

Regional Information Report No. 4K09-06

**Special Project Plan: 2009 Bottom trawl survey of
crab and groundfish: Kodiak, Chignik, South
Peninsula, and Eastern Aleutian Districts**

by

Kally Spalinger

May 2009

Alaska Department of Fish and Game

Divisions of Sport Fish and Commercial Fisheries



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AND GROUND FISH: KODIAK, CHIGNIK, SOUTH PENINSULA, AND
EASTERN ALEUTIAN DISTRICTS**

by
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Division of Sport Fish, Research and Technical Services
333 Raspberry Road, Anchorage, Alaska, 99518-1565

May 2009

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ABSTRACT

This report specifies the objectives and methods for special projects during the Alaska Department of Fish and Game 2009 bottom trawl survey of crab and groundfish in the Kodiak, Chignik, South Peninsula, and Eastern Aleutian districts of the Westward Region. This Special Project Plan is used in conjunction with the Standard Project Operational Plan (Spalinger and Cavin 2004), which describes the annual trawl survey sampling protocols. Special shellfish projects for 2009 include: a legal-size-male Tanner crab tagging project; multiple tows within selected offshore stations in the Kodiak District to assist in determining the variance of Tanner crab population estimates; hemolymph sample collection for genetic analysis to determine bitter crab disease presence; and collection of chela height measurements for male maturity analysis. Special groundfish projects for 2009 include: collection of stomachs from walleye pollock *Theragra chalcogramma*, Pacific cod *Gadus macrocephalus*, flathead sole *Hippoglossoides elassodon*, arrowtooth flounder *Atheresthes stomias*, Pacific halibut *Hippoglossus stenolepis*, northern rock sole *Lepidopsetta polyxystra*, and spiny dogfish *Squalus acanthias* for a National Marine Fisheries Service (NMFS) food habits study, and collection of otoliths and ovaries from various species of female rockfishes *Sebastes spp.* for a reproductive study being conducted by NMFS.

Key words: Tanner crab, shellfish, groundfish, trawl survey, Kodiak, Alaska Peninsula, Chignik, Eastern Aleutian Islands, special projects

INTRODUCTION

From June through September 2009, the Alaska Department of Fish and Game (ADFG) will conduct a bottom-trawl survey in portions of the Westward Region (Figure 1). The survey will focus on waters of historic red king and Tanner crab abundance around Kodiak Island and the Alaska Peninsula from Cape Douglas to False Pass, as well as the Eastern Aleutian Islands. The survey results will be used to estimate the abundance of Tanner crab *Chionoecetes bairdi* and red king crab *Paralithodes camtschaticus* populations and to determine species composition and length frequencies of the groundfish catch by haul and area.

This report details the survey schedule, station maps, and sampling procedures for special projects during the 2009 Westward Region trawl survey. All standard sampling protocols used during the trawl survey are described in detail in the Standard Project Operational Plan (Spalinger and Cavin 2004). Any changes to standard procedures, or special projects associated with the 2009 survey are described in this document.

OBJECTIVES

Special shellfish objectives for the 2009 trawl survey are to tag legal-size-male Tanner crabs from the Northeast and Eastside sections of the Kodiak District, and the Eastern Aleutian District. Tag recoveries show movement between the time of the survey and the winter commercial fishery and also verify shell condition estimates. In addition to the standard collection of hemolymph smears in Alitak Bay to look for bitter crab disease (Spalinger and Cavin 2004) additional hemolymph samples will be collected, preserved, and used to look for the presence of genetic markers consistent with the parasitic dinoflagellate causing the disease. Finally, measurements of chela height collected from male Tanner crabs in Marmot Bay will be expanded to include the Eastside and Westside sections of the Kodiak District.

Special groundfish objectives are to collect whole stomachs and contents from walleye pollock *Theragra chalcogramma*, Pacific cod *Gadus macrocephalus*, flathead sole *Hippoglossoides elassodon*, arrowtooth flounder *Atheresthes stomias*, Pacific halibut *Hippoglossus stenolepis*, northern rock sole *Lepidopsetta polyxystra*, and spiny dogfish *Squalus acanthias* from Marmot and Chiniak bays and to collect otoliths and ovaries from Pacific ocean perch *Sebastes alutus*,

northern rockfish *Sebastes polyspinis*, rougheye rockfish *Sebastes aleutianus*, blackspotted rockfish *Sebastes melanostictus*, and shortraker rockfish *Sebastes borealis* for the National Marine Fisheries Service (NMFS). Beginning in 2009 we will speciate blackspotted rockfish from rougheye rockfish. Blackspotted and rougheye rockfish were previously considered a single species but are now recognized as two separate species (Orr and Hawkins, 2008). For the sixth consecutive year, the sex of each measured spiny dogfish and skate *Raja* and *Bathyraja* will be recorded. Multiple tows for selected stations in Marmot Bay and the Barnabas Gully of the Kodiak District will occur for the purpose of determining the accuracy of Tanner crab station population estimates.

METHODS

SURVEY AREA AND TRAWL PROCEDURES

The 27.4 m ADF&G research vessel *Resolution* will conduct survey trawl tows in areas of known king and Tanner crab habitat throughout the Kodiak, Chignik, and South Peninsula districts of the Westward Region (Figure 1, Appendices A1-15). Akutan Island, Unalaska Bay, Beaver Inlet, Makushin Bay, and Pumicestone Bay in the Eastern Aleutian District will be included in the 2009 survey (Appendices A14 and A15). Tows will be made using a 400-mesh eastern otter trawl.

This year duplicate tows will occur in some of the large offshore stations in the Northeast and Eastside sections of the Kodiak District. Stations were selected based on large Tanner crab population estimates in previous surveys (Spalinger *in prep*, Spalinger 2008, Spalinger 2007, Spalinger 2006). Four stations in Marmot Bay (Appendix A2) and four stations in the Barnabas Gully (Appendix A3) have been divided into four quadrants. In addition to the traditional tow in these stations, which will be sampled according to the Standard Project Operational Plan (Spalinger and Cavin 2004), two to three additional tows, depending on time and weather, will be made in different quadrants of the stations. Stations with multiple tows will be surveyed in the following order of priority: Marmot Bay: 255, MONX, 256, and 283X (Appendix A2); Barnabas Gully: 561, 655, 696, and 589 (Appendix A3). Total catch from the extra tows will be weighed, but only Tanner crabs will be sorted and weighed individually. Crabs will be handled according to the Standard Project Operational Plan (Spalinger and Cavin 2004).

CRAB SAMPLING

Selected legal-size-male Tanner crabs captured from the Northeast and Eastside sections of the Kodiak District (Appendices A1-3), as well as from the Eastern Aleutian District (Appendices A14 and A15), will be tagged. After all the crabs have been measured, preferred legal-size males missing less than two limbs and without reflex impairment will be tagged using the following method. A small hole will be made in the right side of the carapace, above the lower, left corner of the branchial lobe (Figure 2). The hole can be made using either a handmade punch with a short nail attached that will not penetrate deeply into the body cavity, or a tagging gun with an epoxy stopper attached that limits the depth the needle can be inserted. Once the hole is made, a dart with a numbered disc tag attached will be inserted into the hole. The tag number, carapace width, and shell condition will be recorded on the Tanner crab tagging form (Appendix B1) and a dorsal and ventral photo of each crab should be taken, with a label clearly indicating the tag number. The latitude and longitude of the location where the crabs are released should also be recorded on the tagging form. Tagged crabs will be recovered during the January 2010

commercial Tanner crab fishery if population estimates are sufficient for an opening. A detailed operational plan for this project is in development (Sagalkin and Mattes *in prep*).

Samples of hemolymph from Tanner crab in Alitak Bay (Appendix A5) will be preserved in ethanol for future genetic testing to identify parasite DNA (Appendix C1). Samples for this test will be collected in conjunction with the standard hemolymph smears as described in the Standard Project Operational Plan (Spalinger and Cavin 2004). Information should be recorded on an ADF&G Crab Data Form (Appendix B2). After collection, the preserved samples will be stored in a dark location at room temperature until arrangements can be made to have them sent to a genetics laboratory for testing. Results from the genetic testing will be compared to results from the hemolymph smears to determine smear accuracy, and the feasibility of replacing the hemolymph smears with genetic testing in the future.

Chela height measurements from male Tanner crabs will be collected from the Eastside and Westside sections of the Kodiak District (Appendices A3 and A8). Protocol for chela height measurements will follow the standard procedures in Spalinger and Cavin, 2004, with one exception. Measurements will be collected randomly from 50 male Tanner crab >50 mm in carapace width (CW) at each station. The cruise leader may adjust the sampling plan as needed to accommodate high numbers of crabs encountered on the Eastside survey. In this situation the cruise leader may choose to measure chela from every third station, or modify the plan in other ways to allow for timely return of captured crabs to the water. Cruise leaders will detail exact sampling procedures to be kept with data from each haul so methods are repeatable and data analysis can be conducted accordingly.

GROUND FISH SAMPLING

During the Marmot and Chiniak Bay (Appendices A1 and A2) survey tows, stomach samples from walleye pollock, Pacific cod, flathead sole, arrowtooth flounder, Pacific halibut, northern rock sole, and spiny dogfish will be collected. Sample sizes are 15 to 40 stomachs dependent on size group (Appendix D1), with a maximum number of 20 stomachs per species per haul. The goal is to sample two to three species from every haul.

Throughout the survey, otoliths and ovaries from female northern, rougheye, blackspotted, and shortraker rockfish as well as Pacific ocean perch will be removed and preserved. For each five cm size group two samples will be collected (Appendix E1). To assist with speciating rougheye and blackspotted rockfish distinctive differences are shown in Appendix E1. If there is discrete spotting on the first dorsal fin the fish should be called blackspotted rockfish.

In 2009, we will continue to determine the sex of each measured skate and shark. Males are easily identified by the presence of claspers (Figure 3). Small, immature skates and sharks that are difficult to sex will be recorded as unknown.

DATA FORMS AND SAMPLE CUSTODY

Completion and proper disposition of data and samples is the same for the special projects and standard data. It is the responsibility of the cruise leader to ensure all samples and data forms are completed and removed from the boat after each survey leg. Forms are to be organized according to project and put into sequential order by tow, starting with the first tow on top. All data removed from the vessel is to be taken directly to the shellfish office and given to Kally Spalinger, the lead trawl-survey biologist to prevent lost data. Frozen samples must be well

labeled when removed from the R/V *Resolution* freezer and transferred to one of the freezers at the Kodiak Fisheries Research Center, until they can be processed or shipped to their final destination. Samples preserved in formalin should be stored in a location with adequate ventilation until they are shipped. It is also important to inform the lead trawl-survey biologist of the location of all stored samples.

SURVEY EQUIPMENT CHECKLIST

Stomach sampling

- ✓ Specimen forms
- ✓ Specimen labels
- ✓ Five-gallon buckets with lids
- ✓ Formalin
- ✓ Stomach bags
- ✓ One-liter plastic bottles
- ✓ Baking soda
- ✓ Luggage tags
- ✓ 1/8 cup measuring cup
- ✓ Hazardous materials bucket

Tanner tagging

- ✓ Darts with Peterson disc tags
- ✓ Tagging guns with “stops”
- ✓ Handmade nail punch
- ✓ Tagging forms

Genetic hemolymph collection

- ✓ Deep-well plates (96 wells of 1.2 ml capacity)
- ✓ Rubber well caps
- ✓ Syringes
- ✓ ADF&G Crab Data forms

Rockfish ovary and otolith collection

- ✓ Specimen forms
- ✓ Specimen labels
- ✓ Tally sheets
- ✓ Stomach bags
- ✓ Five-gallon buckets with lids
- ✓ Formalin
- ✓ One-liter plastic bottles
- ✓ Baking soda
- ✓ 70% Ethanol
- ✓ Otolith vials
- ✓ Dissecting supplies

PERSONNEL AND SURVEY SCHEDULE

R/V Resolution crew – Captain Denis Cox Jr., Kurt Pederson, Gary Wilson

*Chiniak Bay –
June 16 and 17*

Kally Spalinger (cruise leader)
Dave Gilliland
Collin Hakkinen
Sherry Barker
Lindsey Bidder

*Marmot Bay –
June 18-22*

Kally Spalinger (cruise leader)
Sonya ElMejjati
Dave Gilliland
Collin Hakkinen
Sherry Barker

*Eastside Kodiak –
June 29 to July 16*

Nicholas Sagalkin (cruise leader)
Mark Stichert
Dave Gilliland
Collin Hakkinen
Sherry Barker
Lindsey Bidder (Alitak)

*South Alaska Peninsula, Chignik, and
The Eastern Aleutians -
July 23 to August 28*

*Westside Kodiak and North Mainland –
September 8-18*

-continued-

Kally Spalinger (cruise leader 1)
Mark Stichert (cruise leader 2)
Dave Gilliland
Collin Hakkinen
Sherry Barker

Sonya ElMejjati (cruise leader)
Aaren Ellsworth
Dave Gilliland
Collin Hakkinen
Sherry Barker
Lee Hulbert

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FIGURES

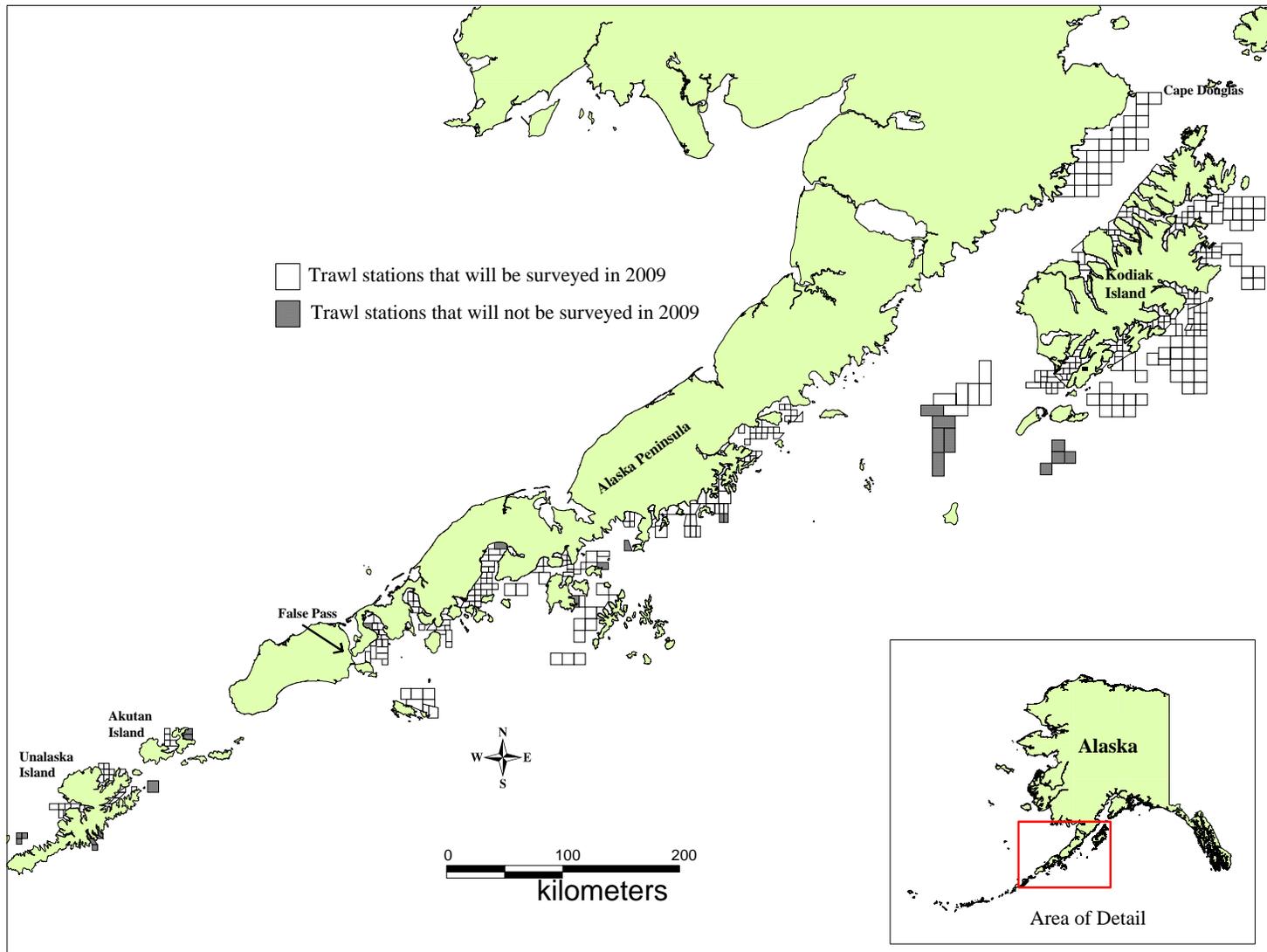


Figure 1.—Westward Region trawl survey area.

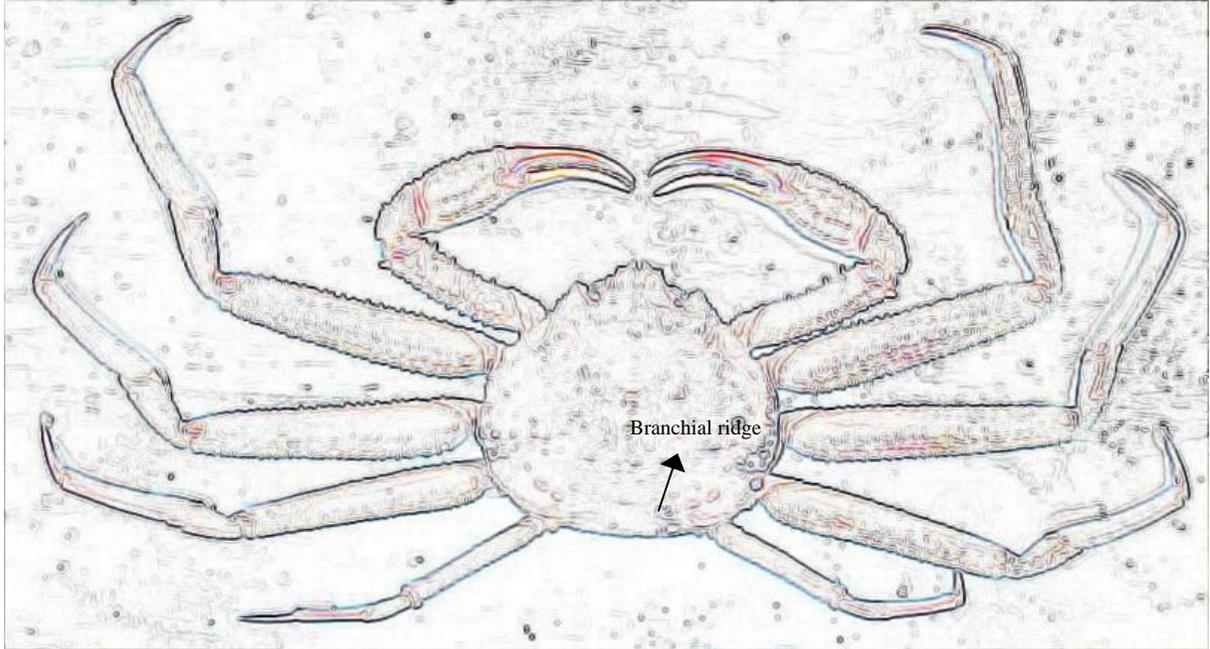


Figure 2.—Diagram of tag insertion location below branchial ridge on Tanner crab.

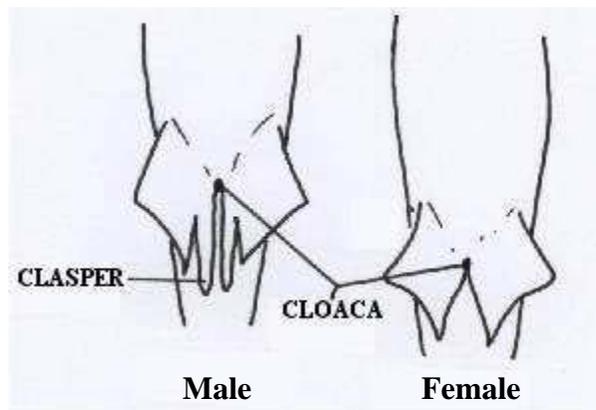
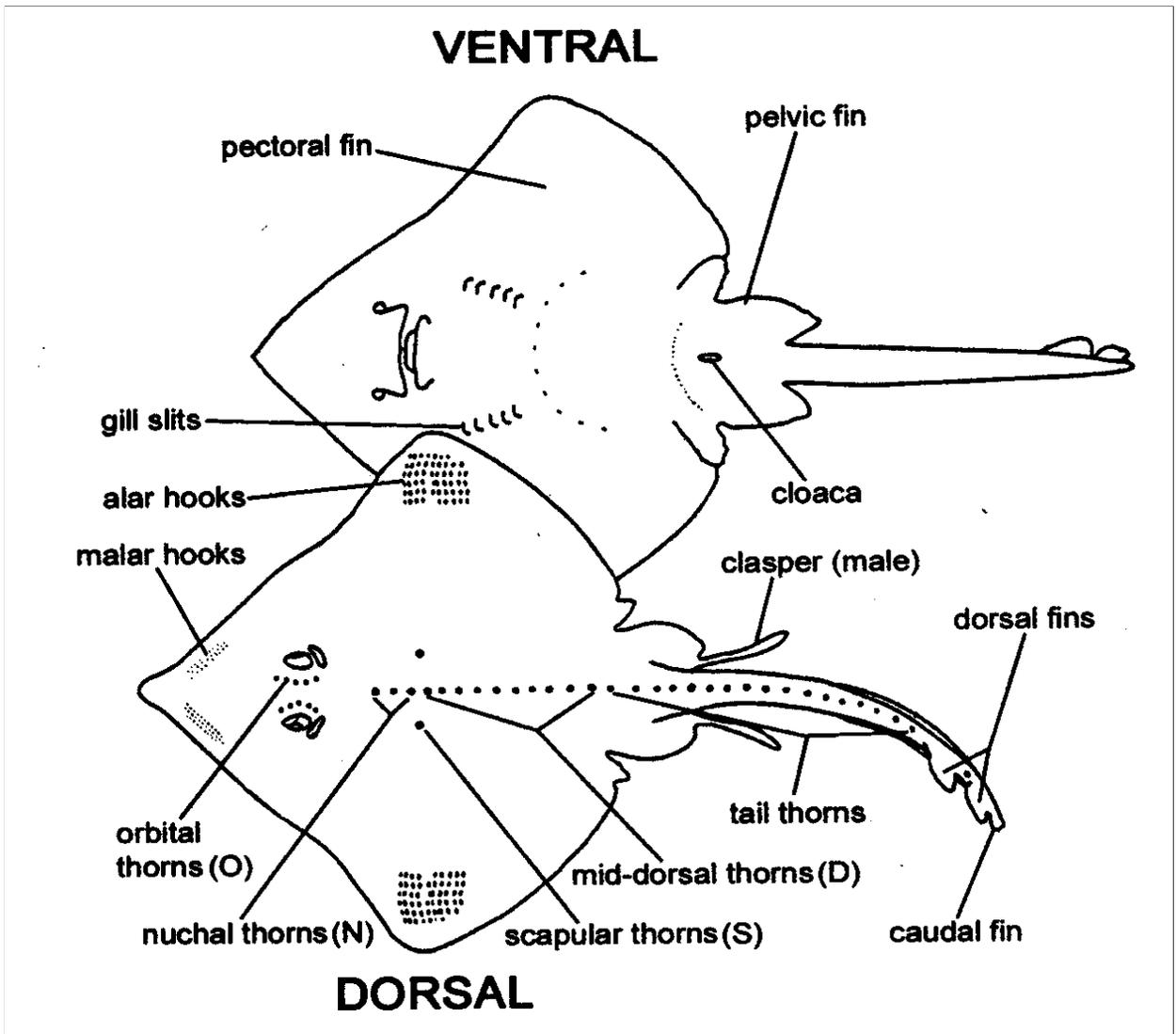
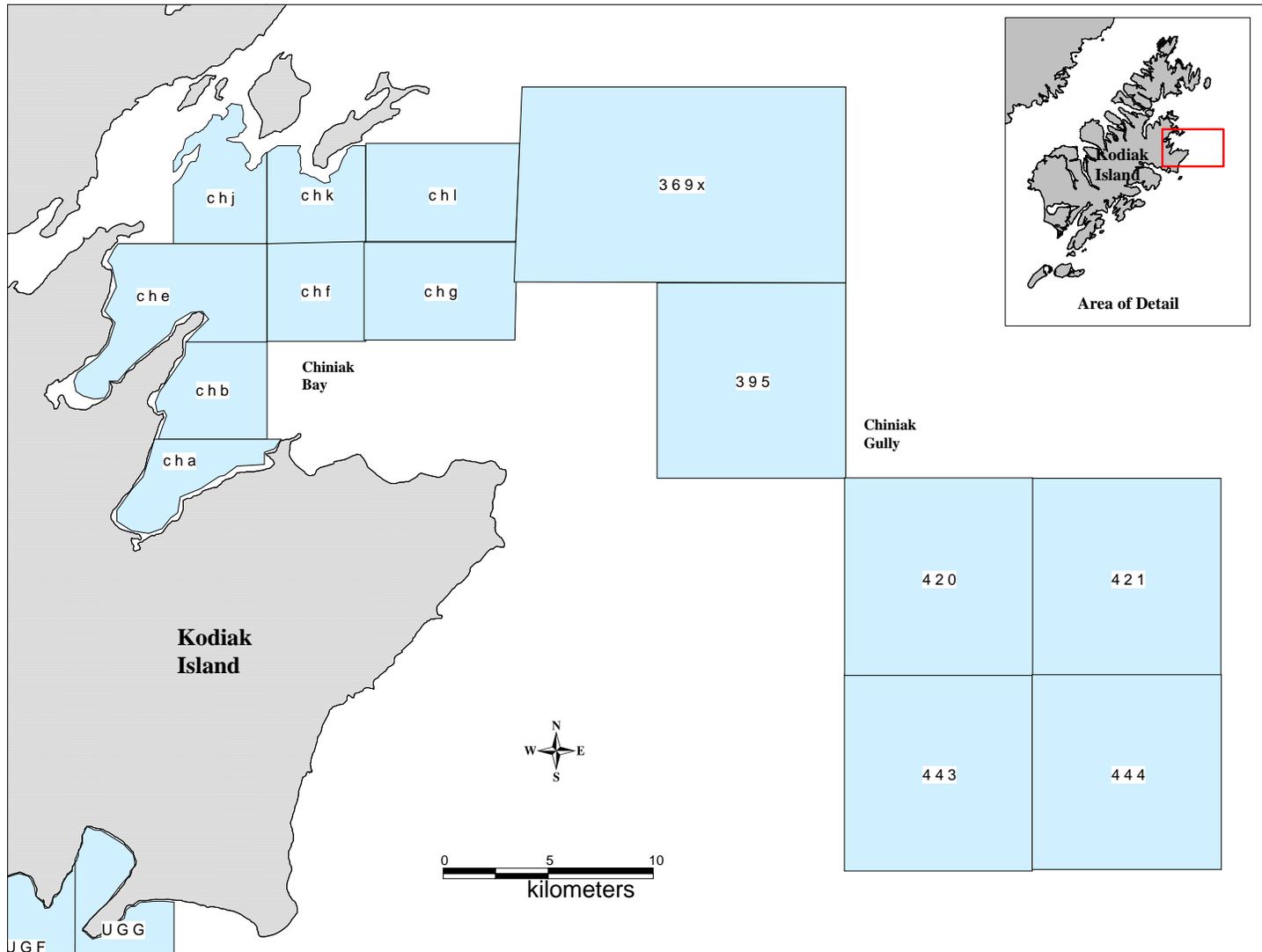
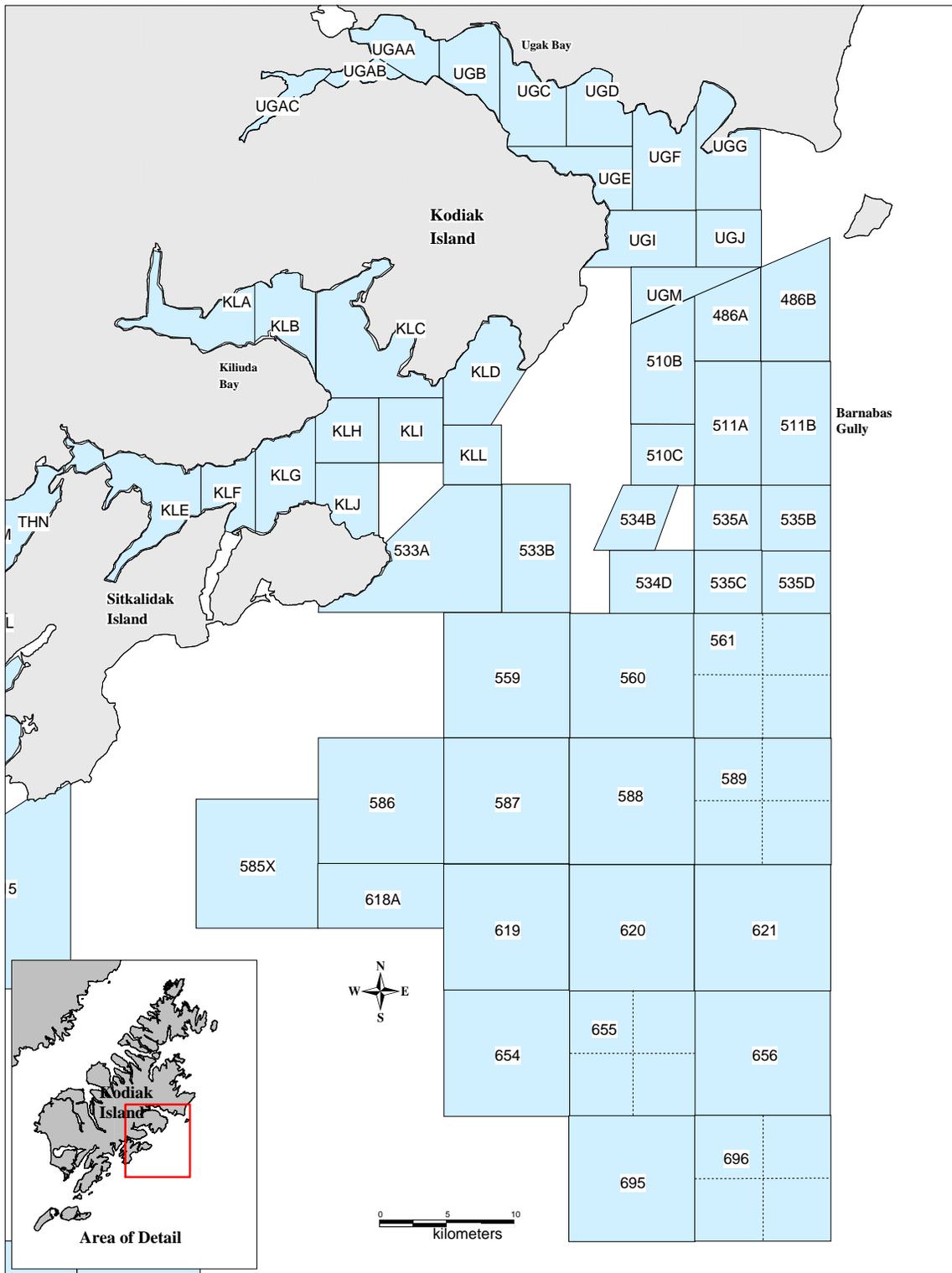


Figure 3.—Basic external skate (top) and shark (bottom) anatomy

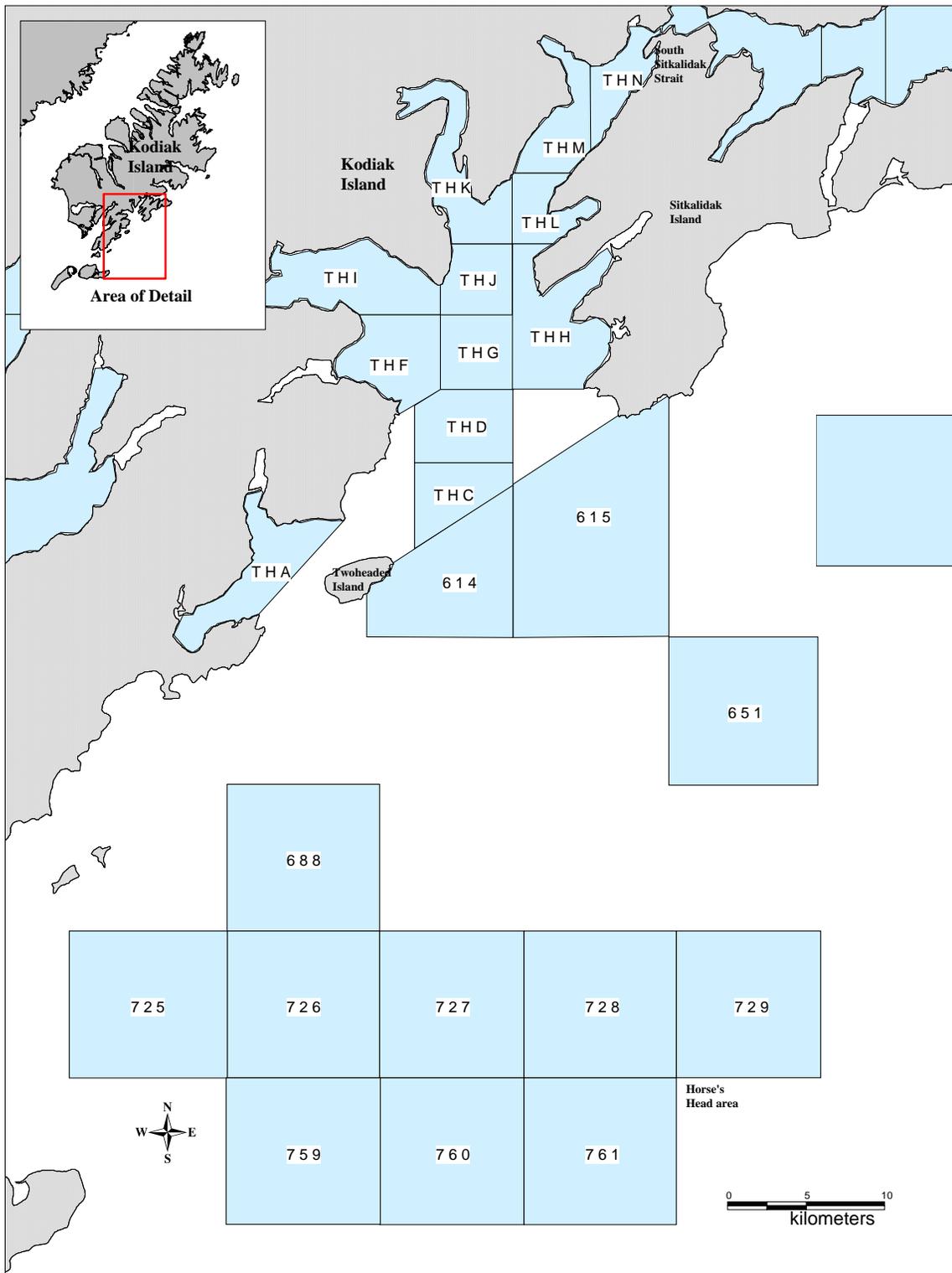
APPENDIX A. TRAWL SURVEY STATION MAPS



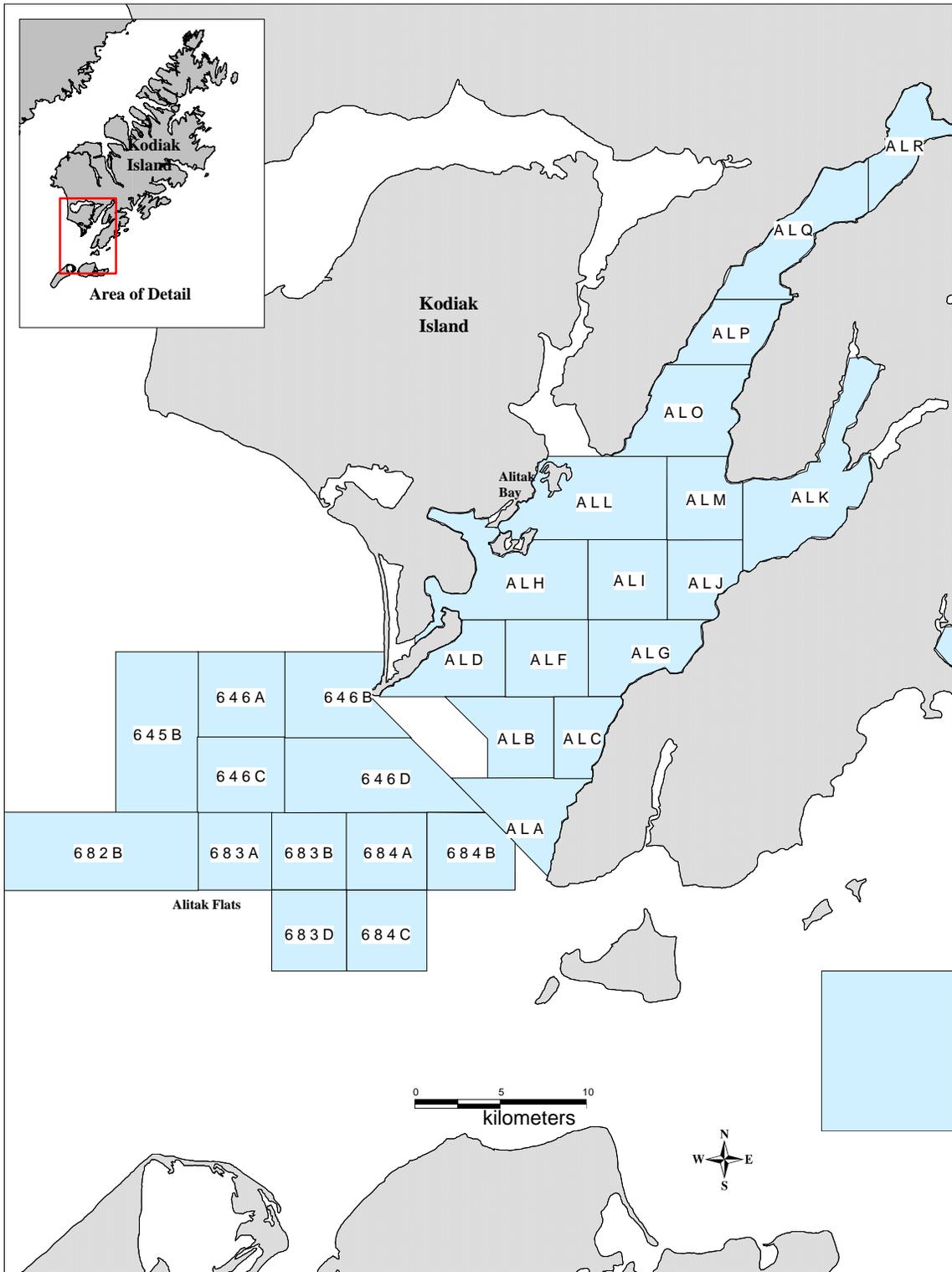
Appendix A1.—Station boundaries and names, Chiniak Bay and Chiniak Gully, 2009 Kodiak District trawl survey.



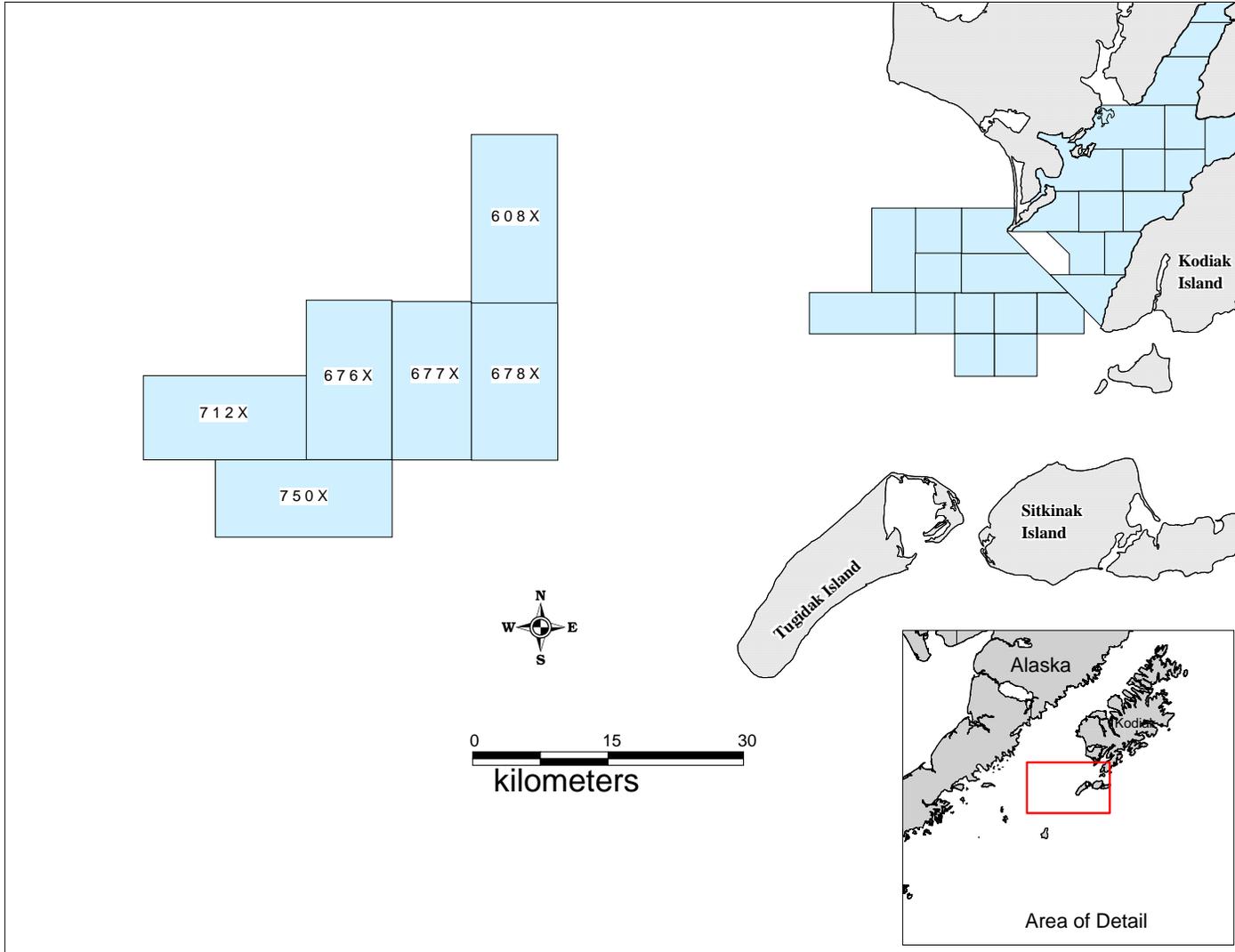
Appendix A3.—Station boundaries and names, Ugak Bay, Kiliuda Bay, and Barnabas Gully, 2009 Kodiak District trawl survey.



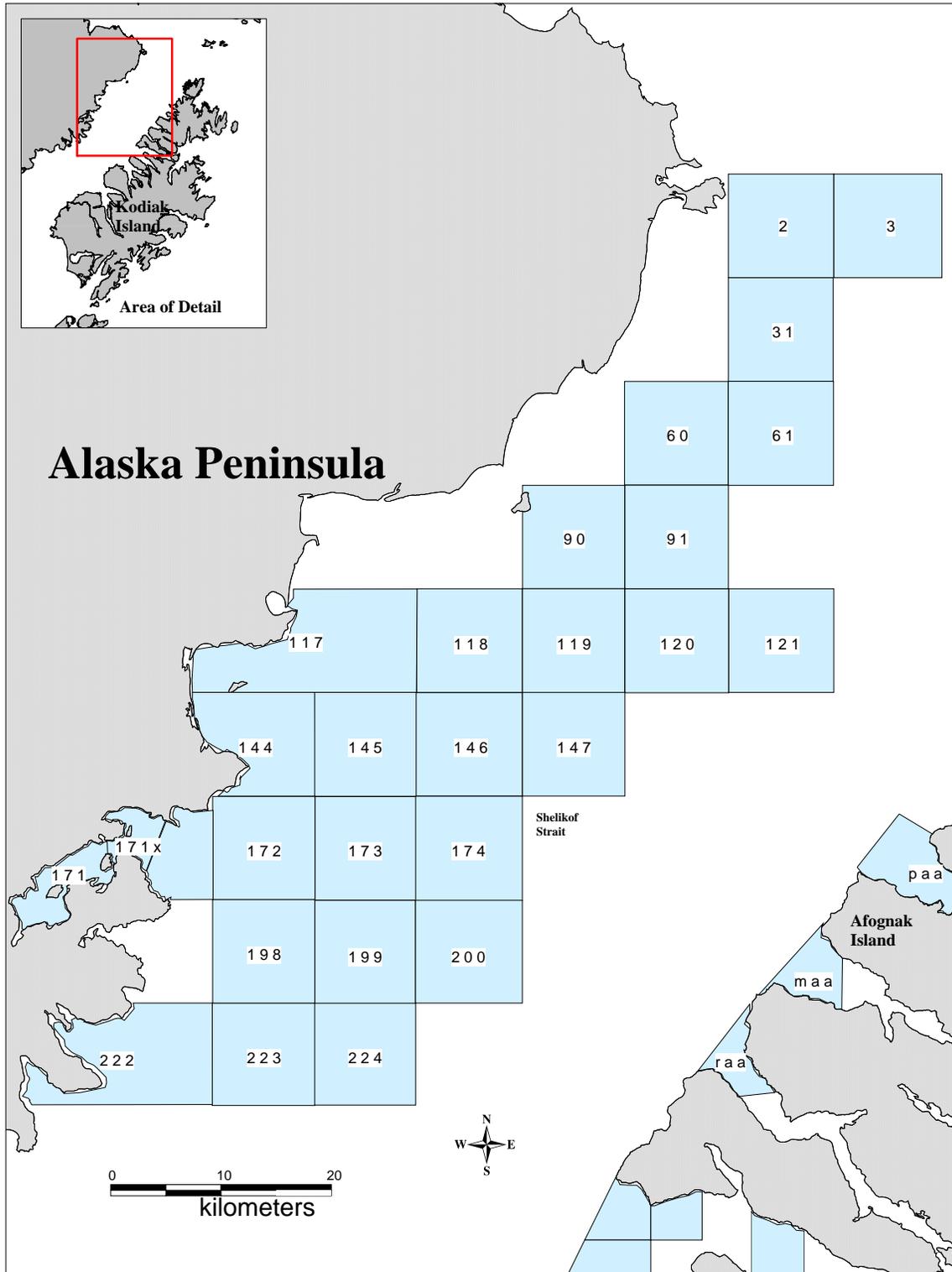
Appendix A4.–Station boundaries and names, South Sitkalidak Strait, Two Headed Island, and Horse’s Head area, 2009 Kodiak District trawl survey.



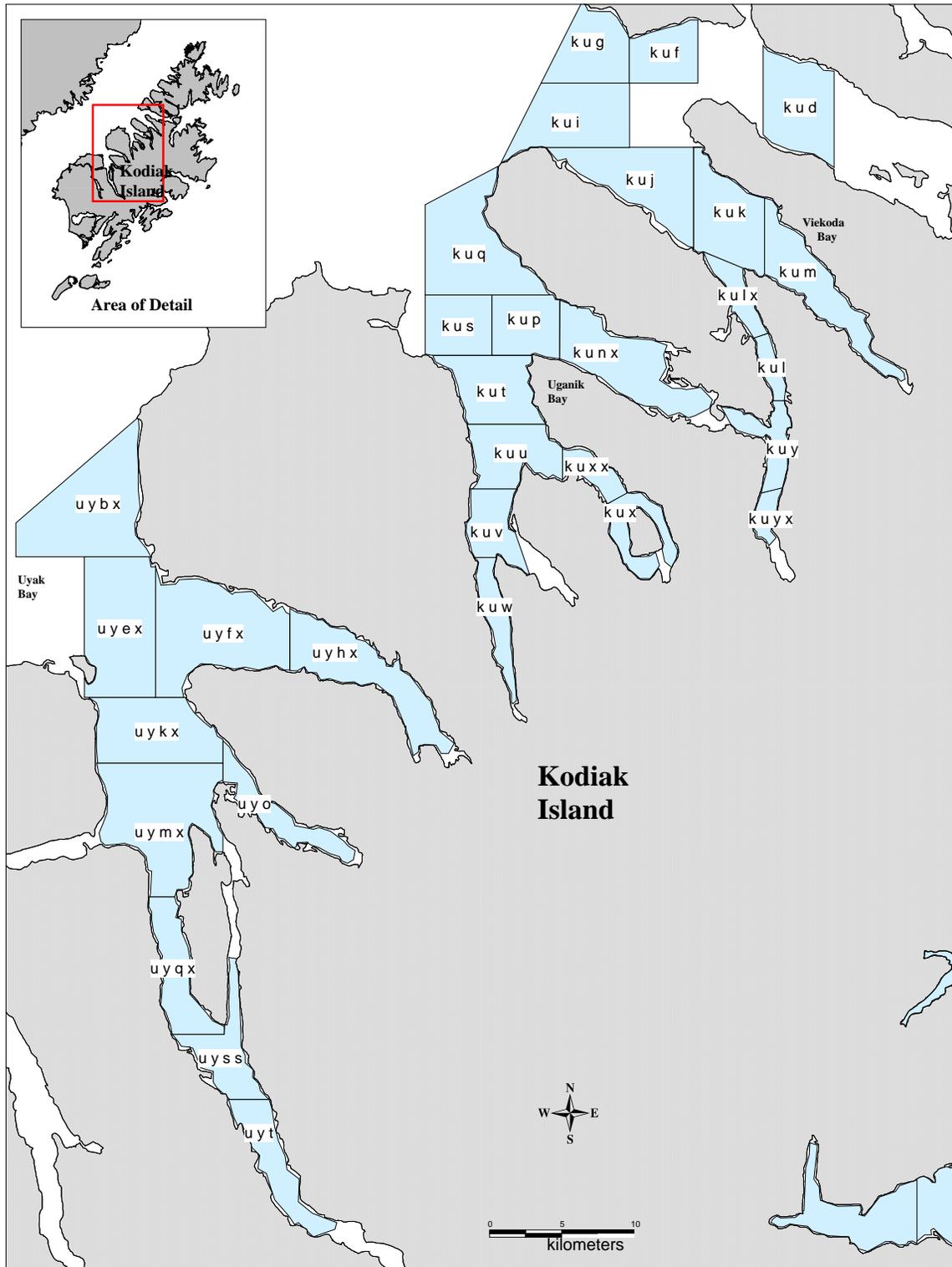
Appendix A5.–Station boundaries and names, Alitak Bay and Alitak Flats, 2009 Kodiak District trawl survey.



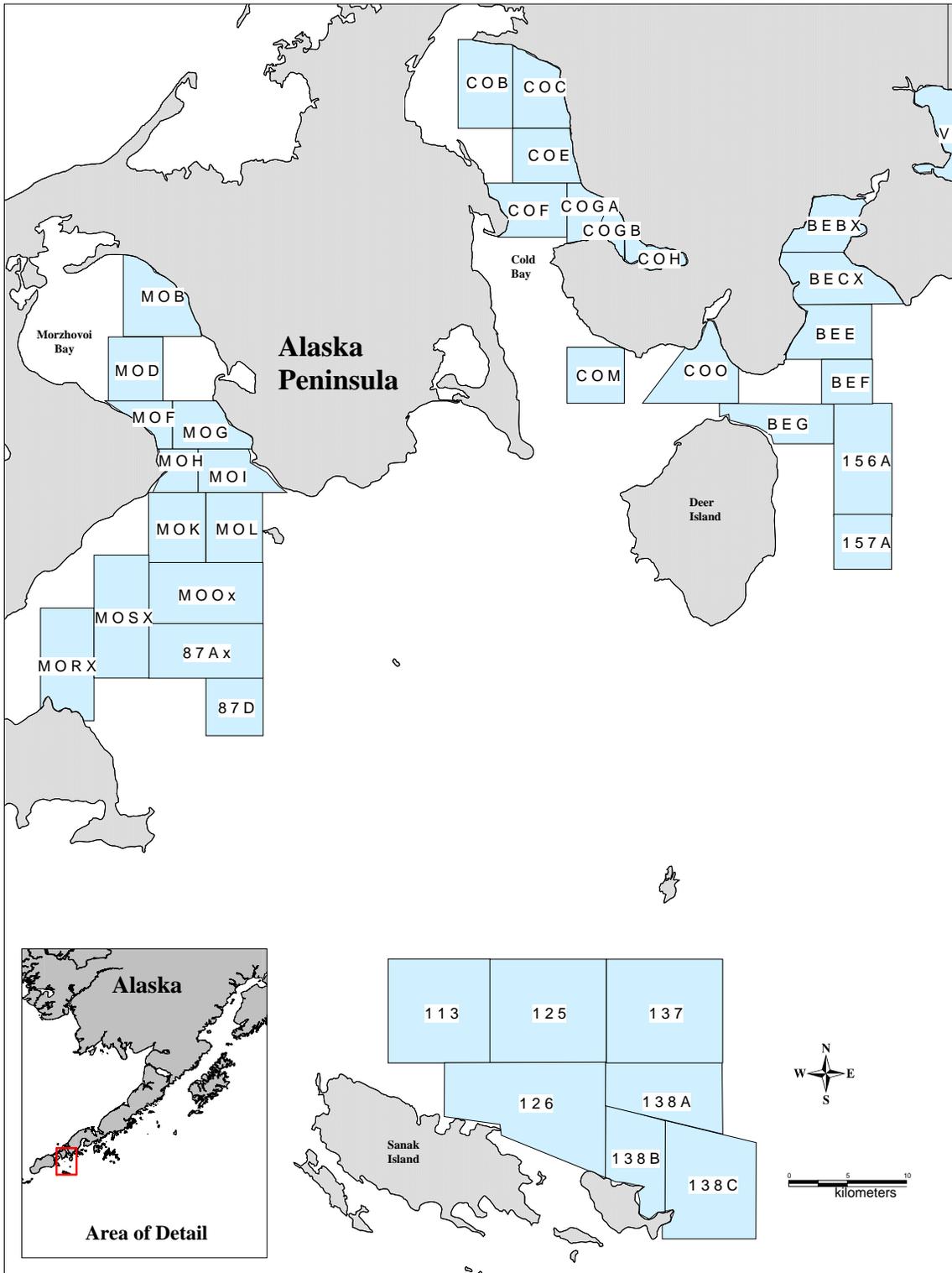
Appendix A6.—Station boundaries and names, Southwest Kodiak offshore, 2009 Kodiak District trawl survey



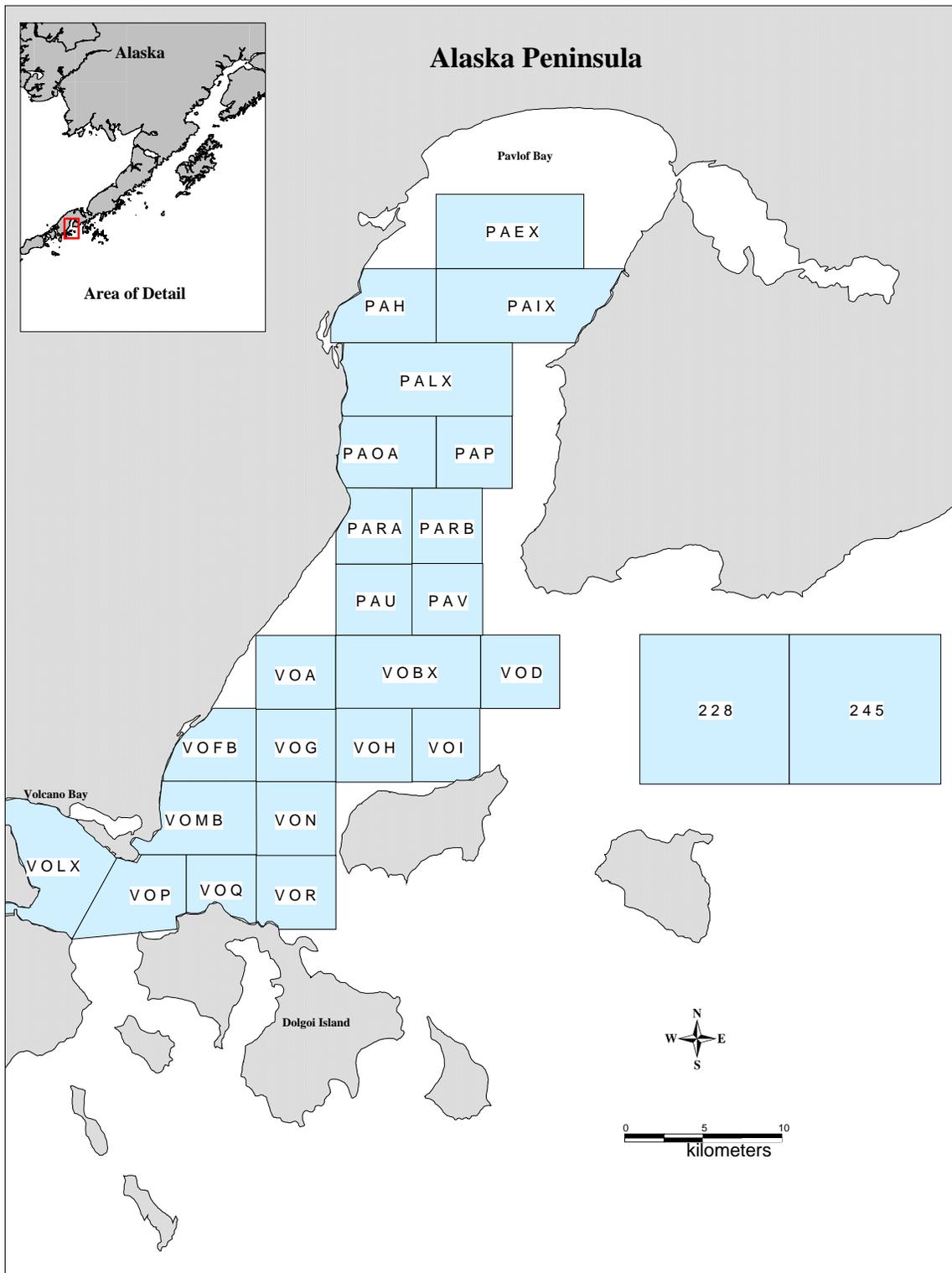
Appendix A7.—Station boundaries and names, Shelikof Strait, 2009 Kodiak District trawl survey.



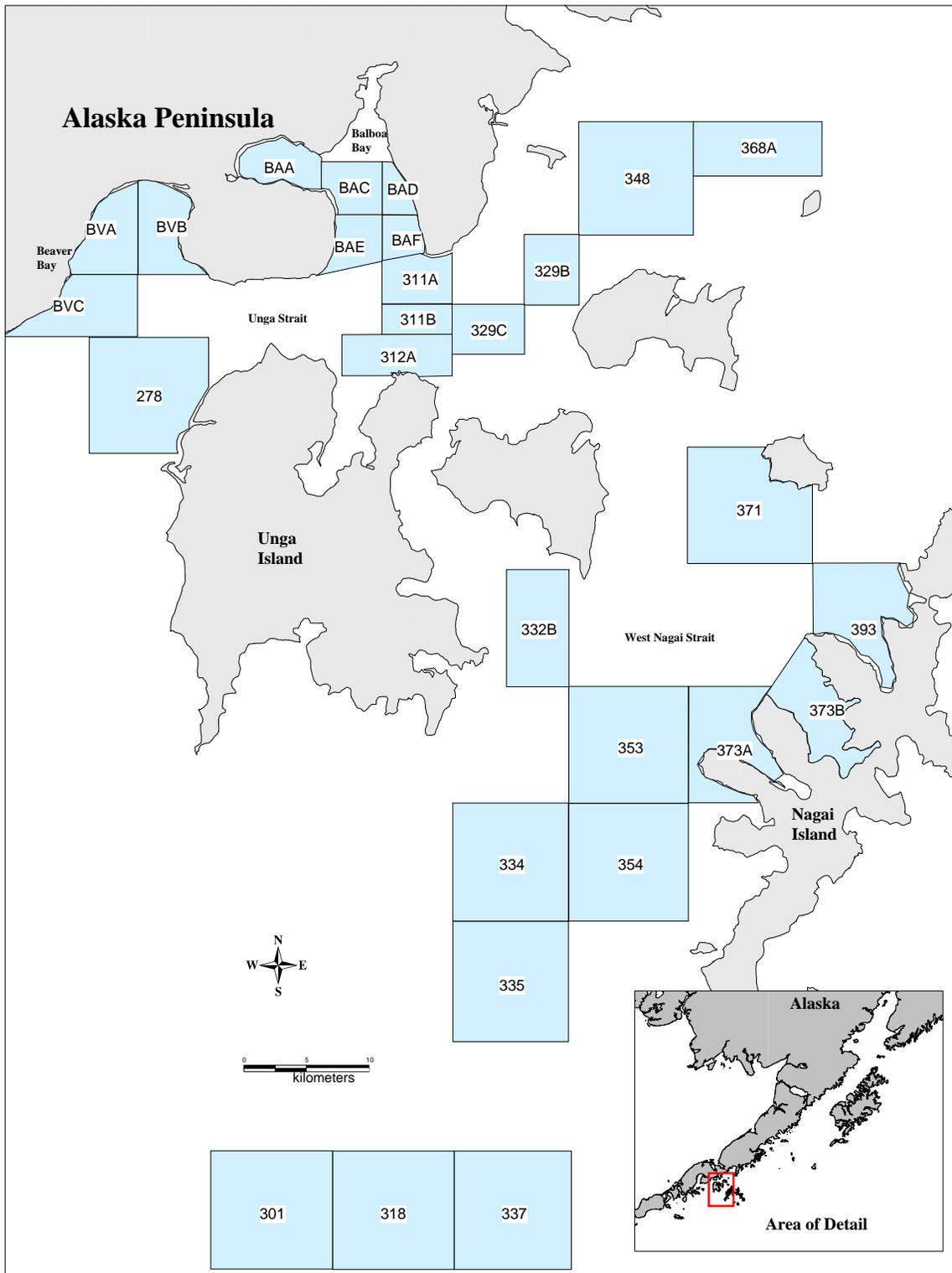
Appendix A8.–Station boundaries and names, Uyak, Uganik, and Viekada bays, 2009 Kodiak District trawl survey.



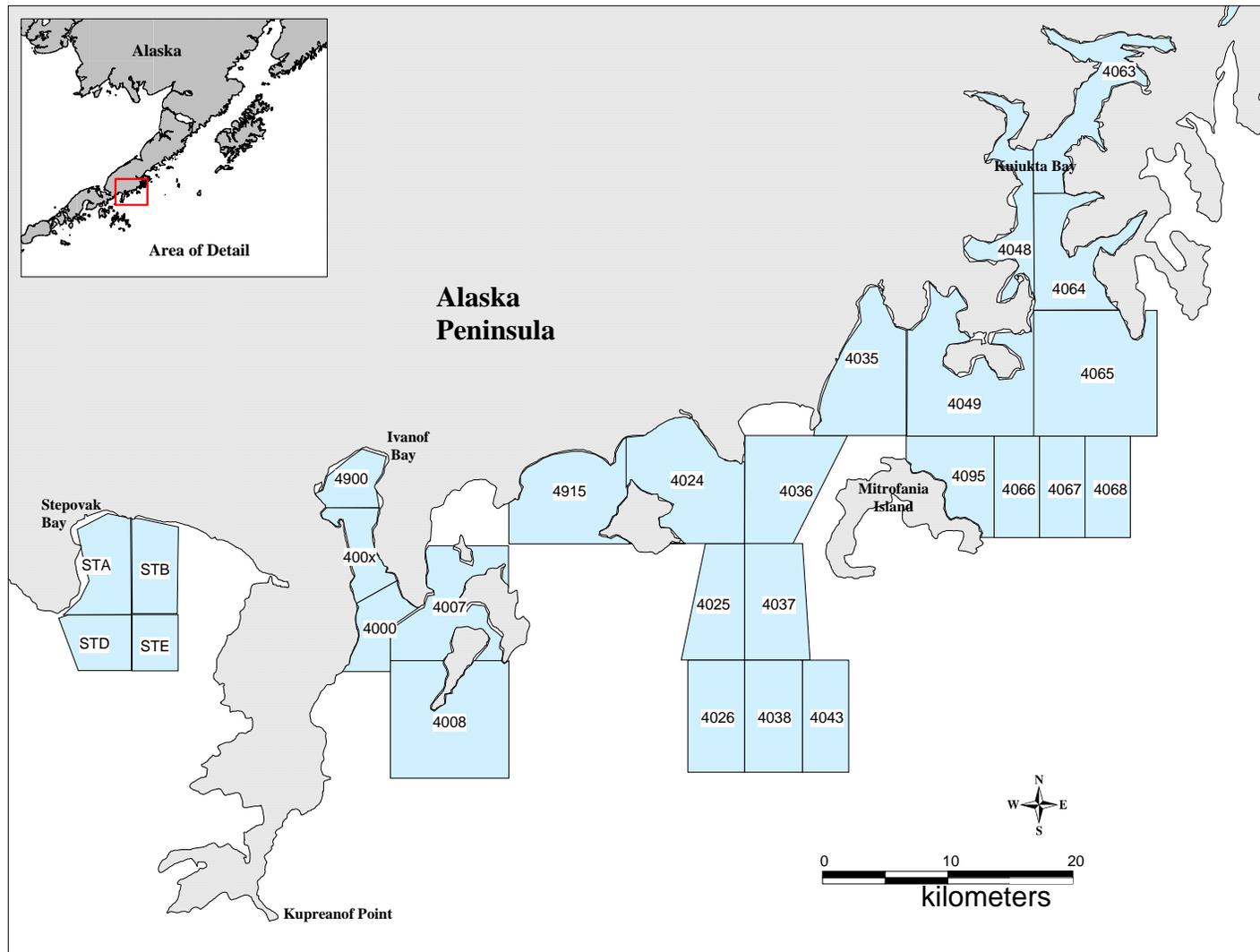
Appendix A9.—Station boundaries and names, Morzhovoi Bay, Cold Bay, Deer Island, and Sanak Island, 2009 South Peninsula District trawl survey.



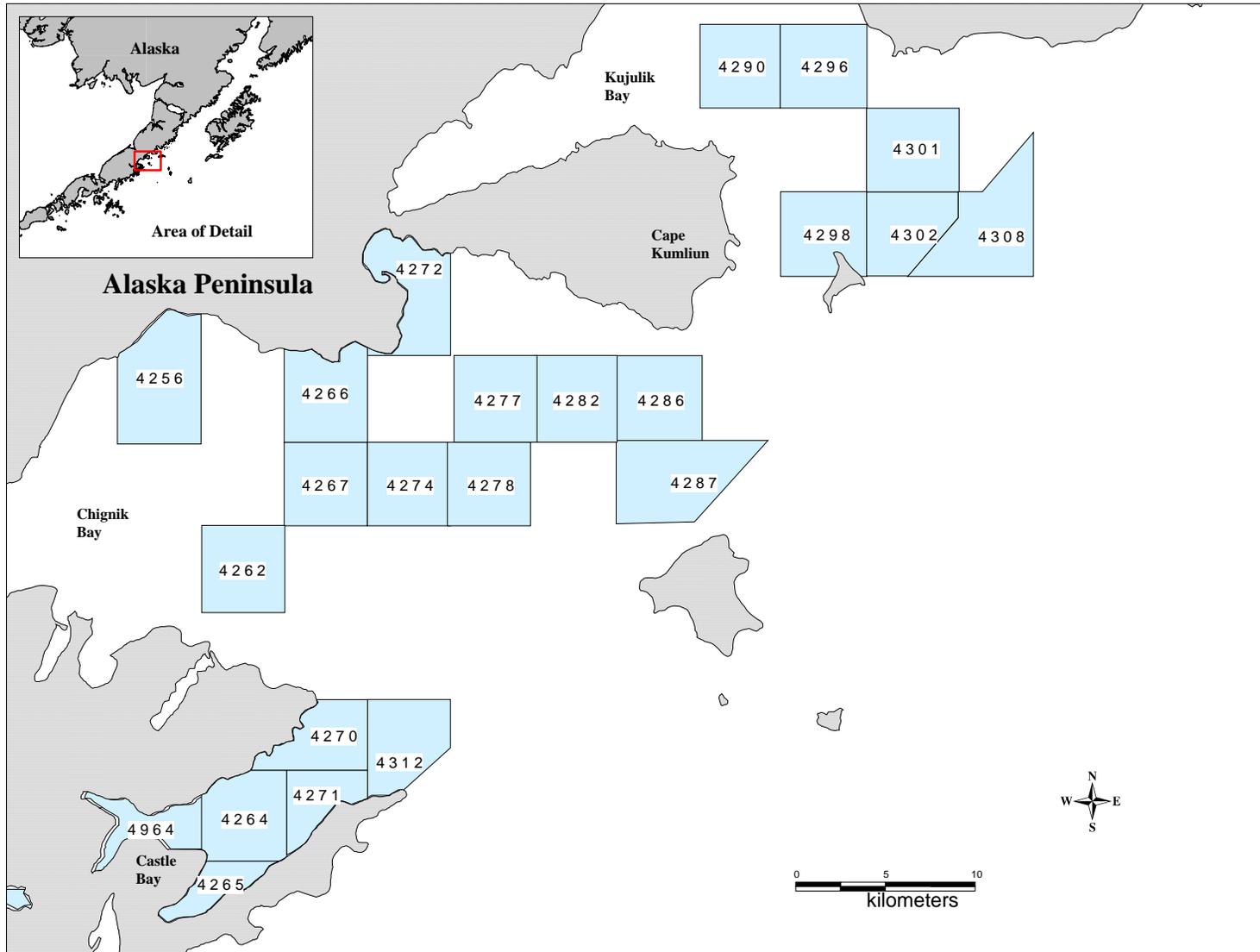
Appendix A10.—Station boundaries and names, Pavlof and Volcano bays, 2009 South Peninsula District trawl survey.



Appendix A11.—Station boundaries and names, Unga Strait, Beaver Bay, Balboa Bay, and West Nagai Strait, 2009 South Peninsula District trawl survey.



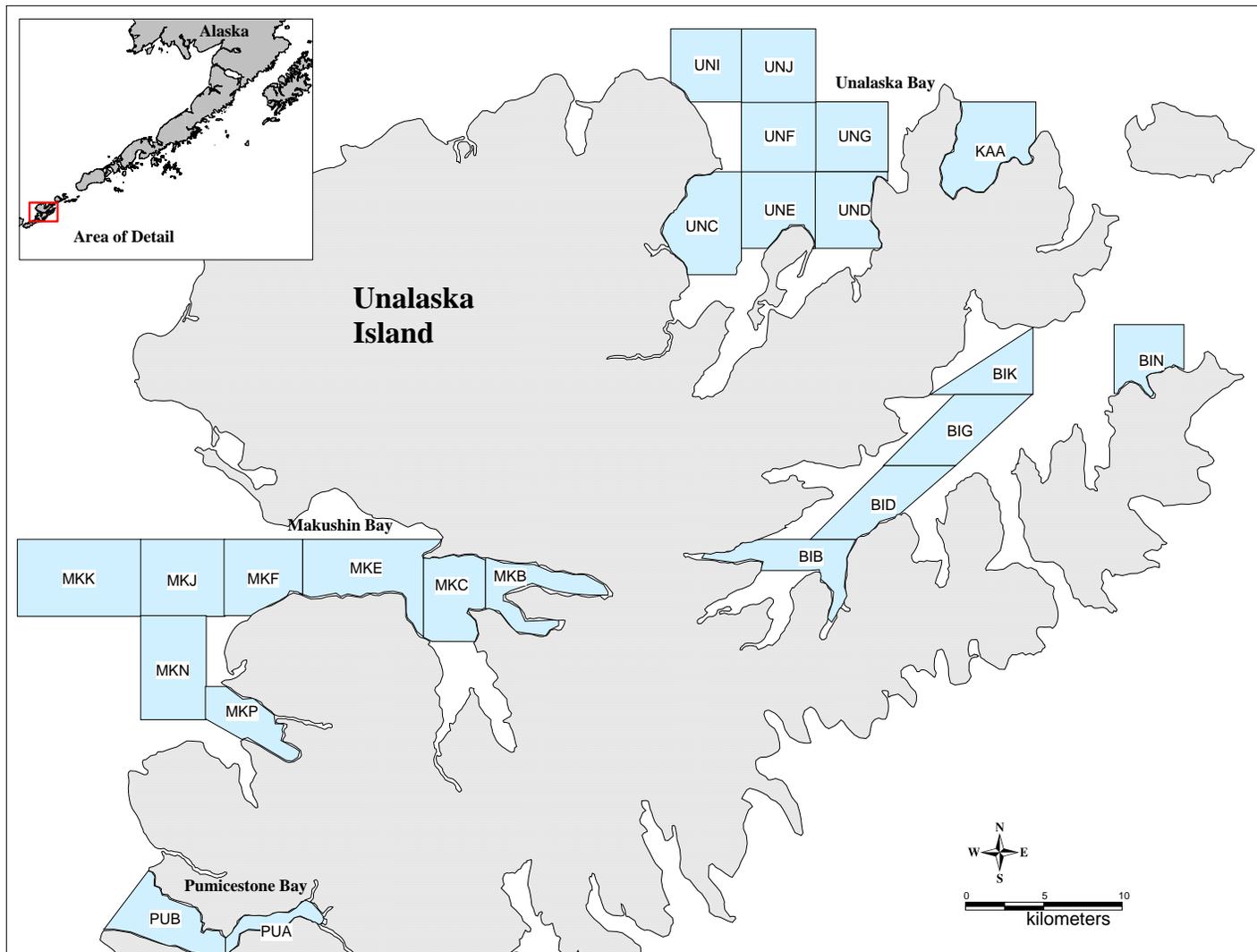
Appendix A12.—Station boundaries and names, Stepovak Bay, Ivanof Bay, Mitrofanina Island, and Kuiukta Bay, 2009 South Peninsula and Chignik District trawl survey.



Appendix A13.–Station boundaries and names, Kujulik, Chignik, and Castle bays, 2009 Chignik District trawl survey.



Appendix A14.—Station boundaries and names, Akutan Bay, 2009 Eastern Aleutian District trawl survey.



Appendix A15.—Station boundaries and names, Beaver Inlet, Unalaska, Makushin, and Pumicestone bays, 2009 Eastern Aleutian District trawl survey.

APPENDIX B. DATA FORMS

Appendix B1.-Tanner crab tagging form and instructions.

Tanner Crab Tagging Form 2009 - Legals											
Beginning tag number = T 0											
TAG #	DATE	HAUL	CARAPACE WIDTH	SHELL CONDITION	RELEASE LOCATION	TAG #	DATE	HAUL	CARAPACE WIDTH	SHELL CONDITION	RELEASE LOCATION
01						26					
02						27					
03						28					
04						29					
05						30					
06						31					
07						32					
08						33					
09						34					
10						35					
11						36					
12						37					
13						38					
14						39					
15						40					
16						41					
17						42					
18						43					
19						44					
20						45					
21						46					
22						47					
23						48					
24						49					
25						50					

-continued-

Tanner crab tagging form

Beginning tag number Write in the thousand and hundred digit from the tag series to keep the sheets from becoming confused.
The tag numbers listed on the sheet only refer to the last two digits of the tag, so it is important to fill in this line.

Date Month and day.

Haul Fill in the haul number where the crabs were captured.

Carapace Width Distance across the carapace between spines, in mm.

Shell Condition 1=soft
2=new
3=old
4=very old

Release Area If the crabs are returned to the water at a location away from the haul site, please record the latitude/longitude of the release location.

Appendix B2.-Example of the ADF&G Crab Data Form.

ADG-Form, prg

-SAMPLE DATA-

ADF&G CRAB DATA FORM

Page 1 of 2

SPECIES C. bairdi
 SEX mixed
 VESSEL Resolution
 DATE 07 10 08

STATION NUMBER			A	L	B
POT ORDER					
BUOY NUMBER					
TRAWL HAUL NUMBER			1	3	2
SAMPLING FACTOR			1		

NO.	SPECIES	SEX CODE	CARAPACE LENGTH (MM)	CARAPACE WIDTH (MM)	SHELL	DISEASE	EGGS			COMMENTS
							% CLUTCH FULLNESS	DEVELOPMENT	CLUTCH	
1	G	1		121	2					Slide# 031 Per# A1
2	G	1		109	1					032 B1
3	G	1		62	1					033 C1
4	G	2		147	1					034 D1
5	G	1		138	2					035 E1
6	G	1		129	3					036 F1
7	G	2		139	1					037 G1
8	G	2		142	1					038 H1
9	G	2		151	1					039 A2
10	G	3		45	1					040 B2
11	G	1		108	1	2				041 C2
12	G	1		112	1	2				042 D2
13	G	1		120	3					043 E2
14	G	1		79	1					044 F2
15	G	1		62	1					045 G2
16	G	2		141	2					046 H2
17	G	1		135	1					047 A3
18	G	3		60	1	2				048 B3
19	G	3		48	1					049 C3
20	G	4		85	1		1/2	1	1	050 D3
21	G	3		45	1					051 E3
22	G	4		90	2		Full	1	1	052 F3
23	G	4		88	2		3/4	1	2	053 G3
24	G	1		110	1					054 H3
25	G	2		140	1					055 A4

TRA

CODE INSTRUCTIONS

- SPECIES**
 1. L. aequispina
 2. P. Camtschatica
 3. P. platypus
 6. C. Bairdi
 7. C. opilio
 9. C. Magister

- SEX CODE**
 1. Sublegal male
 2. Legal male
 3. Juvenile female
 4. Adult female

- SHELL CONDITION**
 0. Soft
 1. New
 2. Old
 3. Very old

- DISEASE CODE**
 1. Black mat
 2. Bitter crab syndrome
 3. Nemertean worm
 4. Parasitic barnacle

- EGG DEVELOPMENT**
 1. Uneyed eggs
 2. Eyed eggs

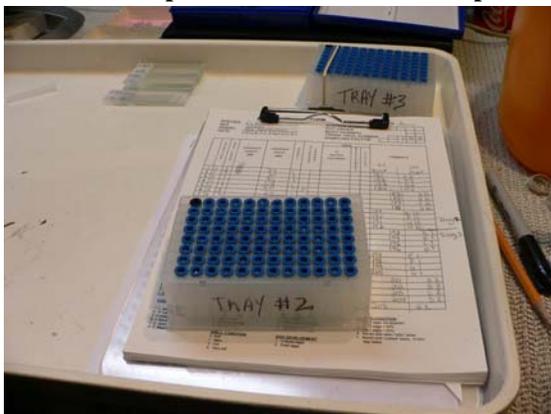
- CLUTCH CONDITION**
 1. Dead eggs not apparent
 2. Dead eggs < 20%
 3. Dead eggs > 20%
 4. Barren with clean "silky" setae
 5. Barren with "matted" setae. Empty egg cases.

-SAMPLE DATA-

**APPENDIX C. TANNER CRAB HEMOLYMPH
COLLECTION: PROTOCOL FOR GENETIC SAMPLING**

Preparation

1. **Pre-fill each well with 0.8 ml of biotech grade ethanol**
2. **Press the rubber caps onto each well**
3. **Label the plates and color code the top left corner - well A1 (with all trays oriented the same way) to denote the starting position**



- a. The plates are labeled down one side with letters and across the top with numbers
4. **Wrap a rubber band around the plate to move over one column at a time as you fill wells with samples**
5. **Completely fill out all applicable header information on the ADF&G Crab Data Form (Appendix B2).**
 - a. Species = *C. bairdi*
 - b. Sex = Mixed
 - c. Vessel = Resolution
 - d. Date = Date of haul

- e. Page _ of _ = Can be filled in after completion of the sample, however if you will be sampling 30 crabs per haul, you will have 2 pages per haul
- f. Station number = You should know which station you were towing in to know if you need to sample for bitter crab or not
- g. Pot order = leave blank
- h. Buoy number = leave blank
- i. Trawl haul number = The haul number of the tow the crabs were from
- j. Sampling factor = leave blank for bitter crab sampling

Sample Collection

1. **Arrange crab on table in way to facilitate sampling and reduce error.**
 - a. Typically I will set up 5 crab at a time. It is easier to keep track of and a handy multiple of 30.
 - b. If you place the crabs carapace up, with their mouths facing right, it will expose the preferred sampling leg, the right cheliped.



2. **Using a new syringe freshly out of its wrapper for each crab, draw hemolymph from the elbow joint of the right cheliped.**
 - a. Slightly raise stopper on syringe before inserting needle
 - b. Wipe the membrane clean with a paper towel so extraneous material does not contaminate the sample.
 - c. Being careful not to puncture yourself insert needle into joint, about halfway, or far enough to ensure angled hole in needle is inside crab tissue.
 - d. Raise stopper just until you see semi-clear fluid being drawn into syringe. If you can

- see it, you have enough. If the crab is heavily infested with BCS it may be more difficult to draw hemolymph.
- e. Small crabs may be more difficult. You may have to try drawing blood from a different leg, particularly if the preferred leg is injured.

-continued-

3. **With the syringe, inject 0.2 ml of hemolymph into each well** (one well per crab)
 - a. If you inject too much hemolymph, or a lot of air, the cap may pop off the well and spray hemolymph/ethanol over adjacent caps.
 - b. To mix the blood and ethanol, invert the plate once in a while
 4. **Complete the information on the ADF&G crab data form.**
 - a. Species = 6
 - b. Sex = Be sure to specify the maturity or legal status of the crab, do not just write down M or F.
 - c. Carapace width
 - d. Shell condition
 - e. Disease codes = enter applicable diseases; if BCS is obvious enter it here, this helps to ground truth the labwork
 - f. Clutch fullness = Use the fractional classifications implemented for the regular sampling
 - g. Development = eyed or uneyed, codes at bottom of form
 - h. Clutch condition = codes at bottom of form
 - i. Comments = Important to write down the PCR sample number here. (This should include both the tray number and the well number containing the sample from that crab.
 5. **Once you have double checked that all information has been recorded and that you have written down the correct sample number in the comments column you can discard the crab.**
 - a. Always check with the on-deck leader before discarding crab over the side.
 6. **At the end of the haul coordinate with the on-deck leader to determine how the data from crab sampled for BCS will be handled.**
 - a. If all crab in the haul were measured and counted (whole-hauled), then enter the data from the BCS sample forms into the crab database.
 1. The easiest way to do this is to use the on-deck computer
 - b. If crab were subsampled, The weight from the BCS sample should be removed from the on-deck form, and only those crab actually in the subsample should be included in the crab database.
 - c. If other sampling methods were used (i.e. %m/f) it is the cruise leaders responsibility to ensure that the crab being sampled for BCS are properly accounted for.
-

**APPENDIX D. GROUND FISH STOMACH SAMPLING
PROTOCOL**

Appendix D1.–Number of stomachs, by species and size groups (cm), to be collected in the 2009 Chiniak and Marmot bays summer survey.

Species	Number	Species	Number
Walleye pollock		Arrowtooth flounder	
< 30 cm	20	< 30 cm	40
30-44	20	30-49	40
45-54	40	≥ 50	40
≥ 55	40	total	120
total	120		
Pacific cod		Pacific halibut	
< 30 cm	20	< 40 cm	15
30-44	20	40-54	15
45-59	40	55-69	30
≥ 60	40	≥ 70	30
total	120	total	90
Flathead sole		Northern rock sole	
< 20 cm	20	< 20 cm	20
20-39	20	20-39	20
≥ 40	20	≥ 40	20
total	60	total	60
Spiny dogfish			
< 40 cm	20		
40-79	20		
≥ 80	20		
total	60		

Appendix D2.–2009 Chiniak and Marmot bays groundfish stomach sampling protocol.

At every haul, after the catch has been dumped in the bin and the major species in the catch are evident, choose two to three species from Appendix D1 which are abundant enough for stomach sampling purposes (about one full basket). With the concurrence of the sorting crew, designate which specimens are to be set aside for stomach dissection after the baskets have been weighed. Set the baskets in a cool, shaded area until the rest of the catch has been processed.

2. Sampling procedures:

- (1) Collect fish that show **no** sign of either net feeding or regurgitation.
*Signs of net feeding and regurgitation (**DO NOT KEEP THESE**):
 - prey items in mouth or gill rakers
 - flaccid (loose and bloated) looking stomach*Signs of "natural" stomachs (**KEEP THESE!**):
 - naturally empty stomachs appear tight and contracted
 - stomachs appear tight around any prey inside
- (2) If the fish is determined to be collectable, measure the fork length, determine the sex and spawning condition, excise the stomach and place in a stomach bag with a label. Try to collect 5 specimens from each size group (e.g. collect 5 stomachs from each of the <30 cm, 30-44 cm, 45-54 cm, and ≥ 55 cm pollock) in one haul. For small fish (≤ 20 cm), do not excise the stomach but instead make a slit in the body cavity to allow penetration of Formalin to the gut. Place the samples of whole fish in a large stomach bag with a label. Submerge samples in a bucket of 10% buffered Formalin. To make the Formalin solution, fill a 5-gallon bucket about half full with sea water, then add one liter 37% Formalin to the bucket. Add one rounded 1/8 cup of baking soda per bucket.
- (3) Each stomach bag should contain a specimen label which records the species, vessel, cruise, haul, specimen number, the fork length of the fish, sex, and the spawning condition (spawning=1 or not spawning=0).
- (4) For each species, start specimen number at "1" and assign a number consecutively until the end of the cruise.
- (5) A specimen form is also filled out for each species in each haul. The specimen form should record the species, vessel, cruise, haul, fork length, sex, spawning condition (spawning or non-spawning), date, and specimen number (individual fish weight does not have to be taken).
- (6) Use the broken lids to cover the bucket each time you add some stomach collections into it. Seal the bucket (by using the unbroken lid) only when the bucket is full or at the end of the cruise.
- (7) Put different species collections in different buckets. Use the permanent mark pen to write the species name, vessel, the address (National Marine Fisheries Service, Food Habits Lab, Bldg. 4, 7600 Sand Point Way NE, Seattle, WA 98115-0070) on the unbroken lid each time you seal a bucket.
- (8) When the cruise is over, please double-check that the lids are completely labeled and add a luggage tag to the bucket handle. The luggage tag should indicate '2009, Marmot Bay, pollock (species), Resolution (boat), and your name'.
- (9) Collect at least 20 stomachs per haul, and you can reach the goal.

End of the Cruise:

At the end of the cruise, the buckets (along with the specimen forms) and the remaining equipment should be taken off the vessel and delivered to NMFS, Kodiak Laboratory in Kodiak. Please inform Mei-Sun Yang or Geoff Lang and they will make arrangements to ship them to Seattle.

**APPENDIX E. ROCKFISH OVARY AND OTOLITH
COLLECTION PROTOCOL**

Appendix E1.– Number of rockfish otoliths and ovaries, by species and size groups (cm), to be collected in the 2009 trawl survey.

Species	Number	Species	Number	Species	Number
Pacific ocean perch		Blackspotted rockfish		Shortraker rockfish	
25-29 cm	2	35-39 cm	2	35-39 cm	2
30-34	2	40-44	2	40-44	2
35-39	2	45-49	2	45-49	2
40-44	2	50-54	2	50-54	2
45-49	2	55-59	2	55-59	2
50-54	2	60-64	2	60-64	2
55-59	2	65-69	2	65-69	2
>60	2	>70	2	70-74	2
total	16	total	16	75-79	2
				80-89	2
				90-99	2
				>100	2
				total	24
Northern rockfish		Rougheye rockfish			
20-24 cm	2	35-39 cm	2		
25-59	2	40-44	2		
30-34	2	45-49	2		
35-39	2	50-54	2		
40-44	2	55-59	2		
45-49	2	60-64	2		
>50	2	65-69	2		
total	14	>70	2		
		total	16		

Project Title: A multi-species rockfish reproductive study in the Gulf of Alaska

Principle Investigator (PI)/Point of Contact: Christina Conrath, Brian Knoth

Affiliation: AFSC, RACE, Groundfish Assessment Program

Address: Kodiak Laboratory, 301 Research Court, Kodiak, AK 99615

Email: Christina.Conrath@noaa.gov

Phone: 907-481-1732

General Description and Justification: Despite the ecological and economic importance of rockfish in the GOA detailed knowledge concerning the reproductive biology of many species is lacking. Reproductive parameters, such length/age at maturity estimates, are critical components of the stock assessment models and estimates of fecundity are important in assessing the reproductive potential of female rockfish. Many rockfish species or species assemblage assessments currently rely on length at maturity estimates that are drawn from small sample sizes or are based exclusively on macroscopic staging (Heifetz et al. 2007, Lunsford et al. 2007). Previous studies have revealed a greater potential for incorrect identification of maturity stages during macroscopic staging when compared to histological evaluations which may bias maturity estimates (Hunter et al. 1992, Zimmerman 1997). In addition, the identification and separation of new rockfish species (*e.g.* roughey and blackspotted) enhances the need for updated biological information. This project will use histological methods to determine the ovary maturities of samples collected through out the year, including this GOA survey collection, commercial fishery observer collections, and other NMFS survey collections.

Detailed collection procedures: Please collect otoliths and ovaries from the following species: Pacific ocean perch, northern rockfish, roughey rockfish, blackspotted rockfish, and shortraker rockfish. Detailed instructions for distinguishing roughey and blackspotted rockfish are provided in Appendix E3. Collect two females within each five cm length group in Appendix E1. Record haul number, date, species name, fork length, and specimen number on a specimen form utilizing the otolith sample number (in vial). Ovaries should be removed, and placed within a cloth stomach bag, together with a specimen label containing the specimen number. Secure the cloth bag by looping the strings once and tighten the strings (no knot). Submerge the bag in a spin top five-gallon bucket half full of the 10% formalin mixture using the bucket opener tool or some other object (not your hands). To make the 10% formalin mixture from 37% formaldehyde, add a one quart (0.945 l) container of 37% formaldehyde (~1" deep layer) into a 5 gallon bucket, add 1/8 cup baking soda (buffer), and add enough water to fill bucket half full. Stir the mixture with a bucket opener tool or long handled knife, then rinse the tool with water. Otoliths should be removed and placed within an otolith sample vial and covered with 70% ethanol.

List of supplies: Specimen forms, tally sheets, specimen labels, stomach bags, 5 gallon DOT approved buckets, 37% Formaldehyde, baking soda for buffering, dissecting supplies, 70% Ethanol.

Hazardous materials: 37% Formaldehyde to be diluted to 10% (3.7%) formalin, 70% Ethanol.

24/7 contact: Christina Conrath and/or Brian Knoth 907-481-1732/907-481-1731.

Rougheye and Blackspotted Rockfish

The recent separation of the rougheye rockfish into two species (rougheye and blackspotted rockfish) necessitates the re-examination of the life history of these species (Orr and Hawkins 2008). *Sebastes melanostictus*, the blackspotted rockfish, is distinguished from *S. aleutianus*, the rougheye rockfish, by the presence of spotting on the spinous dorsal fin and a darker color morph and in general is thought to have a more offshore distribution.

Blackspotted rockfish with distinct spots (not blotches) on the first dorsal fin



Rougheye (top) and blackspotted (bottom) rockfish

