**Regional Operational Plan SF.4A.2019.01**

# Operational Plan: Genetic Sampling of Yelloweye and Black Rockfish from Inside and Outside Waters of Prince William Sound, North Gulf of Alaska, and Southeast Alaska

**by**

**Kathrine G. Howard**

**Chris Habicht**

**Elisa Russ**

**Andrew Olson**

**Jeff Nichols**

**and**

**Martin Schuster**

**January 2019**

**Alaska Department of Fish and Game** **Divisions of Sport Fish and Commercial Fisheries**

# Symbols and Abbreviations

The following symbols and abbreviations, and others approved for the Système International d'Unités (SI), are used without definition in the following reports by the Divisions of Sport Fish and of Commercial Fisheries: Fishery Manuscripts, Fishery Data Series Reports, Fishery Management Reports, and Special Publications. All others, including deviations from definitions listed below, are noted in the text at first mention, as well as in the titles or footnotes of tables, and in figure or figure captions.

### Weights and measures (metric)

| | |
|---|---|
| centimeter | cm |
| deciliter | dL |
| gram | g |
| hectare | ha |
| kilogram | kg |
| kilometer | km |
| liter | L |
| meter | m |
| milliliter | mL |
| millimeter | mm |

### Weights and measures (English)

| | |
|---|---|
| cubic feet per second | $\text{ft}^3/\text{s}$ |
| foot | ft |
| gallon | gal |
| inch | in |
| mile | mi |
| nautical mile | nmi |
| ounce | oz |
| pound | lb |
| quart | qt |
| yard | yd |

### Time and temperature

| | |
|---|---|
| day | d |
| degrees Celsius | °C |
| degrees Fahrenheit | °F |
| degrees kelvin | K |
| hour | h |
| minute | min |
| second | s |

### Physics and chemistry

| | |
|---|---|
| all atomic symbols | |
| alternating current | AC |
| ampere | A |
| calorie | cal |
| direct current | DC |
| hertz | Hz |
| horsepower | hp |
| hydrogen ion activity (negative log of) | pH |
| parts per million | ppm |
| parts per thousand | ppt,<br>‰ |
| volts | V |
| watts | W |

### General

| | |
|---|---|
| Alaska Administrative Code | AAC |
| all commonly accepted abbreviations | e.g., Mr., Mrs., AM, PM, etc. |
| all commonly accepted professional titles | e.g., Dr., Ph.D., R.N., etc. |
| at | @ |
| compass directions: | |
| east | E |
| north | N |
| south | S |
| west | W |
| copyright | © |
| corporate suffixes: | |
| Company | Co. |
| Corporation | Corp. |
| Incorporated | Inc. |
| Limited | Ltd. |
| District of Columbia | D.C. |
| et alii (and others) | et al. |
| et cetera (and so forth) | etc. |
| exempli gratia (for example) | e.g. |
| Federal Information Code | FIC |
| id est (that is) | i.e. |
| latitude or longitude | lat or long |
| monetary symbols (U.S.) | $, ¢ |
| months (tables and figures): first three letters | Jan,...,Dec |
| registered trademark | ® |
| trademark | ™ |
| United States (adjective) | U.S. |
| United States of America (noun) | USA |
| U.S.C. | United States Code |
| U.S. state | use two-letter abbreviations (e.g., AK, WA) |

### Mathematics, statistics

| | |
|---|---|
| *all standard mathematical signs, symbols and abbreviations* | |
| alternate hypothesis | $H_A$ |
| base of natural logarithm | *e* |
| catch per unit effort | CPUE |
| coefficient of variation | CV |
| common test statistics | (F, t, $\chi^2$, etc.) |
| confidence interval | CI |
| correlation coefficient (multiple) | R |
| correlation coefficient (simple) | r |
| covariance | cov |
| degree (angular ) | ° |
| degrees of freedom | df |
| expected value | *E* |
| greater than | > |
| greater than or equal to | ≥ |
| harvest per unit effort | HPUE |
| less than | < |
| less than or equal to | ≤ |
| logarithm (natural) | ln |
| logarithm (base 10) | log |
| logarithm (specify base) | $\log_{2,}$ etc. |
| minute (angular) | ' |
| not significant | NS |
| null hypothesis | $H_O$ |
| percent | % |
| probability | P |
| probability of a type I error (rejection of the null hypothesis when true) | α |
| probability of a type II error (acceptance of the null hypothesis when false) | β |
| second (angular) | " |
| standard deviation | SD |
| standard error | SE |
| variance | |
| population | Var |
| sample | var |

# *REGIONAL OPERATIONAL PLAN SF.4A.2019.01*

# OPERATIONAL PLAN: GENETIC SAMPLING OF YELLOWEYE AND BLACK ROCKFISH FROM INSIDE AND OUTSIDE WATERS OF PRINCE WILLIAM SOUND, NORTH GULF OF ALASKA, AND SOUTHEAST ALASKA

by

Kathrine G. Howard
Alaska Department of Fish and Game, Division of Sport Fish, Anchorage

Chris Habicht
Alaska Department of Fish and Game, Division of Commercial Fisheries, Anchorage

Elisa Russ
Alaska Department of Fish and Game, Division of Commercial Fisheries, Homer

Andrew Olson
Alaska Department of Fish and Game, Division of Commercial Fisheries, Juneau

Jeff Nichols
Alaska Department of Fish and Game, Division of Sport Fish, Juneau

Martin Schuster
Alaska Department of Fish and Game, Division of Sport Fish, Homer

Alaska Department of Fish and Game
Division of Sport Fish and Division of Commercial Fisheries

January 2019

The Regional Operational Plan Series was established in 2012 to archive and provide public access to operational plans for fisheries projects of the Divisions of Commercial Fisheries and Sport Fish, as per joint-divisional Operational Planning Policy. Documents in this series are planning documents that may contain raw data, preliminary data analyses and results, and describe operational aspects of fisheries projects that may not actually be implemented. All documents in this series are subject to a technical review process and receive varying degrees of regional, divisional, and biometric approval, but do not generally receive editorial review. Results from the implementation of the operational plan described in this series may be subsequently finalized and published in a different department reporting series or in the formal literature. Please contact the author if you have any questions regarding the information provided in this plan. Regional Operational Plans are available on the Internet at: http://www.adfg.alaska.gov/sf/publications/

*Kathrine G. Howard,*
*Alaska Department of Fish and Game, Division of Sport Fish,*
*Chris Habicht,*
*Alaska Department of Fish and Game, Division of Commercial Fisheries,*
*333 Raspberry Rd, Anchorage AK 99518-1599, USA*

*Elisa Russ,*
*Alaska Department of Fish and Game, Division of Commercial Fisheries,*
*Martin Schuster,*
*Alaska Department of Fish and Game, Division of Sport Fish,*
*3298 Douglas Pl, Homer, AK 99603-8027, USA*

*Andrew Olson,*
*Alaska Department of Fish and Game, Division of Commercial Fisheries,*
*Jeff Nichols,*
*Alaska Department of Fish and Game, Division of Sport Fish,*
*802 3rd Street (1st Floor), Douglas, AK 99824-5412, USA*

*This document should be cited as follows:*
*Howard, K. G., C. Habicht, E. Russ, A. Olson, J. Nichols, and M. Schuster. 2019. Operational Plan: Genetic sampling of yelloweye and black rockfish from inside and outside waters of Prince William Sound, North Gulf of Alaska, and Southeast Alaska. Alaska Department of Fish and Game, Regional Operational Plan ROP.SF.4A.2019.01, Anchorage.*

The Alaska Department of Fish and Game (ADF&G) administers all programs and activities free from discrimination based on race, color, national origin, age, sex, religion, marital status, pregnancy, parenthood, or disability. The department administers all programs and activities in compliance with Title VI of the Civil Rights Act of 1964, Section 504 of the Rehabilitation Act of 1973, Title II of the Americans with Disabilities Act (ADA) of 1990, the Age Discrimination Act of 1975, and Title IX of the Education Amendments of 1972.

**If you believe you have been discriminated against in any program, activity, or facility please write:**
ADF&G ADA Coordinator, P.O. Box 115526, Juneau, AK 99811-5526
U.S. Fish and Wildlife Service, 4401 N. Fairfax Drive, MS 2042, Arlington, VA 22203
Office of Equal Opportunity, U.S. Department of the Interior, 1849 C Street NW MS 5230, Washington DC 20240

**The department's ADA Coordinator can be reached via phone at the following numbers:**
(VOICE) 907-465-6077, (Statewide Telecommunication Device for the Deaf) 1-800-478-3648,
(Juneau TDD) 907-465-3646, or (FAX) 907-465-6078

**For information on alternative formats and questions on this publication, please contact:**
ADF&G, Division of Sport Fish, Research and Technical Services, 333 Raspberry Rd, Anchorage AK 99518 (907) 267-2375

# SIGNATURE/TITLE PAGE

Project Title: Genetic sampling of yelloweye and black rockfish from inside and outside waters of Prince William Sound, North Gulf of Alaska, and Southeast Alaska

Project leader(s): Kathrine Howard, Chris Habicht, Elisa Russ, Andrew Olson, Jeff Nichols, Martin Schuster

Division, Region and Area: Sport Fish and Commercial Fisheries, Regions I and II, Gulf of Alaska

Project Nomenclature:

Period Covered

Field Dates: January 1–December 31, 2019

Plan Type: Category III

## Approval

| Title | Name | Signature | Date |
|---|---|---|---|
| Project leader | Kathrine Howard | | 12/31/18 |
| Biometrician | Ben Buzzee | | 12/31/18 |
| Fisheries Scientist I | Ben Williams | | 2019-01-15 |

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# ABSTRACT

Previous genetic analyses for black rockfish (*Sebastes melanops*) and yelloweye rockfish (*S. ruberrimus*) throughout their range suggests stock differentiation may support more than one management unit for each of these species in the Gulf of Alaska (GOA). It is believed that stock differentiation would most likely occur where oceanographic conditions or currents diverge. One example is the difference between coastal outside waters versus inside waters, which are less connected by oceanographic currents. The goal of this study is to provide baseline genetic information for black and yelloweye rockfish from inside and outside waters of Southeast Alaska, Prince William Sound, and the northern Gulf of Alaska coast east of the Kenai Peninsula, to be used as a first step in developing future stock assessment and management strategies for these species.

Key words: black rockfish, *Sebastes melanops*, yelloweye rockfish, *Sebastes ruberrimus*, genetic baseline, Prince William Sound, Southeast Alaska, Gulf of Alaska

# INTRODUCTION

The Alaska Department of Fish and Game (ADF&G) recently initiated an interdivisional, interregional effort to develop long-term management and stock assessment strategies for black rockfish (*Sebastes melanops*) and yelloweye rockfish (*S. ruberrimus*) across the Gulf of Alaska (GOA). Defining a “stock” is one of the first steps of any stock assessment, and integral to developing appropriate management criteria and goals for these rockfish species. Population assessment and appropriate management units and measures are informed by an understanding of gene flow across spatial scales.

Low genetic structuring (and therefore less distinction between “stocks”) is often observed in marine animals where mixing of spatially distant individuals (gene flow) occurs. Rockfish (*Sebastes*) tend to be sedentary during their adult life stage but have an extended pelagic larval dispersal stage that may allow for gene flow across large distances. However, stock structure associated with barriers to mixing water masses has been detected for rockfish species. Yelloweye rockfish have been found to be genetically divergent between inshore waters of the Strait of Georgia and outer coastal waters (Siegle et al. 2013), as well as inside waters of Puget Sound and outer coastal waters (Andrews et al. 2018). Inside waters are those that are interior to land masses that restrict water flow and presumably larval transport, whereas outside waters are unrestricted by land masses and subject to coastal ocean currents. Black rockfish have demonstrated genetic breaks associated with major coastal ocean currents and genetic distance correlated with geographic distance along the U.S. West Coast (Lotterhos et al. 2014; Miller et al. 2005) and Gulf of Alaska (Seeb 2007).

Port sampling conducted by both the ADF&G Division of Sport Fish (SF) and the Division of Commercial Fisheries (CF) provides opportunities to collect biological data, tissue samples, associated harvest location, and effort information. These programs are designed to characterize harvest of multiple species caught in their respective fisheries and provide important spatial diversity in fish collections. Port sampling programs have been integral to previous ADF&G genetic sampling projects for a variety of species.

Port sampling programs in Southeast Alaska (SEAK) regularly collect information on black and yelloweye rockfish in 6 groundfish management districts: Northern Southeast Inside (NSEI), Southern Southeast Inside (SSEI), Northern Southeast Outside (NSEO), Central Southeast Outside (CSEO), East Yakutat (EYKT), Southern Southeast Outside (SSEO), and Icy Bay Subdistrict (IBS, black rockfish only) (Figure 1). Sport fishery angler effort, catch, harvest, and biological data are collected from guided and unguided marine boat anglers from 10 ports: Yakutat, Elfin Cove, Gustavus, Juneau, Sitka, Petersburg, Wrangell, Ketchikan, Craig, and Klawock (Jaenicke et al. 2014). Commercial harvests are sampled for effort, catch, and biological data from January through March and from November through December during the directed fishery and from March through November to sample rockfish bycatch during the halibut fishery.

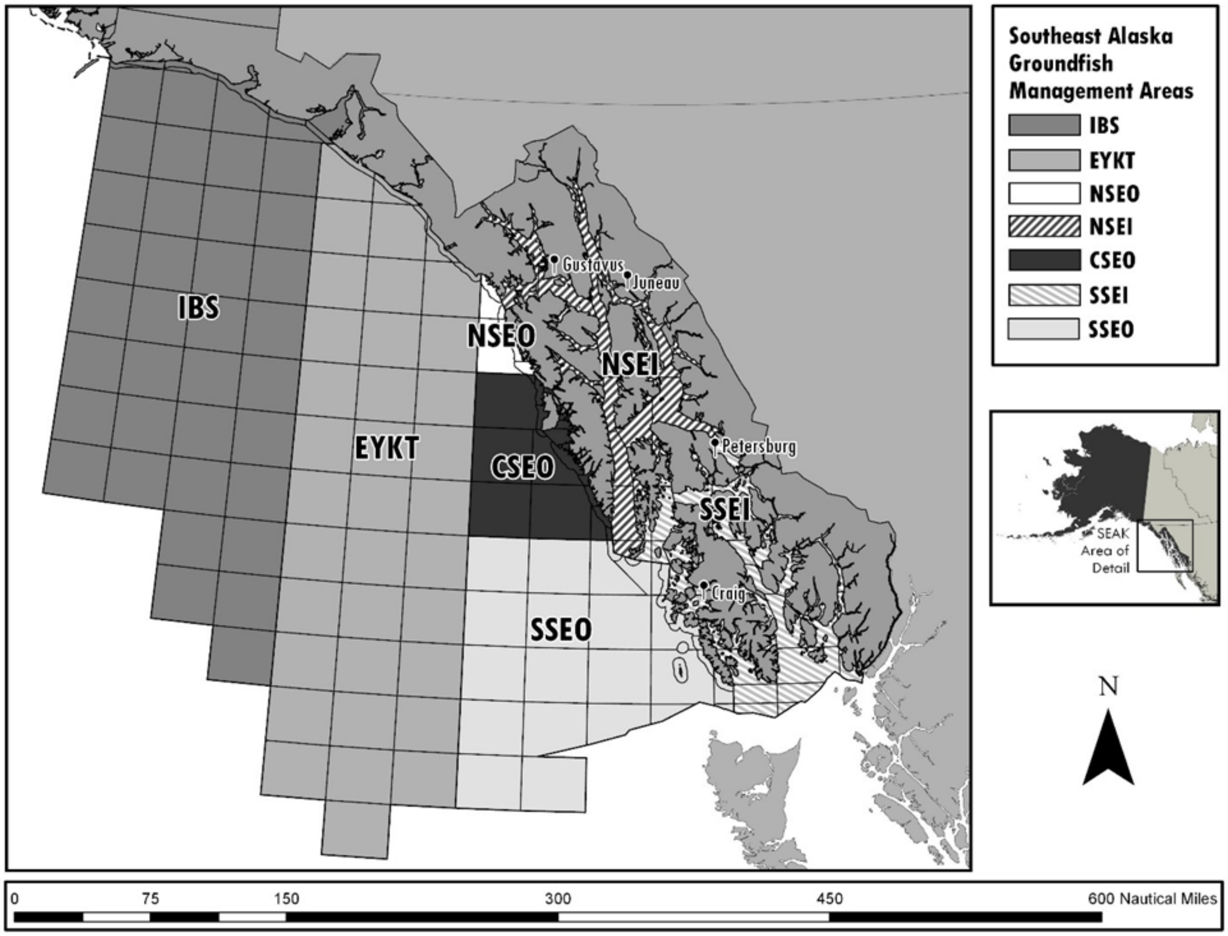

Figure 1.–Southeast Alaska rockfish management areas.

Western and Central Gulf of Alaska port sampling programs collect information on harvest for fisheries occurring west of Yakutat to the Aleutian Islands. Sport fishery catches are assessed for species and age, length, and sex compositions at ports of Homer, Seward, Whittier, Valdez, and Kodiak from May through September annually (Failor 2016). Commercial Fishery management areas E and H include waters inside Prince William Sound (PWS) and adjacent waters outside of PWS and along the North Gulf of Alaska (NG) (Figures 2 and 3). Commercial fisheries are sampled in the ports of Homer, Seward, Whittier, Cordova, Kodiak, Kenai, Valdez, and from buyers in Anchorage. CF sampling occurs throughout the year with sampling goals determined by management area; therefore, effort by port is dependent on fishery dynamics and active landings and buyers.

Port sampling programs in all regions and for both divisions provide an opportunity to assess age, length, and sex compositions, as well as average weight in some cases, for specific species in distinct fisheries. Genetic samples collected as part of this project will have corresponding biological data collected from specimens as part of port sampling program objectives.

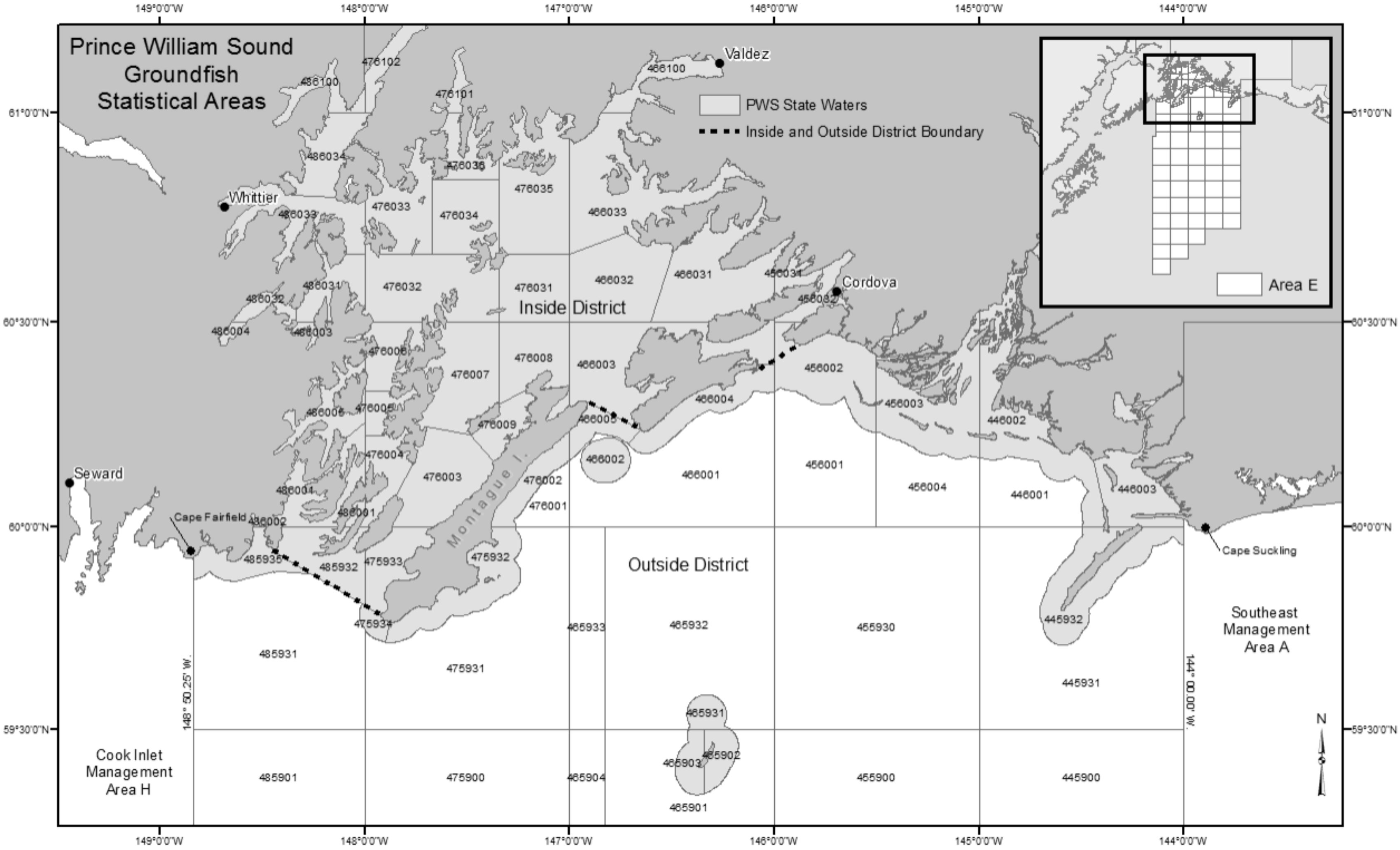

Figure 2.–Prince William Sound (Area E) groundfish management area for commercial fisheries, including districts and statistical areas.

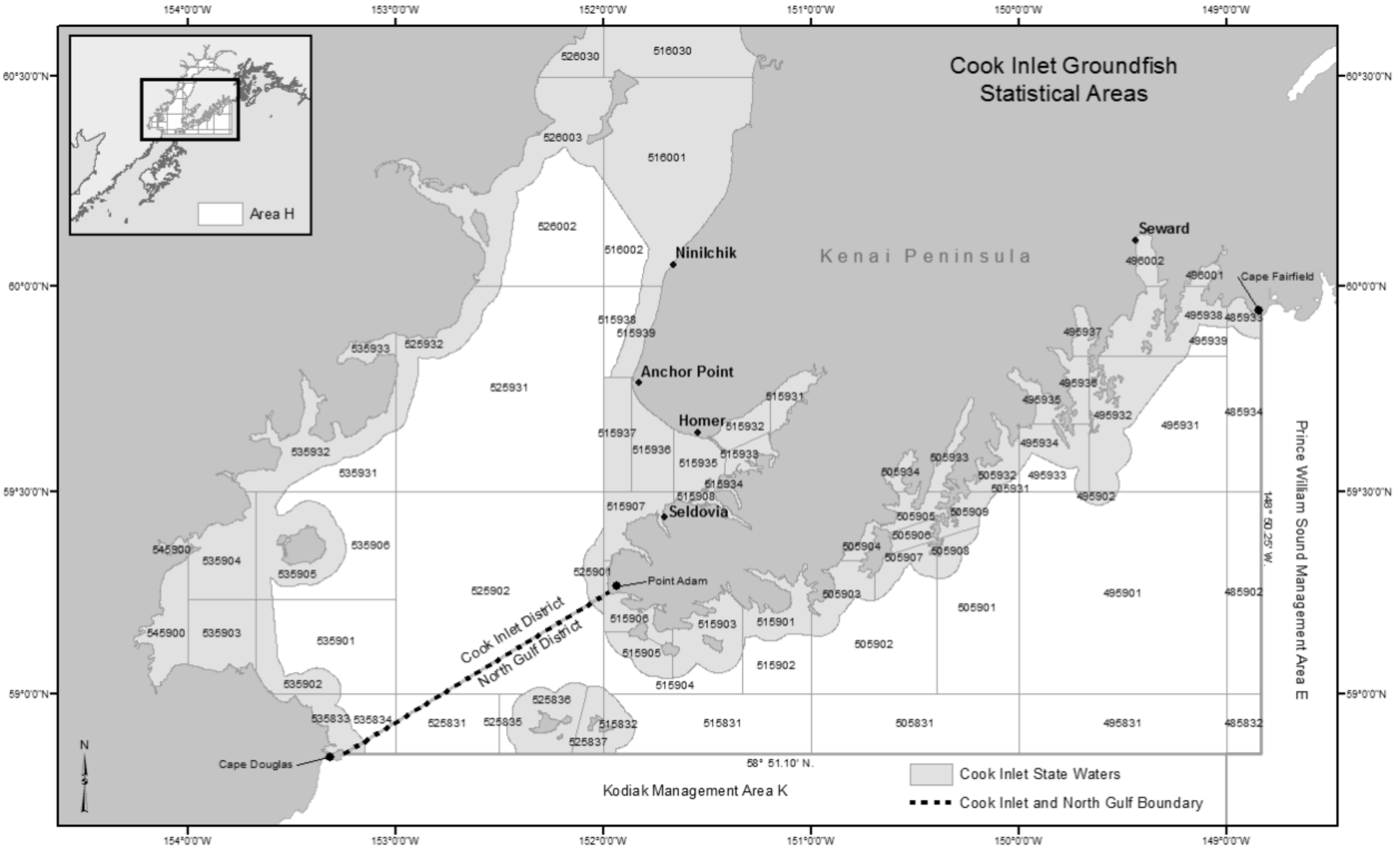

Figure 3.–Cook Inlet (Area H) groundfish management area for commercial fisheries, including districts and statistical areas.

# OBJECTIVES

1) Describe the genetic structure for black rockfish within and between 7 spatial areas representing inside and outside waters of SEAK and PWS–NG.
2) Describe the genetic structure for yelloweye rockfish within and between 7 spatial areas representing inside and outside waters of SEAK and PWS–NG.

# METHODS

This study is designed to achieve objectives while minimizing impacts to existing port sampling programs. Sampling goals are to collect a minimum of 100 genetic tissue samples per species during port sampling for black and yelloweye rockfish from each of the following waters: 1) Northern inside waters of SEAK (NSEI), 2) Southern inside waters of SEAK (SSEI), 3) Northern outside waters of SEAK (NSEO, CSEO, EYKT, and IBS), 4) Southern outside waters of SEAK (SSEO, and CSEO), 5) Inside waters of PWS, 6) Eastern outside waters of PWS–NG, and 7) Western outside waters of PWS–NG.

## SOUTHEAST ALASKA

Inside and outside waters are clearly demarcated by management area in SEAK and these management areas will form the basis of the sampling design. To obtain diverse spatial coverage of inside and outside waters, sampling strata will include northern (NSEI) and southern (SSEI) components of the inside waters as well as northern (NSEO, CSEO, EYKT, IBS) and southern (SSEO, CSEO) components of outside waters (Figure 1).

*Genetic tissue samples will be collected from the first 100 biological samples for each species and management area from commercial fisheries sampling in SEAK.*

The commercial fisheries port sampling program currently targets 550 biological samples from each SEAK groundfish management area for each of these rockfish species. Given sampling results from recent years, this program can provide ample samples for fulfilling this study's sample size objectives for yelloweye rockfish in outside waters, but limited samples for black rockfish from outside waters, and no samples from inside waters (Table 1).

Table 1.–Sample sizes obtained for biological sampling of black and yelloweye rockfish in commercial fisheries, 2016–2018.

| | Black rockfish | | | Yelloweye rockfish | | |
|---|---|---|---|---|---|---|
| Management area | 2016 | 2017 | 2018 | 2016 | 2017 | 2018 |
| NSEO | 100 | 0 | 0 | 575 | 410 | 378 |
| EYKT | 0 | 0 | 0 | 589 | 572 | 560 |
| CSEO | 50 | 450 | 0 | 559 | 560 | 738 |
| SSEO | 0 | 0 | 0 | 155 | 31 | 11 |

*All black and yelloweye rockfish will be sampled for genetic tissue by SF port samplers in the ports of Craig–Klawock, Petersburg, Gustavus, and Juneau in SEAK.*

Sampling of black rockfish from inside-water management areas (NSEI and SSEI) as well as from outside waters will be provided by SF port sampling. Sampling in the ports of Craig–Klawock, Petersburg, Gustavus, and Juneau should provide complementary samples to those obtained from commercial fisheries to meet sample size objectives (Table 2). Port samplers cannot easily distinguish sample origin by management area in the field for northern and southern inside and outside waters without adversely affecting the existing port sampling program.

Table 2.–Sample sizes obtained for biological sampling of black and yelloweye rockfish in sport fisheries from Craig–Klawock, Juneau, Gustavus, and Petersburg, 2016–2018.

| | Black rockfish | | | Yelloweye rockfish | | |
|---|---|---|---|---|---|---|
| Management area | 2016 | 2017 | 2018 | 2016 | 2017 | 2018 |
| SSEI | 73 | 69 | 54 | 100 | 68 | 119 |
| NSEI | 115 | 73 | 129 | 129 | 94 | 137 |
| SSEO | 202 | 298 | 358 | 232 | 394 | 432 |
| NSEO | 119 | 58 | 176 | 80 | 51 | 64 |

## PRINCE WILLIAM SOUND–NORTH GULF

Commercial and sport fishermen who return to ports in and adjacent to PWS (Valdez, Whittier, Cordova, and Seward) fish in both inside and outside waters. To provide a broad geographic sampling distribution of fish including both outside waters of PWS and the adjacent North Gulf (NG), samples will be categorized by 1 spatial group representing the inside waters of PWS, and 2 spatial groups representing neighboring outside waters (CF Areas E and H divided at Cape Fairfield). For the purposes of this study, inside waters of PWS will be defined by statistical areas that are interior to land masses and where little water transport with coastal ocean currents is expected. In order to demarcate the inside and outside waters more distinctly than CF districts, PWS (Area E) statistical areas were assigned to inside, outside, and border categories based on expected exposure to coastal ocean currents (Table 3, Figure 2). Border statistical areas will be excluded from the analysis because it is unclear whether samples from those areas should be designated as inside or outside waters. Neighboring statistical areas in NG (Area H, Figure 3) will provide an additional outside stratum to obtain broad geographic sampling of areas exposed to coastal ocean currents in the northern GOA in the vicinity of PWS and allow genetic analysis of populations west of PWS.

*Genetic tissue samples will be collected from the first 100 biological samples for each species and spatial stratum from commercial fisheries sampling in PWS and NG.*

The commercial fisheries port sampling program currently targets 550 biological samples from each of PWS (Area E) and NG (Area H) for each rockfish species. Based on recent years, this program is expected to provide ample samples for fulfilling sample size objectives for yelloweye rockfish from inside and outside waters of PWS, and outside waters of NG, and black rockfish from NG. If targeted effort is increased on rockfish bycatch in PWS, this program may be able to fulfill sample size objectives for outside waters of PWS; however, no black rockfish samples have been collected from commercial fisheries for PWS inside waters in recent years (Table 4).

Table 3.–Category designation for Prince William Sound, Area E, statistical areas.

| Inside Waters | Border Waters | Outside Waters | |
|---|---|---|---|
| 476034 | 466005 | 445830 | 476001 |
| 476009 | 475933 | 445900 | 476002 |
| 476033 | 485932 | 445931 | 485831 |
| 476032 | 486001 | 445932 | 485901 |
| 476031 | | 446001 | 485931 |
| 476007 | | 446002 | 485935 |
| 476008 | | 446003 | 486002 |
| 476004 | | 455830 | |
| 476003 | | 455900 | |
| 466100 | | 455930 | |
| 466033 | | 456001 | |
| 466032 | | 456002 | |
| 476006 | | 456003 | |
| 466031 | | 456004 | |
| 476005 | | 465830 | |
| 466003 | | 465901 | |
| 456031 | | 465902 | |
| 456032 | | 465903 | |
| 476035 | | 465904 | |
| 476036 | | 465931 | |
| 476101 | | 465932 | |
| 476102 | | 465933 | |
| 486003 | | 466001 | |
| 486004 | | 466002 | |
| 486005 | | 466004 | |
| 486031 | | 475830 | |
| 486032 | | 475900 | |
| 486033 | | 475931 | |
| 486034 | | 475932 | |
| 486100 | | 475934 | |

*Note*: See Figure 2 for location of statistical areas.

Table 4.–Sample sizes obtained for biological sampling of black and yelloweye rockfish from PWS and NG in commercial fisheries, 2015–2017.

| Species | Year | PWS inside | PWS outside | PWS border | PWS total | NG (outside) |
|---|---|---|---|---|---|---|
| Black rockfish | 2015 | 0 | 53 | 0 | 53 | 799 |
| | 2016 | 0 | 98 | 8 | 106 | 641 |
| | 2017 | 0 | 5 | 0 | 5 | 555 |
| Yelloweye rockfish | | | | | | |
| | 2015 | 407 | 165 | 26 | 598 | 372 |
| | 2016 | 188 | 194 | 15 | 397 | 561 |
| | 2017 | 277 | 106 | 6 | 389 | 579 |

*All black rockfish will be sampled for genetic tissue by SF port samplers in the ports of Valdez and Whittier.*

To accommodate for the paucity of samples of PWS black rockfish (both inside and outside waters), SF port sampling programs will be utilized. Sport fish harvest from the ports of Valdez and Whittier provide good spatial distribution of black rockfish from inside and outside waters of PWS (Table 5). Although samples are collected in Seward from both PWS and NG outside waters, there is a mix of harvest locations due to the proximity of that port to the boundary between management areas which cannot be resolved from sampling data, and therefore, port sampling from Seward will be excluded from this project. Additionally, port samplers cannot easily distinguish sample origin by management area in the field for inside and outside waters without adversely affecting the existing port sampling program; however, due to the proximity of Valdez and Whittier to inside as well as outside waters of PWS, there is a higher likelihood that these geographic locations, will be sampled (versus NG).

In addition to these port sampling sources, other research projects conducted by ADF&G in PWS and NG have collected tissue samples for use in genetic analyses, primarily for yelloweye rockfish. These additional samples and associated sample locations will also contribute to the sample objectives of this study.

Table 5.–Sample sizes obtained for biological sampling of black rockfish in sport fisheries at Valdez and Whittier, 2015–2017.

| Year | Port | Inside | Outside | Total |
|---|---|---|---|---|
| 2015 | Valdez | 125 | 545 | 670 |
| | Whittier | 25 | 243 | 268 |
| 2016 | Valdez | 62 | 246 | 308 |
| | Whittier | 43 | 283 | 326 |
| 2017 | Valdez | 16 | 252 | 268 |
| | Whittier | 11 | 145 | 156 |

## TISSUE COLLECTION AND DATA REDUCTION

Black and yelloweye rockfish will be harvested either by mechanical jig, hand troll, or longline gear from commercial fisheries and by rod and reel from sport fisheries. Hook and line sampling by ADF&G staff for other research projects will also supplement port sampling effort. Target sample size is set at 100 individuals per species for each of the 7 locations described under the objectives. Fin clips from individual fish will be excised, placed on and stapled to Whatman[1] sample cards, placed in an airtight Pelican case with desiccant beads, desiccated in the field, and shipped to the ADF&G Gene Conservation Laboratory (GCL) for analysis.

Data files associated with tissue collections (date, location, species, length, sex, genetic identification number, etc.) will be sent to the GCL in Anchorage along with tissue samples. Metadata for the sample collection will be provided by project leaders for the GCL's database. Genetic identification numbers and metadata will also be maintained in respective port sampling databases. The GCL will curate tissues, associated data, and genotype information in their archives for any future use.

## GENETIC ANALYSIS

Total genomic DNA will be isolated from 20 to 30 mg of the fin sample using a NucleoSpin 96 Tissue Kit by Macherey-Nagel (Düren, Germany). PCR will be carried out in 10 µl reaction volumes (10 mM Tris-HCl, 50 mM KCl, 0.2 mM each dNTP, 0.5 units Taq DNA polymerase [Promega, Madison, WI]) using an MJ research PTC-225 thermal cycler.

Black rockfish populations will be distinguished using up to 10 loci: two dinucleotide loci (*Sma1 and Sma3*) developed from *S. maliger* (Wimberger et al. 1999), as well as 8 tri- and tetranucleotide loci (*Sme2, 3, 4, 5, 8, 9, 11,* and *14*) developed from *S. melanops* (Seeb and Seeb 2007). Amplification of loci will be conducted both in multiplexes and singularly. Thermal cycler profiles for the two annealing temperatures will be as follows: 1) 92°C (5 min); 25 cycles of 92°C (30s) + 56°C (30 sec) + 1°C/s to 72°C + 72°C (20s); 72°C (30min) and 2) 92°C (5 min); 25 cycles of 92°C (30s) + 58°C (30 sec) + 1°C/s to 72°C + 72°C (20s); 72°C (30min).

Yelloweye rockfish populations will be distinguished using up to 9 loci previously used for examination of population structure in this species (Siegle et al. 2013). Two loci (*Sal1* and *3*) were developed by Miller et al. (2000), 5 loci (*Sme3, 5, 8, 12,* and *13*) by Seeb et al. (L.W. Seeb, E. J. Kretschmer, and J. B. Olsen, ADF&G, Characterization of microsatellite loci derived from black rockfish (*Sebastes melanops*), unpublished manuscript), and 1 locus (*Sru9*) by Miller et al. (K. M. Miller, A. D. Schulze, and R. E. Wither, Department of Fisheries and Oceans Canada, A novel approach to individual and species identification of rockfish, unpublished manuscript), and 1 locus (*Sru20*) by Miller et al. (K. M. Miller, A. D. Schulze, and R. E. Withler, Department of Fisheries and Oceans Canada, unpublished data). Thermal cycler profiles for the two annealing temperatures will follow Siegle et al. (2013)

Microsatellites will be size fractionated using an ABI 377-96 automated DNA sequencer operated in GeneScan mode on a 5% denaturing polyacrylamide gel (ABI 1996). The instrument will be run under Plate Check, Prerun, and Run modules of 36C-2400. Sample cocktail for loading will include 2.4 µL deionized formamide, 0.4 µL GeneScanTM500TAMRATM size standard, 0.4 µL loading dye (included in size standard box), and 0.5 µL PCR product. Data will be analyzed using the internal lane sizing standard and local Southern sizing algorithm in the GeneScan software v. 3.2 (ABI 1998). Alleles for each locus will be scored and data will be tabulated for importing into statistical software with Genotyper software, v. 2.5 (ABI 1996).

Allele frequencies and Hardy-Weinberg equilibrium will be calculated using GENEPOP version 3.4 (Raymond and Rousset 1995) and GenAlEx 6 (Peakall and Smouse 2005). To evaluate the

---

[1] Product names used in this publication are for completeness and do not constitute product endorsement.

relationships among spatial areas, $F_{ST}$ (proportion of genetic variance contained in a subpopulation) will be calculated among all spatial area collections as well as pairwise between spatial area collections using *FSTAT* version 2.93 (Goudet 2001). A matrix of pairwise $F_{ST}$ values between all pairs of spatial areas will be computed. Population structure will be visualized using multidimensional scaling (MDS) of the pairwise values as implemented in *NtSYS* (Exeter Software, Setauket, NY) to reduce the dimensionality of the interpopulation distances to 3-dimensional space. A log-likelihood *G* statistic test implemented in *FSTAT* will be used to test for population (spatial areas) differentiation on a pairwise basis. The test does not assume random mating within samples and will be based on 1000 randomizations. The distribution of variation as measured by AMOVA will be calculated using GeneAlEx 6. *FSTAT* will be also used to estimate allelic richness per locus and spatial area, over all spatial areas, and comparatively between spatial areas. Allelic richness is a measure of the number of alleles independent of sample size and will allow for a relative comparison of the quantity of alleles among populations or groups of populations with differing sample sizes.

An isolation-by-distance analysis will be also conducted to evaluate whether the genetic similarity among spatial areas decreases as the geographic distance between them increases. Mantel tests as implemented in the computer program IBDWS Version 2.1 (Bohonak et al. 2005) will be used to evaluate distance matrices for association between genetic divergence and geographic distance in 2 km units over the range of the study. Geographic distance will be measured as shortest coastal point to point distance. Point locations for each spatial area will be the geographic center of the area.

# SCHEDULE AND DELIVERABLES

| Dates | Activity | Responsibility |
|---|---|---|
| December 2018-April 2019 | Sample supply kits and instructions provided to project leaders | Habicht |
| December 2018-November 2019 | Sample collection | Russ, Olson, Arthur, Schuster, Nichols |
| September-November 2019 | Send tissue samples to ADF&G Gene Conservation Lab | Russ, Olson, Arthur, Schuster, Nichols |
| December 2019-May 2020 | Genetic analysis | Habicht |
| December 2020 | Report of results | Howard, Habicht, Russ, Olson, Arthur, Schuster, Nichols |

If sample size objectives are not met during this allotted timeframe, sampling will be continued in subsequent years to achieve adequate sample sizes for analysis to meet objectives. Results from this study will be documented in an Alaska Department of Fish and Game Fishery Data Series Report and (or) an external peer-reviewed publication. Analysis and a draft report will be completed by December 2020.

# RESPONSIBILITIES

Kathrine Howard, Fisheries Scientist I (SF)
*Duties*: Overall coordination of sampling, budget administration, subsampling collections to the desired sample size using statistical area information when applicable prior to genetic analysis, and reporting.

Christ Habicht (or new geneticist) (CF)
*Duties*: Oversee provision of all genetic tissue samples supplies, laboratory processing of samples and analysis, and author or co-author final report.

Elisa Russ, Fishery Biologist II (CF)
*Duties*: Oversee commercial fisheries sampling in PWS–NG and contribute to final report.

Andrew Olson, Fishery Biologist III (CF)
*Duties*: Oversee commercial fisheries sampling in SEAK and contribute to final report.

Jeff Nichols, Fishery Biologist IV (SF)
*Duties*: Oversee sport fisheries sampling in SEAK and contribute to final report.

Martin Schuster, Fishery Biologist II (SF)
*Duties*: Oversee sport fisheries sampling in PWS–NG areas and contribute to final report.

Donnie Arthur, Fishery Biologist I (SF)
*Duties*: Collect genetic tissue samples of yelloweye rockfish in PWS and contribute to final report.

# BUDGET SUMMARY

Primary costs of this project are anticipated to be the genetic sample analysis and technician time to assist with port sampling. For 100 samples × 7 spatial strata × $35/sample × 2 species, genetic sample analysis is expected to cost approximately $49,000 (split between lines 100 ($30,000) and 400 ($19,000). Additional port sampler staff time may be required to reduce impacts on project operations with the addition of genetic sampling tasks: $8,000 (split between lines 100 ($7,000) and 200 ($1,000) will be provided to support PWS–NG commercial fisheries sampling, and $15,000 (line 100) will be provided to support SEAK SF sampling.

Projected FY19–FY20 costs:

| Line Item | Category | Budget ($K) |
|---|---|---:|
| 100 | Personnel | 52 |
| 200 | Travel | 1 |
| 300 | Contractual | 0 |
| 400 | Commodities | 19 |
| 500 | Equipment | 0 |
| Total | | 72 |

# REFERENCES CITED

ABI (Applied Biosystems Inc.). 1996. Genotyper 2.0 users' manual. Perkin-Elmer Corporation, Foster City, CA.

ABI (Applied Biosystems Inc.). 1998. ABI Prism 377 DNA sequencer 96-lane upgrade. Perkin-Elmer Corporation, Foster City, CA.

Andrews, K. S., K. M. Nichols, A. Elz, N. Tolimieri, C. J. Harvey, R. Pacunski, D. Lowry, K. L. Yamanaka, and D. M. Tonnes. 2018. Cooperative research sheds light on population structure and listing status of threatened and endangered rockfish species. Conservation Genetics 19(4): 865-878.

Bohonak, A. J., J. L. Jensen, S. T. Kelley, and E. C. Ngan. 2005. Isolation by distance, web service. BMC Genetics 6: 13. http://phage.sdsu.edu/~jensen/

Failor, B. 2016. Operational Plan: Assessment of Pacific halibut and groundfish sport harvest in Southcentral Alaska, 2016–2018. Alaska Department of Fish and Game, Regional Operational Plan. ROP.SF.2A.2016.20, Anchorage.

Goudet, J. 2001. FSTAT (Version 1.2): A computer program to calculate F-statistics. Journal of Heredity 86: 485-486.

Jaenicke, M., D. Tersteeg, and S. J. H. Power. 2014. Southeast Alaska marine boat sport fishery harvest studies, 2015–2016. Alaska Department of Fish and Game, Regional Operational Plan SF.1J.2015.06, Anchorage.

Lotterhos, K. E., S. J. Dick, and D. R. Haggarty. 2014. Evaluation of rockfish conservation area networks in the United States and Canada relative to the dispersal distance for black rockfish (*Sebastes melanops*). Evolutionary Applications 7(2):238-259.

Miller, K. M., A. D. Schulze, and R. E. Wither. 2000. Characterization of microsatellite loci in *Sebastes alutus* and their conservation in congeneric rockfish species. Molecular Ecology 9(2), 240-242.

Miller, J. A., M. A. Banks, D. Gomez-Uchida, and A. L. Shanks. 2005. A comparison of population structure in black rockfish (*Sebastes melanops*) as determined with otolith microchemistry and microsatellite DNA. Canadian Journal of Fisheries and Aquatic Science 62: 2189-2195.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288-295. Available from http://www.anu.edu.au/BoZo/GenAlEx/

Raymond, M., and F. Rousset. 1995. GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248-249.

Seeb, L. W., and J. E. Seeb. 2007. Investigations of genetically important conservation units of species inhabiting the EVOS Area II: Black Rockfish (*Sebastes melanops*). Restoration Project 99252 Final Report to Exxon Valdez Oil Spill Trustee Council, Anchorage Alaska.

Seeb, L. W. 2007. Genetic markers distinguish populations of black rockfish in the Gulf of Alaska. Pages 57-60 [In] E. A. Berntson, P. S. Levin, and P. C. Moran (editors). Conservation of North Pacific rockfishes: ecological genetics and stock structure. Proceedings of the workshop, March 2-3, 2004, Seattle, WA. U.S. Dept. Commer., NOAA Tech. Memo. NMFS-NWFSC-80.

Siegle, M. R., E. B. Taylor, K. M. Miller, R. E. Withler, and K. L. Yamanaka. 2013. Subtle population genetic structure in yelloweye rockfish (*Sebastes ruberrimus*) is consistent with a major oceanographic division in British Columbia, Canada. PLoS ONE. 8(8): e71083.

Wimberger, P., J. Burr, A. Gray, A. Lopez and P. Bentzen. 1999. Isolation and characterization of twelve microsatellite loci for rockfish (*Sebastes*). Marine Biotechnology, 1(3): 311-315.