Regional Operational Plan DF.#R.YY-XX

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

by

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Month Year

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

Symbols and Abbreviations

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**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 ft3/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 pH

(negative log of)

parts per million ppm

parts per thousand ppt,

‰

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 HA

base of natural logarithm *e*

catch per unit effort CPUE

coefficient of variation CV

common test statistics (F, t, χ2, 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) log2, etc.

minute (angular) '

not significant NS

null hypothesis HO

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 DF.#R.YY-XX

**Title**

by

Author Name

Alaska Department of Fish and Game, Division, City

Alaska Department of Fish and Game  
Division

Month Year

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Author name,

Alaska Department of Fish and Game, Division,

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Signature/Title Page

|  |  |
| --- | --- |
| Project Title: |  |
| Project leader(s): |  |
| Division, Region and Area |  |
| Project Nomenclature: |  |
| Period Covered |  |
| Field Dates: |  |
| Plan Type: | Category III |

**Approval**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Title |  | Name |  | Signature |  | Date |
| Project leader |  |  |  |  |  |  |
| Biometrician |  |  |  |  |  |  |
| Research Coordinator |  |  |  |  |  |  |
| Regional Supervisor |  |  |  |  |  |  |

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# 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 be most likely between inside waters and outside 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 Prince William Sound and Southeast Alaska.

Key words: black rockfish, yelloweye rockfish, genetic baseline, Prince William Sound, Southeast Alaska

# Introduction

The Alaska Department of Fish and Game (ADF&G) recently initiated an interdivisional, inter-regional effort focused on developing 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 definition of appropriate management units and measures are informed by understanding of gene flow across spatial scales.

Low genetic structuring is often expected 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 water masses mixing has also 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, while 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 conducting by Sport Fish (SFD) and Commercial Fisheries (CFD) divisions provide opportunities to collect biological data and tissue samples, along with associated statistical area of harvest. 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 genetic sampling projects for a variety of species.

Port sampling programs in Southeast Alaska (SEAK) regularly collect information on black and yelloweye rockfish in six groundfish management areas: Northern Southeast Inside (NSEI), Southern Southeast Inside (SSEI), Northern Southeast Outside (NSEO), Central Southeast Outside (CSEO), East Yakutat (EYKT), Southern Southeast Outside (SSEO), Icy Bay Subdistrict (IBS, black rockfish only) (Figure 1). 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). Biological characteristics of commercial harvest are sampled from January–March and November–December annually for the directed fishery and from March–November for bycatch samples collected during the halibut fishery (CITE).

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, 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 Coast (NGC) (Figure 2).

# Objectives

1. Collect a minimum of 100 genetic tissue samples during port sampling of each species (black and yelloweye rockfish) from each of:
   1. Northern inside waters of SEAK – NSEI
   2. Southern inside waters of SEAK – SSEI
   3. Northern outside waters of SEAK–NSEO/ CSEO/ EYKT/IBS
   4. Southern outside waters of SEAK–SSEO/ CSEO
   5. Inside waters of PWS
   6. Eastern outside waters of PWS/NGC–Area E
   7. Western outside waters of PWS/NGC–Area H
2. Assess genetic structure within and among each of the sample groups for each species.

# Methods

Study design will endeavor to achieve objectives while minimizing impacts to existing port sampling programs.

## Southeast Alaska

Inside and outside waters are clearly demarcated by management area in SEAK and these management areas will form the basis of sample design. To get diverse spatial coverage of inside and outside waters, sample 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).

The commercial fisheries port sampling program currently targets 550 biological samples from each SEAK groundfish management area for each of these rockfish species. In recent years this program would provide ample samples for fulfilling 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). *Genetic tissue samples will be collected from the first 100 biological samples for each species and spatial group from commercial fisheries sampling in SEAK.*

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 |

Sampling of inside waters management areas (NSEI and SSEI) as well as outside water black rockfish will be provided by SFD port sampling. Sampling in just 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. *Therefore, all black and yelloweye rockfish will be sampled for genetic tissues by SFD port samplers in the ports of Craig/Klawock, Petersburg, Gustavus, and Juneau in SEAK*.

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 | | |
|  | 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 Coast

Commercial and sport fishermen who return to ports in and adjacent to PWS (Valdez, Whittier, Cordova, Seward) fish in both inside and outside waters. One spatial group will represent inside waters of PWS, and two spatial groups will represent neighboring outside waters (Areas E and H) for broad geographic distribution of fish from the NGC adjacent to PWS. 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. As management areas do not clearly define inside and outside waters for 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 as it is unclear whether samples from those areas should be designated as inside or outside waters. Neighboring statistical areas in Area H (NGC) will provide an additional outside stratum to obtain broad geographic sampling of areas exposed to coastal ocean currents in the vicinity of PWS.

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

|  |  |  |
| --- | --- | --- |
| Inside Waters | Border Waters | Area E Outside Waters |
| 476034 | 466005 | 445830 |
| 476009 | 475933 | 445900 |
| 476033 | 485932 | 445931 |
| 476032 | 486001 | 445932 |
| 476031 |  | 446001 |
| 476007 |  | 446002 |
| 476008 |  | 446003 |
| 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 |
|  |  | 476001 |
|  |  | 476002 |
|  |  | 485831 |
|  |  | 485901 |
|  |  | 485931 |
|  |  | 485935 |
|  |  | 486002 |

The commercial fisheries port sampling program currently targets 550 biological samples from each of Area H and Area E for each rockfish species. In recent years this program would provide ample samples for fulfilling sample size objectives for inside waters of PWS, and outside waters from Area E and Area H, with the exclusion of black rockfish from Area E (Table 4). *Genetic tissue samples will be collected from the first 100 of these biological samples for each species and spatial stratum from commercial fisheries in the PWS/NGC area.*

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Year | Black Rockfish | | Yelloweye Rockfish | |
| Area E | Area H | Area E | Area H |
| 2015 | 53 | 799 | 598 | 372 |
| 2016 | 106 | 641 | 397 | 561 |
| 2017 | 5 | 555 | 389 | 579 |

To accommodate for the paucity of samples of Area E black rockfish (both inside and outside PWS), SFD port sampling programs will be utilized. Sport fish harvest from ports of Valdez and Whittier provide good spatial distribution of black rockfish from inside and outside waters of Area E (Table 5). 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. *Therefore, all black rockfish will be sampled for genetic tissues by SFD port samplers in the ports of Valdez and Whittier in the PWS/NGC area*.

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | Inside | Outside | Total |
| 2015 | Valdez | 125 | 545 | 670 |
|  | Whittier | 25 | 243 | 268 |
|  | 2015 Total | 150 | 788 | 938 |
| 2016 | Valdez | 62 | 246 | 308 |
|  | Whittier | 43 | 283 | 326 |
|  | 2016 Total | 105 | 529 | 634 |
| 2017 | Valdez | 16 | 252 | 268 |
|  | Whittier | 11 | 145 | 156 |
|  | 2017 Total | 27 | 397 | 424 |

In addition to these port sampling sources, research projects in PWS 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.

## Tissue Collection

Black and yelloweye rockfish will be collected either by hook and line or from commercial and sport fish catches. Hook and line sampling is expected to be generally non-lethal. Target sample size was set at 100 individuals per species for each of the seven statistical areas. Fin clips from individual fish will be excised, placed on and stapled to Whatman sample cards, placed in an airtight Pelican case with desiccant beads, desiccated in the field, and shipped to the laboratory for analysis.

## Genetic Analysis

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

For black rockfish, populations will be distinguished using up to ten loci. Two dinucleotide loci, *Sma1 and Sma3* developed from *S. maliger* (Wimberger et al. 1999), as well as eight 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) 92oC (5 min); 25 cycles of 92oC (30s) + 56oC (30 sec) + 1oC/s to 72oC + 72oC (20s); 72oC (30min) and 2) 92oC (5 min); 25 cycles of 92oC (30s) + 58oC (30 sec) + 1oC/s to 72oC + 72oC (20s); 72oC (30min).

For yelloweye, populations will be distinguished using up to nine loci previously used for examination of population structure in this species (Siegle et al. 2013). Two loci (*Sal1* and *3)* were developed byMiller et al. (2000), five loci (*Sme3, 5, 8, 12,* and *13)* by Seeb et al. (1999), and one locus *(Sru9)* by Miller et al. (2006), and one locus *(Sru20)* by Miller et al. 2000). Thermal cycler profiles for the two annealing temperatures will be as 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 2.4uL deionized formamide, 0.4uL GeneScanTM500TAMRATM size standard, 0.4uL loading dye (included in size standard box), and .5uL 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 relationships among statistical areas, *FST* will be calculated among all statistical area collections as well as pairwise between statistical area collections using *FSTAT* version 2.93 (Goudet 2001). A matrix of pairwise FST values between all pairs of statistical 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 three-dimensional space. A log-likelihood *G* statistics test as implement in *FSTAT* will be used to test for population (statistical 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 statistical area, over all statistical areas, and comparatively between statistical areas. Allelic richness is a measure of the number of alleles independent of sample size and will allow for comparison of the quantity 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 statistical areas decrease 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 distances matrices for association between genetic divergence and geographic distance 2 (km) over the range of the study. Geographic distance will be measured as shortest coastal point to point distance. Point locations for each statistical 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 continue to achieve adequate sample sizes for analysis in the subsequent year until objectives are met. 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 draft report will be completed by December 2020.

# RESPONSIBILITIES

Kathrine Howard, Fisheries Scientist I (SFD)

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) (CFD)

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 (CFD)

Duties: Oversee commercial fisheries sampling in the PWS/NGC areas and contribute to final report.

Andrew Olson, Fishery Biologist III (CFD)

Duties: Oversee commercial fisheries sampling in SEAK and contribute to final report.

Jeff Nichols, Fishery Biologist IV (SFD)

Duties: Oversee sport fisheries sampling in SEAK and contribute to final report.

Martin Schuster, Fishery Biologist II (SFD)

Duties: Oversee sport fisheries sampling in the PWS/NGC areas and contribute to final report.

Donnie Arthur, Fishery Biologist I (SFD)

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 genetic sample analysis and technician time to assist with port sampling. For 100 samples x 7 spatial strata x $35/sample x 2 species, genetic sample analysis is expected to cost approximately $49,000. Additional port sampler staff time may be required to reduce impacts on project operations with the addition of genetic sampling tasks. X hrs x X staff = $X

Projected FY19/FY20 costs:

|  |  |  |
| --- | --- | --- |
| Line Item | Category | Budget ($K) |
| 100 | Personnel | 15 |
| 200 | Travel | 0 |
| 300 | Contractual | 0 |
| 400 | Commodities | 49 |
| 500 | Equipment | 0 |
| Total |  | 64 |

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# Figures

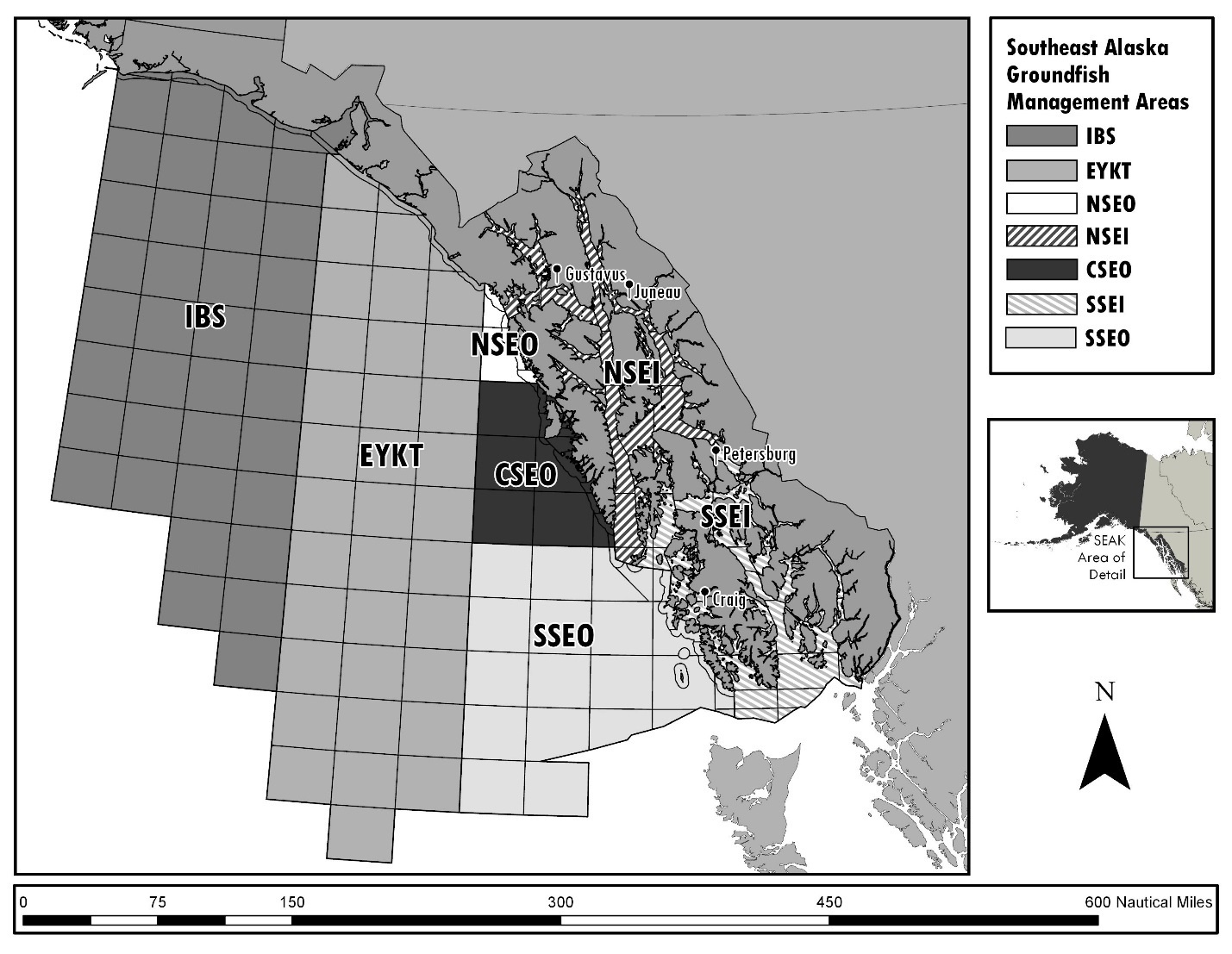


Figure 1. Southeast Alaska rockfish management areas.