OCS Study
BOEM 2014-050

Arctic Cod Pilot Genomics Study: Preliminary Results from Analyses of Mitochondrial DNA

US Department of the Interior
Bureau of Ocean Energy Management
Alaska Region

BOEM
Bureau of Ocean Energy Management

OCS Study
BOEM 2014-050

Arctic Cod Pilot Genetics Study: Preliminary Results from Analyses of Mitochondrial DNA

Sandra L. Talbot, Research Geneticist stalbot@usgs.gov (907) 786-7188
George K. Sage, Geneticist ksage@usgs.gov (907) 786-7189
Sarah A. Sonsthagen, Geneticist ssonsthagen@usgs.gov (907) 786-7054
Megan C. Fowler mfowler@usgs.gov (907) 786-7024
Biological Sciences Technician,

Prepared under USGS Offshore Research Funds Account

US Department of the Interior
Bureau of Ocean Energy Management
Alaska Region

Prepared by
Alaska Science Center
U.S. Geological Survey
4210 University Drive,
Anchorage, AK 99508

US Department of the Interior
Bureau of Ocean Energy Management
Alaska Region
January 2014

BOEM
Bureau of Ocean Energy Management

ABSTRACT

Arctic cod (*Boreogadus saida*) plays a critical ecological role as key prey species and the primary pathway through which lower trophic production is transferred to other marine vertebrates. Thus, an understanding of Arctic cod dynamics is critical when evaluating impacts of predicted and observed changes in ice concentration in Arctic ecosystems. Nevertheless, information required for the conservation and management of the Arctic cod population in this region is scant. Prior analyses of mitochondrial DNA (mtDNA) cytochrome b (cytb) sequence data, gathered as part of a pilot study to assess the genetic characteristics of Arctic cod in the Beaufort Sea region of the Nearctic relative to the adjacent Chukchi Sea and more distant Atlantic population in St. Lawrence Bay, failed to uncover significant structuring across the Nearctic overall, based on variance in haplotype frequency (F_{ST} = 0.017, P = 0.073; Φ_{ST} = -0.022, P = 0.962). However, we did uncover a signal of substructuring between the Beaufort and Chukchi sea populations of Arctic cod (F_{ST} = 0.043, P = 0.018), and this pattern conformed to preliminary results from microsatellite loci, which differentiated between Chukchi and Beaufort seas (western populations), but also show differentiation between the western populations and eastern populations (Gulf of St. Lawrence, Baffin Bay, and Trinity Bay). Since the mtDNA genetic data rejected the null hypothesis of panmixia in Alaskan (Southern Beaufort Sea and Chukchi Sea), we recommended that future sampling in the Beaufort Sea include sampling of both the eastern and western margins of the Beaufort Sea, to better assess the boundaries of the Beaufort Sea population, and also that microgeographic structuring within the Beaufort Sea be conducted. Here, we assay slope and shelf populations within the central Beaufort Sea Arctic cod distribution to determine whether there is structuring among populations within the region, and between slope and shelf habitat. Our analyses failed to detect a significant signal of differentiation between Arctic cod sampled from the shelf, and those sampled from the slope, suggesting Arctic cod breeding within the Beaufort Sea belong to a single, interbreeding unit. We are currently increasing sample sizes so lack of differentiation cannot be attributed to a Type 2 statistical error.

INTRODUCTION

The Arctic cod (*Boreogadus saida*) is a key species in Arctic food webs and occupies nearly all depths during its life cycle. The species has adapted to icy, freezing (-1.9º C) habitats, in part due to the evolution of one of the four structurally diverse antifreeze proteins found among polar and north-temperate teleosts (Chen et al. 1997). This adaptation to polar life is reflected in the species' physiology (Enevoldsen et al. 2003) and distribution relative to sea ice (Lønne and Gullikesen 1989). It is unclear whether Arctic cod will be driven to extinction as the Arctic ice retreats, as posited by Cheung et al. (2008). However, in the face of environmental changes that exceed current physiological tolerances, the Arctic cod has the same strategies available to other Arctic species: they can shift their range to track changing conditions, persist in refugial habitats, adapt rapidly to changing conditions, hybridize with a closely-related but more generalist species, or go extinct. Genomics and transcriptomic analyses can shed light on whether Arctic cod are truly ice dependent, or whether there is potential to adapt to retreating ice through differential expression of existing genes. However, transcriptomic data are best interpreted when population genetics and phylogeographic relationships are well-understood.

Few population genetic and phylogeographic studies have been performed from Arctic cod occupying western and eastern locales in northern North America, in part due to the difficulty of sampling species that are distributed in high Arctic marine waters (Lønne and Gulliksen 1989). Basic life history information required for the conservation and management of the Arctic cod population occupying marine habitats in Arctic Alaska, including quantitative estimates of population size, vital rates, and/or status, is scarce, and until recently, distributional data were largely limited to studies conducted in the Beaufort Sea, primarily in 2002 (Gradinger and Bluhm 2004). Sea ice conditions changed dramatically since that time; sea ice extent and thickness have continued to decrease in recent decades (Durner et al. 2009) and further changes are predicted (Stroeve et al. 2007, 2010; Tietsche et al. 2011; Wang and Overland 2009, 2012). The absence of basic information on distribution patterns, not only of the Chukchi and Beaufort population(s) as a whole, but also within these locales relative to continental or deep-sea habitats, precludes the development of robust studies to determine the status of the population and its response to perturbations. This is further complicated by the challenges of sampling a widely distributed Arctic population.

Identifying the boundaries of biologically meaningful populations remains challenging, particularly for Arctic marine species. Traditional population genetic and phylogeography studies, particularly when combined with other methods, such as stable isotope and/or heavy metal analyses (Jay et al. 2008) and direct tagging estimates, can help to identify local and regional spatial use patterns (Scribner et al. 2005), detect both historical and recent changes in demography (Weckworth et al. 2005, 2010), delineate populations and assess levels and polarity of evolutionary dispersal (gene flow) (Sonsthagen et al. 2009, Howes et al. 2009). Results of such studies provide a critical foundation for the design of comparative genomics and transcriptomics research efforts, which due to high costs typically assess more genetic material than traditional genetics methods, but for fewer individuals. To provide necessary foundational data to design future transcriptomics research efforts, we are conducting a pilot study that uses neutral genetic data to determine whether Arctic cod occupying northern Alaskan waters comprise a single population, or occur in multiple genetically distinct stocks. Marine species are often expected to demonstrate genetic homogeneity among populations (Waples 1998), due in

part to the lack of obvious barriers to dispersal in ocean habitats, and patterns of differentiation vary among Arctic gadoids, including *B. saida* elsewhere within the species' range (Pálsson et al. 2009).

During FY2011, we initiated a pilot study to 1) develop and test molecular markers in Arctic cod sampled from the Chuckchi and Beaufort Sea; 2) compare with data from Arctic cod sampled there with those from more geographically distant populations to 3) determine whether there is any signature of structuring among the three populations based on mtDNA (see Figure 1). Given the results of the preliminary analyses conducted in FY2011 (Talbot et al. 2011), we then developed protocols and sampling supplies for field collection of central Beaufort Sea Arctic cod genetic samples in August 2012, and to provide information to help develop recommendations for a pan-Arctic genetic stock separation and genomics study of Arctic cod that will contribute toward an understanding of Arctic cod adaptation across the species' Holarctic distribution.

The FY2011 genetic study assessed the genetic characteristics of Arctic cod in the Beaufort Sea region of the Nearctic relative to the adjacent Chukchi Sea and more distant Atlantic population in St. Lawrence Bay (Talbot et al. 2011). The analyses failed to uncover significant structuring across the Nearctic overall, based on variance in haplotype frequency (F_{ST} = 0.017, P = 0.073; Φ_{ST} = -0.022, P = 0.962). However, we did uncover a signal of differentiation between the Beaufort and Chukchi sea populations of Arctic cod (F_{ST} = 0.043, P = 0.018). This pattern was corroborated by preliminary results from microsatellite loci (J. Nelson, U. Victoria, pers. comm.), which differentiated between Chukchi and Beaufort seas (western populations), but, unlike the mtDNA analyses, also showed differentiation between the western populations and eastern populations (Gulf of St. Lawrence, Baffin Bay, and Trinity Bay).

To further examine levels of population differentiation within the Beaufort Sea, during FY2012 we gathered and analyzed genetic data from Arctic cod sampled in August, 2011, from two general habitat types – slope and shelf habitats --- along three generally longitudinal transects (Figure 2) that aimed to examine also the influence of freshwater input on population structure. Here, we report preliminary results of this effort. We also incorporate these results into the FY2011 results to reassess regional levels of structure.

METHODS

Sample collection and laboratory analyses

Fin clips from 194 Arctic cod collected along longitudinal transects in the central Beaufort Sea (Table 1, Figure 2), collected in August, 2011 and stored in 100% ethanol, were forwarded to the Alaska Science Center (ASC) Molecular Ecology Laboratory (MEL) in July, 2012. Whole genomic DNA was extracted using sans-phenol extraction procedures routinely employed in the MEL and outlined in Handel et al. (2006). We used primers developed during FY2011, using information available in public databases (GenBank: http://www.ncbi.nlm.nih.gov) to target the maternally-inherited mitochondrial DNA *cytochrome b* (cytb) gene, which has already been demonstrated to be polymorphic in Arctic cod globally and in the Beaufort Sea, as well as in sister species (Pálsson et al. 2009, Talbot et al. 2011). These primers targeted for amplification

and sequencing a ca. 878 base pair (bp) portion of the cytb gene for individuals sampled from 5-10 individuals from each of two populations representing target sampling locales in each habitat type. Polymerase chain amplification and sequencing procedures used to collect nucleotide data from the cytb gene were similar to those outlined in Handel et al. (2006).

Data analyses

We used ARLEQUIN ver. 2.0 (Schneider et al. 2000) to estimate haplotype (*h*) and nucleotide (π) diversity (Nei 1987, Eq. 8.4 and 10.6, respectively) at the mtDNA cytb gene. We tested the hypothesis of selective neutrality, and for historical fluctuations in population demography, using Fu's F_S (Fu 1997) and Tajima's *D* (Tajima 1989), implemented in ARLEQUIN. We applied critical significance values of 5%, which requires a *P*-value of below 0.02 for Fu's F_S (Fu 1997). An unrooted phylogenetic tree of cytb haplotypes was constructed using NETWORK 4.6 (Fluxus Technology Ltd. 2011) employing the Reduced Median network method (Bandelt et al. 1995), to illustrate possible reticulations in the gene tree because of homoplasy. Distance matrices were generated, using MEGA 3.1(Kumar et al. 2004), using the close-neighbor interchange option and a search level of 1. Homologous sequence data from a representative of the sister species, *Arctogadus glacialis* (Brienes et al. 2008; GenBank Accession No. AM919429), were included in the analysis.

We further assessed fluctuations in historical demography by generating mismatch distributions (Rogers and Harpending 1992) and raggedness indices (*rg*) of the observed distribution (Harpending 1994) using ARLEQUIN and based on a spatial and sudden expansion model at the population level, and overall. We also tested for evidence of population fluctuations using FLUCTUATE (parameters: 10 short chains, sampling increments = 200, steps per chain = 2,000; 10 long chains, sampling increments = 200, steps per chain = 20,000; random starting tree; starting value of $g = 1$; Kuhner 2006). Because standard deviations are only approximate, and computations may show an upward bias, we used *g* to indicate population growth if $g > 3$ SD.

Levels of population structuring was assessed by testing for differences in the spatial variation in haplotype frequency based on analogs of the *F*-statistics, F_{IS} (Wright 1951) and F_{ST} (Excoffier et al. 1992), which describe the apportionment of haplotypic variance among individuals within and among populations, respectively. Single locus estimates of F_{ST} variance for mtDNA (Φ) overall were obtained using ARLEQUIN. Confidence intervals were used to determine if Φ among populations is significantly greater than zero. Estimates of interpopulational variance (Φ_{ST}) were derived, also using ARLEQUIN. Pairwise estimates for mtDNA was weighted by incorporating a model of evolution determined using Modeltest 3.06 (Posada and Crandall 1998). Significance of Φ_{ST} values were based on random permutation tests ($n = 10{,}000$), whereby haplotypes are randomly permutated between populations.

We used hierarchical analyses of molecular variance (AMOVA; Excoffier et al. 1992) to test for significance of geographic partitioning of slope versus shelf aggregations; these represented *a priori* hypothesized genetic units. Analyses of variance incorporated genetic distances (AMOVA), and incorporated frequencies alone (ANOVA). Support for the genetic partitioning of shelf vs. slope aggregations would be based on significant values of among-habitat (group) variance (Φ_{CT}). However, since significant genetic partitions can occur for

multiple reasons, we also tested other, *a posteriori* partitions that might indicate regional substructuring, such as along a longitudinal gradient, or other arbitrary groupings. We assumed that maximal Φ_{CT} values that were significantly different from random distributions of individuals were the most probable geographical subdivisions among hypotheses tested. Thus, given concordance between the distribution of genetic subdivisions at the neutral genetic markers and subspecies delineations, Φ_{CT} should be significant, and account for more among-group variation than for alternative groupings.

RESULTS AND DISCUSSION

We amplified and sequence the cytb gene from 108 individuals (n = 4 – 10 for each of 2 populations along the northern and southernmost end of the 3 transects; see Table 1, Figure 2). For some analyses, we incorporated information gathered during FY2011 from 90 individuals (n = 30 for each Beaufort Sea 2008 samples, BS; middle Chukchi Sea 2008 samples, MC; St. Lawrence Bay, SL; see Figure 1, Table 2). The amplified 878 bp fragment consistently yielded 707 bp sequence product for all individuals, and all analyses were conducted on this 707 bp fragment.

Forty-six haplotypes (CB02-CB47) were observed across all 3 locales (Table 1), almost doubling the number of haplotypes observed across the BS, MC and SL populations assayed during FY2011. Haplotypes CB02, CB04, CB05, CB06 and CB07 were the most common, occurring in >68% of all samples assayed (Table 1). Forty-one of the 707 nucleotide sites consistently recovered across all samples were variable; of these, 16 substitutions were parsimony informative, 39 were transitions, 1 was a transversion, and 1 was multistate (Table 2).

A visual representation of the genealogical relationships among haplotypes is shown in Figure 3; the distribution of haplotypes by aggregation (SL, MC, BS 2008, BS2011), is shown in Figure 4. As found in the samples assayed in FY2011, we observed two groups within the genealogy of Arctic cod, Group 1 and Group 2 (Figures 3, 4), and new haplotypes were added to both groups. Pairwise differences Group 2 haplotypes (CB07, 08, 09, 22, 29, 30, 31, 32, 38, 43) and Group 1 haplotypes averaged 0.012, whereas the average number of pairwise differences within the two groups was 0.005 (Group 1) and 0.003 (Group 2). Haplotype Group 2 is the only group that received bootstrap support of >70% in the minimum evolution phylogenetic tree shown in Figure 3 (here, 76% BP), even when samples from Iceland and Greenland, obtained from GenBank, were included for comparison (data not shown). All populations included individuals carrying haplotypes from both Group 1 and Group 2; that is, there appears to be no simple concordance between the distribution of Group 1 and 2 haplotypes and geography.

Genetic diversity and demographic fluctuations

Haplotype diversity (*h*) is relatively high across populations, with both haplotype and nucleotide (π) diversity highest in the Beaufort Sea and lowest in the middle Chukchi Sea (Table 3). Fu's F_S and Tajima's *D* values significantly negative for all populations, suggesting the portion of the mtDNA cytb gene assayed conforms to neutral expectations (Table 3) but the populations underwent expansion, historically. However, we observed no signal of population expansion based on significantly large *g* (not observed, Table 3; an exception is BS2011). Raggedness

values (*rg*) range from 0.023 to 0.036 (Table 3), and although distributions were not significantly ragged, all populations demonstrated a bimodal distribution in mismatch distributions, suggesting either population stability, or population structuring, perhaps due to amalgamation of several populations (data not shown). This result is generally concordant with the results based on a smaller fragment size from cytb, reported by Pálsson et al. (2009). They also observed a bimodal mismatch distribution, gathered from populations elsewhere in the species' range, although they observed a signal of recent expansion within a "Bering Strait" sample. Pálsson et al. (2009) suggest that Arctic cod may have occupied a wider distribution at lower latitudes in North America and the Pacific Ocean, similar to the current range of the closely-related *Arctogaus glacialis*, and that previously segregated, well-defined lineages of Arctic cod may be mixed in current populations.

Population differentiation

Analyses of molecular variance (AMOVA) failed to detect a significant signal of differentiation among populations (Φ_{SC} = 0.025, P = 0.201; F_{SC} = -0.003, P = 0.323) or between Arctic cod sampled from the shelf, and those sampled from the slope, whether incorporating a model of evolution, or based on frequencies only (Φ_{CT} = -0.018, P = 0.989; F_{CT} = 0.011, P = 0.08, respectively). Similarly, AMOVA failed to detect differentiation between Arctic cod collected along the 3 longitudinal transects (Φ_{CT} = -0.002, P = 0.399; F_{CT} = -0.004, P = 0.645, respectively: see Figure 2). These results suggest Arctic cod aggregating within the central Beaufort Sea belong to a single, interbreeding unit. We are currently augmenting these analyses with data from an additional 78 individuals across the slope and shelf populations, to ensure that the failure to detect differentiation between either the latitudinal or longitudinal transects is due to a Type 2 statistical error.

However, we found significant, although shallow, differentiation between Arctic cod occupying the central Beaufort Sea region sampled in 2011, and those sampled farther to the west in 2008 (F_{ST} = 0.023, P = 0.024; Table 4). Exploratory analyses rejected the hypothesis that increased sample size for the 2011 effort was responsible for the observed signal of differentiation between samples collected in 2008 (n = 30) and those collected during 2011 (n = 108). Surprisingly, however, we failed to reject the null hypothesis of panmixia between the central Beaufort Sea (2011 samples) and the Chukchi Sea population (F_{ST} = -0.010, P = 0.89; Table 4), collected in 2008 (Figure 1) Population structuring within the Beaufort Sea Arctic cod aggregations appears to be complex, and analyses from nuclear genes (microsatellite loci) might be needed to better understand the relationships within the Arctic cod aggregations there.

LITERATURE CITED

Bandelt H. J., P. Forster, B. C. Sykes, and M. B. Richards. 1995. Mitochondrial portraits of human populations.Genetics 141:743-753.

Breines, R., A. Ursvik, M. Nymark, S. D. Johansen, and D. H. Coucheron. 2008. Complete mitochondrial genome sequences of the Arctic Ocean codfishes *Arctogadus glacialis* and *Boreogadus saida* reveal oriL and tRNA gene duplications. Polar Biol. 31:1245-1252.

Chen, L., A. L. DeVries, and C.-H. C. Cheng. 1997. Convergent evolution of antifreeze glycoproteins in Antarctic notothenioid fish and Arctic cod. Proc. Natl. Acad. Sci. USA 94:3817-3822.

Cheung, W.W.L., V.W.Y. Lam, and D. Pauly, *Dynamic bioclimate envelop model to predict climate-induced changes in distribution of marine fishes and invertebrates.*, in *Modelling Present and Climate-shifted Distribution of Marine Fishes and Invertebrates. Fisheries Centre Research Report*, W.W.L. Cheung, V.W.Y. Lam, and D. Pauly, Editors. 2008, The Fisheries Centre, University of British Columbia: Vancouver. p. 5-50.

Durner, G.M., D. C. Douglas, R. M. Nielson, R.M., S. C. Amstrup, T. L. McDonald, I. Stirling, M. Mauritzen, E. W. Born, E.W., Ø Wiig, E. DeWeaver, M. C. Serreze, S. Belikov, M. Holland, J. A. Maslanik, J. Aars, D. Bailey, and A. E. Derocher. 2009. Predicting 21st-century polar bear habitat distributionfrom global climate models. Ecological Monographs 79:25–58.

Enevoldsen, L.T., Heiner, I., and DeVries, A.L. 2003. Does fish from the Disko Bay area of Greenland possess antifreeze proteins during the summer? Polar Biol. 26:365–370.

Excoffier, L., P. E. Smouse, and J. M. Quatro. 1992. Analysis of molecular variance from metric distances among DNA haplotypes: applications to human mitochondrial DNA restriction data. Genetics 131:479-491.

Fluxus Technologies Inc. 2011. NETWORK Ver. 4.6. Available online at <www.fluxus-engineering.com> (April 14, 2011).

Fu, Y. X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selections. Genetics 147:915-925.

Gradinger, R., and B. Bluhm. 2004. In-situ observations on the distribution and behaviour of amphipods and Arctic cod (*Boreogadus saida*) under the sea ice of the High Arctic Canada Basin. Polar Biol. 27:595-603.

Handel, C. M., L. M. Pajot, S. L. Talbot and G. K. Sage. 2006. Use of buccal swabs for sampling DNA from nestling and adult birds. Wildlife Society Bulletin 34:1094-1100.

Harpending, H. C. 1994. Signature of ancient population growth in a low-resolution mitochondrial mismatch. Hum. Biol. 66:591-600.

Howes, B. J., J. W. Brown, H. L.Gibbs, T. B. Herman, S. W. Mockford, K. A. Prior, and P. J. Weatherhead. 2009. Directional gene flow patterns in disjunct populations of the black ratsnake (*Pantheropis obsoletus*) and Blanding's turtle (*Emydoidea blandingii*). Conservation Genetics 10: 407-417.

Jay, C. V., P. M. Outridge, and J. L. Garlich-Miller. 2008. Indication of two Pacific walrus stocks from whole tooth elemental analysis. Polar Biol. 31:933.

Kuhner, M. 2006. LAMARC 2.0: Maximum likelihood and Bayesian estimation of population parameters. Bioinformatics 22:768-770.

Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Briefings in Bioinformatics 5:150-163.

Lønne, O.J., and B. Gulliksen 1989. Size, age and diet of Polar cod, *Boreogadus saida* (Lepechin 1773), in ice covered waters. Polar Biol. **9**: 187–191.

Nei, M., T. Maruyama, and R. Chakraborty. 1975. The bottleneck effect and genetic variability in populations. Evolution 29: 1-10.

Pálsson, S., T. Källman, J. Paulsen, and E. Árnason. 2009. An assessment of mtDNA variation in Arctic gadoids. Polar Biology 32: 471-479.

Posada, D., and K.A. Crandall. 1998. MODELTEST: testing the model of DNA substitution. – Bioinformatics 14: 817-818.

Rogers, A. R. 1995. Genetic evidence for a Pleistocene population explosion. Evolution 49:608-

615.
Rogers, A. R., and H. Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. 9: 552-569.
Schneider, S., D. Roessli, and L. Excoffier. 2000. ARLEQUIN version 2.0: a software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Geneva.
Scribner, K. T., J. A. Blanchong, D. J. Bruggeman, B. K. Epperson, C-Y Lee, Y-W Pan, R. I Shorey, H. H. Prince, S. R. Winterstein, and D. R. Luukkonen. 2005. Geographical genetics: conceptual foundations and empirical applications of spatial genetic data in wildlife management. Journal of Wildlife Management 69: 1434-1453.
Sonsthagen, S.A., S.L. Talbot, R.B. Lanctot, K. Scribner, and K.G. McCracken. 2009. Hierarchical spatial genetic structure of Common Eiders (Somateria mollissima) along a migratory corridor. Auk. 126:744–754.
Stroeve, J., M.M. Holland, W. Meier, T. Scambos, M. Serreze. 2007: Arctic sea ice decline: Faster than forecast. Geophysical Research Letters, 34, L09501, [Available online at http://www.agu.org/pubs/crossref/2007/2007GL029703.shtml]
Stroeve, J.C., M.C. Serreze, M.M. Holland, J.E. Kay, J. Malanik, A.P. Barrett. 2011: The Arctic's rapidly shrinking sea ice cover: a research synthesis. Climatic Change, 110, 1005-1027. [Available online at http://www.springerlink.com/index/10.1007/s10584-011-0101-1]
Talbot, S. L., G. K. Sage, and S. A. Sonsthagen. 2011. Arctic cod genomics study: Preliminary results from analyses of mitochondrial DNA. U. S. Geological Survey interim report to BOEM, 8 July, 2011.
Tajima, F. 1989. The effect of change in population size on DNA polymorphism. Genetics 123: 597-601.
Tietsche, S., D. Notz, J.H. Jungclaus, J. Marotzke. 2011: Recovery mechanisms of Arctic summer sea ice. Geophysical Research Letters, 38, L02707. [Available online at http://www.agu.org/pubs/crossref/2011/2010GL045698.shtml]
Wang, M., J. E. Overland. 2009: A sea ice free summer Arctic within 30 years. Geophysical Research Letters, 36, 5, [Available online at http://www.agu.org/pubs/crossref/2009/2009GL037820.shtml]
Wang, M.,J.E. Overland. 2012: A sea ice free summer Arctic within 30 years: An update from CMIP5 models. Geophysical Research Letters, 39, L18501
Waples, R. D. 1998. Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. J. Hered. 98: 438-450.
Weckworth, B. V., S. L. Talbot, G. K. Sage, J. A. Cook. 2005. A signal for independent coastal and continental histories among North American wolves. Molecular Ecology 14: 917-931.
Weckworth, B. V., S. L. Talbot and J. A. Cook. 2010. Phylogeography of wolves in the Pacific Northwest. Journal of Mammalogy 91: 363-375.
Wright, S. 1951. The genetical structure of populations. Ann. Eugen. 15: 323-354.

Table 1. Samples collected in August 2011 (see Figure 2 for site location) and analyzed in this study.

Site	Habitat	Number of Samples Provided	Number of Samples Analyzed
CB06	shelf	25	12
CB08	shelf	9	7
CB26	slope	18	10
CB27	slope	15	10
EB08	shelf	27	9
EB14	shelf	4	4
EB23	slope	20	10
EB32	slope	12	10
WB02	slope	19	10
WB05	slope	14	10
WB30	shelf	26	11
WB35	shelf	5	5
Total	**n/a**	**194**	**108**

Table 2. Nucleotide sequence differences among 46 mitochondrial DNA *cytochrome b* haplotypes and distribution of haplotypes among 30 individuals sampled from St. Lawrence Bay (SL), the Beaufort Sea during 2008 (BS 2008) and the middle Chuckchi Sea (MC), and 108 individuals sampled from the central Beaufort Sea during 2011 (BS 2011). Variable site position numbers (read vertically) refer to the location of each variable site in a 707 base pair sequence. Dots indicate similarity with haplotype CB02.

	Variable Site Positions	Populations				
	0000000111122223333334455555555555566666666					
	0234579033815780111275723335667899002347 9	SL	BS		MC	**Total**
Haplotype Designation	3138123228062623358426721272489447361022 0		2008	2011		
CB02	CTCCCTATTCGAATATGCTCGAACTCCGAGTCATTGATGGA	4	3	6	3	**16**
CB03	....T......G.............T..............	–	1	–	–	**1**
CB04	.....	9	7	20	6	**42**
CB05	.....T.....	7	1	29	11	**48**
CB06	.....G..	1	3	8	3	**15**
CB07	.....C.C.T.......T.................A.CG..	2	5	10	3	**20**
CB08	.....C.C.T.......T.................ATCG..	1	–	–	–	**1**
CB09	...A.C.C.T.......T.................A.CG..	1	1	1	–	**3**
CB10	..T.....T.....	1	–	–	–	**1**
CB11	.........T.....A..G..	2	–	3	1	**6**
CB12	.....C.....	1	–	1	–	**2**
CB13	.....T.......C.....	–	2	1	–	**3**
CB14	.....TT.....	–	1	–	–	**1**
CB15	.....C.....T.....	–	1	–	–	**1**
CB16	.....T..G.....	–	1	–	–	**1**
CB17	.....T.....A.....A..G..	1	–	–	–	**1**
CB18	.....A.....	–	1	1	–	**2**
CB19	.....T.....A.G..	–	1	1	–	**2**
CB20	.....T.....A..GA.	–	–	1	1	**2**
CB21	.....A.....	–	–	1	1	**2**
CB22	...T.C.C.T.......T.................A.CG..	–	–	–	1	**1**
CB23	.....A.....T....A.G..	–	1	1	–	**2**

CB24	.....G..T.....	–	1	1	–	**2**
CB25	.....A....	–	–	1	–	**1**
CB26	.....A.....G	–	–	1	–	**1**
CB27	.....G	–	–	1	–	**1**
CB28	.....T.....G.....A.....A..G..	–	–	1	–	**1**
CB29	.....CGC.T.....T.....A.CG..	–	–	1	–	**1**
CB30	.C...C.C.T.....T.....A.CG..	–	–	1	–	**1**
CB31	.....C.C.T.....T.....T.....A.CG..	–	–	1	–	**1**
CB32	.....C.T.....T.....A..G..	–	–	1	–	**1**
CB33	.....C.....	–	–	1	–	**1**
CB34	.....T..G.....A..G..	–	–	1	–	**1**
CB35	.....C.....	–	–	1	–	**1**
CB36	.....T.....G.....	–	–	1	–	**1**
CB37	T.....T.....	–	–	1	–	**1**
CB38	.....C.CCT.....T.....A.CGA.	–	–	1	–	**1**
CB39	.....G.....A.G..	–	–	1	–	**1**
CB40	.....G.....T.....	–	–	1	–	**1**
CB41	.....A.....A.G..	–	–	1	–	**1**
CB42	.....C.....	–	–	1	–	**1**
CB43	.....C.C.T.....T.....C.....A.CG..	–	–	1	–	**1**
CB44	.....C.....G..	–	–	1	–	**1**
CB45	.....T.....	–	–	1	–	**1**
CB46	.....A.....	–	–	1	–	**1**
CB47	.....T.....C.....	–	–	1	–	**1**

Table 3. Genetic diversity and historical fluctuations in population demography at the mtDNA *cytochrome b* gene among Arctic cod sampled from St. Lawrence Bay (SL), the Beaufort Sea 2008 (BS 2008) and 2011 (BS 2011), and the middle Chuckchi Sea (MC). *h* = haplotype diversity (Nei 1987); π = nucleotide diversity (Nei 1987), *g* (SD) = growth and standard deviation (Kuhner 2006), Θ (SD) = mutation paramteter (Rogers 1995); τ = time to demographic expansion (Rogers 1995), *rg* = raggedness index (Harpending 1994), *n* = sample size per population.

	Population			
Parameter	SL	BS 2008	BS 2011	MC
h	0.851	0.913	0.881	0.818
π	0.0046	0.0057	0.0050	0.0038
Tajima's *D*	**-0.121**	**-0.705**	**-1.095**	**-0.731**
Fu's F_S	**-2.065**	**-4.649**	**-22.053**	**-0.237**
g (SD)	226.9	538.4	**-19.4**	13.4
	(161.9)	(192.4)	(4.353)	(168.7)
Θ (SD)	0.0052	0.0151	0.0214	0.0039
	(0.0011)	(0.0030)	(0.0019)	(0.0009)
τ	8.2	9.0	8.6	8.1
	(1.04–12.54)	(2.40–13.36)	(1.18–12.44)	(0.19–11.78)
rg	0.039	0.023	0.024	0.041
n	30	30	108	30

Table 4. Results of pairwise tests for population differentiation based on mtDNA haplotype data: F_{ST} above the diagonal and Φ_{ST} (K81 uf + I (rates = equal, Pin = 0.8725) below. Significant comparisons are in bold text.

	SL	**BS 2008**	**BS 2011**	**MC**
SL	–	0.013	-0.003	-0.005
BS 2008	-0.023	–	**0.023**	**-0.044**
BS 2011	-0.017	0.012	–	-0.010
MC	-0.021	-0.007	-0.014	–

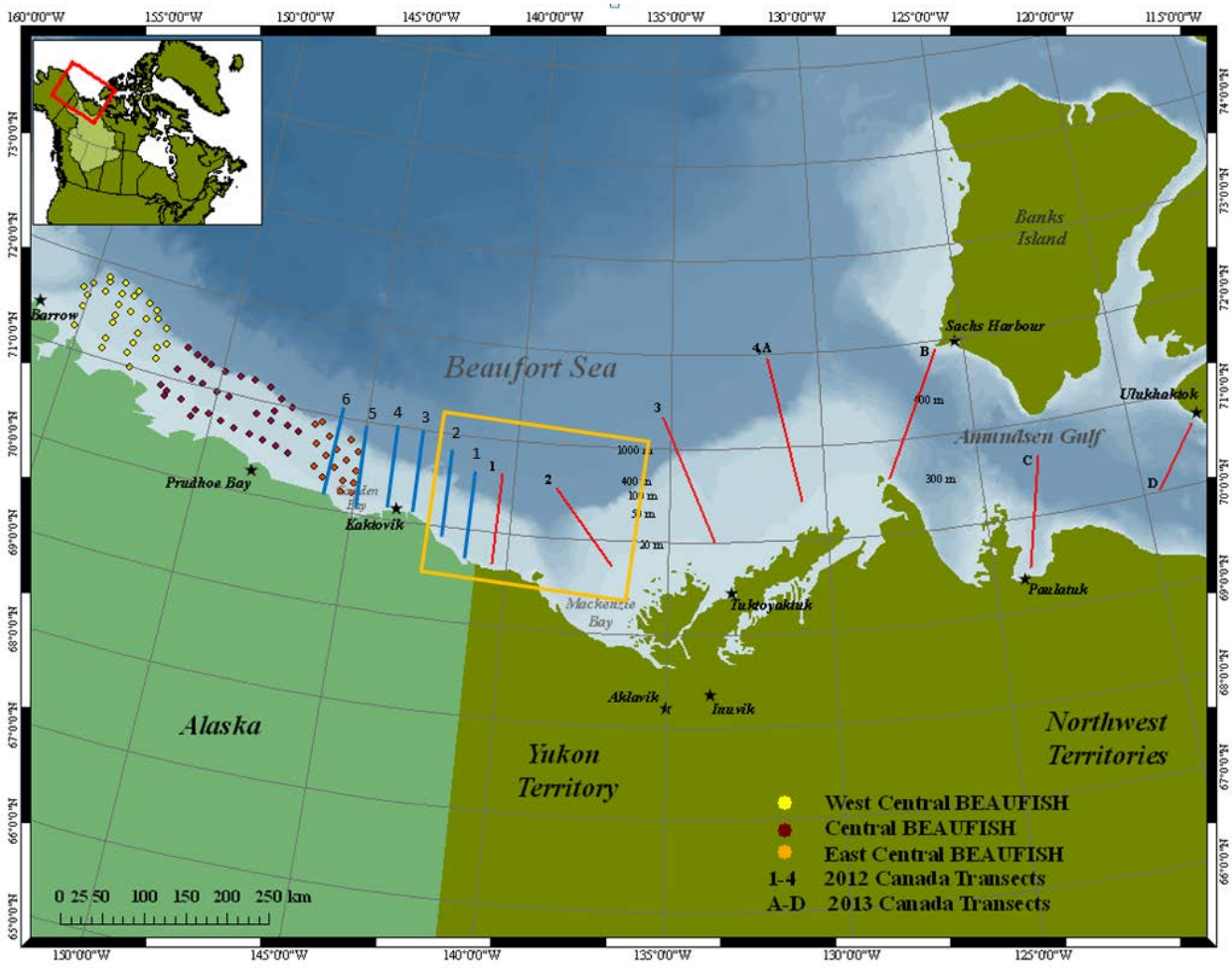

Figure 1. Location of sampling sites for 2008 (yellow dots), 2011 (red dots), and 2012 (orange box). Sample transects to the east are planned for 2013. Image courtesy of K. Wedemeyer, BOEM. See Table 1 for sample sizes.

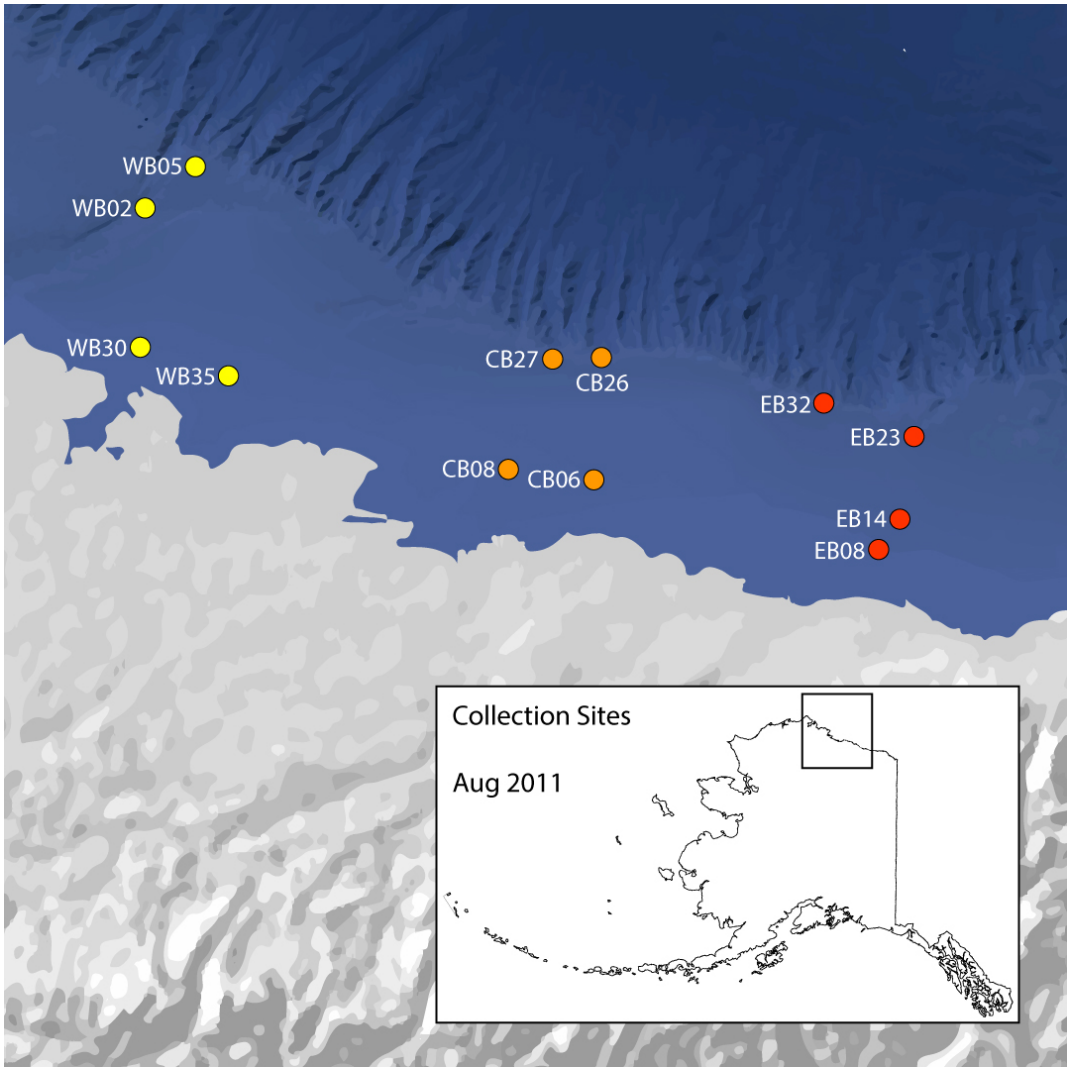

Figure 2. Sites in the Central Beaufort Sea where Arctic cod were sampled during August, 2011. Slope populations included WB02, WB05, CB26, CB27, EB23 and EB32. Shelf populations included WB30, WB35, CB06, CB08, EB08 and EB14.

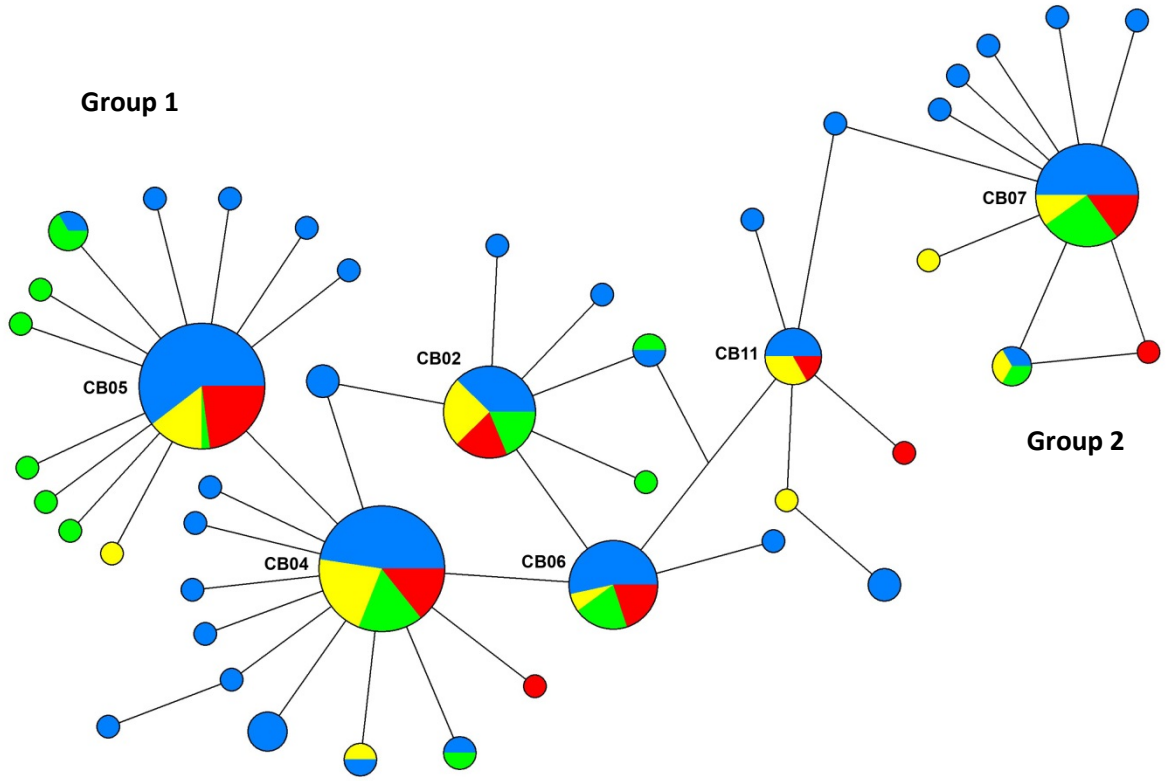

Figure 3. Parsimony network illustrating the relationship of 46 mtDNA *cytochrome b* haplotypes (CB2–CB47) assayed from Arctic Cod. Size of the node corresponds to the frequency of each haplotype. SL haplotypes are illustrated in yellow, BS 2008 in green, BS2011 in blue, and MC in red. All line segments represent a difference at one nucleotide position between neighboring haplotypes.

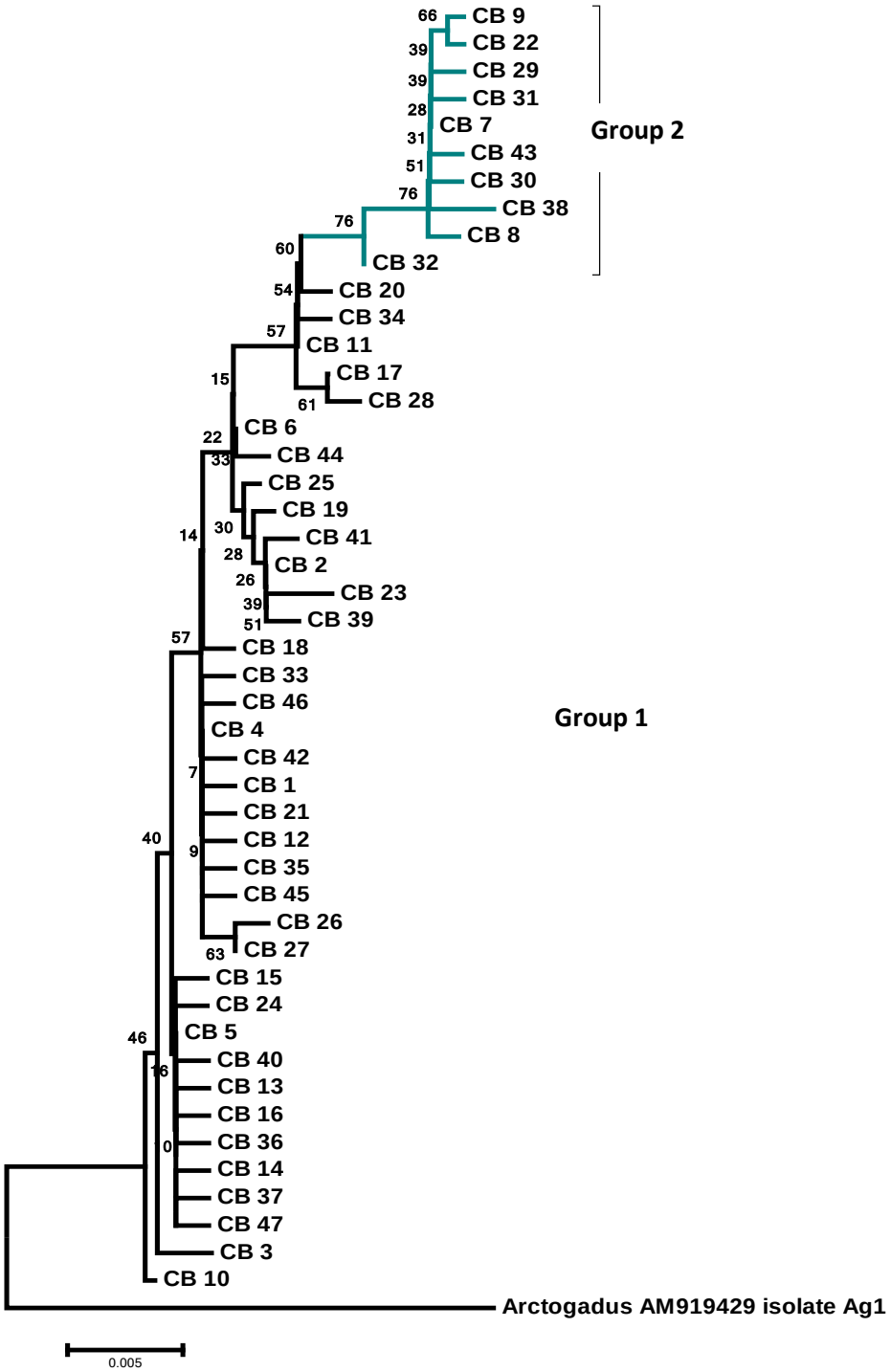

Figure 4. Minimum evolution distance tree showing phylogenetic relationships among 47 mtDNA *cytochrome b* haplotypes (CB2–CB47) assayed from Arctic Cod. Numbers in nodes indicate bootstrap support for indicated cluster. The line at the base of the tree represents K81 uf + I (rates = equal, Pin = 0.8725) distance lengths. CB1 here represents Arctic cod (*Boreogadus saida*) cytochrome b sequences obtained from GenBank (Accession No. AM919428; Brienes et al. 2008). *Arctogadus glacialis* (GenBank Accession No. AM919429; Brienes et al. 2008*)* is included as an outgroup.

DISCLAIMER

Study concept, oversight, and funding were provided by the US Department of the Interior, Bureau of Ocean Energy Management, Environmental Studies Program, Washington, DC. This report has been technically reviewed by BOEM and it has been approved for publication. The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the opinions or policies of the US Government, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

REPORT AVAILABILITY

To download a PDF file of this Gulf of Mexico OCS Region report, go to the US Department of the Interior, Bureau of Ocean Energy Management, Environmental Studies Program Information System website and search on OCS Study BOEM 2014-050.

This report can be viewed at select Federal Depository Libraries. It can also be obtained from the National Technical Information Service; the contact information is below.

US Department of Commerce
National Technical Information Service
5301 Shawnee Rd.
Springfield, VA 22312
Phone: (703) 605-6000, 1(800)553-6847
Fax: (703) 605-6900
Website: http://www.ntis.gov/

CITATION

Sandra L. Talbot, George K. Sage, Sarah A. Sonsthagen, Megan C. Fowler. 2014. Arctic Cod Pilot Genomics Study: Preliminary Results from Analyses of Mitochondrial DNA. US Dept. of the Interior, Bureau of Ocean Energy Management, Alaska BOEM region Anchorage, Alaska. OCS Study BOEM 2014-050. 19 pp.

The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under US administration.

BOEM
Bureau of Ocean Energy Management

The Bureau of Ocean Energy Management

As a bureau of the Department of the Interior, the Bureau of Ocean Energy (BOEM) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS) in an environmentally sound and safe manner.

The BOEM Environmental Studies Program

The mission of the Environmental Studies Program (ESP) is to provide the information needed to predict, assess, and manage impacts from offshore energy and marine mineral exploration, development, and production activities on human, marine, and coastal environments.