dogfish (*Squalus suckleyi*) fishery and Lingcod (*Ophiodon elongatus*) fishery is less than 0.1% (Fisheries and Oceans Canada 2012). Rougheye and Blackspotted Rockfish harvest is managed jointly as a single aggregate stock using individual vessel quotas (IVQ's) and catch limits with an annual catches fluctuating around 1,000 tonnes.

Until 2005, Rougheye and Blackspotted Rockfish were considered one species, *Sebastes aleutianus*, with light- and dark-coloured morphs. Genetic research has since confirmed two distinct species with Blackspotted Rockfish being distinguished from Rougheye Rockfish by a combination of characteristics including a spotted body and spinous-dorsal fin, an overall dusky colour, a longer first and fourth dorsal-fin spine, and longer and more numerous gill rakers (Gharrett et al. 2005, Orr & Hawkins 2008).

Despite these distinguishing characteristics, DNA analysis is the only known method to distinguish the two species accurately (Fisheries and Oceans Canada 2012). Consequently, catch records include pooled contributions from both Blackspotted and Rougheye Rockfish and there is little information about the distribution, stock structure, life history characteristics (e.g. maximum age and growth rate), and historical landings for each species.

The lack of species-specific information represents a potential risk because of the possible loss of unrecognized biological diversity (Fisheries and Oceans Canada 2012), which in turn can reduce the ecosystem's adaptability to variable conditions and impede maintenance of ecosystem functions and services (Mangel et al. 1996). The temporal stability of ecosystem services provided by diverse communities is often referred to as the 'portfolio effect' similar to the effects of asset diversity on the stability of financial portfolios. For example, the portfolio effect substantially reduces interannual variability experienced by the Bristol Bay Sockeye Salmon (*Oncorhynchus nerka*) commercial fishery with an estimated tenfold increase in the frequency of fishery closures in absence of population and life history diversity (Schindler et al. 2010). The benefits from conserving biodiversity are widely accepted in fisheries management, but conserving biodiversity is difficult to implement in practice when distinguishing catches of morphologically similar species is too expensive or impractical to do consistently and reliably.

Rougheye and Blackspotted Rockfish are long-lived and low-fecundity species with an estimated generation time of approximately 50 years, making the species vulnerable to overfishing and population collapse (COSEWIC 2007, Shotwell et al. 2015). In 2007, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) designated Rougheye and Blackspotted Rockfish as species of special concern, indicating they may become threatened or endangered because of a combination of biological characteristics and identified threats (COSEWIC 2007). In 2012, Department of Fisheries and Oceans Canada (DFO) developed a management plan for Rougheye and Blackspotted Rockfish in response to COSEWIC's designation, but a formal stock assessment has not yet been conducted in Canada.

Although the Rougheye/Blackspotted species complex is not considered overfished in Alaska or the US West Coast (Hicks et al. 2013, Spencer & Rooper 2014),

contributions of Alaskan and US West Coast stocks of Rougheye and Blackspotted Rockfish to the BC stock are poorly understood. Many rockfish (*Sebastes* spp.) species are sedentary as adults, but widespread dispersal is possible through pelagic larvae (Berntson et al. 2007). Northeast Pacific Rockfishes consist of a diverse group of over 70 species and while some species show widespread gene flow, others exhibit fine-scale population structure (Berntson et al. 2007). Thus, we cannot assume the stock structure and dynamics of Rougheye and Blackspotted Rockfish are the same as other rockfish species, or to what degree US stocks contribute to the BC stock.

The unknown status of Rougheye and Blackspotted Rockfish, combined estimated declines in abundance of many other rockfish species including bocaccio (*S. paucispinis*), Pacific ocean perch (*S. alutus*), canary rockfish (*S. pinniger*), cowcod (*S. levis*), widow rockfish (*S. entomelas*), yelloweye rockfish (*S. ruberrimus*), and darkblotched rockfish (*S. crameri*), has motivated a genetic sampling program for Rougheye and Blackspotted Rockfish caught on government-sponsored fishery independent research surveys. The distribution and stock structure information gleaned from species-level classification is intended to improve future assessments by accounting for potential differences in exploitation and inform potential avoidance efforts by commercial fleets.

2. Methods

I assembled candidate models using depth, survey set location, and bottom ruggedness as set-specific predictors. I used AIC_c to compare model fit, ranked models using AIC weights, and examined top ranked models for the presence of uninformative variables. I also used a weighted median polish algorithm to assess the effect of large-scale spatial variables on the proportion of Blackspotted Rockfish. I compared the two methods, using R^2 and root mean squared error as measures of fit, to examine if large-scale spatial trend accounted for most of the variation in the proportion of Blackspotted Rockfish, or if set-specific variables could provide better insight into the distribution of Blackspotted and Rougheye Rockfish. I used the median polish algorithm to predict relative compositions of Blackspotted and Rougheye Rockfish in historical commercial catch records and included a brief summary of observer misclassification rates of Blackspotted and Rougheye Rockfish.

2.1. Data

Rougheye and Blackspotted Rockfish tissue samples were collected during DFO groundfish indexing surveys including longline trap, bottom trawl, and longline hook from 2010 to 2012 inclusive. Either a fin clip or piece of brain no larger than 5mm by 5mm was taken from 3,304 fish over 321 survey sets and stored in individual vials of alcohol. Two diagnostic procedures were used to distinguish Rougheye and Blackspotted Rockfish: microsatellite analysis at the *Sma6* microsatellite alleles as described by Gharrett et al. (2005) and single-nucleotide polymorphism (SNP) analysis at the *Cfo* I SNP as described by Garvin et al. (2011). Species-specific microsatellite markers (*Sma6*) and SNPs were concordant in 2153 Blackspotted Rockfish and 676 Rougheye Rockfish, while evidence of hybridization was found for 220 fish. The analyses failed for 255 samples. Sample set locations, sample sizes, and proportions of Blackspotted Rockfish are summarized in Figure 1 (all maps were generated using the PBSmapping

package (Schnute et al. 2015) in R version 3.1.2 (R Core Team 2013)). Hybrids were excluded from the analysis, but catches of first-generation (F1) and second-generation (F2) hybrids are illustrated in Figure 2.

Although exact spatial coordinates of the survey sets were available for the survey data used to predict the response, spatial coordinates of commercial set locations cannot be released because of data confidentiality restrictions. Rather, the commercial data set locations are described by a 0.5 degrees longitude by 0.5 degrees latitude grid cell (Figure 3). Grid cells that did not contain catches from at least three vessels were excluded from the dataset. The catches include bottom trawl, longline hook, and longline trap commercial fisheries from 1996 to 2015.

2.2. Point-based analysis

I estimated the effects of point variables on the proportion of Blackspotted Rockfish using logistic regression as implemented by the function `<glmer()>` in the `lme4` package (Bates et al. 2015) in R version 3.1.2 (R Core Team 2013). The estimated proportion of Blackspotted Rockfish per set was calculated as:

$$Y = \frac{b}{T} \tag{1}$$

where b indicates the total number of Blackspotted Rockfish caught in a set and T indicates the total number of Rougheye Rockfish and Blackspotted Rockfish caught in a set. The model assumes that Y is a random variable with mean value and variance of the form:

$$E(Y) = \mu , \quad \text{Var}(Y) = \frac{\mu(1-\mu)}{T} \tag{2}$$

μ in Eq. (2) represents a proportion about which the response value, Y , is distributed. The form of the variance, $\text{Var}(Y)$, recognizes that the amount of variation will be small if μ is near 0 or 1, and larger if it is closer to 0.5. A logit transformation of Y serves as the response variable since Y is bounded between 0 and 1, and assumes that the predictors act linearly such that

$$\log\left(\frac{\mu}{1-\mu}\right) = \eta = \beta_0 + \beta_1 x_1 + \dots + \beta_p x_p \quad (3)$$

where η is the unbounded response variable and x_1, \dots, x_p are predictor variables defined below.

2.2.1. Location predictors

I calculated the approximate set location (latitudinal and longitudinal coordinates) as the midpoint between the start and end coordinates of longline trap, bottom trawl, and longline hook sets (S_{lat} and S_{lon}) (Table 1). I also created a new spatial variable describing the distance of a set from the northwest corner of the study area (S_{dist}) to account for the diagonal orientation of the samples along the coast. This allowed me to account for latitudinal and longitudinal effects simultaneously without including an interaction term and over-parameterizing the model. I also used the Pacific Marine Fisheries Commission (PMFC) management areas (S_{area}) (Figure 4) to estimate large-scale spatial trend. Candidate models were fit using one spatial variable to avoid redundant information.

2.2.2. Other predictors

Mean set depth (D) was found by averaging the observed depth at one-minute recording intervals between anchors. I calculated bottom ruggedness, or heterogeneity, using the terrain ruggedness index (TRI). Using an elevation raster layer, TRI measures the sum change in elevation between a grid cell and its eight neighbour grid cells (Riley et al. 1999). I rasterized topography data (obtained from <http://topex.ucsd.edu/cgi->

bin/get_data.cgi) using a resolution of 0.029° longitude by 0.024° latitude and estimated TRI values for each set with the function `<terrain()>` from the raster package (Hijmans 2015) in R version 3.1.2 (R Core Team 2013).

2.2.3. Model fitting

I used a generalized linear mixed-effects modelling (GLMM) approach to estimate the effects of location, depth, and TRI on the proportion of Blackspotted Rockfish in a survey set. GLMMs combine the properties of generalized linear models (GLMs), which can handle binomial responses using a logit link function, and linear mixed-effects models, which incorporate random effects to account for between-subject variation in the value of the dependent variable. Initial analyses of the data using GLMs revealed residual variation among sets that was about 4 times larger than expected under the binomial distribution. Adding a normally distributed random intercept term to the model for each binomial count can account for such overdispersion (Warton & Hui 2011), so I considered set ID a random effect in all models. The final model for the linear predictor was specified as:

$$\eta_i = \beta_0 + D_i\beta_1 + TRI_i\beta_2 + S_{n,i}\beta_3 + \kappa_i \tag{4}$$

where $\kappa_i \sim N(0, \sigma_i^2)$, independently, and represents the random effect for set. $S_{n,i}$ describes the spatial variable used, where n is latitude, longitude, area, or distance.

Latitudinal and longitudinal predictors were highly correlated (-0.92) and to avoid collinearity, I did not include both latitude and longitude in any of the candidate models. Candidate models were fit by maximum likelihood using the Laplace Approximation. To alleviate convergence problems, all predictor variables were centered and scaled. Candidate model selection was performed using second-order Akaike information criterion (AIC_c) rather than Akaike information criterion (AIC) because AIC_c increases the relative penalty for model complexity with small datasets and is recommended when the sample size divided by the number of model parameters is less than 40 (Burnham & Anderson 2002). Wald Chi-square tests were used to compute p-values for the

significance of fixed effects in the top-ranked candidate models to detect uninformative variables.

2.3. Grid-based analysis

To match the spatial scale of the aggregated commercial catch data, I overlaid a grid with a resolution of 0.5° longitude by 0.5° latitude over survey catch locations to derive large-scale spatial predictors. Catches of Blackspotted and Rougheye Rockfish were aggregated within each grid cell, resulting in a two-way table of 14 row factors and 19 column factors (Figure 3). Large-scale spatial trend was modelled by fitting additive row and column effects:

$$Y_{ij} = \alpha + r_i + c_j + \epsilon_{ij} \tag{5}$$

where Y_{ij} is the *log* transformed proportion of Blackspotted Rockfish for the i^{th} row and j^{th} column of the grid, α is the overall mean, r_i is the i^{th} row effect ($i=1, \dots, 14$), c_j is the j^{th} column effect ($j=1, \dots, 19$), and ϵ_{ij} represents random error. I used a smoothing algorithm known as median polish to obtain an additive decomposition like Eqn. 5, where row medians and column medians are removed from the response variables repeatedly until the row and column medians approach zero. The algorithm is used as an exploratory data analysis technique used to quantify large-scale spatial trend and is useful over other decomposition procedures that utilize means, like ANOVA, for its robustness to outliers (Cressie 1993). The medians were weighted by the square root of total Blackspotted and Rougheye Rockfish caught in a cell because the aggregated catches differed greatly between cells; some cells only contained one rockfish, while others had over 300. The square root transformation allowed grid cells with larger samples to have a greater influence on the calculated row and column medians rather than all cells having equal weight.

2.4. Observer misclassification rates

Observer misidentification rates have been estimated at 34% for adult Blackspotted Rockfish and 9% for adult Rougheye Rockfish (Garvin et al. 2011). The dataset for my research included observer classifications of Blackspotted and Rougheye Rockfish for 273 trawl and longline trap sets, so I provided a summary of observer misclassification rates in British Columbia to assess whether future classification of Rougheye and Blackspotted Rockfish could rely solely on visual classification. I also graphically assessed possible correlations of misclassification rates to sex, fork length, weight, and maturity stage of the fish.

3. Results

3.1. Point-based analysis

Longitude and management area were the strongest predictors of proportions of Blackspotted Rockfish, while the non-spatial variables—ruggedness and depth—were the weakest predictors (Table 2). The best-fit candidate model included ruggedness, longitude, and depth, although dropping depth from the model did not cause a large decrease in explained variation. Proportions of Blackspotted Rockfish appeared strongly correlated to longitude (chi-square value of 79, p -value < 0.0001) and terrain ruggedness (chi-square value of 29, p -value < 0.0001), but not set depth (chi-square value of 2, p -value = 0.112). Using longitude and ruggedness as predictors, the $R^2 = 0.88$ and RMSE = 0.12 (Figure 5). The model coefficients suggest that the predicted ratio of Blackspotted Rockfish to Rougheye Rockfish in a set decreases by 83% for a one degree increase in longitude, holding ruggedness constant. For a one-unit increase in ruggedness, the predicted ratio of Blackspotted Rockfish to Rougheye Rockfish in a set increases by 183%, holding longitude constant (Table 3). The 95% confidence interval for longitude on the odds ratio scale is $\exp(-2.26) = 0.10$ to $\exp(-1.35) = 0.26$, which excludes 1 (the null value), providing further evidence of a longitudinal effect on the proportion of Blackspotted Rockfish. Similarly, the 95% confidence interval on the odds ratio scale for ruggedness excludes the null value, $\exp(0.30) = 1.35$ to $\exp(1.08) = 2.94$, providing evidence that ruggedness is also a significant predictor of the proportion of Blackspotted Rockfish.

3.2. Grid-based analysis

Taking only latitude and longitude effects into account, the weighted median polish method explained 49.5% of the variation in the proportion of Blackspotted

Rockfish, with the root mean squared error equal to 0.24 (Figure 6). This approach did not fit the data as well as the point-based approach, but still performed well given that the sample size was reduced from 321 to 47 when the data was aggregated.

The estimates from Eqn. 5 were used to predict proportions of Blackspotted and Rougheye Rockfish in historical catch records. However, one area in the commercial data extended beyond survey area and the predicted response for this area had to be extrapolated from the median polish estimates. The cell, $s = (x_{20}, y_2)$, was east of the study area, but within latitudinal bounds. I therefore defined the estimates of this cell as

$$\bar{\mu}(s) = \bar{a} + \bar{r}_2 + \bar{c}_{19} + \left(\frac{x - x_{19}}{x_{18} - x_{19}} \right) (\bar{c}_{18} - \bar{c}_{19})$$

(6)

3.3. Partitioning catch weight by species

I used the median polish algorithm to generate a surface for partitioning commercial catch weight by species (Figure 7). The estimated catch weights from 1996 to 2015 show that catches of Blackspotted Rockfish were on average 70% of the total Blackspotted/Rougheye Rockfish catch before 2005 and 78% after 2005 (Figure 8). In 2006, commercial groundfish fisheries implemented 100% dockside and at-sea monitoring, and individual vessel accountability for all catch. Thus, the majority of recorded commercial catch data for the Rougheye/Blackspotted Rockfish complex prior to 2006 is from the groundfish trawl fleets. Large contributions to the overall Rougheye/Blackspotted Rockfish complex catch from longline hook fleets are not shown until after the catch accounting reforms were implemented (Figure 9). The inclusion of the longline hook data after 2005 and subsequent jump in estimated proportion of Blackspotted Rockfish caught suggests that changes in effort and monitoring standards of different fisheries can alter the species composition of the annual catch.

3.4. Visual Classification Performance

Based on observer classifications, the total number of Rougheye Rockfish caught was overestimated by 55% compared to the true catch, whereas Blackspotted Rockfish was underestimated by 14%. Out of 2649 fish used to assess observer misidentification rates, observers correctly classified 86% of fish (Table 4). Of the total number of Blackspotted Rockfish caught, 16% were misidentified, whereas 5% of the total Rougheye Rockfish catch was misidentified. I did not find evidence that fork length, weight, or sex of a fish had an effect on observer accuracy of Blackspotted or Rougheye Rockfish.

4. Discussion

The presence of cryptic species can hinder accurate species identification, which presents problems for catch accounting in fisheries. My results show that although Rougheye and Blackspotted Rockfish are both found throughout the Pacific Northwest, Aleutian Islands, and Bering Sea, the species exhibit local differences in their distributions that can be described by ruggedness, latitude, and longitude. While spatial and bathymetric variables may only serve as proxies for environmental gradients or processes that directly influence distributions of Rougheye and Blackspotted Rockfish, they are easily observable predictors, available for most historical data, and account for spatial changes in effort rather than assuming a constant selectivity pattern over time.

Depth was not a significant factor in predicting proportions of Blackspotted Rockfish, contrary to previous publications by Gharrett et al. (2007) and Orr & Hawkins (2008), and may be an artefact of the sampling design. For the longline trap and trawl surveys, which recorded the beginning and end depths of the sets, the average difference in depth at the start and end of the set was 7 m. However, some sets differed in start and end depth by as much as 276 m, particularly the trap survey sets (Figure 10). The wide depth coverage by the sets may hinder our ability to detect an effect on the proportion of Blackspotted Rockfish caught. I also looked at only trawl sets for a possible depth effect because the start and end depths did not differ as much on average as in the trap sets, but did not detect a depth effect. Given that the sampling design was similar to those used in previous studies, there may not be a depth preference between Blackspotted and Rougheye Rockfish in B.C.

Other studies of rockfish distribution in B.C. suggest that ocean currents may play an important role in larval dispersion and retention. Distinct genetic populations of Pacific ocean perch (*Sebastes alutus*) along the west coast of Vancouver Island and throughout Queen Charlotte Sound and Dixon's Entrance could not be described by

isolation by distance, suggesting that other variables probably contribute to genetic isolation (Withler 2001). For example, the direction and magnitude of surface currents may be useful in understanding the distribution of Blackspotted and Rougheye Rockfish, but are, unfortunately, not available for partitioning historical landings between the two species. Additionally, oceanographic variables will change throughout the year (e.g. surface water flows in the opposite direction through Hecate Strait in the winter versus the summer) and we do not know enough about the life history of Rougheye and Blackspotted Rockfish to infer their response to seasonal changes. Surveys that intercept Blackspotted and Rougheye Rockfish occur in the same months every year, thus it is difficult to distinguish effects of time, gear, or other variables that change seasonally without having seasonal contrast in the surveys from year-to-year.

The spatial resolution of estimating catches of Rougheye and Blackspotted Rockfish is an important consideration for future management. My comparison of point-based versus grid-based approaches to modelling the spatial data showed that management areas and large-scale grid-based approach did not predict proportions of Blackspotted Rockfish as well as the point-based approach using ruggedness and longitude. Although I used a weighting scheme to compensate for the differing sample sizes of grid cells when aggregating catches, information on the variability among sets was lost by pooling the samples. Hurlbert (1984) identifies at least three problems with this approach: (1) the pooled fish are not independent because they are part of correlated observations within sets, (2) there is no proper way to assess the significance of environmental variables without accounting for the variability among sets, and (3) the pooled sets are not given equal weight because sets that catch many fish will dominate the results. Thus, spatially aggregating the sets is not statistically sound approach and should not be used in future efforts to partition commercial catch weights of Blackspotted and Rougheye Rockfish, as point-based methods avoid these issues and provide more accurate estimates.

Limitations of the point-based analysis may be more apparent as management decisions are implemented for the Rougheye/Blackspotted Rockfish complex. Currently, a single coastwide quota is issued for the complex (Fisheries and Oceans Canada 2015). Without distinguishing quotas by area or species, we may ultimately be more

interested in finding areas where Rougheye and Blackspotted Rockfish do not occur, rather than the relative proportions of each species. However, my work has shown that survey catches of Rougheye Rockfish were much less common than Blackspotted Rockfish and that the species exhibit different local distributions. Catches of Rougheye Rockfish are concentrated off the west coast of Vancouver Island and within Goose Island Gully and Mitchell's Gully. These may be areas of concern in terms of fishing effort if Rougheye Rockfish require additional conservation. Another limitation of this study is that the response variable, proportion of Blackspotted Rockfish, did not account for the presence of hybrids. Given the low-level of hybridization found (6% of all samples), I did not find it necessary to include hybrids in the analysis. However, the presence of hybrids in B.C. is six times greater than those found in the US West Coast and Alaska from analyses with allozyme data (Hawkins et al. 2005) and DNA markers (Gharrett et al. 2005). This could indicate that hybridization of the species is more common in B.C. or it could result from the improvement in our ability to detect hybrids. Kochzius (2009) notes that allozyme analyses have a lower resolution than direct assessment of DNA and that mitochondrial DNA (mtDNA) cannot distinguish species that hybridize if hybrids backcross and the maternally inherited mitochondrial genome passes from one species to another. The samples in this study were classified using both mtDNA and SNP analyses, which may account for the increase in detected hybrids.

The point-based model I developed, and the proportions of Rougheye and Blackspotted Rockfish predicted from it, provide information to support future stock assessments of the species. Rougheye and Blackspotted Rockfish are currently managed together as a species complex along the U.S. West Coast, B.C., and GOA because there is no method to distinguish the species in catches. The most recent GOA assessment stated that we must continue to manage Rougheye and Blackspotted Rockfish as a complex until observers and survey biologists can reliably identify both species (Shotwell et al. 2011). I showed that identification of Blackspotted and Rougheye Rockfish should not rely on visual observer classification because observers are more likely to misclassify Blackspotted Rockfish and largely overestimate the number of Rougheye Rockfish caught. However, there are other inexpensive tools to help distinguish species that can improve our understanding of their distributions, stock structure, and historical population trends. My research has taken the first steps in

implementing these tools for Rougheye and Blackspotted Rockfish populations in B.C. and can serve as a foundation for other regions to build on as genetic databases grow.

5. Tables

Table 1. Parameters used to fit the candidate models.

Variable	Description
latitude (S_{lat})	Degrees latitude ranging from 48.3 to 54.7
longitude (S_{lon})	Degrees longitude ranging from -134.1 to -125.9
distance (S_{dist})	Distance (m) of a set from the northwest corner of the study area
management area (S_{area})	Factor levels describing PMFC management areas: 3C, 3D, 5A, 5B, 5C, 5D, 5E
depth (D)	Continuous values describing the mean depth (m) at one-minute recording intervals between anchors for a single set
ruggedness (TRI)	Continuous values quantifying bottom heterogeneity based on the terrain ruggedness index

Table 2. Second-order AIC (AIC_c) comparison of candidate models for point analysis.

Model	K	AIC _c	Delta AIC _c	AIC _c weight	log-Likelihood
1 TRI_lon_depth	5	923.17	0.00	0.54	-456.49
2 TRI_lon	4	923.62	0.45	0.43	-457.75
3 TRI_dist	4	930.67	7.50	0.01	-461.27
4 TRI_dist_depth	5	931.99	8.82	0.01	-460.90
5 TRI_area	9	934.93	11.76	0.00	-458.18
6 TRI_area_depth	10	936.40	13.23	0.00	-457.84
7 TRI_lat	4	939.97	16.80	0.00	-465.92
8 TRI_lat_depth	5	941.99	18.82	0.00	-465.90
9 lon	3	952.49	29.32	0.00	-473.21
10 area	8	957.27	34.10	0.00	-470.41
11 dist	3	969.97	46.80	0.00	-481.95
12 lat	3	983.91	60.74	0.00	-488.92
13 TRI	3	1013.15	89.98	0.00	-503.54
14 depth	3	1018.39	95.22	0.00	-506.16

Variables are: TRI= Terrain Ruggedness Index, lon= longitude, depth= mean set depth, dist= distance from the northwest corner of study area, area= major management

area, lat= latitude. K describes the number of parameters in the model. Models are ranked using AIC_c and two measures are used to compare models relative to the highest ranked model: ΔAIC_c and AIC_c weight. The log-likelihood reflects the overall fit of the model, with smaller values indicating a worse fit.

Table 3. GLMM coefficients, standard error estimates, and 95% confidence intervals. All values are on the log-odds scale.

Parameter	Estimate	SE	Lower CL	Upper CL	P-value
intercept	1.54	0.19	1.18	1.94	6.83E-16
longitude	-1.78	0.20	-2.21	-1.45	<2E-16
ruggedness	1.04	0.20	0.67	1.45	1.39E-7

Table 4. Observer misidentification contingency table comparing the number of Rougheye and Blackspotted Rockfish according to visual classification (i.e. guessed) versus genetically confirmed identifications of Rougheye and Blackspotted Rockfish.

	confirmed BS	confirmed RE	total
guessed RE	303	520	823
guessed BS	1744	29	1773
total	2047	549	2596

6. Figures

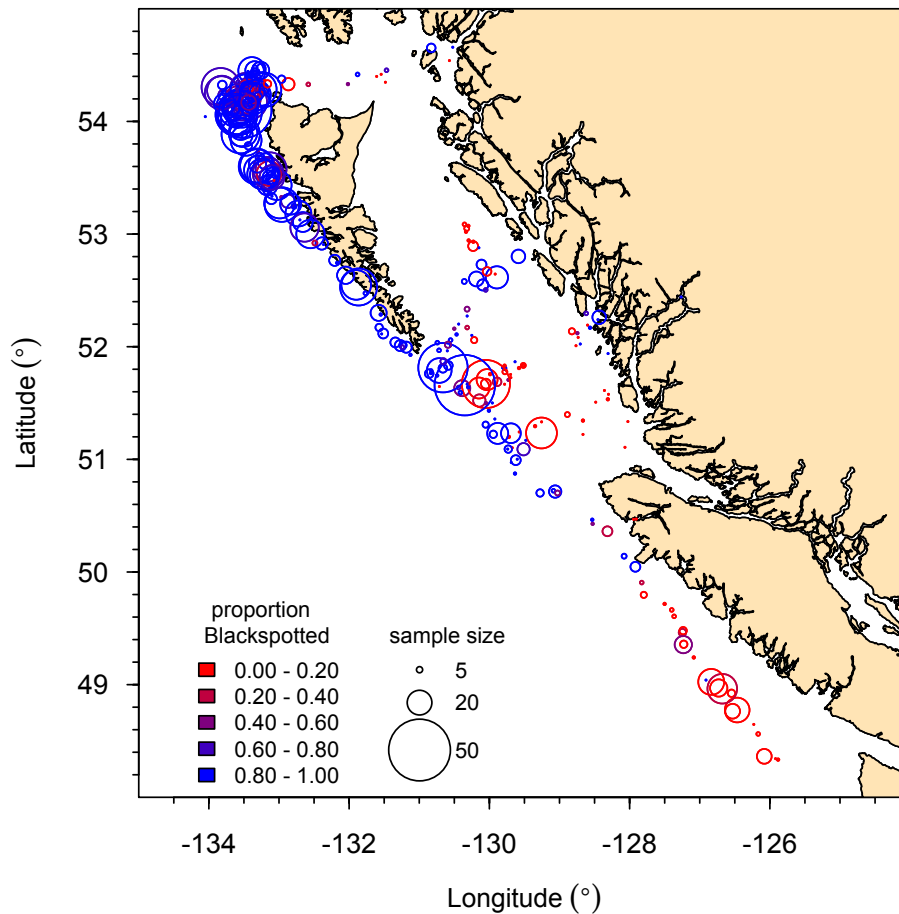


Figure 1 Combined fishery independent survey data from hook longline (IPHC and PHMA), trap longline, and trawl aggregated over 2010-2012. The point size indicates the total number of Blackspotted Rockfish and Rougheye Rockfish caught in a set, while color indicates the proportion of Blackspotted Rockfish out of the total Blackspotted/Rougheye Rockfish catch.

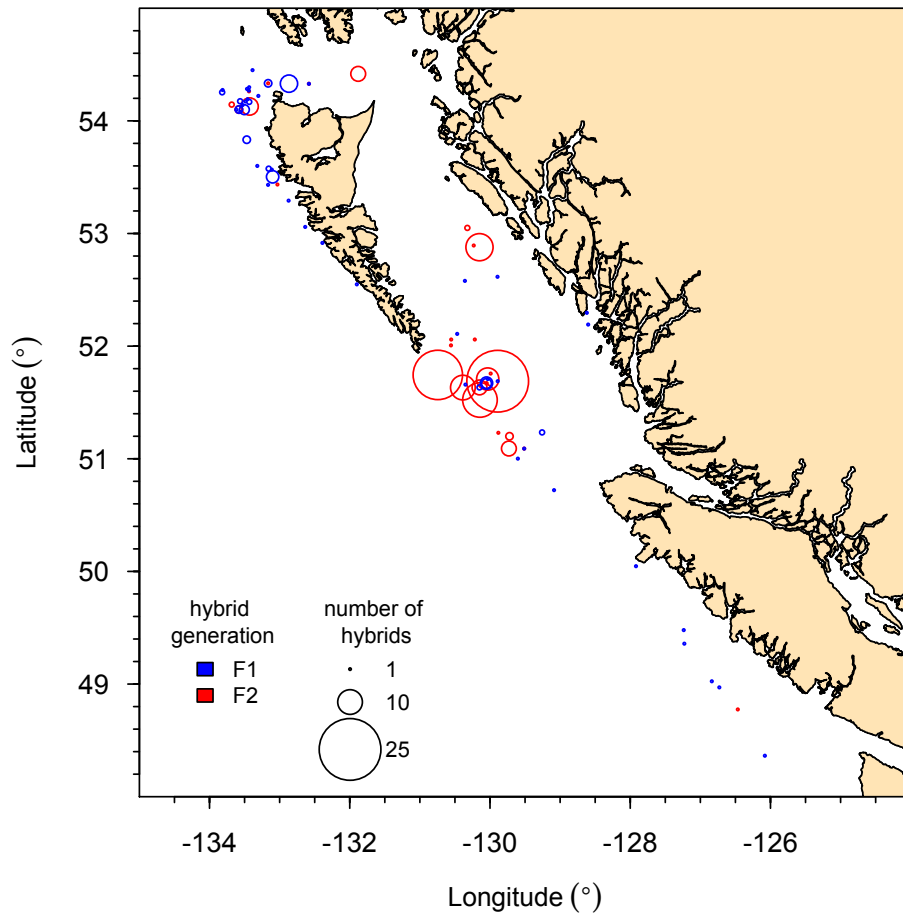


Figure 2 Combined fishery independent survey data from hook longline (IPHC and PHMA), trap longline, and trawl aggregated over 2010-2012. The point size indicates the total number of hybrids caught in a set, while color indicates the hybrid generation.

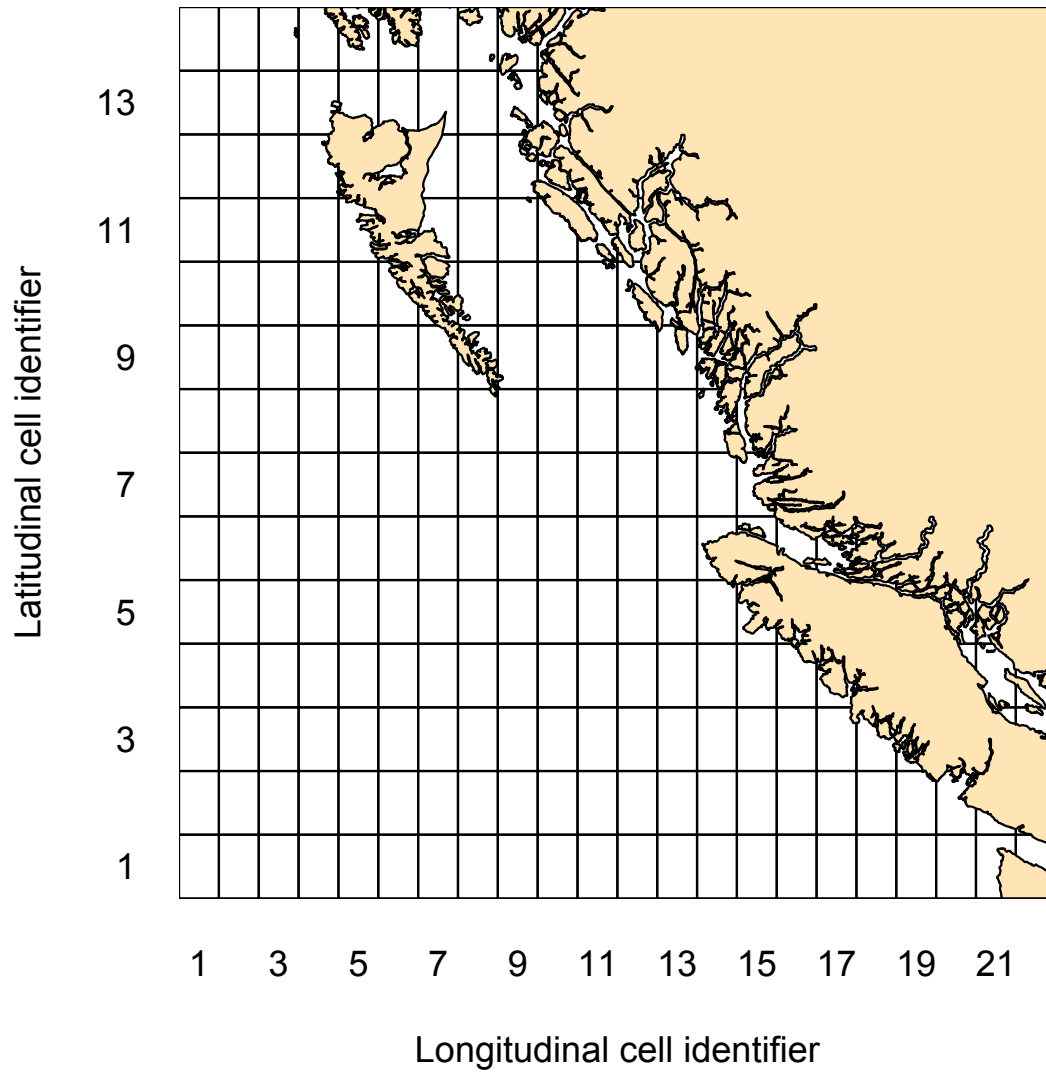


Figure 3 Spatial grid used to define latitudinal and longitudinal coordinates of sets. Intervals are spaced at 0.5 degrees latitudinally and longitudinally.

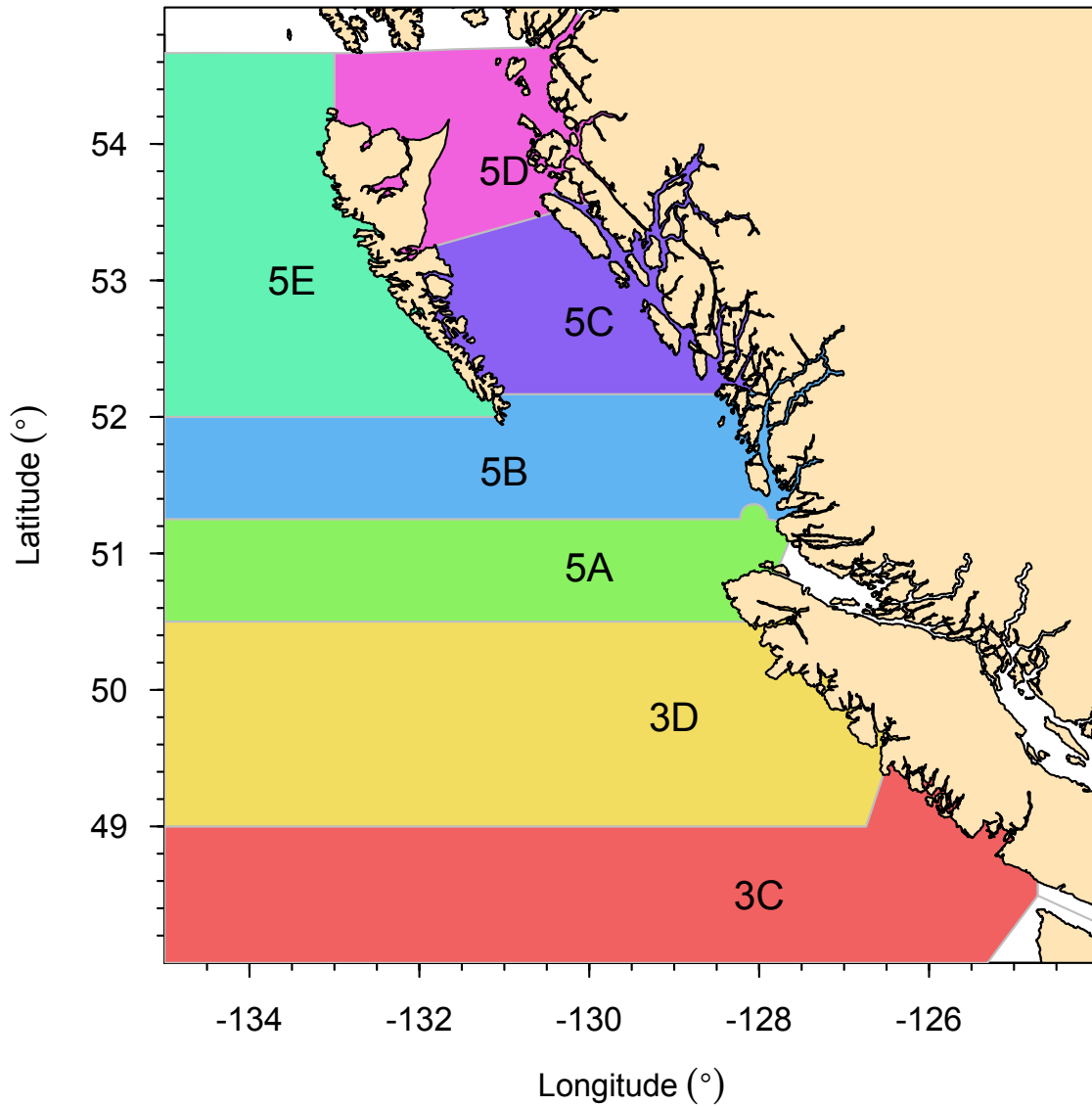


Figure 4 Pacific Marine Fisheries Commission (PMFC) major management areas. Area 4B (Strait of Georgia) was not included in this study, as no Rougheye or Blackspotted Rockfish were encountered there.

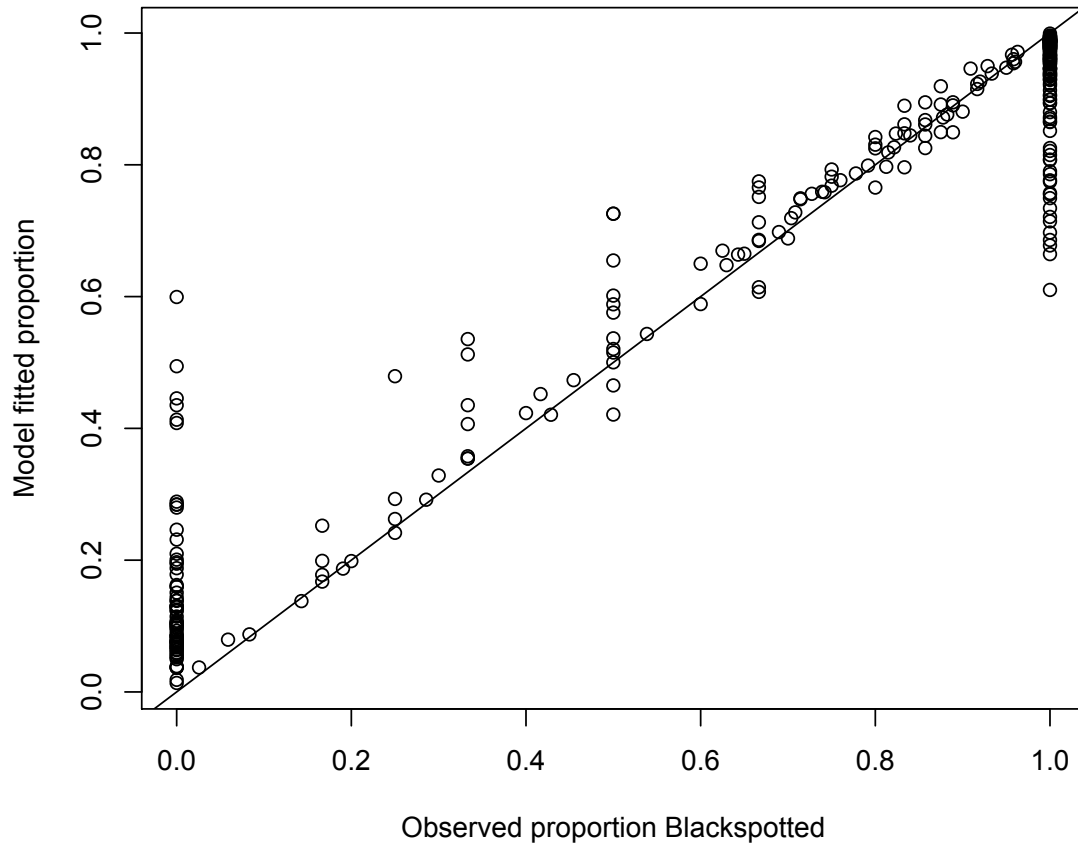


Figure 5 Observed proportions of Blackspotted Rockfish versus the GLMM fitted proportions. The solid diagonal line indicates where the points would fall if the model perfectly fitted the observed data

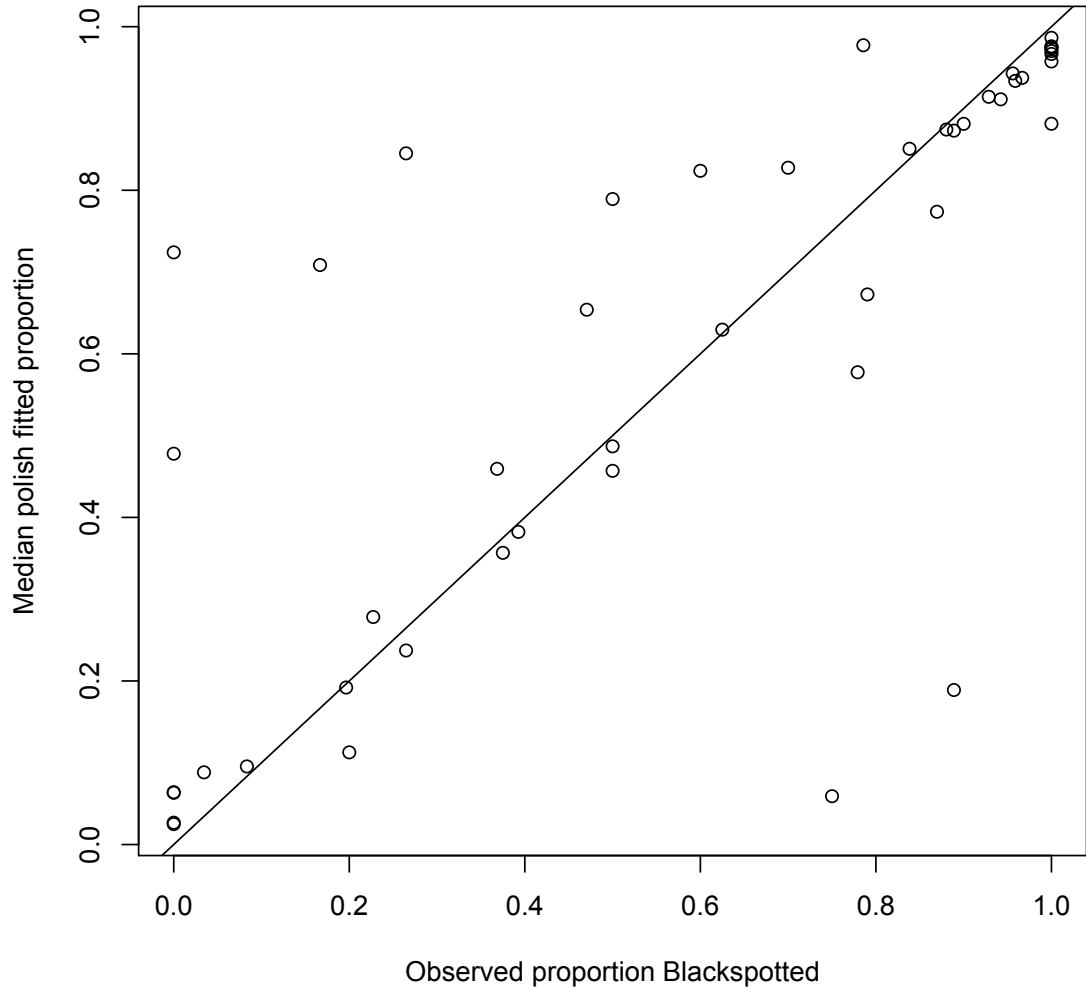


Figure 6 Observed proportion of Blackspotted Rockfish versus weighted median polish fitted proportions. The solid diagonal line indicated where the points would fall if the median polish perfectly fitted the observed data.

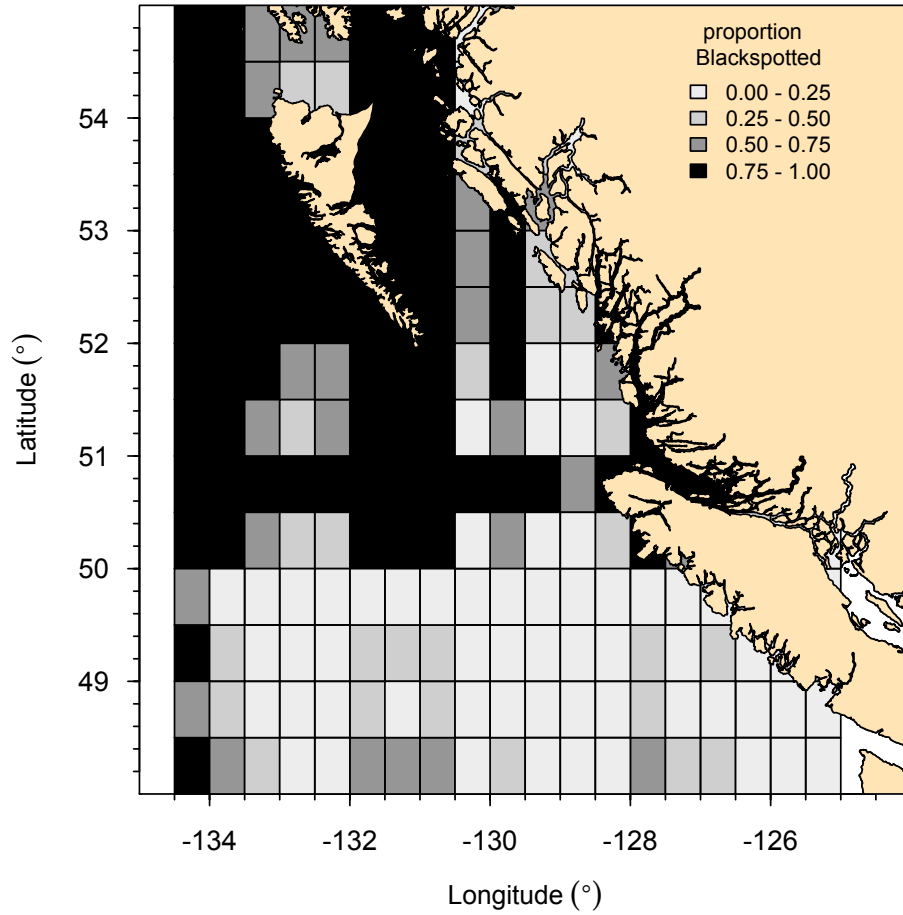


Figure 7 Median polish fitted surface used to estimate catch weights of Blackspotted Rockfish in spatially aggregated commercial catch records of Blackspotted and Roughey Rockfish.

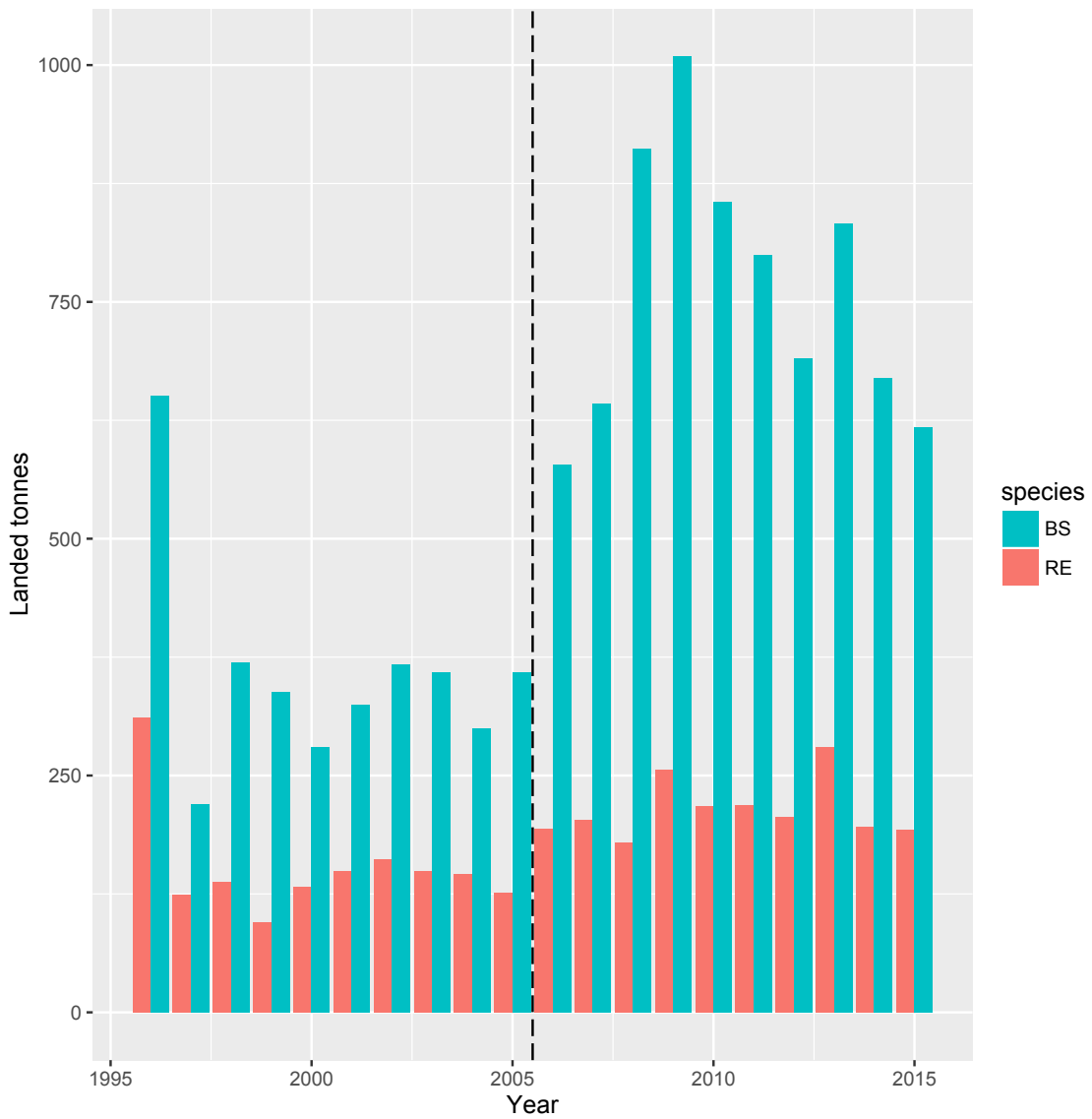


Figure 8 Estimated landed tonnes of Blackspotted Rockfish and Rougheye Rockfish based on median polish effects. The dashed vertical line indicates when full catch accounting was implemented.

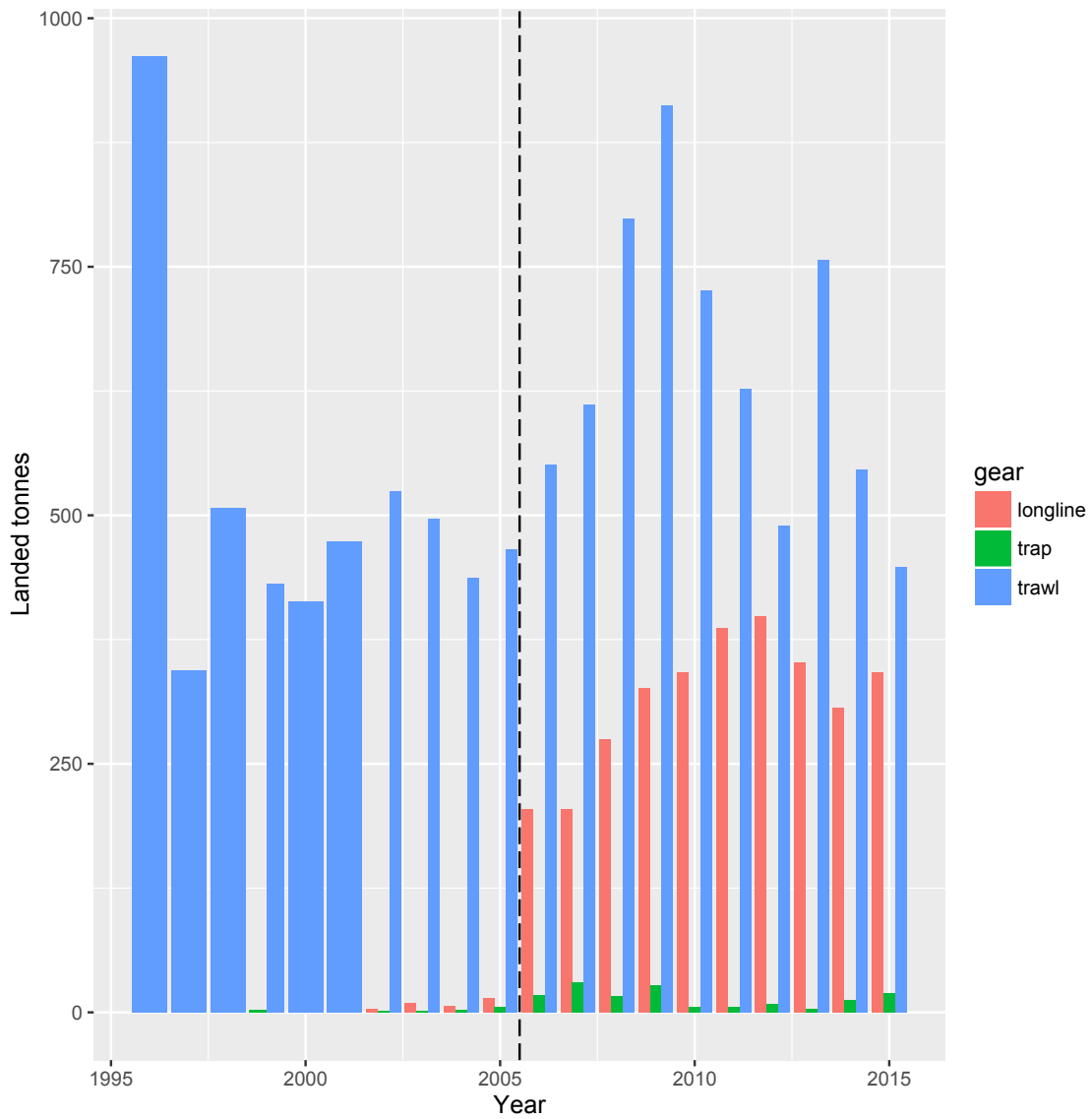


Figure 9 Historical commercial catch records for Rougheye Rockfish and Blackspotted Rockfish colored by gear type. The dashed vertical line indicates when full catch accounting was implemented.

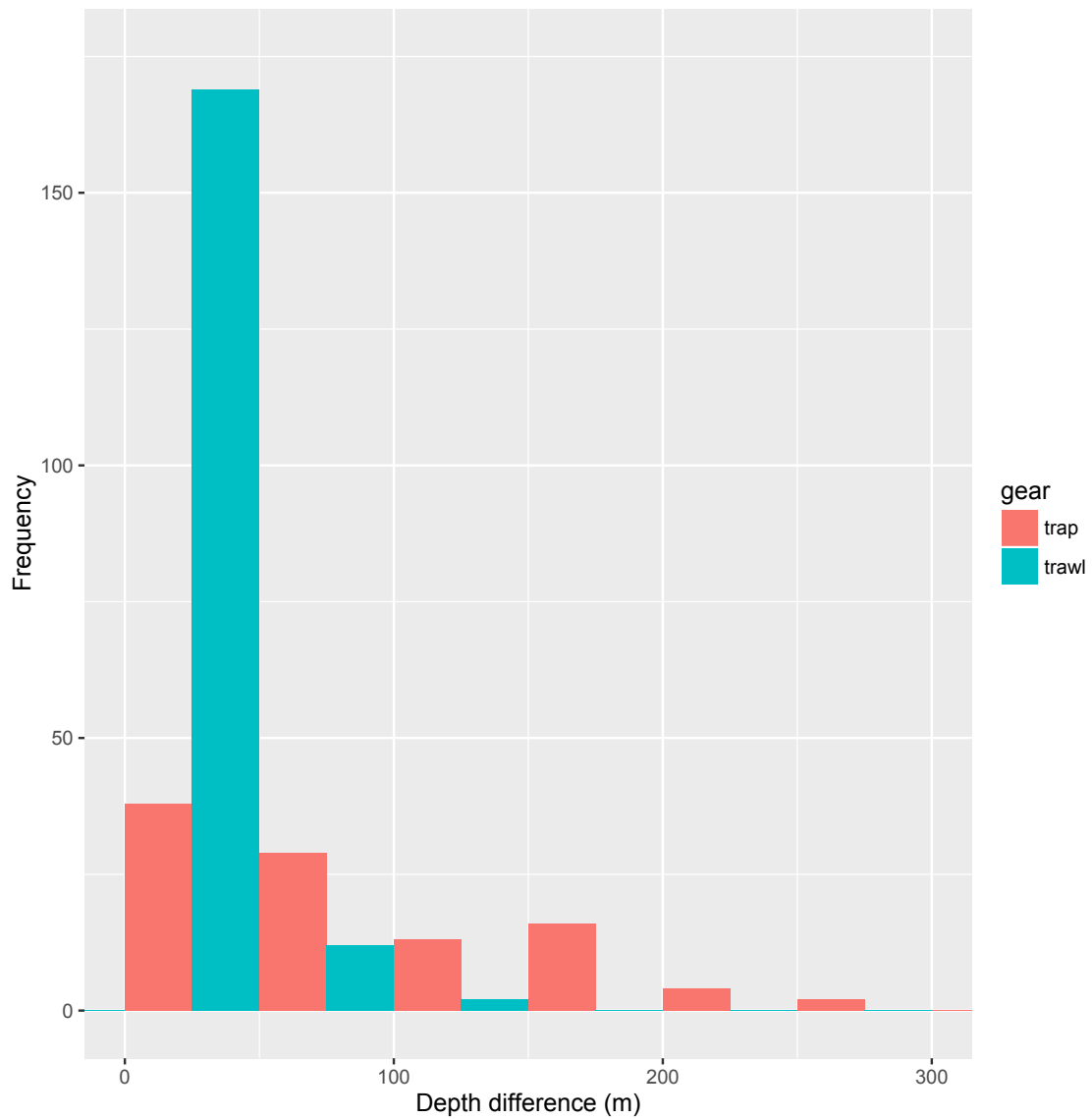


Figure 10 Distribution of the difference between the start and end depths of longline trap and trawl surveys. The start and end depths were not available for the hook longline survey data.

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Appendix A: Supplementary Survey Data

Description:

The accompanying text file shows the raw fisheries independent survey data. Each survey set has a unique identification number indicated by the column heading "FISHING_EVENT_KEY". Under "TRIP_TYPE", there are three different groundfish trawl synoptic surveys: West Coast of Haida Gwaii (WCHG), Hecate Strait (HS), and Queen Charlotte Sound (QCS). There are three different hook longline surveys: the International Pacific Halibut Commission (IPHC), the Pacific Halibut Management Association (PHMA) North, and PHMA South. The longline trap survey is labeled as "sablefish survey". The seven management areas shown in Figure 4 are under the heading "MAJOR_AREA". Total counts of Blackspotted Rockfish, Rougheyeye Rockfish, F1 hybrids, and F2 hybrids for each set are indicated by the headings "BS", "RE", "F1", and "F2", respectively. For some tissue samples, the genetic assay failed. The "FAIL" column heading indicates these.

Filename:

CreamerJulie_survey_data.txt

Appendix B. Supplementary Commercial Data

Description:

The accompanying text file shows the commercial data from 1996 – 2015. Each commercial set has a unique identification number indicated by the column heading “FISHING_EVENT_KEY”. Under “GEAR”, hook longline is indicated as “LONGLINE”, while trap longline is listed as “TRAP”. “LANDED_ROUND_KG” represents the total landed weight of combined Rougheye Rockfish and Blackspotted Rockfish for a set. “PID” is the longitudinal cell identifier shown in Figure 3 and “SID” is the latitudinal cell identifier.

Filename:

CreamerJulie_commercial_data.txt

Appendix C. Supplementary Observer Data

Description:

The accompanying text file shows the observer visual classifications for some of the fish sampled. For the column heading "SPECIMEN_SEX_CODE", a "0" indicates missing data, "1" is male, "2" is female, and "3" is undetermined. For "MATURITY_CODE", "1" is immature, "2" and "3" are maturing, "4" is mature, "5" is ripe, "6" is spent, and "7" is resting. Under the heading "SPECIES_GUESS", "16" means that the observer classified the fish as a Rougheye Rockfish, while "17" means Blackspotted Rockfish. The column labeled "SMA6" shows the results from the *Sma6* microsatellite analysis with "23" indicating Blackspotted Rockfish, "24" is Rougheye Rockfish, "25" is both species, and "26" is failure. Results from the SNP analysis are listed under the heading "MTSNP", with "27" indicating Blackspotted Rockfish, "28" is Rougheye Rockfish, and "29" is failure. The "RESOLVED_GENETIC_ID" lists Blackspotted Rockfish as "18", Rougheye Rockfish as "19", F1 hybrids as "20", F2 hybrids as "21", and failure as "22".

Filename:

CreamerJulie_observer_data.txt

Appendix D. Supplementary Topography Data

Description:

The accompanying text file was acquired from Scripps Institution of Oceanography's satellite geodesy database (http://topex.ucsd.edu/cgi-bin/get_data.cgi). The first column lists longitudinal coordinates as positive values (i.e. -90° would be represented as 270), the second column lists latitudinal values, and the third column give the elevation in meters.

Filename:

CreamerJulie_topo_data.txt