Regional Operational Plan SF.1J.2019.XX

Operational Plan: Reproductive and Biological Sampling of Black Rockfish (*Sebastes melanops*) from Southeast Alaska and the Southern Gulf of Alaska

by

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July 2019

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

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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

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July 2019

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Signature/Title Page

|  |  |
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# Abstract

There is a limited understanding of the reproductive biology of black rockfish (*Sebastes melanops*) in Alaskan waters. The primary purpose of this project is to test the feasibility of attaining adequate sample sizes of mature black rockfish, which would provide the basis to estimate maturity schedules (length and age at maturity) and fecundity (both absolute and relative) in Southeast Alaska. An increased understanding of the reproductive biology of this rockfish species is necessary for science-based management and population dynamics modeling for stock assessment purposes. This project will be an important first step for generating estimates of these important biological parameters for these rockfish at the northernmost extent of their range. Sampling will take place in Southeast Alaska and the southern Gulf of Alaska.

Key words: Black rockfish, *Sebastes melanops*, Southeast Alaska, Gulf of Alaska, maturity, gonads, fish condition.

# INTRODUCTION

## Purpose

Recent interdivisional and interregional rockfish workshops have led the Alaska Department of Fish and Game (ADF&G) to focus efforts 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). During these workshops, the reproductive parameters of black rockfish and yelloweye rockfish were identified as a priority research topic.

The overall purpose of this project is to test the feasibility of attaining adequate sample sizes of female black rockfish to estimate length and age maturity schedules for Southeast Alaska (SEAK). This includes documenting sampling protocols for future collection, identification, preservation, and processing of black rockfish gonads; and collecting samples related to a population’s reproductive potential (i.e., age, length, fecundity, fish condition, etc.). Yelloweye rockfish will be sampled opportunistically during this study. Efforts will be divided between inside and outside waters in SEAK, which includes the southern Gulf of Alaska.

## Background

In general, rockfish grow slowly, mature late, and exhibit low rates of natural mortality (Love et al. 2002). These life history characteristics are more extreme in some rockfish species than others. A latitudinal effect on life history characteristics such as maturity has been documented in several fish species, including several rockfish species (*Sebastes* spp*.*)(Haldorson and Love 1991; Ni and Sandeman 1994; Hannah et al. 2009). Consequently, reproductive traits documented in more southerly locations may not be representative of those traits in more northern stocks. The Gulf of Alaska (GOA) represents the northernmost distribution of black rockfish (Mecklenburg et al. 2002) and estimates of maturity schedules are lacking from the area (Haldorson and Love 1991).

The GOA ecosystem is dynamic and undergoes regime shifts (Combes et al. 2009; Pozo Buil and Lorenzo 2015) that can affect species productivity and rates of growth and maturity (Anderson et al. 1997; Anderson and Piatt 1999). Maturity of fish in a population, which can vary spatially and temporally, and which is affected by environmental and fishing selective pressures that also vary in space and time (Trippel 1995; Law 2000; Hutchings 2005), can strongly affect a stock’s vulnerability to overfishing. Populations like rockfish, that can be harvested at a young age and mature at an old age, are vulnerable to overfishing (Myers and Mertz 1998) and therefore need informed management.

Length or age at maturity is an important life history parameter for population dynamic modeling and for estimating reproductive potential or spawning stock biomass (Clark 1991; Goodyear 1993). Rockfish species require robust management strategies given their life history attributes and significance to sport and commercial fisheries; however, data critical to implementing such strategies, such as age at maturity, is lacking for black rockfish in their northernmost range. The project described in this operational plan will further the work currently being done by ADF&G to begin identifying age and length and maturity, in addition to other reproductive parameters, for black rockfish and yelloweye rockfish in Prince William Sound and the northern GOA (Blain-Roth et al. 2019).

Although methods for estimating rockfish length or age at maturity have varied in the past (Wyllie-Echeverria 1987; Kronlund and Yamanaka 2001; Yamanaka et al. 2011), there is now a consensus that some portion of adult rockfish populations may skip spawn; these mature individuals can be confused with immature fish, and therefore histological methods are preferred over macroscopic methods for determining maturity status (Rideout et al. 2005; Rideout and Tomkiewicz 2011; Conrath 2017). The use of histological evaluation of thin-sectioned ovarian tissue (Wyllie-Echeverria 1987; Hannah et al. 2009) and sampling during peak ovary development (Westrheim 1975; Gunderson et al. 1980) are believed to decrease error in assigning maturity status to fish. These methods will be used to accomplish the primary objectives of this project.

# Objectives

## Primary Objective

1. Generate length and age at maturity schedules for black rockfish in 3 different spatial strata in SEAK that can be utilized for the Southern Southeast Inside (SSEI), Southern Southeast Outside (SSEO), and Central Southeast Outside (CSEO) groundfish management areas).

## Secondary Objectives

1. Target length distributions that are likely to encompass black rockfish *L*50 and *A*50 are as follows:
   1. 20% of samples with length <35 cm, 60% of samples with length 35–50 cm, 20% of samples with length >48 cm.
2. Determine the stage of gonad development using histology sampling.
3. Collect ovaries for estimating the mean density of oocytes or embryos per gram of ovary for each mature female.

### Tasks

1. Collect biological data on fish condition including:
   1. lengths, weights, and otoliths;
   2. liver weights;
   3. tissue samples; and
   4. stomach content samples.
2. Record location, depth of capture, fishing effort (time), and other environmental variables at fishing locations.

# Methods

## Study Design and Data Collection

### Study Area

Black rockfish will be collected in 3 of the 7 groundfish management areas, specified for commercial fisheries in SEAK (Figure 1). Sampling trips for black rockfish will be based out of the following 3 SEAK ports: Sitka (CSEO), Craig (SSEO), and Ketchikan (SSEI). The location of sampling from each port will be dictated by weather conditions, presence of other fishing effort, and past fishing experience. To avoid local depletion, an attempt will be made to distribute fishing effort as evenly as possible across the study area.

#### Sitka - CSEO

The outside waters of the Sitka area roughly correspond to the CSEO groundfish management area (Figure 1). CSEO includes all the waters west of Chichagof and Baranof islands and is bound on the northern border at 57°30.000’ latitude and the south by a line projecting south of Cape Ommaney. Waters in the area are characterized by a diversity of coastal habitat that is often defined by high-relief rocky islands, tidally influenced bays, and multiple sounds and straits. Many of these areas are exposed to strong tidal currents. While the water and land around Sitka exhibit a complex scheme, much of the waters of CSEO are open ocean. The waters of CSEO are a mixing point for inland nutrient rich waters and the warm currents coming up along the outer coast.

#### Craig - SSEO

The western shore of Prince of Wales (POW) Island is comprised of numerous islands that separate it from outside waters of the Pacific Ocean. Two groundfish management areas occur on western POW, which are the SSEO and SSEI areas (Figure 1). Waters in the SSEO management area are generally the waters to the west of the western most islands of the coast of POW and is bound on the northern border by Cape Lynch at 56°N latitude and the south by a line projecting south of Cape Muzon into Dixon Entrance. Waters in the SSEI are the remaining waters surrounding POW, including the western shore of POW to the boundary islands of the SSEO management area. For this project, the fishing based out of Craig will primarily occur in the SSEO management area.

#### Ketchikan - SSEI

The inside waters of the Ketchikan area (SSEI; Figure 1) includes all waters east and north of a line from the southernmost tip of Caamano Point to 54° 40’ N latitude, 131° 45’ W longitude, and north of a line from 54° 40’ N latitude, 131° 45’ W longitude to 54° 42.48’ N latitude, 130° 36.92’ W longitude. This area includes the islands of Revillagigedo, Gravina, Annette, and Duke, as well as the Alaska mainland to the east, from the middle of the Cleveland Peninsula south to Portland Canal. These waters are protected from the outer coast to the west by POW Island (6,674 km2). The inside waters of the Ketchikan area connect to the open ocean along the southern Alaska-Canada boundary at Dixon Entrance. These waters are characterized by a diversity of coastal habitat that is often defined by high relief rocky islands, tidally influenced bays and estuaries, and a network of passages and channels.

### Field Collection

Black rockfish will be collected onboard ADF&G vessels by hook and line from July - October 2019, until sample sizes are obtained, or weather prohibits sampling. A minimum of a 2-person crew will capture and sample fish. Sampling will occur opportunistically and will be spread spatially across the study area. Females will be the targeted sex; however, a subsample of <20% males is expected due to misidentification in the field. The length distribution of black rockfish samples will follow the approach employed by Blain et al. (2019). Sampling goals for predetermined size bins of black rockfish are as follows:

1. 20% of samples with length <35 cm
2. 60% of samples with length 35–50 cm
3. 20% of samples with length >50 cm.

Data will be recorded on the datasheets provided in Appendices A1 and A2. Datasheets will be printed on Rite in the Rain or weatherproof paper.

#### Timing of Collection

Samples will be collected starting in July 2019 and will continue to be collected until sample size goals for Objective 1 are met (see “Sample Size” section, below) or until weather conditions prohibit sampling. Fecundity samples (i.e., density of oocytes and embryos per gram of ovary) for Secondary Objective 3 will be collected from fish when available.

Field collection will occur from ADF&G vessels using hook-and-line sampling. When a target species is caught, the following will occur:

1. Record capture depth (m) from the vessel’s depth finder and GPS location (Decimal Degrees) from the vessel’s chart plotter at the time that the fish is hooked.
2. For fish that are retained, a numbered Floy tag[[1]](#footnote-2) or zip tie will be attached to the dead fish to assign the fish an ID number that pairs data and samples for each fish.

#### External assessment

The following external feature data will be collected immediately following capture of all black rockfish, regardless of whether the fish is released or retained:

1. The presence of esophageal eversion, a barotrauma injury, will be documented for each retained rockfish.
2. Sex will be identified by external genitalia (Figure 2) and macroscopic observation of the stage of gonads (if applicable; Table 1). Notes on each fish will be recorded on the datasheet, including if the fish is losing eggs or larvae due to the change of pressure. If loss is occurring, the fish will immediately be placed in a 2-gallon plastic bag or dissected for ovary extraction immediately.
3. Fork length (FL) and total length (TL) will be measured for each rockfish, to the nearest millimeter, using a measuring board.
4. If regurgitation is not observed, stomach contents will be collected and stored in a Ziplock bag labeled with fish number and species.
5. A fin clip for genetic processing will be collected following methods of Howard et al. (2019). Final samples will be delivered to the ADF&G Genetics lab.

#### Internal Assessment and Field Processing

If an individual (black or yelloweye rockfish) is to be sacrificed, it will be subdued with a concussive blow to the top of the head and the following data will be collected:

1. Sex will be verified via internal gonad examination and recorded.
2. Whole fish body weight will be determined to the nearest 0.01 kg using a motion-calibrated scale and recorded.
3. Gonad stage will be identified following methods of Westrheim (1975), described in Table 1, removed (see methods described in “Gonad Identification and Removal” section below), and recorded.
4. The liver will be removed, and liver weight will be recorded to the nearest 0.001 kg. (per request from Ben Williams, ADF&G, Fisheries Scientist I, Juneau).
5. Following liver, organ, and gonad removal, fish will be reweighed to determine somatic weight.
6. A tissue sample (with no skin) will be collected from the left upper dorsal area of the fish (Figure 3). The tissue sample will be put in a Ziplock bag and frozen for future analysis in a separate project. Samples will be stored with SF staff until project is complete and samples can be sent to the appropriate location for stable isotope analysis.
7. Both otoliths will be removed from each fish. The excess tissue will be removed from the otoliths and they will be stored in 48-deep well plates.
8. A sample of the stomach contents will be collected whenever possible. The entire stomach and its contents will be removed and stored in a whirl pack and frozen for future analysis. If stomach contents are collected, this will be noted. Samples will be stored at the Juneau ADF&G office following collection (per request from Ben Williams, Fisheries Scientist I, ADF&G, Juneau).

#### Gonad Identification and Removal

An incision will be made from the vent along the ventral side of the fish to remove the gonads. Flesh and skin along the side of the gut cavity will be cut away carefully, exposing the organs. Gonads can be removed by separating the gonads from membranous connective tissue. Gonads must not be punctured. Whole gonads will temporarily be stored in plastic bags labeled with the corresponding fish ID number and chilled in a cooler or refrigerator. The gonads will never be placed directly in contact with ice or in a freezer to avoid cell lysis due to freezing. A photograph will be taken of each set of gonads with the fish number visible in the photo, and notes describing the macroscopic stage of gonads will be recorded. A fresh gonad weight will be collected whenever possible. The gonads will then be transferred to storage jars and fixed in a formalin-based fixative (Formal-Fixx) with a ratio of 10:1 of fixative to tissue volume.

### Sample Size—Length and Age at Maturity (Objective 1)

To assess the precision of estimates for length and age at maturity and determine appropriate sample sizes, a series of simulations were conducted using results obtained by Hannah et al. (2009). The simulations consisted of the following steps:

1. For a given sample size, lengths (ages) were simulated from a uniform distribution.
2. Maturity status was simulated using parameter estimates from the logistic regression performed in Hannah et al. (2009).
3. The same techniques described in the Data Analysis section below were employed, resulting in a credible interval for *L50* or *A50*.
4. Recorded the width of the interval in terms of percent of estimate where

|  |  |
| --- | --- |
|  | (1) |

The procedure described above was performed 1,000 times, and the maximum percent width recorded served as a reasonable upper bound on the possible width of credible intervals obtained, using a given sample size.

After performing the above procedure for both lengths and ages, it was concluded that a sample size of 80 female black rockfish per strata will be required. The sampling region will be stratified into 3 areas: CSEO, SSEO, and SSEI, which will require a minimum of 240 female black rockfish to be harvested for this project.

## Data Reduction

For field-collected samples, data will be recorded on field datasheets (Appendices A1 and A2) printed on Rite in the Rain or weatherproof paper. The project leader will ensure that all field data are entered in a Microsoft Excel spreadsheet upon return from the field. The fields and data in the Excel file will be set up so that input into R software (R Core Team 2017) can be done efficiently. A separate datasheet will be used for data collected in the laboratory (e.g., fecundity, development stage from histology, and fixed ovary weight). These data will be simultaneously entered into Excel and recorded on the datasheet, which will be a backup hard copy. Datasheets will be retained and filed for future data quality control. Data will be uploaded to the ADF&G data warehouse OceanAK.

## Data Analysis

Analyses of samples collected during this project will occur as part of a Master of Science project that is anticipated to begin in the fall of 2020.

### Lab and Other Non-field Processing

#### Gonads–Histology and Fecundity

Collected ovaries will be assessed for maturity (mature = 1 and immature = 0) and stage of ovary development (stages 1–7; Table 1) in the lab. Crude maturity will be assigned based on macroscopic observations using criteria adapted from Westrheim (1975; Table 1). Histological examinations will be used to validate maturity values and stage of development based on the stage of the most advanced oocyte or embryo described in keys from Bowers (1992) and Bobko and Berkeley (2004).

To prepare histological slides, sections will be prepped from preserved ovaries by embedding them in paraffin wax, thin-sectioning to 5–7 µm, and then staining with hematoxylin and eosin Y while on a slide (West 1990; Hannah et al. 2009). Histological preparation will be conducted through Veterinary Services at the University of Alaska Fairbanks or will be put out to bid if the cost is >$5,000. Slides will be viewed under a compound microscope. The final determination of maturity and reproductive activity will be assessed from histological observations following the methods of Bowers (1992) and Bobko and Berkeley (2004).

#### Fecundity

Fecundity will be estimated using the gravimetric method (Murua et al. 2003). Each ovary will be weighed in the lab to produce a fixed total weight (*wi*) in grams. Three spatial subsamples from each ovary will be used to assess fecundity for each ovary (Bobko and Berkeley 2004). A subsample will be taken from the anterior, interior, and posterior sections of each ovary. Subsamples will be weighed to the nearest 0.001 g. Eggs will be spread apart on a gridded petri-dish using a fine brush, and oocytes or embryos for each subsample will be enumerated using an automated particle counter. The number of oocyte or embryos (*oi*) for each subsample will be divided by the subsample weight (*wi*) to produce a density of eggs per gram of ovary () and summed over the number of samples to produce a mean density:

|  |  |
| --- | --- |
|  | (2) |

where *n* is the number of subsamples (at least 3).

Subsamples (*n*) will continue to be collected until a coefficient of variation (CV) of 5% or less is achieved for the mean density of oocytes or embryos per gram of ovary (Kjesbu 1989; Murua et al. 2003):

|  |  |
| --- | --- |
|  | (3) |

where *s* is the standard deviation of mean density of eggs per gram of ovary.

The mean density will then be multiplied by the total weight of the ovary (*Wo*) to yield an estimate of absolute fecundity (*AF*) (Murua et al. (2003)):

|  |  |
| --- | --- |
|  | (4) |

Absolute fecundity will be estimated for stages 3–5 (mature through ripe) with the possibility that each stage will be treated separately in the analysis of fecundity to account for the possibility of fecundity downregulation (aborted eggs over the course of development).

Relative fecundity (*RF*) will be estimated by dividing the absolute fecundity by fish weight (*Wfish*):

|  |  |
| --- | --- |
|  | (5) |

### Length and Age at Maturity (Objective 1)

The maturity values from histological examination of black rockfish will be used to estimate the probability of maturity as a function of length (age). A Bayesian analysis will be performed to obtain estimates and credible intervals for *L50* and *A50*. Probability of maturity will be modeled as a Bernoulli(*p*) random variable where

|  |  |
| --- | --- |
|  | (6) |

Weakly informative Normal(0, 100) priors will be used for both . Draws from the posterior distribution of will be obtained using Program R (R Core Team 2017) and the rjags package (Plummer 2016). After obtaining draws for , draws from the posterior distribution of *L50* (*A50*) will be calculated as follows:

|  |  |
| --- | --- |
|  | (7) |

A 90% central credible interval will be calculated from the posterior distribution. The median of the posterior distribution will be used as the estimate. The model will be fit once using length as the explanatory variable and once using age, resulting in unique estimates and credible intervals for *L50* and *A50*.

##### Otolith-based Age

Ages will be determined from otoliths using the break-and-burn method (Chilton and Beamish 1982) by ADF&G Commercial Fisheries Division staff in Juneau, Alaska..

##### Hepatosomatic Index (HSI)

As a measure of energy reserves, a hepatosomatic index (HSI) will be calculated following the methods of Busacker et al. (1990). The liver will be removed and weighed. Somatic weight (grams) will be calculated after removing the organs, liver, and ovaries (i.e., gutted weight):

|  |  |
| --- | --- |
| HSI = (liver weight/somatic weight) × 100. | (8) |

# Schedule and Deliverables

The timeline for this project does not include specific dates for field or office activities; actual sampling dates (for each location) will depend on a variety of factors, including weather conditions, staff availability, etc.

|  |  |
| --- | --- |
| Dates | Activity |
| July-October 2019 | Prepare sampling supplies and vessels.  Sampling trips begin; length will vary from 1–7 days; data editing and entry into spreadsheets will occur as the season progresses. |
| Fall 2020 | Master of Science student begins graduate project through UAF. Samples will be processed in the lab as part of the graduate student’s project. |

# responsibilities

*Kercia Schroeder, Fishery Biologist II, Co-Principal Investigator*

Duties: Co-author on operational plan. Supervises overall project and data compilation. Edits, analyzes, and reports data.

*Troy Tydingco, Fishery Biologist III, Sitka Area Management Biologist, Co-Principal Investigator*

Duties: Co-author on operational plan and reports; assists with field work coordination for sampling out of Sitka, purchasing sampling supplies, and data entry.

*Craig Schwanke, Fishery Biologist III, Craig Area Management Biologist, Co-Principal Investigator*

Duties: Co-author on operational plan and reports; assists with field work coordination for sampling out of Craig, purchasing sampling supplies, and data entry.

*Kelly Reppert, Fishery Biologist III, Ketchikan Area Management Biologist, Co-Principal Investigator*

Duties: Co-author on operational plan and reports; assists with field work coordination for sampling out of Ketchikan, purchasing sampling supplies, and data entry.

*Ben Buzzee, Biometrician*

Duties: Provides input to sampling design and operational plan. Provides support during data analysis and reporting.

*Ben Williams, Fishery Scientist*

Duties: Provides input and guidance on analytical and laboratory methodology, as well as being a member of the UAF graduate committee.

*Jeff Nichols, Regional Research Coordinator*

Duties: This position reviews the operational plan and the FDS report and assists in obtaining funding for the project.

# Budget summary

FY 17

|  |  |  |
| --- | --- | --- |
| Line item | Category | Budget ($K) |
| 100 | Personal Services | 3 |
| 200 | Travel | 6 |
| 300 | Contractual | 4 |
| 400 | Commodities | 15 |
| 500 | Equipment | 0 |
| Total |  | 28 |

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# Tables

Table 1.–Macroscopic observation of rockfish ovary for assessment of maturity and development stage.

|  |  |  |
| --- | --- | --- |
| Stage of development | | Macroscopic description of ovaries |
| 1 | Immature | small, translucent, and peach or yellow in color |
| 2 | Maturing (immature) | small to medium, firm, translucent or opaque, yellowish in color |
| 3 | Mature | large, translucent or opaque, yellow in color |
| 4 | Fertilized | large, may be hydrated or loose (not firm), translucent, yellow-orange in color |
| 5 | Ripe | large, hydrated or loose, yellow or gray in color with black dots (presence of eye pigment from embryos or larvae) |
| 6 | Spent | medium to large, flaccid, gray, red, or purple in color, residual larvae may be present |
| 7 | Resting | medium, firm, gray-red, dark or black blotches |

*Source*: Westrheim 1975.

# Figures

A picture containing text, map

Description automatically generated

Figure 1.–Southeast Alaska groundfish management areas designated for commercial fisheries.



Figure 2.–Identification of external genitalia of rockfish.



Figure 3.–Location of tissue sample collection.

# Appendix A: Field data sheets

Appendix A1.–Field data sheet for capture and biological information.



Appendix A2.–Field data sheet for catch and effort.



1. Product names used in this publication are included for completeness but do not constitute product endorsement. [↑](#footnote-ref-2)